Deciphering common and rare genetic effects on reading ability

ISBN: 978-90-76203-78-2
Printed and bound by Ipskamp Drukkers

Deciphering common and rare genetic effects on reading ability

Proefschrift
ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op maandag 7 november 2016
om 12.30 uur precies

door
Amaia Carrión Castillo
geboren op 22 november 1988
te Donostia (Spanje)

Promotor
Prof. dr. Simon E. Fisher

Copromotor
Dr. Clyde Francks (MPI)

Manuscriptcommissie
Prof. dr. Anneke I. den Hollander
Prof. dr. Juha Kere (Karolinska Institutet, Zweden)
Prof. dr. Gerd Schulte-Körne (Ludwig-Maximilians-University Munich, Duitsland)

Deciphering common and rare genetic effects on reading ability

Doctoral Thesis
to obtain the degree of doctor
from Radboud University Nijmegen
on the authority of the Rector Magnificus prof. dr. J.H.J.M. van Krieken,
according to the decision of the Council of Deans
to be defended in public on Monday, November 7, 2016
at 12.30 hours

by
Amaia Carrión Castillo
Born on 22 November 1988
in Donostia (Spain)

Supervisor
Prof. dr. Simon E. Fisher

Co-supervisor
Dr. Clyde Francks (MPI)

Doctoral Thesis Committee
Prof. dr. Anneke I. den Hollander
Prof. dr. Juha Kere (Karolinska Institutet, Sweden)
Prof. dr. Gerd Schulte-Körne (Ludwig-Maximilians-University Munich, Germany)

Etxekoei

CONTENTS

1

GENERAL INTRODUCTION

Reading is a paradigmatic case of a human cultural achievement. It is a fairly new development: the earliest written records date back a few thousand years (Rogers, 2004), and draw a boundary between history and prehistory. Scripts are thought to have evolved independently in different cultures (Coulmas, 2003), such as the cuneiform script in Mesopotamia, the Chinese script in Asia (Boltz, 1986), and the Olmec script in Mesoamerica (Rodriguez Martinez et al., 2006). The ability to encode language into a written form, and to decode the written language by reading, has shaped recent human societies and history. Despite this central role, being able to read is neither a necessary or defining skill for humans: not all cultures have it, and even in the ones that developed a writing system, mastering this skill was typically restricted to a minority of the population.

Notwithstanding that it is a learned cultural trait, the ability to read and write is an instantiation of specific neurobiological and cognitive systems that are in place in the human species. The main aim of the present thesis is to study the genes that underlie those systems, and to see how genetic variation influences variation in reading ability.

1.1 THE LEARNING-TO-READ BRAIN

Reading consists of deciphering information that is in written form back to linguistic form. So as to access the content of the linguistic information, reading requires the reader to find a correspondence between the written characters (graphemes) and representations of speech sounds (phonemes) or other units of language that convey meaning (e.g. morphemes). Hence, language is a prerequisite for learning this highly specialized skill (Peterson and Pennington, 2015), although this relationship becomes less hierarchical as reading co-occurs and feeds back into some aspects of language

acquisition in school-age children. Consequently, several language-related cognitive processes play an important role and co-occur during the reading process. Coming to an explicit awareness of the sound structures of words (phoneme awareness) is required to enable the automatic matching of letters to sounds (van der Leij et al., 2013; Peterson and Pennington, 2015). For example, to decode the word 'cat', you need to identify and manipulate the corresponding speech sounds (the phonemes /k/, /æ/ and /t/). Phoneme awareness can be assessed using tasks such as phoneme deletion, where participants are asked to identify how a word would be said if one sound were omitted (e.g. how would *cat* sound without the /k/? /æt/). Fast processing speed is also important for timing mechanisms involved in reading (de Jong and van der Leij, 2003; Pennington, 2006). Processing speed can be assessed using rapid naming tasks where participants have to name a number of highly familiar symbols (which can be digits, letters, colours or pictures) as fast as possible (de Jong and van der Leij, 2003). Performance on such tasks correlate with reading ability, and have varying predictive powers depending on age and reading experience (van der Leij et al., 2013), and whether the way in which the words of a language are spelled (their orthographic system) is more or less consistent (Landerl et al., 2013; Caravolas et al., 2013). The consistency of orthography varies across languages. For example, the sound /u/ is systematically written as 'u' in different contexts in Basque ('su', 'zugan', 'gu', 'lur') or Spanish ('burro', 'bruja', 'bruno') and as 'oe' Dutch ('koe', 'moe', 'goed', 'boer'), whereas the sound /uː/ [1] is written in multiple forms in English ('too', 'true', 'shoe', 'flew', 'through').

A highly organized cortical system that integrates information on several aspects of words (i.e. the written form, the speech sounds, and the meaning) has been described in the adult literate brain (Price, 2012). It includes three main areas which are usually co-located in the left hemisphere: a posterior region in the dorsal inferior parietal lobule (IPL), a posterior ventral occipitotemporal region (OT), and an anterior area around the inferior frontal gyrus (IFG) (see Figure 1.1). The same brain networks are usually involved in skilled readers across different languages and writing systems (Rueckl et al., 2015), and are likely constrained by networks underlying processing of spoken language. Nevertheless, they are not hard-wired: learning to read exemplifies brain plasticity as the relevant circuitry is refined upon acquisition of literacy (Dehaene et al., 2015).

1 In English, the closest equivalent of the phoneme /u/ of the other three languages is specified as being long, hence the different phonetic symbol used, i.e. /uː/.

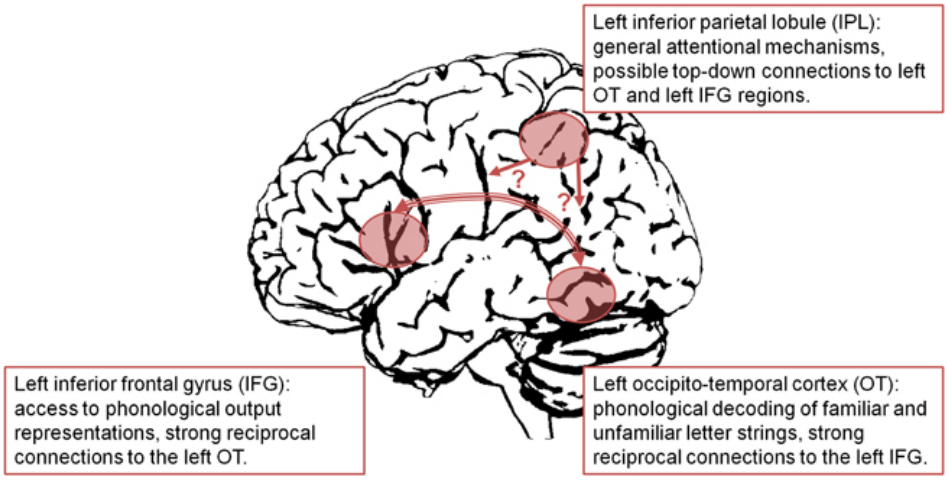


Figure 1.1: Left hemisphere reading network, taken from Richlan (2012).

1.2 VARIATION IN READING ABILITY

Mastering reading is not equally challenging for everyone. Reading ability is a continuously distributed trait in the population, and it has been proposed that the underlying causal mechanisms are similar across the distribution. Individuals that fall at the low end of the distribution are categorized as dyslexic (Shaywitz et al., 1992).

The dichotomization of any continuous trait relies on establishing a threshold which, as in the case of dyslexia, can be arbitrary (Bishop, 2015; Peterson and Pennington, 2015). For research purposes, a threshold reading performance of 1.5 standard deviations (SD) below the normative mean for a child's age has been used (Shaywitz et al., 1990; Peterson and Pennington, 2015), although this cut-off varies across studies (usually between -2SDs and -1SDs). An alternative diagnostic criterion is defined by the discrepancy between the observed performance and the predicted level of proficiency given IQ (Peterson and Pennington, 2015), although the use of IQ for dyslexia diagnosis is currently debated.

The definition of dyslexia is based on the exclusion of other possible causes that could explain the difficulty in mastering reading (e.g. intellectual disability, inadequate exposure to reading, or other obvious causes like comorbid neurological conditions or a history of head injury) (Grigorenko, 2001). The prevalence of dyslexia is one of the highest for neurodevelopmental disorders, with estimates ranging from 5-10% (Shaywitz et al., 1990), although these figures vary across countries and sex

(male:female ratio 3.5-4.01 in epidemiological samples) (Pennington, 1990), and are dependent on the diagnostic criteria. Despite the relative arbitrariness of the definition, dyslexia highlights the importance of reading in current modern societies since the dyslexic person is at a disadvantage compared to normal readers, resulting in relatively reduced educational and professional achievements. According to the UK's Dyslexia Institute, undiagnosed dyslexic people also have a cost for society, since they are more likely to be excluded from school or unemployed for long periods of time.

1.3 COMPLEX AND MULTIFACTORIAL AETIOLOGY

Multiple factors influence reading ability. On the one hand, environment plays a crucial role: the most drastic example being that without exposure to text, no reading ability can develop. Important environmental factors include home literacy environment, socio-economic status, and parental education (Peterson and Pennington, 2015). Genetic factors also affect variation: reading difficulties are familial, and heritability studies have estimated that 0.40-0.80 of the liability to dyslexia is due to genetic variation (DeFries et al., 1987). Genetic influences are not restricted to the diagnosis of dyslexia, but also affect normal variation in reading performance (heritabilities estimated from 0.57-0.67) (Harlaar et al., 2007; de Zeeuw et al., 2015), as well as several skills that support reading ability, including rapid naming (0.46) and phonological awareness (0.61) (Petrill et al., 2006).

The molecular genetic framework underlying this genetic component is complex and heterogenic. Many genes are likely to contribute to the liability of reading difficulties. As for other complex traits (Gottesman and Gould, 2003) a multifactorial threshold model is assumed; it is thought that most genetic risk variants each explain a very small fraction ($<1\%$) of the total variance and are common in the general population (i.e. with a minor allele frequency (MAF) higher than 5%). People below the diagnostic threshold for dyslexia are enriched for those variants that act as additive risk factors. However, common variation might not be able to explain the heritability estimates entirely by itself. There may also be variants that are rare in the general population (MAF$<1\%$) but that have a substantial penetrance for the carriers. These can possibly explain the dominant Mendelian inheritance pattern of dyslexia that has been observed in some families. Hence, both common and rare variants potentially contribute to complex traits such as reading (dis)ability (Schork

et al., 2009), and the genetic risk factors for dyslexia liability are heterogeneous (Figure 1.2). Moreover, these risk factors might interact non-additively with each other (epistasis) (Mascheretti et al., 2015) and with the environment (gene x environment interaction) (Mascheretti et al., 2013).

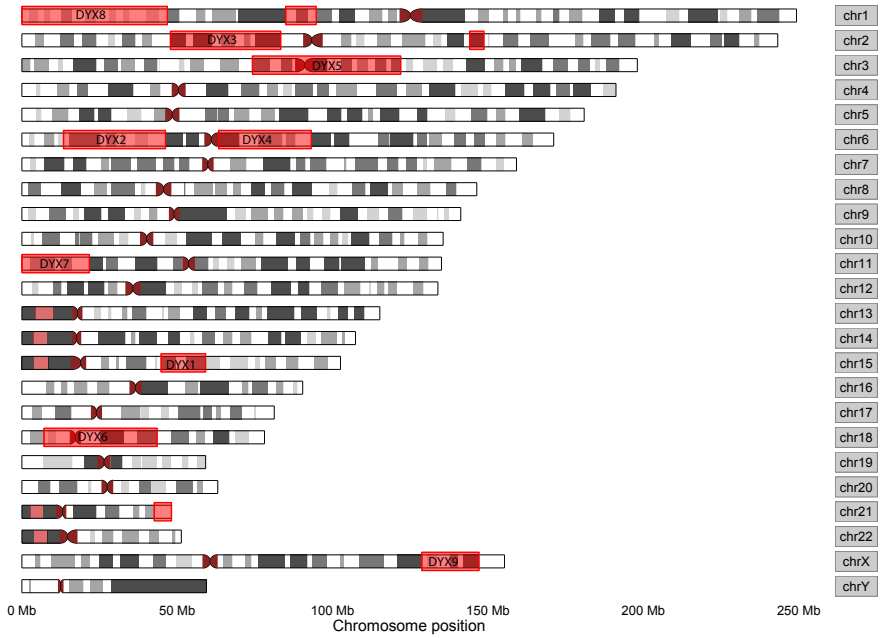


Figure 1.2: Ideogram of the genome, with dyslexia linked regions highlighted in red, and the most prominent susceptibility loci labelled as DYX1-DYX9.

As discussed in more detail later in this thesis, multiple dyslexia susceptibility loci in the genome (DYX1-DYX9) have been identified through linkage analysis studies and/or chromosomal rearrangements cosegregating with dyslexia in families (summarized in Figure 1.2). Candidate genes within some of these loci have been proposed, including *DYX1C1* (Taipale et al., 2003) and *CYP19A1* (Anthoni et al., 2012) in DYX1 (chr15q); *KIAA0319* (Francks et al., 2004; Cope et al., 2005) and *DCDC2* (Meng et al., 2005) in DYX2 (chr6p); *MRPL19* and *C2ORF3* (Anthoni et al., 2007) in DYX3 (chr2p); and *ROBO1* in DYX5 (chr3) (Hannula-Jouppi et al., 2005). Variation within these genes has been associated with dyslexia and reading-related quantitative traits in independent studies, and functional investigations of the proteins they encode has revealed common biological pathways in which several of these genes are implicated including axon guidance, dendrite outgrowth and ciliary biology (Adler et al., 2013;

Lamminmaki et al., 2012; Ivliev et al., 2012; Peschansky et al., 2010), further discussed in later chapters of this thesis.

In the past few years, there have been several technological advances in molecular methods. On the one hand, high throughput genotyping (SNP-chips) have become cost-effective, which have enabled moving from the 'candidate-gene' association studies to a whole-genome oriented approach to assess common genetic variation in reading abilities and dyslexia through genome-wide association scans (GWAS). Several GWAS studies have been performed with the aim to identify other common genetic variants associated with variation in the reading phenotype. These studies did not yet yield any associations that exceed standard thresholds for genome-wide significance, most likely as a consequence of low power due to limited sample size. Nonetheless, they have opened a promising avenue, identifying new suggestive association signals on chromosomes 1p13.1, 3p24.3, 4q26, 5q35.1, 7q32.1, 13q34, 16q22.3, 19p13.3, 21q11.3 and 22q12.3 (Gialluisi et al., 2014; Eicher et al., 2013; Field et al., 2013; Luciano et al., 2013).

On the other hand, the use of massively parallel sequencing technology, or next generation sequencing (NGS), has permitted geneticists to obtain a complete overview of the variation within the whole exome (i.e. protein coding regions) or the whole genome. This approach makes it possible to study both common and rare variation in samples of interest. The study of low frequency genetic variation, which cannot be captured easily by chips used for GWAS, is particularly important because it is an aspect of human genetic variation that had been underexplored until very recently. Through NGS studies we now have a better characterization of the whole spectrum of genetic variation at the individual and population level, and of how these rare variants affect human traits in health and disease (Walter et al., 2015). This technology has enabled researchers to resolve the underlying genetic causes of multiple previously unsolved Mendelian disorders as well as to increase understanding of a number of complex diseases (as reviewed in Bamshad et al. (2011)). With respect to dyslexia, a whole exome sequencing (WES) study resulted in the identification of a rare coding variant in *CEP63* that co-segregates with dyslexia in a multiplex family (Einarsdottir et al., 2015).

In sum, reading ability has an important and complex genetic component, for which some key players have been identified so far. However, in order to build a comprehensive picture of the genetic factors underlying reading (dis)ability and to understand the mechanistic causal relation from the genes to the behaviour, we will

need to (a) identify new genes and (b) to evaluate the relative relevance and biological role of each of these key elements.

1.4 AIM OF THIS THESIS

The main aim of this thesis was to improve our understanding of the genetic underpinnings of reading (dis)ability. To this end, I investigated common and rare genetic variants that might affect reading-related quantitative traits and dyslexia.

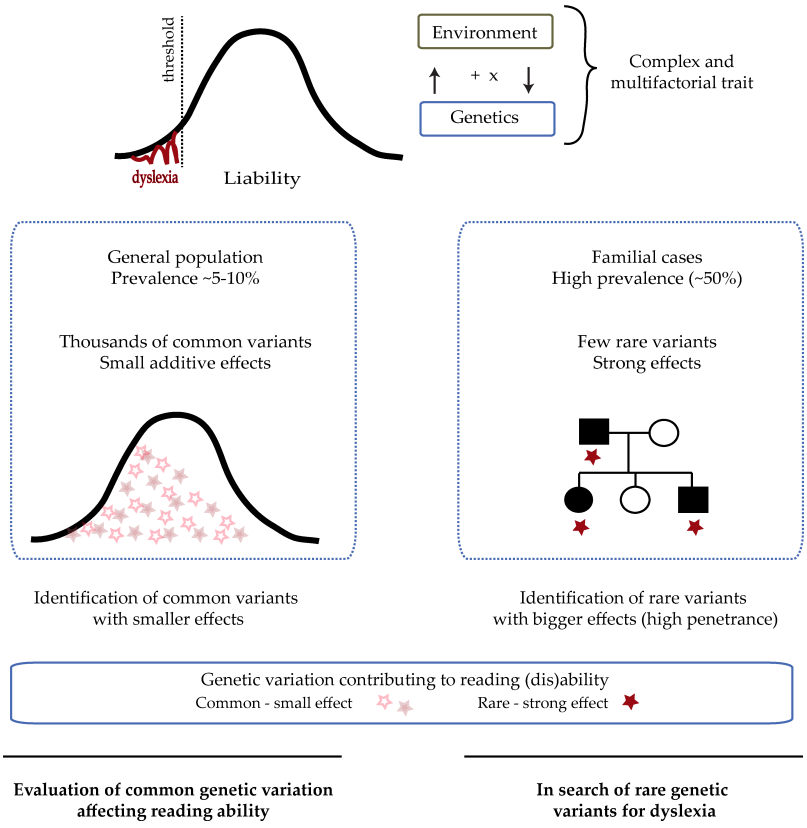


Figure 1.3: Schematic representation of possible different types of genetic contribution to the reading phenotype, and their relationship to this thesis.

First, in Chapter 2, I carried out a systematic review of the literature on the molecular genetics of dyslexia. I focused mainly on some of the most prominently studied

candidate genes for dyslexia susceptibility (e.g. *DYX1C1, KIAA0319, DCDC2, ROBO1*), describing the history of these candidate genes from their initial genetic mapping, through identification of associated gene variants, to characterization of gene function in cellular and animal model systems. I also provided an overview of additional genes and loci that have been suggested as potential risk factors. The role of behavioural and brain related intermediate phenotypes was discussed. Finally, the Dutch Dyslexia Program (DDP) was also introduced in this chapter, since several datasets deriving from this program are used in the studies of later chapters.

In Chapter 3, I performed a longitudinal analysis of some of the most intensively studied candidate single nucleotide polymorphisms (SNPs) for dyslexia susceptibility. This study used a Dutch dataset (from the DDP) characterized with several reading-related quantitative measures over multiple developmental stages. I carried out longitudinal association analyses, to evaluate these well-known candidate dyslexia SNPs in a longitudinal context.

In Chapter 4, I reviewed the first genome-wide association scan (GWAS) studies of the field and selected the top association signals from these studies for further investigation. I performed multivariate and univariate association analyses of such SNPs in an entirely novel population-based dataset characterized with several reading-related quantitative measures.

In Chapter 5, I re-analysed a family that had previously been linked to chromosome Xq27, taking advantage of the new possibilities offered by NGS technology. Whole exome and/or whole genome sequencing was performed for key members in this family, and the linkage analysis was re-visited with the additional data. The combination of linkage analysis and NGS enabled me to define genomic regions of interest, and to evaluate the possible contributions of coding, noncoding and structural genetic variants.

In Chapter 6, I adopted a similar NGS-based approach to study another large multigenerational family with recurrent cases of dyslexia. Linkage analysis was performed for the first time on this family, and whole genome sequenced individuals were used to identify putative rare variants with substantial penetrance. The findings suggested new candidates which connect with biological pathways that are already suspected to be important for dyslexia.

Finally, in Chapter 7 I have summarized and reviewed the main findings of the experimental chapters (i.e. Chapters 3 to 6), and discussed the state of the art and

future perspectives of the genetics of reading abilities, in relation to the complementary approaches that were considered within these studies.

REFERENCES

Adler WT, Platt MP, Mehlhorn AJ, Haight JL, Currier TA, et al. (2013) Position of neocortical neurons transfected at different gestational ages with shRNA targeted against candidate dyslexia susceptibility genes. PLoS ONE 8: e65179.

Anthoni H, Sucheston LE, Lewis BA, Tapia-Paez I, Fan X, et al. (2012) The aromatase gene CYP19A1: several genetic and functional lines of evidence supporting a role in reading, speech and language. Behav Genet 42: 509–527.

Anthoni H, Zucchelli M, Matsson H, Muller-Myhsok B, Fransson I, et al. (2007) A locus on 2p12 containing the co-regulated MRPL19 and C2ORF3 genes is associated to dyslexia. Hum Mol Genet 16: 667–677.

Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, et al. (2011) Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 12: 745–755.

Bishop DV (2015) The interface between genetics and psychology: lessons from developmental dyslexia. Proc Biol Sci 282.

Boltz WG (1986) Early Chinese writing. World Archaeology 17: 420–436.

Caravolas M, Lervag A, Defior S, Seidlova Malkova G, Hulme C (2013) Different patterns, but equivalent predictors, of growth in reading in consistent and inconsistent orthographies. Psychol Sci 24: 1398–1407.

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, et al. (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76: 581–591.

Coulmas F (2003) Writing systems: An introduction to their linguistic analysis. Cambridge University Press.

de Jong P, van der Leij A (2003) Developmental changes in the manifestation of a phonological deficit in dyslexic children learning to read a regular orthography. Journal of Educational Psychology 95: 22–40.

de Zeeuw EL, de Geus EJ, Boomsma DI (2015) Meta-analysis of twin studies highlights the importance of genetic variation in primary school educational achievement. Trends in Neuroscience and Education pp. –, URL http://www.sciencedirect.com/science/article/pii/S2211949315000198.

DeFries JC, Fulker DW, LaBuda MC (1987) Evidence for a genetic aetiology in reading disability of twins. Nature 329: 537–539.

Dehaene S, Cohen L, Morais J, Kolinsky R (2015) Illiterate to literate: behavioural and cerebral changes induced by reading acquisition. Nat Rev Neurosci 16: 234–244.

Eicher JD, Powers NR, Miller LL, Akshoomoff N, Amaral DG, et al. (2013) Genome-wide association study of shared components of reading disability and language impairment. Genes Brain Behav 12: 792–801.

Einarsdottir E, Svensson I, Darki F, Peyrard-Janvid M, Lindvall JM, et al. (2015) Mutation in CEP63 co-segregating with developmental dyslexia in a Swedish family. Hum Genet 134: 1239–1248.

Field LL, Shumansky K, Ryan J, Truong D, Swiergala E, et al. (2013) Dense-map genome scan for dyslexia supports loci at 4q13, 16p12, 17q22; suggests novel locus at 7q36. Genes Brain Behav 12: 56–69.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, et al. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75: 1046–1058.

Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, et al. (2014) Genome-wide screening for DNA variants associated with reading and language traits. Genes Brain Behav 13: 686–701.

Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160: 636–645.

Grigorenko EL (2001) Developmental dyslexia: an update on genes, brains, and environments. J Child Psychol Psychiatry 42: 91–125.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Harlaar N, Dale PS, Plomin R (2007) From learning to read to reading to learn: substantial and stable genetic influence. Child Dev 78: 116–131.

Ivliev AE, 't Hoen PA, van Roon-Mom WM, Peters DJ, Sergeeva MG (2012) Exploring the transcriptome of ciliated cells using in silico dissection of human tissues. PLoS ONE 7: e35618.

Lamminmaki S, Massinen S, Nopola-Hemmi J, Kere J, Hari R (2012) Human ROBO1 regulates interaural interaction in auditory pathways. J Neurosci 32: 966–971.

Landerl K, Ramus F, Moll K, Lyytinen H, Leppanen PH, et al. (2013) Predictors of developmental dyslexia in European orthographies with varying complexity. J Child Psychol Psychiatry 54: 686–694.

Luciano M, Evans DM, Hansell NK, Medland SE, Montgomery GW, et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav 12: 645–652.

Mascheretti S, Bureau A, Battaglia M, Simone D, Quadrelli E, et al. (2013) An assessment of gene-by-environment interactions in developmental dyslexia-related phenotypes. Genes Brain Behav 12: 47–55.

Mascheretti S, Facoetti A, Giorda R, Beri S, Riva V, et al. (2015) GRIN2B mediates susceptibility to intelligence quotient and cognitive impairments in developmental dyslexia. Psychiatr Genet 25: 9–20.

Meng H, Smith SD, Hager K, Held M, Liu J, et al. (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci USA 102: 17053–17058.

Pennington BF (1990) The genetics of dyslexia. J Child Psychol Psychiatry 31: 193–201.

Pennington BF (2006) From single to multiple deficit models of developmental disorders. Cognition 101: 385–413.

Peschansky VJ, Burbridge TJ, Volz AJ, Fiondella C, Wissner-Gross Z, et al. (2010) The effect of variation in expression of the candidate dyslexia susceptibility gene homolog Kiaa0319 on neuronal migration and dendritic morphology in the rat. Cereb Cortex 20: 884–897.

Peterson RL, Pennington BF (2015) Developmental dyslexia. Annu Rev Clin Psychol 11: 283–307.

Petrill SA, Thompson LA, Deater-Deckard K, Dethorne LS, Schatschneider C (2006) Genetic and Environmental Effects of Serial Naming and Phonological Awareness on Early Reading Outcomes. J Educ Psychol 98: 112–121.

Price CJ (2012) A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. Neuroimage 62: 816–847.

Richlan F (2012) Developmental dyslexia: dysfunction of a left hemisphere reading network. Front Hum Neurosci 6: 120.

Rodriguez Martinez MdelC, Ortiz Ceballos P, Coe MD, Diehl RA, Houston SD, et al. (2006) Oldest writing in the New World. Science 313: 1610–1614.

Rogers H (2004) Writing Systems: A Linguistic Approach (Blackwell Textbooks In Linguistics). {Blackwell Publishers}.

Rueckl JG, Paz-Alonso PM, Molfese PJ, Kuo WJ, Bick A, et al. (2015) Universal brain signature of proficient reading: Evidence from four contrasting languages. Proc Natl Acad Sci USA 112: 15510–15515.

Schork NJ, Murray SS, Frazer KA, Topol EJ (2009) Common vs. rare allele hypotheses for complex diseases. Curr Opin Genet Dev 19: 212–219.

Shaywitz SE, Escobar MD, Shaywitz BA, Fletcher JM, Makuch R (1992) Evidence that dyslexia may represent the lower tail of a normal distribution of reading ability. N Engl J Med 326: 145–150.

Shaywitz SE, Shaywitz BA, Fletcher JM, Escobar MD (1990) Prevalence of reading disability in boys and girls. Results of the Connecticut Longitudinal Study. JAMA 264: 998–1002.

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, et al. (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci USA 100: 11553–11558.

van der Leij A, van Bergen E, van Zuijen T, de Jong P, Maurits N, et al. (2013) Precursors of developmental dyslexia: an overview of the longitudinal Dutch Dyslexia Programme study. Dyslexia 19: 191–213.

Walter K, Min JL, Huang J, Crooks L, Memari Y, et al. (2015) The UK10K project identifies rare variants in health and disease. Nature .

2

MOLECULAR GENETICS OF DYSLEXIA: AN OVERVIEW

Dyslexia is a highly heritable learning disorder with a complex underlying genetic architecture. Over the past decade, researchers have pinpointed a number of candidate genes that may contribute to dyslexia susceptibility. Here, we provide an overview of the state of the art, describing how studies have moved from mapping potential risk loci, through identification of associated gene variants, to characterization of gene function in cellular and animal model systems. Work thus far has highlighted some intriguing mechanistic pathways, such as neuronal migration, axon guidance, and ciliary biology, but it is clear that we still have much to learn about the molecular networks that are involved. We end the review by highlighting the past, present and future contributions of the Dutch Dyslexia Programme to studies of genetic factors. In particular, we emphasize the importance of relating genetic information to intermediate neurobiological measures, as well as the value of incorporating longitudinal and developmental data into molecular designs.
Keywords: molecular genetics, dyslexia, review

This chapter has been published as:
Carrion-Castillo, A., Franke, B., & Fisher, S. E. (2013). Molecular genetics of dyslexia: An overview. Dyslexia, 19(4), 214-240. doi:10.1002/dys.1464

2.1 INTRODUCTION

Over the past decade or so, advances in molecular technologies have enabled researchers to begin pinpointing potential genetic risk factors implicated in human neurodevelopmental disorders (Graham and Fisher, 2013). A significant amount of work has focused on developmental dyslexia (specific reading disability). The search for risk genes underlying dyslexia is well motivated; a wealth of prior information from familial clustering and twin studies suggests a substantial inherited component. For example, the proportion of variance in reading skills that is explained by genetic endowment is high, with heritability estimates ranging from 0.4-0.8 (Schumacher et al., 2007). At the same time, it is clear that the genetic architecture underlying dyslexia must be complex and multifactorial, involving a combination of polygenicity (two or more genes contribute to the phenotype) and heterogeneity (the same disorder can be caused by multiple origins in different individuals). Moreover, it is likely that many of the genetic risk factors will have small effect sizes, or only be implicated in rare cases.

Crucially, the success of tracking down the molecular basis of a disorder depends not only on the available genomic methodologies, but also on the strategies used to ascertain and characterize the phenotype of interest. Developmental dyslexia is typically defined as a severe difficulty in the mastery of reading and/or spelling skills that cannot be explained by impaired intelligence, socioeconomic factors, or other obvious causes like comorbid neurological conditions or a history of head injury (Grigorenko, 2001). Such a definition is largely exclusive (i.e. it relies on exclusion of other possible causes), based on an unexpected discordance between predicted levels of proficiency (for example, calculated from chronological age and/or IQ) and the observed performance. As a consequence, a categorical diagnosis of dyslexia (affected versus unaffected) can be highly sensitive to the nature of the assessment procedures, including which tests are administered and how the diagnostic thresholds are set.

Faced with the limitations of categorical definitions, many genetic investigations of dyslexia make direct use of data from psychometric measures for assessing relationships between molecular factors and the disorder in cohorts under study (Fisher and DeFries, 2002). Some of these quantitative traits, such as a person's performance on single word reading or spelling tests, directly index the defining difficulties. Others tap into particular underlying cognitive processes that are hypothesized to contribute to reading and spelling proficiency, including orthographic processing, pho-

neme awareness, rapid automatized naming, and phonological short-term memory (Table 2.1). The associated psychometric measures can be considered as examples of endophenotypes: quantitative indices that are closer to the underlying biological phenomena, and that are conceivably easier to link with the genetic factors (Gottesman and Gould, 2003). A good characterization of endophenotypes can aid in understanding the critical biological mechanisms, and in pinpointing the genes that are involved, especially for genetically complex traits (Kendler and Neale, 2010). In the past couple of years, some studies (Czamara et al., 2011; Darki et al., 2012; Peyrard-Janvid et al., 2004; Pinel et al., 2012; Roeske et al., 2011; Wilcke et al., 2012) have moved beyond the behavioural measures described above, attempting to define brain imaging or neurophysiological measures as endophenotypes for dyslexia genetics (e.g. event-related potentials from electrophysiology, effects on cortical volumes). Although this field is still emerging and most of the findings await replication, neuroimaging endophenotypes are a promising step for building bridges between genetic information and behavioural output.

Orthographic processing	Reflects orthographic knowledge. Can be measured using orthographic choice tasks of phonologically similar letter strings.
Phoneme awareness	The ability to identify and manipulate the sounds in spoken words, which reflects phonological processing. Can be measured using phonemic deletion tasks.
Rapid automatized naming	Rapid naming of highly familiar visual symbols, which reflects speed of processing. Can be measured by several rapid naming tests of symbols (digits/letters), colours, or pictures.
Phonological short-term memory	Recall for a period of several seconds to a minute without rehearsal. Hypothesized to tap phonological processing. Can be measured using nonsense word repetition tasks

Table 2.1: Key cognitive skills underlying reading, and associated psychometric tests.

As noted above, there are inherent problems with conceptualizing dyslexia as a dichotomous trait (affected or unaffected). Indeed, it has been proposed that dyslexia may not constitute a qualitatively distinct disorder at all, but could simply reflect the lower end of normal variation in reading ability (Shaywitz et al., 1992). In this context, recent genetic studies have not only investigated cohorts of people with dyslexia, but also extended their analyses to reading-related phenotypes in unselected populations from large epidemiological samples (birth cohorts, twin studies, biobanking initiatives, and so on). The substantial numbers of samples available from these kinds of general population collections can improve statistical power for detecting contributions of common genetic risk factors, which are expected to have individual effect sizes that are rather small (Paracchini, 2011). Despite the challenges of genetic complexity, there are at least 9 reported candidate regions of interest for dyslexia in the human genome (DYX1-DYX9), and up to 14 individual candidate genes with varying degrees of supporting evidence (Poelmans et al., 2009). The suggested candidates include some that have been implicated in specific biological processes such as migration of neurons during early brain development, or outgrowth of dendrites and axons (e.g. *ROBO1, KIAA0319, DCDC2, DYX1C1*). Indeed, a molecular framework that attempts to synthesize these different findings has been formulated by researchers from the Dutch Dyslexia Programme (DDP) (Poelmans et al., 2011), attributing a central role to a signaling network involved in neuronal migration and neurite outgrowth. Efforts have also been made to merge the genetic findings with a neuropsychological framework (Giraud and Ramus, 2012), proposing that abnormal neuronal migration might lead to anomalous brain oscillations, disturbing the sampling of the auditory signal, and thereby affecting phonological processing.

Thus, important progress has been made, but the picture is still far from complete. The present paper will outline research that has been carried out at several levels in order to understand the genetic basis of dyslexia, from multiple different research laboratories across the world, including the contributing findings from the DDP. We give an overview of the main dyslexia susceptibility loci that are currently known, in each case starting from the initial linkage mapping (see Glossary in Section 2.11 for definition of this and other technical terms), moving to the support provided by association studies and then zooming into the candidate genes and their functional roles. We end by considering future perspectives for the field, and providing examples of how further molecular work with the DDP can help to fill in the gaps between genotype and phenotype.

2.2 FIRST CLUES: THE DYX1 LOCUS

A connection between specific reading disability and genetic markers on chromosome 15 was perhaps the earliest finding for the field (Smith et al., 1983). Subsequent studies have repeatedly highlighted linkage to this chromosome, with peak signals mostly located around genetic markers in 15q21, dubbed the DYX1 locus (Grigorenko et al., 1997; Schulte-Korne et al., 1998; Chapman et al., 2004; Platko et al., 2008), although a signal at another location, 15q15, has also been reported (Morris et al., 2000; Schumacher et al., 2008). Genome-wide linkage analyses of reading and spelling quantitative traits in an unselected twin sample (Bates et al., 2007) found replication-level support for linkage of regular word spelling to DYX1.

2.2.1 *Discovery of DYX1C1 gene*

A candidate gene in DYX1, subsequently named *DYX1C1*, was first identified through studies of a Finnish family in which a chromosomal rearrangement, a translocation involving chromosomes 2 and 15, co-segregated with reading and writing difficulties (Nopola-Hemmi et al., 2000). The chromosome 15 breakpoint of this translocation was located within the DYX1 region that had previously been linked to dyslexia in other studies. Precise mapping of the breakpoint demonstrated that it directly disrupted the *DYX1C1* gene, which encodes a 420 amino acid protein with three protein-protein interaction domains (tetratricopeptide repeats, TPR) (Taipale et al., 2003). The gene was shown to be expressed in a subset of human glial and neuronal cells. Furthermore, two *DYX1C1* sequence changes (single-nucleotide polymorphisms or SNPs) were found to be associated with dyslexia in additional Finnish families. Both these changes were proposed as putative functional alleles: $-3G > A$ creates a potential new binding site for a transcription factor known as Elk-1, while $1249G > T$ introduces a premature stop codon that shortens the encoded protein by four amino acids.

In subsequent work, multiple groups have tested for association between dyslexia (and related traits) and these two *DYX1C1* variants, but the results remain inconclusive. Marino et al. (2007) reported that the -3A allele was significantly associated with deficits on a measure of short-term memory (Single Letter Backward Span), as was a haplotype combining -3A with 1249T. However, in some *DYX1C1* investigations, opposite patterns of effects were observed; the major alleles of these variants (i.e. the

non-risk alleles from the original study) were associated with a diagnosis of dyslexia (Brkanac et al., 2007; Wigg et al., 2004), or with deficits on orthographic choice tasks (Scerri et al., 2004). Several other studies, both in dyslexia cohorts and in the general population, failed to replicate the original associations with these *DYX1C1* putative risk alleles or their haplotype (Bates et al., 2010; Bellini et al., 2005; Tran et al., 2013).

Other markers in *DYX1C1* have been reported to show associations with categorical dyslexia (Dahdouh et al., 2009; Wigg et al., 2004), with short-term memory performance in females with dyslexia (Dahdouh et al., 2009), or with quantitative measures of reading-related traits in the general population (Bates et al., 2010). A recent neuroimaging genetics study of 79 people (Darki et al., 2012) included *DYX1C1* markers from these prior studies. One of the markers, previously associated with irregular and nonword reading performance by Bates et al. (2010), was found to correlate with white matter volume locally, in the left temporo-parietal region, and also on the global brain level. However, the marker in question was not significantly correlated with reading performance in this same sample, which was not selected for dyslexia.

The lack of consistency of the *DYX1C1* associations has been recently assessed using meta-analyses. One of them (Zou et al., 2012) integrated case-control and family-based association test studies to examine the -3G/A variant, concluding that there is no statistical evidence of an association between this SNP and dyslexia risk. Another meta-analysis assessed cumulative evidence from 10 independent studies of -3G/A and 1249G/T, and found low credibility of evidence for both SNPs, due to high levels of heterogeneity between studies (Tran et al., 2013).

2.2.2 *Investigating DYX1C1 functions*

To gain insights into the potential roles of *DYX1C1* (and putative dyslexia risk alleles) in brain development, the gene and its encoded protein have been functionally characterized. Variants in the promoter region of *DYX1C1*, including the -3G/A SNP, have been suggested to mediate allele-specific binding of transcription factors (such as TFII-I and Sp1) and/or to be associated with different expression levels of the gene. Investigations of the DNA fragment spanning the -3G/A SNP identified that it was bound by TFII-I, PARP (poly ADP-ribose polymerase 1) and a splicing factor known as SFPQ (Tapia-Paez et al., 2008).

In a developmental study of the rodent orthologue, *Dyx1c1*, expression levels of this gene were knocked down in embryonic neocortex of the rat (Wang et al., 2006;

Currier et al., 2011; Adler et al., 2013). This experimental manipulation led to an aberrant migration pattern of the treated neurons, in which the cells accumulated in the multipolar stage of migration. Effects were non-autonomous; they were not limited to the cells that had been knocked down for Dyx1c1, but also disturbed some other neighbouring cells (Currier et al., 2011). Overexpression of Dyx1c1 rescued migration, confirming that the knockdown was causing the aberrant phenotype; and it was found that the TPR domains were sufficient for this rescue (Wang et al., 2006). However, the study did not support a functional role for the $1249G > T$ SNP that creates a premature stop codon, because overexpression of the truncated variant also rescued migration.

When neurons were subjected to embryonic knockdown of Dyx1c1 levels, they migrated past their expected laminar targets (Adler et al., 2013). These overmigration observations have been confirmed in a subsequent investigation using live-cell imaging of human neuroblastoma cells, where knockdown of *DYX1C1* led to increased migration rates compared to controls, and this was dependent not only on the TPR domains, but also on another novel highly conserved motif, referred to as a DYX1 domain (Tammimies et al., 2013). Analysis of changes in global gene expression levels after perturbation of *DYX1C1*, by overexpression or knockdown in these cell lines, uncovered a group of genes that was enriched for known functions, including "cellular component movement", "cell migration" and "nervous system development", as well as a pathway involved in focal adhesion (Tammimies et al., 2013).

Further studies have shown that the rat orthologue of *DYX1C1* interacts with estrogen receptors in primary rat neurons (Massinen et al., 2009). Based on these data, it has been proposed that *DYX1C1* negatively regulates estrogen receptor levels in a dose-dependent manner, decreasing their transcriptional activity and stability. As the estrogen pathway is known to be important for brain development, interaction of *DYX1C1* with sex hormones has been postulated as a potential contributor to the often reported sex difference prevalence of dyslexia.

Tammimies and colleagues specifically assessed whether *DYX1C1* can interact with other proteins implicated in neuronal migration and/or associated with dyslexia susceptibility, including *DCDC2* and *KIAA0319* (see next section) (Tammimies et al., 2013). It was found that *DYX1C1* interacts with *LIS1* (a protein implicated in lissencephaly, a rare brain disorder caused by severely disrupted neuronal migration) and *DCDC2*, but not with *KIAA0319*. Several new interactions with *DYX1C1* were also reported, with a significant overrepresentation of proteins that are components

of the cytoskeleton, three of which (TUBB2B, TUBA1 and Ataxin1) were further validated.

Recent evidence suggests that *DYX1C1* and other candidate dyslexia genes, such as *DCDC2* and *KIAA0319*, might be involved in the growth and function of cilia (Ivliev et al., 2012). These tiny hairlike structures line the surfaces of many types of cells and can move in rhythmic waves. There is a growing realization of the important roles that cilia play in early brain patterning and homeostasis. The zebrafish orthologue of *DYX1C1* is expressed in many ciliated tissues and its knockdown leads to multiple ciliopathy-related phenotypes (Chandrasekar et al., 2013). *Dyx1c1-* mutant mice are reported to display ciliary motility defects (Tarkar et al., 2013). Finally, recessive loss of function mutations of *DYX1C1* have been identified in human patients with Primary Ciliary Diskinesia, a disorder characterized by chronic airway disease, laterality defects and male infertility (Tarkar et al., 2013).

2.3 TWO GENES FOR THE PRICE OF ONE: THE DYX2 LOCUS

Cardon et al. (1994) described the first evidence for a chromosome-6 quantitative trait locus involved in dyslexia susceptibility, spanning the Human Leukocyte Antigen complex on $6p21.3$. Linkage at $6p21 - 23$ (the DYX2 locus) has since been reported by multiple further studies, with several reading-related traits, using a variety of approaches and sampling strategies (Grigorenko et al., 1997; Fisher et al., 2002; Kaplan et al., 2002; Platko et al., 2008; Fisher et al., 1999). A linkage study of Specific Language Impairment (SLI) that targeted known candidate regions for dyslexia, identified support for involvement of the DYX2 region, suggesting impacts beyond diagnostic boundaries (Rice et al., 2009), but earlier studies of other SLI cohorts have not found evidence of linkage to this locus (Consortium, 2002; Bartlett et al., 2002).

DYX2 is one of the most replicated dyslexia susceptibility loci to date, but still a number of reports have failed to find support for this region. For example, one of the early studies that focused on spelling disability in German families did not find evidence for DYX2 linkage (Schulte-Korne et al., 1998), and this locus did not show any signal in a genome-wide linkage scan of reading abilities in the general population (Bates et al., 2007). Moreover, the DDP analysed a set of 108 families with at least two affected children, assessing categorical status and also key quantitative traits, including word reading, phonological decoding, verbal competence, nonsense-

word repetition, and rapid automatized naming (de Kovel et al., 2008), but did not detect any linkage to DYX2 in the cohort.

2.3.1 *The KIAA0319 gene*

Building on prior findings of linkage to DYX2, several research teams used association analysis to narrow the region of interest and to zoom in on a convincing candidate gene or genes. In one investigation of five phenotypes that measured orthographic and phonologic skills in dyslexia families from the U.S. (Deffenbacher et al., 2004), associations were reported with markers in five genes from the DYX2 region: *VMP*, *DCDC2*, *KIAA0319*, *TTRAP*, and *THEM2*. In another quantitative trait association study, involving sets of families from the U.K. and the U.S., Francks et al. (2004) narrowed the focus to a small (70kb) region spanning *THEM2*, *TTRAP* and *KIAA0319*, with the main risk haplotype being identified by a SNP marker (rs2143340) upstream of *KIAA0319*, in the *TTRAP* locus. Cope et al. (2005), investigating an independent U.K. sample, similarly reported an enrichment of dyslexia-associated SNPs in this interval, although they did not replicate the rs2143340 finding.

Following these initial indications of *KIAA0319* involvement, several other studies have suggested that markers and haplotypes (rs4504469-rs2038137-rs2143340 [1-1-2], rs4504469-rs6935076 [2-1]) in this gene are associated with categorical dyslexia and/or with quantitative traits, not only in people with dyslexia, but also in general population samples (Harold et al., 2006; Luciano et al., 2007; Paracchini et al., 2008; Couto et al., 2010; Newbury et al., 2011; Venkatesh et al., 2013). However, as observed for other dyslexia candidate genes, a number of studies did not find evidence for biased transmission of *KIAA0319* markers in their dyslexic samples (Brkanac et al., 2007; Ludwig et al., 2008; Schumacher et al., 2006a). Despite these negative reports, a recent meta-analysis that focused on the $931C > T$ polymorphism (rs4504469) of *KIAA0319* concluded that the minor T allele is significantly associated with dyslexia risk (Zou et al., 2012).

Investigations have also assessed whether *KIAA0319* alleles might have broad impacts across different neurodevelopmental disorders. In a study that assessed association of candidate genes in relation to dyslexia and other frequently comorbid disorders such as Attention Deficit Hyperactivity Disorder (ADHD) and SLI (Scerri et al., 2011), *KIAA0319* variants were associated with reading and spelling scores. SNPs rs6935076 and rs9461045 appeared to have a specific effect on dyslexia, whereas

rs2143340 was associated with general reading ability - the effect did not dilute when widening the analysis to the general population. Another study reported that several variants in the region upstream of *KIAA0319* (rs4504469-C, rs761100-G, rs6935076-T) were associated with reading and language phenotypes in a SLI sample (Rice et al., 2009). However, the replication levels of these studies are difficult to evaluate, because even when the SNP markers are the same, the risk alleles are often not consistent, with different directions of effect (increasing susceptibility in one study, but showing a protective effect in another). For example, the specific risk alleles of rs761100 and rs6935076 that were correlated with reduced expressive language scores in one dyslexic cohort (Newbury et al., 2011), were those that had correlated with increased reading and language performance in other studies (Harold et al., 2006).

Variants within the *THEM2-TTRAP-KIAA0319* region have been tested for association with neuroimaging phenotypes in small samples from the general population, again with differing effects in different studies. Using functional MRI, Pinel and colleagues reported that a SNP in *THEM2* (rs17243157) was associated with asymmetry of activation at the temporal lobe during a reading task (Pinel et al., 2012). However, the other DYX2 SNPs tested in this study were not found to be associated with any activation pattern in the brain regions of interest. Another study found that the rs2143340 SNP in *TTRAP* was associated with activation in the right and left anterior inferior parietal lobe during phonologic processing tasks (Cope et al., 2012). In their recent structural imaging study, Darki et al. (2012) reported that rs6935076 in *KIAA0319* had a significant effect on the white matter volume of the left temporo-parietal region, but not with reading scores in that sample (Darki et al., 2012).

Cell-based approaches have been used to identify a potential functional basis of genetic associations in the *THEM2-TTRAP-KIAA0319* region. The first such study focused on one established risk haplotype for dyslexia (rs4504469- rs2038137-rs2143340 [1-1-2]), and showed that it was associated with lower expression levels of KIAA0319, indicating that regulatory sequence variants could be affecting transcriptional regulation of this gene (Paracchini et al., 2006). In a follow-up investigation of this effect, Dennis et al. (2009) tested various versions of the promoter of KIAA0319, carrying different dyslexia-associated SNP alleles, via reporter gene assays. They zoomed in on a particular functional SNP, rs9461045, finding that the minor allele yields reduced expression of reported genes in neuronal and non-neuronal cell lines. This variant creates a binding site for a transcription factor, known as OCT-1, that could explain

reduced expression of *KIAA0319* from the risk haplotype. Indeed, when OCT-1 was knocked down, the expression levels of the risk allele were shown to recover.

The *KIAA0319* gene has several variants due to alternative splicing: A, B and C, encoding different versions of the protein (Velayos-Baeza et al., 2007). Version A of the protein localizes in the plasma membrane of the cell; it has a single domain that spans the membrane and it forms dimers - two molecules of the protein bind to each other to form a functional unit. This protein variant undergoes modifications (addition of carbohydrate groups, also known as glycosylation) that typically contribute to protein folding, stability, cell adhesion, and cell-cell interaction. Therefore, it has been proposed that it could be involved in the interaction of neurons and glial fibers during neuronal migration (see below), most probably mediated by specific interaction domains that are present in its central region (Velayos-Baeza et al., 2008). The other two protein variants (B and C) lack the transmembrane domain and are localized in the endoplasmic reticulum of the cell. Only variant B has been detected in the extracellular medium, and its size suggests that, like variant A, it is glycosylated. It has thus been speculated that the *KIAA0319* gene may have a wider functional spectrum that also includes signaling (Velayos-Baeza et al., 2008, 2010; Levecque et al., 2009).

The expression pattern of *KIAA0319* in the developing neocortex is consistent with its hypothesized role in neuronal migration (Paracchini et al., 2006). The gene is also expressed in the adult brain, being relatively abundant in the cerebellum, the cerebral cortex, the putamen, the amygdala and the hippocampus (Peschansky et al., 2010; Velayos-Baeza et al., 2007). Studies of cortical tissue reported highest expression in the superior parietal cortex, primary visual cortex and occipital cortex (Meng et al., 2005).

When the expression of *Kiaa0319* (the rodent orthologue of *KIAA0319*) was experimentally knocked down in embryonic rat neocortex, this disturbed neuronal migration, by reducing the migration distances from the ventricular zone towards the cortical plate (Peschansky et al., 2010; Paracchini et al., 2006; Adler et al., 2013). Periventricular heterotopias (clusters of disorganized neurons along the lateral ventricles of the brain) were found in three quarters of the animals, containing large numbers of neurons that did not migrate properly and formed clumps around the ventricles. The effects of knockdown appeared to be non-cell autonomous, disturbing both radially and tangentially migrating neurons (Peschansky et al., 2010; Adler et al., 2013). *Kiaa0319* knockdown also led to enlargement of apical dendrites of

the treated neurons, which could be rescued by overexpression of the human gene (Peschansky et al., 2010). The longer term effects of embryonic *Kiaa0319* knockdown on specific brain structures of the brain have also been studied. After the gene had been knocked down embryonically in a lateral ventricle, adult male rats displayed a reduced midsagittal area of the corpus callosum, but no difference in volume of the cortex and hippocampus (Szalkowski et al., 2013). The authors pointed out that the area affected in their rodent studies has previously been associated with phonological processing deficits in humans with dyslexia.

2.3.2 *The DCDC2 gene*

DCDC2, another gene in the DYX2 region, was first proposed as a dyslexia candidate gene based on association of SNPs with one quantitative index of dyslexia severity (discrepancy between expected and observed reading scores) in a set of U.S. families (Meng et al., 2005), overlapping with the cohort analysed in some of the *KIAA0319* studies described above. Meng et al. (2005) also characterized a small (2.4kb) deletion within the *DCDC2* locus that contained a short tandem repeat (STR), referred to as BV677278. The STR was highly variable with multiple alleles, and by combining the deletion with the 10 minor alleles of the STR, the authors were able to show association with another quantitative phenotype in the cohort, performance on a homonym choice task. A number of subsequent reports have described association of this STR marker with a categorical definition of dyslexia (Schumacher et al., 2006a), and with quantitative measures of reading and memory (Marino et al., 2012). However, other studies could find only weak and inconsistent evidence of association, for example Harold et al. (2006).

Experimental studies suggest that the BV677278 STR is bound by a transcription factor called ETV6 (Powers et al., 2013) expressed in human brain (Meng et al., 2011), and that different STR alleles might affect gene regulation. The STR is in high linkage disequilibrium with a haplotype block that is associated with phonological awareness and a composite language measure (Powers et al., 2013). This *DCDC2* risk haplotype seems to interact in a non-additive manner with a known *KIAA0319* risk haplotype (Francks et al., 2004; Scerri et al., 2011; Paracchini et al., 2008); individuals carrying both dyslexia risk haplotypes had a significantly worse performance than expected (Powers et al., 2013).

Additional SNP markers in *DCDC2* have also been associated with dyslexia (rs-807724, rs793862, rs807701) (Schumacher et al., 2006a; Wilcke et al., 2009; Newbury et al., 2011) and with quantitative measures such as reading fluency and nonsense word repetition (Scerri et al., 2011). In contrast to the effects of certain *KIAA0319* markers, whose putative effects extend to the general population, it has been proposed that *DCDC2* variants may contribute specifically to reading (dis)ability in people with dyslexia, as associations do not hold when widening the sample to include SLI, ADHD, or non-affected individuals (Scerri et al., 2011). Some studies fail to find support for effects of *DCDC2* markers even within dyslexic cohorts (Brkanac et al., 2007; Zuo et al., 2012; Venkatesh et al., 2013).

A recent meta-analysis (Zhong et al., 2013), including 8 publications and a total of 941 cases and 1183 controls, assessed association with dyslexia for the most consistently reported *DCDC2* markers (rs807701, rs793862, rs807724, rs1087266 and the 2.4kb deletion). Overall, allele C of rs807701 was significantly associated with the risk of dyslexia, while the other markers showed no evidence of association. However, sensitivity analysis suggested that the results were of low reliability and should be treated with caution.

Association between the 2.4kb deletion within *DCDC2* and gray matter distribution in the brain was tested in a small sample of healthy individuals (Meda et al., 2008). It was proposed that the heterozygous subjects had higher grey matter volume in the superior, medial and inferio temporal-gyri, the fusiform gyrus, the hippocampus, the uncus, the parahippocampal, the occipito-parietal and the inferior and middle frontal gyri. In their functional imaging study of DYX2 candidates, Cope and colleagues reported that the BV677278 STR of *DCDC2* was associated with activation of the superior anterior cingulate gyrus, posterior cingulate gyrus, left paracentral lobule and the left inferior frontal gyrus during phonological processing tasks (Cope et al., 2012). Additional imaging genetics projects have investigated other markers in *DCDC2* (Jamadar et al., 2011; Darki et al., 2012). For example, Darki et al. (2012) reported that rs793842 was associated with variation in white matter volume of the temporo-parietal region.

DCDC2 encodes a protein that contains two doublecortin domains. These domains are named after a related protein (doublecortin or *DCX*) that has been implicated in lissencephaly, and they are thought to mediate interactions with the cytoskeleton of the cell. Two isoforms are produced by alternative splicing, with the larger version being expressed in adult and fetal brain (Schumacher et al., 2006a). Screening

of adult human brain tissues suggests that it is most highly expressed in the entorhinal cortex, the inferior and medial temporal cortex, the hypothalamus, the amygdala and the hippocampus (Meng et al., 2005). The protein localizes in primary cilia, neurites and cytoplasm of hippocampal neurons (Massinen et al., 2011), and associates with a protein known as Kif3a at the primary cilium, in a manner that depends on both the doublecortin domains. There is bioinformatic support for the implication of *DCDC2* in cilia (Ivliev et al., 2012) and overexpression of the gene increases the average length of a cilium to approximately twice the normal length (Massinen et al., 2011). Studies in nematode worm models (*C. elegans*) suggest that it is important for neuronal morphology (Massinen et al., 2011).

Similar to findings for *Dyx1c1* and *Kiaa0319*, knockdown of *Dcdc2* expression in utero in rats yielded disturbed migration of neuronal precursors from the ventricular surface towards the pial surface (Meng et al., 2005; Adler et al., 2013). By contrast, studies of knockout mice that lack *Dcdc2* did not find any defects in brain morphology, function, or behaviour; in particular, the structure, number and length of neuronal cilia in neocortex and hippocampus did not differ between knockout animals and the wild-type mice (Wang et al., 2011). However, in utero knockdown of the related gene, *Dcx* (doublecortin) caused more developmental disruption in *Dcdc2* knockouts than in wild-type mice: subcortical heterotopias and disruptions of dendritic growth (Wang et al., 2011). This suggests that there may be partial functional redundancy of these two genes in regulating neuronal migration and dendritic growth in the mice. A follow-up study of the *Dcdc2* knockout mice reported reduced performance in visual discrimination tasks (which had not been evident in the earlier study) as well as impairments in long-term working memory, despite the absence of any deficits in neuronal migration (Gabel et al., 2011). The *Dcdc2* mutated mice also learned less efficiently, which is intriguing given that dyslexia is primarily a learning disorder (Gabel et al., 2011).

2.4 THE DYX3 LOCUS: A CONNECTION WITH IQ?

By studying a large multigenerational family from Norway in which dyslexia appeared to be inherited in a simple dominant manner, Fagerheim et al. (1999) identified a candidate locus (DYX3) on chromosome 2 (2p12-16). Over a decade later, the identity of the putative causative mutation in this family remains unknown. Nevertheless, further investigations in other samples have supported linkage of the 2p12-16

region with dyslexia (Kaminen et al., 2003) and with several reading-related quantitative traits in dyslexic samples, including the sibling pairs of the DDP (Fisher et al., 2002; Francks et al., 2002; Petryshen et al., 2002; de Kovel et al., 2008). A nearby region on 2q22.3 has also been linked to phonemic decoding efficiency in families with dyslexia (Raskind et al., 2005), and to reading of irregular words and regular spelling in the general population (Bates et al., 2007).

The first candidate genes proposed for DYX3 (*SEMA4F*, *OTX1* and *TACR1*) did not contain risk variants that could account for the evidence of linkage to this chromosomal region (Francks et al., 2002; Peyrard-Janvid et al., 2004). Subsequently, a two-stage study pinpointed a small interval of interest within 2p12, containing two overlapping haplotypes that were associated with dyslexia in two populations (Anthoni et al., 2007). The region defined by the haplotypes lay between a hypothetical gene, *FLJ13391* and two other candidates, *MRPL19* and *C2ORF3*. In studies of lymphocyte cells, heterozygous carriers of the putative risk haplotypes had significantly lower expression levels of *MRPL19* and *C2ORF3* than people who carried only non-risk alleles. However, other studies in an SLI cohort (Newbury et al., 2011) and a general population sample (Scerri et al., 2011) did not replicate the association between variants in *MRPL19/C2ORF3* and language and reading traits.

Most recently, the relevant markers on 2p12 were found to be significantly associated with verbal and performance IQ in an investigation that examined the impact of multiple different candidate dyslexia and SLI risk factors on general cognitive abilities (Scerri et al., 2012). One of the highlighted SNPs in *MRPL19* (rs917235) was also associated with variation in white matter volume in the posterior corpus callosum and the cingulum, brain regions that have been shown to be connecting sections of the parietal, occipital and temporal cortices. Thus, the authors proposed that the *MRPL19/C2ORF3* gene findings are more likely to be related to general cognition than having a specific effect on reading or language skills.

2.5 DYX5, THE *ROBO1* GENE AND AXON GUIDANCE

Nopola-Hemmi and colleagues described a four generation Finnish family in which profound reading difficulties were inherited in a manner that was consistent with involvement of a single dominant gene, which they mapped to the 3p12-q13 region, named the DYX5 locus (Nopola-Hemmi et al., 2001). Support for this region was found in a genome-wide scan of quantitative reading-related traits in dyslexia fami-

lies (Fisher et al., 2002), as well as with irregular word reading in the general population Bates et al. (2007). A case-control study in the Afrikaner population also found suggestive association between dyslexia and markers in 3q13 (Platko et al., 2008).

Although *5HT1F* and *DRD3* were first proposed as candidate genes in the DYX5 region (Nopola-Hemmi et al., 2001), *ROBO1* in 3p12 was soon identified to be disrupted in a dyslexic case with a de novo chromosomal translocation affecting this locus (Hannula-Jouppi et al., 2005). Intriguingly, *ROBO1* encodes a protein that acts as an axon guidance receptor. Moreover, on returning to the original four-generation family that first showed linkage to DYX5, Hannula-Jouppi uncovered a putative risk haplotype of *ROBO1* that co-segregated with dyslexia in 19 of the 21 dyslexic family members. No protein-coding change could be identified, but the dyslexia-associated alleles of the risk haplotype had attenuated expression of *ROBO1* in lymphocytes from affected individuals, suggesting that altered regulation of this gene could be a potential causal mechanism (Hannula-Jouppi et al., 2005). Other SNPs in *ROBO1* have been reported to be associated with performance on measures of short-term memory (nonsense word repetition and digit span) but not with tests of reading in the general population (Bates et al., 2011).

ROBO1 is strongly expressed in developing and adult brain tissue (Lamminmaki et al., 2012). Studies of animal orthologues have shown that the encoded protein acts as a receptor for molecular guidance cues during cellular migration and axonal navigation, playing an important role in crossing of axons across the midline between brain hemispheres. To investigate potential effects on axon crossing in humans, Lamminmaki et al. (2012) used magnetoencephalography to study dyslexic individuals carrying the *ROBO1* risk haplotype, taken from the original family studied by Nopola-Hemmi et al. (2001) and Hannula-Jouppi et al. (2005). On assessing the strength of auditory pathways using a binaural suppression endophenotype, they found that the control group had a significantly smaller response to binaural than to monaural stimulation, whilst the risk haplotype group did not (Lamminmaki et al., 2012). The strength of the ipsilateral suppression in both hemispheres also correlated with *ROBO1* expression levels in blood. However, the risk haplotype group did not show any significant difference from controls in total biallelic expression of this gene (i.e. expressed from both chromosomal copies). Nevertheless, the authors proposed that partially reduced levels of *ROBO1* expression might be causing dyslexia in this family, by affecting the auditory processing and brain development as shown by their defective interaural interaction.

2.6 ADDITIONAL DYSLEXIA SUSCEPTIBILITY LOCI

The loci discussed above are the most well studied ones thus far. Even so, a number of other regions in the genome have also been proposed to harbour susceptibility genes, and have been designated as DYX loci, as briefly covered below.

DYX4 A region of 6q11.2-q12 (named the DYX4 locus) was linked to phonological coding in dyslexia and related quantitative traits (Petryshen et al., 2001). This linkage has not been replicated, although one study has reported a suggestive linkage of 6q15 to spelling of irregular words in a general population sample (Bates et al., 2007).

DYX6 In two parallel genome-wide linkage screens of quantitative traits in independent sets of dyslexia families, the most significant markers for performance on single-word reading tests coincided, implicating a region on 18p11.2, DYX6 (Fisher et al., 2002). A study of German families did not detect linkage to chromosome 18p11-q12 (Schumacher et al., 2006b). However, in other samples DYX6 has been linked to phonological and orthographic coding measures (Bates et al., 2007) and to reading performance (Seshadri et al., 2007). Four potential candidate genes (*MC5R*, *DYM*, *NEDD4L* and *VAPA*) have been proposed in this region (Scerri et al., 2010).

DYX7 Evidence for DYX7 (11p15) comes from a suggestive linkage with phonological awareness in the genome-wide linkage screens conducted by Fisher and colleagues (Fisher et al., 2002), followed by targeted analyses of the region spanning *DRD4*, the dopamine D4 receptor, an extensively studied ADHD candidate gene on 11p15.5 (Hsiung et al., 2004). Although this latter study observed linkage to the region, the authors could not detect any association between known allelic variants of *DRD4* and dyslexia susceptibility.

DYX8 The short arm of chromosome 1 was implicated in one of the earlier findings of the field; a translocation affecting 1p22 was reported to co-segregate with severe writing and reading difficulties in a small family (Rabin et al., 1993). Subsequent studies have provided support for a dyslexia susceptibility locus in a slightly different location on this chromosome, 1p34-p36 (named DYX8) via linkage analysis of qualitative phenotypes and quantitative measures (Grigorenko et al., 2001). Further evidence has come from the sibling pair studies

of the DDP, in which the strongest linkage peak for categorical dyslexia was located at 1p36 (de Kovel et al., 2008) as well as from a later report of 1p36 linkage in families with SLI (Rice et al., 2009). *KIAA0319L* in 1p34 has been proposed as a candidate gene in the DYX8 region, since it is a likely homologue of *KIAA0319*, displaying 61% similarity at the protein level (Couto et al., 2008). Embryonic knockdown of *Kiaa0319l* expression in rats caused a similar phenotype to that observed in the earlier experiments targeting *Kiaa0319*: aberrant migration patterns with heterotopias and non-cell autonomous effects (Platt et al., 2013).

DYX9 In addition to recruiting families in which at least two first-degree relatives had a history of reading problems, the DDP identified a number of large three-generation Dutch pedigrees with multiple affected individuals (de Kovel et al., 2004). A genome-wide linkage scan of categorical dyslexia was carried out in one particularly interesting family, in which 15 of 29 available members could be classified as affected, based on reading tests. The study identified a genome-wide significant peak of linkage on the X chromosome, in Xq27.3, around the marker DXS8043, with a risk haplotype shared by 12 of the 15 affected family members (de Kovel et al., 2004). All four males who carried this haplotype were severely affected based on their reading scores - note that males only carry a single X chromosome, while females carry two copies. The eight female carriers with a categorical diagnosis of dyslexia showed greater variability in phenotype, and there was also an additional female carrier who was unaffected. This is consistent with a putative causative mutation in this region having a dominant mode of action, but with reduced penetrance and more variable effects in females. Analysis of the coding sequence of four candidate genes within this shared region (*FMR1*, *Cxorf1 (TMEM257)*, *DKFZp574M2010*, and *KIAA1854 (SLITRK2)*) did not reveal any mutations, and the causative gene in this family remains undiscovered.

Analyses of the separate DDP sibling pair sample did not find supporting evidence for DYX9 (de Kovel et al., 2004), suggesting that the involvement of the putative risk gene might be limited to rare mutations of large effect. Nevertheless, there are hints from other studies that there might indeed be common risk variants located in this part of the X chromosome. Linkage between Xq26 and reading-related measures was reported in the earlier genome-wide screens by Fisher and colleagues (Fisher et al., 2002). Xq27.3 markers showed suggestive

association with dyslexia in the female sub-sample of a study in an Afrikaner population (Platko et al., 2008), and suggestive linkage to a nonsense-word spelling phenotype in a general population sample (Bates et al., 2007). Finally, a recent study of French dyslexic families also supported the DYX9 locus, with maximal linkage at Xq27.3 (Huc-Chabrolle et al., 2013). The authors proposed that variants affecting *FMR1*, a gene implicated in fragile X syndrome (the most common genetic cause of intellectual disability), might be involved, although sequencing of this gene and six other candidates (*CXORF1, CXORF51, SLITRK2, FMR2, ASFMR1, FMR1NB*) failed to identify any mutation or polymorphisms co-segregating with dyslexia.

In addition to those described above, several additional loci have been described only once. Of particular interest are areas of the genome implicated through chromosomal aberrations which segregate with disorder in multiple members of a family. One of these is 21q22.3, which was found to segregate with dyslexia in a father and his two affected sons (Poelmans et al., 2009).

2.7 SHARED GENETIC AETIOLOGY BETWEEN DYSLEXIA AND LANGUAGE IMPAIRMENTS?

Given that many people with dyslexia show subtle underlying problems with aspects of linguistic processing, it is interesting to consider whether there might be some shared genetic mechanisms that are common to reading disability and more overt forms of language disorder. To test this at the molecular level, candidate genes identified from studies of language-impaired cohorts have been tested with respect to dyslexia as well as reading-related traits in the general population.

SLI, one of the most common forms of language impairment, is highly comorbid with dyslexia ($43-55\%$) (Newbury et al., 2011). Based on association analyses of SLI families, Newbury et al. (2009) proposed *CMIP* (16q23.2-q23.3) and *ATP2C2* (16q24.1) as candidate genes that modulate phonological short-term memory (measured by nonsense word repetition tasks) specifically in children with language impairment. A subsequent study confirmed a lack of association of *CMIP* and *ATP2C2* with nonsense word repetition in the unselected general population, but at the same time observed support for the contribution of *CMIP* variants to aspects of reading performance, including single word reading and spelling skills, across the normal range of abilities (Scerri et al., 2011).

Investigations of families with SLI have also identified association of language measures, particularly nonsense word repetition, with SNPs in *CNTNAP2* (7q35-36) (Vernes et al., 2008). This gene encodes a neurexin protein with multiple important functions in the central nervous system, and it is downregulated by *FOXP2*, a gene involved in rare single-gene forms of speech and language disorder (Vernes et al., 2008). Suggestive linkage to 7q35 has been found for speech and reading measures in independent SLI cohorts (Rice et al., 2009), and the SLI-associated *CNTNAP2* SNPs are correlated with assessments of early language performance (at age 2 years) in a general population sample (Whitehouse et al., 2011). In one study of dyslexia families using SNPs from all three SLI candidate genes (*CNTNAP2*, *ATP2C2*, *CMIP*) associations were only weak and sporadic, leading the authors to argue against pleiotropic effects of these loci (Newbury et al., 2011). However, association of *CNTNAP2* SNPs with nonsense word repetition has been described in a different dyslexia sample (Peter et al., 2011), while a copy number variant affecting *CNTNAP2* was recently reported in an individual with severe problems in reading–spelling tests and naming tasks (Veerappa et al., 2013).

As noted above, rare mutations of the *FOXP2* transcription factor have been found to cause a severe speech and language disorder, characterized by speech apraxia and multiple deficits in expressive and receptive language (Fisher and Scharff, 2009). To investigate whether common polymorphisms of *FOXP2* might affect the processing of written language in dyslexia, Wilcke et al. (2012) carried out a case-control association study of variants in this gene and detected nominal association for one SNP, rs21533005. They further studied this SNP in a small fMRI genetics study of phonological processing, proposing that non-carriers of the risk allele showed overactivation of temporo-parietal areas such as the angular and supramarginal gyri. They also suggested an interaction between dyslexia and genetic risk for the rolandic operculum, a brain region which is involved in motoric speech production. Another functional imaging study reported that SNPs of *FOXP2* were significantly associated with reading-related activation in two frontal regions of the left hemisphere: the inferior frontal gyrus and the dorsal part of the precentral gyrus (Pinel et al., 2012).

2.8 EXPLORING NEW ENDOPHENOTYPES: MISMATCH NEGATIVITY

As noted above, moving towards brain-based endophenotypes of dyslexia is likely to be important for the future of the field, opening up new avenues for genetic inves-

tigation. One potential endophenotype of interest is auditory mismatch negativity (MMN), a well-established component of auditory event related potential (ERP) that is elicited by any discriminable change in some repetitive aspect of the on-going auditory stimulation. It is an automatic response, and thus does not require attention, with a peak that is usually 100-250ms after stimulus onset (Naatanen, 2001). This ERP component provides an objective measure of discrimination accuracy, as it has been correlated with behavioural performance. MMN elicitation depends on short term memory, which has been shown to be reduced in dyslexics. The DDP longitudinal study has found that this phenotype is already disturbed very early in life in children from dyslexia risk families (van Leeuwen et al., 2008; van Zuijen et al., 2012), (also see paper on Precursors of developmental dyslexia, this issue). A later component of the MMN (referred to as late MMN or lMMN), with latency between 300-700ms, has also been proposed as a potential endophenotype, and it has been found to be reduced in the dyslexic population when compared to a control group (Neuhoff et al., 2012).

Roeske et al. (2011) carried out a genome-wide screen for association with MMN measures in children with dyslexia, and identified markers on 4q32.1 that were consistently associated with lMMN. The associated variants lie in a "gene desert" (a chromosomal region containing very few protein-coding genes). However, the markers were found to show significant association with levels of messenger RNA expressed from another gene located on another chromosome - *SLC2A3* in chromosome band 12p13. *SLC2A3* encodes the predominant facilitative transporter of glucose in neurons. The authors postulated that the identified SNPs on 4q32.1 exert regulatory effect on the *SLC2A3* locus (known as transregulation, since it lies on a different chromosome). They proposed that altered levels of *SLC2A3* protein could lead to glucose deficits in neurons of children with dyslexia, contributing to their smaller MMN during passive listening tasks. Another report described association between lMMN and three rare variants in high linkage disequilibrium in DYX2 on chromosome 6 (Czamara et al., 2011); one within *DCDC2* and the other two in the intergenic region between *DCDC2* and *KIAA0319*. It has been hypothesized that effects on lMMN in dyslexic readers reflect "intact auditory discrimination ability but alterations at later stages of auditory/ phonological processing" (Giraud and Ramus, 2012). Given the promising findings above, the lMMN endophenotype should be further pursued for genetic studies.

2.9 GENETICS AND THE DUTCH DYSLEXIA PROGRAM: PAST, PRESENT AND FUTURE

The DDP has already stimulated, and will continue to contribute to, molecular genetic research in at least three different related areas, considered below.

First, the DDP assembled a cohort of Dutch nuclear families with dyslexia in which 219 sibling pairs were phenotyped using key quantitative measures including nonsense word repetition, nonsense word reading, word reading, and rapid automatized naming. As demonstrated by de de Kovel et al. (2008), a robustly characterized sample such as this can be used to independently evaluate contributions of candidate dyslexia susceptibility loci identified in other studies. Replication of linkage and association findings is especially important for conditions like dyslexia, in which the genetic underpinnings are complex and multifactorial. Interestingly, work with the DDP cohort has replicated effects of DYX3 on chromosome 2 and DYX8 on chromosome 1, while at the same time finding no evidence for linkage to what is perhaps the most consistently replicated locus, DYX2 on chromosome 6. Evidence for linkage to DYX1 on chromosome 15, another commonly reported locus, was only found for the nonsense word repetition quantitative trait, which had low correlation with dyslexia categorical status in the DDP sample. de Kovel et al. (2008) point out that while quantitative traits are often used in dyslexia genetics, in the hope of getting closer to the molecular causes, the evidence provided by such measurements is seldom as simple to interpret as we wish. They note that "linkage peaks at the same locus are associated with different quantitative traits in different studies", which complicates the reliability of distinct findings that are often considered "replications".

It is clear that the dyslexia susceptibility loci that have been suggested by studies thus far can only account for a small proportion of the total heritability of this trait. Thus, there are likely to be multiple additional genetic risk factors waiting to be discovered. Based on experiences with other complex brain-related phenotypes, to successfully identify common risk variants of small effect size it will be necessary to carry out particularly large-scale screens of the genome. This can perhaps best be achieved by combining a number of independent well-phenotyped samples from multiple different research teams, in which case the existing DDP cohorts can make a key contribution to future gene discovery efforts.

Second, research by the DDP illustrates the potential of identifying rare multigenerational families in which many individuals are affected, in order to gain novel in-

sights into the biological underpinnings of disorder. In particular, as described above, the DDP reported identification of a Dutch family of almost 30 members, in which half were defined as dyslexic, and discovered a region of the X chromosome with significant linkage (de Kovel et al., 2004). This study again highlights the genetic heterogeneity of dyslexia. Although in most families/cases there will be complex genetic architecture with many risk variants, each contributing to a small amount of the total variance of reading ability, there are also unusual examples where the inheritance pattern appears consistent with a rare mutation of just a single gene, with a large effect size. Making use of recent technological advances in the field of genetics, we are currently using next-generation DNA sequencing technologies to zoom in on the putative causal mutation in this X-linked family. As has been shown by prior investigations of the *FOXP2* gene in speech and language disorders (Fisher and Scharff, 2009) rare single-gene effects offer exciting molecular windows into the aetiological pathways underlying more common cases of disorder. Two additional large pedigrees collected by DDP with similar segregation patterns to the reported family also await analysis (unpublished data).

Third, almost every molecular genetic study of dyslexia thus far has adopted a fixed perspective on behavioural and cognitive skills, failing to acknowledge that this is a developmental trait, a moving target in terms of phenotypic definition. A crucial factor for the future is to consider developmental trajectories for reading- and language-related skills of the children who are being studied, and relate them to the underlying genetics. As detailed in accompanying papers (see paper on Precursors of developmental dyslexia, this issue), the DDP assembled a very extensively phenotyped longitudinal sample of children from families who are at-risk of dyslexia, as well as matched non-risk families. These children have been carefully evaluated at multiple developmental timepoints from birth to 10 years of age, using a range of electrophysiological, behavioural and cognitive measures, motivated by hypotheses about the biological underpinnings of dyslexia. DNA has been collected from the majority of children and parents from the longitudinal cohort, providing a unique resource for association analyses with dyslexia endophenotypes; we are currently carrying out such studies for the top candidate SNPs from prior literature, described in our review above. As well as being able to assess the molecular contributions to several quantitative traits, including electrophysiological endophenotypes, this new upcoming research can also account for the temporal structure of the longitudinal assessments, assessing how profiles change with development and how this relates

to genotype. Such cohorts offer unprecedented opportunities to tap into central questions about the mechanistic and dynamic links that connect genes to dyslexia.

2.10 DISCUSSION

In sum, the relationship between genetic information and reading skills (as with other behavioural outputs) is not straightforward, especially when the genetic bases are known to be highly complex and heterogenic. Hence, there is a large amount of data that needs to be accumulated and integrated in order to reach a coherent understanding that will enable us disentangle the environmental and genetic effects on dyslexia. Endophenotypes such as behavioural traits and neuroimaging measurements enable a quantitative assessment of the dyslexic phenotype and their relation to genetic variants, which will be essential for us to gain better understanding of its biology. The evidence from existing candidates points towards molecular pathways affecting neuronal migration and axon guidance, which in turn may compromise the brain architecture affecting phonological processing (for example). It is likely that multiple susceptibility genes remain to be discovered, and it will be interesting to find out whether they implicate similar neuronal mechanisms to those candidates suggested so far, or lead us into novel molecular pathways.

Most of the genetic variants that have been related to dyslexia in prior work have been non-coding (not altering sequences of encoded proteins), and as such they have been proposed to have regulatory effects on the genes, although in the majority of cases direct functional evidence has not been demonstrated. On the other hand, the loss of function of several of the candidate genes in animal models has shown impacts on brain development. Nevertheless, the observed phenotypes in these model systems can often be quite subtle.

Note that the dyslexic category is specific to a literate culture, and that most of the affected people would not have a disability if they were not required to read. Reading depends on years of explicit instruction and practice in order to develop this highly specialized skill. Hence, the genetic effects that underlie reading abilities and disabilities must be widespread in nature, given that they are affecting the fine tuning of an otherwise robust molecular and cognitive system.

The ultimate goal of this area of research is not only to break down the genetic components of dyslexia, but also to build bridges from the subcellular molecular pathways to manifestations of reading problems. To do so, we will need to step into

the brain, trying to understand the effects of dyslexia candidate genes on structures and functions of key neural circuits, and on temporal processing of language, as well as how these relate to the behavioural traits on which dyslexia is defined.

2.11 GLOSSARY OF MOLECULAR GENETIC TERMS

ALLELES Alternative variants of the same gene or genomic position, originally arising due to mutation.

ASSOCIATION ANALYSIS Testing for non-random correlations between a phenotypic trait (qualitative or quantitative) and specific allelic variants. Can be carried out for just a single genetic marker, or used to screen multiple sites within a gene, or even across the entire genome, assuming appropriate adjustments are made for multiple testing when evaluating significance of results.

CHROMOSOMAL BAND Each human chromosome has a short arm ('p') and long arm ('q'), separated by a centromere. Each chromosome arm is divided into regions, or cytogenetic bands, that can be seen using a microscope and special stains. These bands are labeled p1, p2, p3, q1, q2, q3, etc., counting from the centromere outwards. At higher resolutions, sub-bands can be seen within the bands, also numbered from centromere outwards. For example, the cytogenetic map location of the *DYX1C1* gene is 15q21.3, which indicates it is on chromosome 15, q arm, band 2, sub-band 1, and sub-sub-band 3.

COPY NUMBER VARIANT A structural alteration of a chromosome in which there are an abnormal number of copies of a particular section of DNA, as a consequence of regions of the genome being deleted or duplicated.

CYTOSKELETON The cellular scaffolding of a cell. It has an essential and dynamic role in many cellular processes, such as cellular division, migration, and intracellular transport.

DELETION Loss of genetic material. The size of the missing region may range from just a single nucleotide of DNA, all the way to a large part of a chromosome.

DOMINANT INHERITANCE In studies of genetic disorders, when one abnormal copy of a gene from a single parent gives rise to the disorder, even though the copy inherited from the other parent is normal.

ENDOPHENOTYPE A measurable intermediate trait that is assumed to provide a closer link to the biological substrate of a disorder.

EXPRESSION Process in which genetic information contained in DNA is used to synthesize a functional gene product, such as a protein. For example, if a protein-coding gene has high expression in a particular tissue, it means that large amounts of the encoded protein are being produced in that tissue.

GENOTYPE The genetic constitution of an individual. Can refer to the entire complement of genetic material, a specific gene, or a set of genes.

HAPLOTYPE A specific combination of several adjacent polymorphisms on a chromosome that are inherited together.

HERITABILITY The proportion of variability in a particular characteristic that can be attributed to genetic influences. It is a statistical description that applies to a specific population and so it can change if the environment is altered.

LINKAGE MAPPING The use of polymorphic genetic markers (such as short tandem repeats) to identify the approximate genomic location of a gene responsible for a given trait. This technique relies on tracking the inheritance of the genetic markers in families and testing whether they co-segregate with the trait of interest, in a manner that is unlikely to have occurred by chance.

LINKAGE DISEQUILIBRIUM A property of physically close regions of the genome, which tend to be inherited together, resulting in the non-random association of specific allelic variants at the neighbouring loci.

ORTHOLOGUE Corresponding versions of the same gene found in different species, having arisen from a single gene present in the last common ancestor of those species. Orthologues in different species may be denoted with distinct symbols, and human genes are typically referred to with uppercase letters. For example, the first reported dyslexia candidate gene has the symbol *DYX1C1* in humans, but Dyx1c1 in rodents.

PENETRANCE The proportion of individuals with a particular gene variant who also express an associated trait (phenotype). Complete penetrance means that every individual with the same specific genotype manifests the phenotype.

PHENOTYPE The appearance of an individual in terms of a particular characteristic; physical, biochemical, physiological etc., resulting from interactions between genotype, environment and random factors.

PLEIOTROPY When a single gene has effects on multiple unrelated phenotypes.

POLYMORPHISM A position in the genome that contains variation in the population and therefore has more than one possible allele. At present, the most commonly studied of these are single nucleotide polymorphisms (SNPs) involving a single nucleotide at a specific point in the genome. When a SNP is discovered in the human population, it is given a unique identifier, a number beginning with "rs", so that it can be consistently described in different studies. For example, rs4504469 is a SNP within the *KIAA0319* gene with two known alleles, either a C (the most common allele) or a T.

PROMOTER A region at the start of each gene that is responsible for its regulation, allowing it to be switched on/off in different cell types and developmental stages (i.e. determining when and where the gene is expressed). This process depends on transcription factors that bind to these regions.

RECESSIVE INHERITANCE In studies of genetic disorders, when the disorder is only manifested if both copies of a gene are abnormal (one copy inherited from each parent).

REPORTER GENE ASSAY A test of gene function that can be carried out in cells grown in the laboratory, used to assess the role of specific variants in the regulatory regions of genes (such as promoters). The region of interest is placed next to a reporter gene and inserted into the cells being studied. The amount of gene product from the reporter gene can be measured.

SHORT TANDEM REPEAT Repeating sequences of 2-6 nucleotides, one after another in the genome. In many cases, the number of repeats is variable in different members of a population and hence can be used as a polymorphic marker.

SPLICING When genetic information at the DNA level is converted into a gene product, such as protein, it occurs via an intermediate molecule, known as messenger RNA. This intermediate molecule undergoes an editing process in which certain sections are removed and remaining pieces joined together, a cellular process that is referred to as splicing.

STOP CODON A small section within a gene that signals the endpoint (termination) of the protein that it encodes. If a mutation results in an early stop codon, midway within a gene, then the encoded protein is truncated and may not function properly.

TRANSLOCATION Genetic rearrangement in which part of a chromosome breaks and becomes attached to another part of the same chromosome, or to a different chromosome.

TRANSCRIPTION FACTOR A DNA-binding protein that regulates gene expression.

References

Adler WT, Platt MP, Mehlhorn AJ, Haight JL, Currier TA, et al. (2013) Position of neocortical neurons transfected at different gestational ages with shRNA targeted against candidate dyslexia susceptibility genes. PLoS ONE 8: e65179.

Anthoni H, Zucchelli M, Matsson H, Muller-Myhsok B, Fransson I, et al. (2007) A locus on 2p12 containing the co-regulated MRPL19 and C2ORF3 genes is associated to dyslexia. Hum Mol Genet 16: 667–677.

Bartlett CW, Flax JF, Logue MW, Vieland VJ, Bassett AS, et al. (2002) A major susceptibility locus for specific language impairment is located on 13q21. Am J Hum Genet 71: 45–55.

Bates TC, Lind PA, Luciano M, Montgomery GW, Martin NG, et al. (2010) Dyslexia and DYX1C1: deficits in reading and spelling associated with a missense mutation. Mol Psychiatry 15: 1190–1196.

Bates TC, Luciano M, Castles A, Coltheart M, Wright MJ, et al. (2007) Replication of reported linkages for dyslexia and spelling and suggestive evidence for novel regions on chromosomes 4 and 17. Eur J Hum Genet 15: 194–203.

Bates TC, Luciano M, Medland SE, Montgomery GW, Wright MJ, et al. (2011) Genetic variance in a component of the language acquisition device: ROBO1 polymorphisms associated with phonological buffer deficits. Behav Genet 41: 50–57.

Bellini G, Bravaccio C, Calamoneri F, Donatella Cocuzza M, Fiorillo P, et al. (2005) No evidence for association between dyslexia and DYX1C1 functional variants in a group of children and adolescents from Southern Italy. J Mol Neurosci 27: 311–314.

Brkanac Z, Chapman NH, Matsushita MM, Chun L, Nielsen K, et al. (2007) Evaluation of candidate genes for DYX1 and DYX2 in families with dyslexia. Am J Med Genet B Neuropsychiatr Genet 144B: 556–560.

Cardon LR, Smith SD, Fulker DW, Kimberling WJ, Pennington BF, et al. (1994) Quantitative trait locus for reading disability on chromosome 6. Science 266: 276–279.

Chandrasekar G, Vesterlund L, Hultenby K, Tapia-Paez I, Kere J (2013) The zebrafish orthologue of the dyslexia candidate gene DYX1C1 is essential for cilia growth and function. PLoS ONE 8: e63123.

Chapman NH, Igo RP, Thomson JB, Matsushita M, Brkanac Z, et al. (2004) Linkage analyses of four regions previously implicated in dyslexia: confirmation of a locus on chromosome 15q. Am J Med Genet B Neuropsychiatr Genet 131B: 67–75.

Consortium S (2002) A genomewide scan identifies two novel loci involved in specific language impairment. Am J Hum Genet 70: 384–398.

Cope N, Eicher JD, Meng H, Gibson CJ, Hager K, et al. (2012) Variants in the DYX2 locus are associated with altered brain activation in reading-related brain regions in subjects with reading disability. Neuroimage 63: 148–156.

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, et al. (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76: 581–591.

Couto JM, Gomez L, Wigg K, Cate-Carter T, Archibald J, et al. (2008) The KIAA0319-like (KIAA0319L) gene on chromosome 1p34 as a candidate for reading disabilities. J Neurogenet 22: 295–313.

Couto JM, Livne-Bar I, Huang K, Xu Z, Cate-Carter T, et al. (2010) Association of reading disabilities with regions marked by acetylated H3 histones in KIAA0319. Am J Med Genet B Neuropsychiatr Genet 153B: 447–462.

Currier TA, Etchegaray MA, Haight JL, Galaburda AM, Rosen GD (2011) The effects of embryonic knockdown of the candidate dyslexia susceptibility gene homologue Dyx1c1 on the distribution of GABAergic neurons in the cerebral cortex. Neuroscience 172: 535–546.

Czamara D, Bruder J, Becker J, Bartling J, Hoffmann P, et al. (2011) Association of a rare variant with mismatch negativity in a region between KIAA0319 and DCDC2 in dyslexia. Behav Genet 41: 110–119.

Dahdouh F, Anthoni H, Tapia-Paez I, Peyrard-Janvid M, Schulte-Korne G, et al. (2009) Further evidence for DYX1C1 as a susceptibility factor for dyslexia. Psychiatr Genet 19: 59–63.

Darki F, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T (2012) Three Dyslexia Susceptibility Genes, DYX1C1, DCDC2, and KIAA0319, Affect Temporo-Parietal White Matter Structure. Biol Psychiatry 72: 671–676.

de Kovel CG, Franke B, Hol FA, Lebrec JJ, Maassen B, et al. (2008) Confirmation of dyslexia susceptibility loci on chromosomes 1p and 2p, but not 6p in a Dutch sib-pair collection. Am J Med Genet B Neuropsychiatr Genet 147: 294–300.

de Kovel CG, Hol FA, Heister JG, Willemen JJ, Sandkuijl LA, et al. (2004) Genome-wide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. J Med Genet 41: 652–657.

Deffenbacher KE, Kenyon JB, Hoover DM, Olson RK, Pennington BF, et al. (2004) Refinement of the 6p21.3 quantitative trait locus influencing dyslexia: linkage and

association analyses. Hum Genet 115: 128–138.

Dennis MY, Paracchini S, Scerri TS, Prokunina-Olsson L, Knight JC, et al. (2009) A common variant associated with dyslexia reduces expression of the KIAA0319 gene. PLoS Genet 5: e1000436.

Fagerheim T, Raeymaekers P, T?nnessen FE, Pedersen M, Tranebjaerg L, et al. (1999) A new gene (DYX3) for dyslexia is located on chromosome 2. J Med Genet 36: 664–669.

Fisher SE, DeFries JC (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. Nat Rev Neurosci 3: 767–780.

Fisher SE, Francks C, Marlow AJ, MacPhie IL, Newbury DF, et al. (2002) Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. Nat Genet 30: 86–91.

Fisher SE, Scharff C (2009) FOXP2 as a molecular window into speech and language. Trends Genet 25: 166–177.

Fisher SE, Stein JF, Monaco AP (1999) A genome-wide search strategy for identifying quantitative trait loci involved in reading and spelling disability (developmental dyslexia). Eur Child Adolesc Psychiatry 8 Suppl 3: 47–51.

Francks C, Fisher SE, Olson RK, Pennington BF, Smith SD, et al. (2002) Fine mapping of the chromosome 2p12-16 dyslexia susceptibility locus: quantitative association analysis and positional candidate genes SEMA4F and OTX1. Psychiatr Genet 12: 35–41.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, et al. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75: 1046–1058.

Gabel LA, Marin I, LoTurco JJ, Che A, Murphy C, et al. (2011) Mutation of the dyslexia-associated gene Dcdc2 impairs LTM and visuo-spatial performance in mice. Genes Brain Behav 10: 868–875.

Giraud AL, Ramus F (2012) Neurogenetics and auditory processing in developmental dyslexia. Curr Opin Neurobiol 23: 1–6.

Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160: 636–645.

Graham SA, Fisher SE (2013) Decoding the genetics of speech and language. Curr Opin Neurobiol 23: 43–51.

Grigorenko EL (2001) Developmental dyslexia: an update on genes, brains, and environments. J Child Psychol Psychiatry 42: 91–125.

Grigorenko EL, Wood FB, Meyer MS, Hart LA, Speed WC, et al. (1997) Susceptibility loci for distinct components of developmental dyslexia on chromosomes 6 and 15. Am J Hum Genet 60: 27–39.

Grigorenko EL, Wood FB, Meyer MS, Pauls JE, Hart LA, et al. (2001) Linkage studies suggest a possible locus for developmental dyslexia on chromosome 1p. Am J Med Genet 105: 120–129.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Harold D, Paracchini S, Scerri T, Dennis M, Cope N, et al. (2006) Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia. Mol Psychiatry 11: 1085–1091.

Hsiung GY, Kaplan BJ, Petryshen TL, Lu S, Field LL (2004) A dyslexia susceptibility locus (DYX7) linked to dopamine D4 receptor (DRD4) region on chromosome 11p15.5. Am J Med Genet B Neuropsychiatr Genet 125B: 112–119.

Huc-Chabrolle M, Charon C, Guilmatre A, Vourc'h P, Tripi G, et al. (2013) Xq27 FRAXA Locus is a Strong Candidate for Dyslexia: Evidence from a Genome-Wide Scan in French Families. Behav Genet 43: 132–140.

Ivliev AE, 't Hoen PA, van Roon-Mom WM, Peters DJ, Sergeeva MG (2012) Exploring the transcriptome of ciliated cells using in silico dissection of human tissues. PLoS ONE 7: e35618.

Jamadar S, Powers NR, Meda SA, Gelernter J, Gruen JR, et al. (2011) Genetic influences of cortical gray matter in language-related regions in healthy controls and schizophrenia. Schizophr Res 129: 141–148.

Kaminen N, Hannula-Jouppi K, Kestila M, Lahermo P, Muller K, et al. (2003) A genome scan for developmental dyslexia confirms linkage to chromosome 2p11 and suggests a new locus on 7q32. J Med Genet 40: 340–345.

Kaplan DE, Gayan J, Ahn J, Won TW, Pauls D, et al. (2002) Evidence for linkage and association with reading disability on 6p21.3-22. Am J Hum Genet 70: 1287–1298.

Kendler KS, Neale MC (2010) Endophenotype: a conceptual analysis. Mol Psychiatry 15: 789–797.

Lamminmaki S, Massinen S, Nopola-Hemmi J, Kere J, Hari R (2012) Human ROBO1 regulates interaural interaction in auditory pathways. J Neurosci 32: 966–971.

Levecque C, Velayos-Baeza A, Holloway ZG, Monaco AP (2009) The dyslexia-associated protein KIAA0319 interacts with adaptor protein 2 and follows the

classical clathrin-mediated endocytosis pathway. Am J Physiol, Cell Physiol 297: C160–168.

Luciano M, Lind PA, Duffy DL, Castles A, Wright MJ, et al. (2007) A haplotype spanning KIAA0319 and TTRAP is associated with normal variation in reading and spelling ability. Biol Psychiatry 62: 811–817.

Ludwig KU, Roeske D, Schumacher J, Schulte-Korne G, Konig IR, et al. (2008) Investigation of interaction between DCDC2 and KIAA0319 in a large German dyslexia sample. J Neural Transm 115: 1587–1589.

Marino C, Citterio A, Giorda R, Facoetti A, Menozzi G, et al. (2007) Association of short-term memory with a variant within DYX1C1 in developmental dyslexia. Genes Brain Behav 6: 640–646.

Marino C, Meng H, Mascheretti S, Rusconi M, Cope N, et al. (2012) DCDC2 genetic variants and susceptibility to developmental dyslexia. Psychiatr Genet 22: 25–30.

Massinen S, Hokkanen ME, Matsson H, Tammimies K, Tapia-Paez I, et al. (2011) Increased expression of the dyslexia candidate gene DCDC2 affects length and signaling of primary cilia in neurons. PLoS ONE 6: e20580.

Massinen S, Tammimies K, Tapia-Paez I, Matsson H, Hokkanen ME, et al. (2009) Functional interaction of DYX1C1 with estrogen receptors suggests involvement of hormonal pathways in dyslexia. Hum Mol Genet 18: 2802–2812.

Meda SA, Gelernter J, Gruen JR, Calhoun VD, Meng H, et al. (2008) Polymorphism of DCDC2 Reveals Differences in Cortical Morphology of Healthy Individuals-A Preliminary Voxel Based Morphometry Study. Brain Imaging Behav 2: 21–26.

Meng H, Powers NR, Tang L, Cope NA, Zhang PX, et al. (2011) A dyslexia-associated variant in DCDC2 changes gene expression. Behav Genet 41: 58–66.

Meng H, Smith SD, Hager K, Held M, Liu J, et al. (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci USA 102: 17053–17058.

Morris DW, Robinson L, Turic D, Duke M, Webb V, et al. (2000) Family-based association mapping provides evidence for a gene for reading disability on chromosome 15q. Hum Mol Genet 9: 843–848.

Naatanen R (2001) The perception of speech sounds by the human brain as reflected by the mismatch negativity (MMN) and its magnetic equivalent (MMNm). Psychophysiology 38: 1–21.

Neuhoff N, Bruder J, Bartling J, Warnke A, Remschmidt H, et al. (2012) Evidence for the late MMN as a neurophysiological endophenotype for dyslexia. PLoS ONE 7:

e34909.

Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, et al. (2011) Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behav Genet 41: 90–104.

Newbury DF, Winchester L, Addis L, Paracchini S, Buckingham LL, et al. (2009) CMIP and ATP2C2 modulate phonological short-term memory in language impairment. Am J Hum Genet 85: 264–272.

Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, et al. (2001) A dominant gene for developmental dyslexia on chromosome 3. J Med Genet 38: 658–664.

Nopola-Hemmi J, Taipale M, Haltia T, Lehesjoki AE, Voutilainen A, et al. (2000) Two translocations of chromosome 15q associated with dyslexia. J Med Genet 37: 771–775.

Paracchini S (2011) Dissection of genetic associations with language-related traits in population-based cohorts. J Neurodev Disord 3: 365–373.

Paracchini S, Steer CD, Buckingham LL, Morris AP, Ring S, et al. (2008) Association of the KIAA0319 dyslexia susceptibility gene with reading skills in the general population. Am J Psychiatry 165: 1576–1584.

Paracchini S, Thomas A, Castro S, Lai C, Paramasivam M, et al. (2006) The chromosome 6p22 haplotype associated with dyslexia reduces the expression of KIAA0319, a novel gene involved in neuronal migration. Hum Mol Genet 15: 1659–1666.

Peschansky VJ, Burbridge TJ, Volz AJ, Fiondella C, Wissner-Gross Z, et al. (2010) The effect of variation in expression of the candidate dyslexia susceptibility gene homolog Kiaa0319 on neuronal migration and dendritic morphology in the rat. Cereb Cortex 20: 884–897.

Peter B, Raskind WH, Matsushita M, Lisowski M, Vu T, et al. (2011) Replication of CNTNAP2 association with nonword repetition and support for FOXP2 association with timed reading and motor activities in a dyslexia family sample. J Neurodev Disord 3: 39–49.

Petryshen TL, Kaplan BJ, Fu Liu M, de French NS, Tobias R, et al. (2001) Evidence for a susceptibility locus on chromosome 6q influencing phonological coding dyslexia. Am J Med Genet 105: 507–517.

Petryshen TL, Kaplan BJ, Hughes ML, Tzenova J, Field LL (2002) Supportive evidence for the DYX3 dyslexia susceptibility gene in Canadian families. J Med Genet 39:

125–126.

Peyrard-Janvid M, Anthoni H, Onkamo P, Lahermo P, Zucchelli M, et al. (2004) Fine mapping of the 2p11 dyslexia locus and exclusion of TACR1 as a candidate gene. Hum Genet 114: 510–516.

Pinel P, Fauchereau F, Moreno A, Barbot A, Lathrop M, et al. (2012) Genetic variants of FOXP2 and KIAA0319/TTRAP/THEM2 locus are associated with altered brain activation in distinct language-related regions. J Neurosci 32: 817–825.

Platko JV, Wood FB, Pelser I, Meyer M, Gericke GS, et al. (2008) Association of reading disability on chromosome 6p22 in the Afrikaner population. Am J Med Genet B Neuropsychiatr Genet 147B: 1278–1287.

Platt MP, Adler WT, Mehlhorn AJ, Johnson GC, Wright KA, et al. (2013) Embryonic disruption of the candidate dyslexia susceptibility gene homolog Kiaa0319-like results in neuronal migration disorders. Neuroscience 248C: 585–593.

Poelmans G, Buitelaar JK, Pauls DL, Franke B (2011) A theoretical molecular network for dyslexia: integrating available genetic findings. Mol Psychiatry 16: 365–382.

Poelmans G, Engelen JJ, Van Lent-Albrechts J, Smeets HJ, Schoenmakers E, et al. (2009) Identification of novel dyslexia candidate genes through the analysis of a chromosomal deletion. Am J Med Genet B Neuropsychiatr Genet 150B: 140–147.

Powers NR, Eicher JD, Butter F, Kong Y, Miller LL, et al. (2013) Alleles of a Polymorphic ETV6 Binding Site in DCDC2 Confer Risk of Reading and Language Impairment. Am J Hum Genet 93: 19–28.

Rabin M, Wen XL, Hepburn M, Lubs HA, Feldman E, et al. (1993) Suggestive linkage of developmental dyslexia to chromosome 1p34-p36. Lancet 342: 178.

Raskind WH, Igo RP, Chapman NH, Berninger VW, Thomson JB, et al. (2005) A genome scan in multigenerational families with dyslexia: Identification of a novel locus on chromosome 2q that contributes to phonological decoding efficiency. Mol Psychiatry 10: 699–711.

Rice ML, Smith SD, Gayan J (2009) Convergent genetic linkage and associations to language, speech and reading measures in families of probands with Specific Language Impairment. J Neurodev Disord 1: 264–282.

Roeske D, Ludwig KU, Neuhoff N, Becker J, Bartling J, et al. (2011) First genome-wide association scan on neurophysiological endophenotypes points to trans-regulation effects on SLC2A3 in dyslexic children. Mol Psychiatry 16: 97–107.

Scerri TS, Darki F, Newbury DF, Whitehouse AJ, Peyrard-Janvid M, et al. (2012) The dyslexia candidate locus on 2p12 is associated with general cognitive ability and

white matter structure. PLoS ONE 7: e50321.

Scerri TS, Fisher SE, Francks C, MacPhie IL, Paracchini S, et al. (2004) Putative functional alleles of DYX1C1 are not associated with dyslexia susceptibility in a large sample of sibling pairs from the UK. J Med Genet 41: 853–857.

Scerri TS, Morris AP, Buckingham LL, Newbury DF, Miller LL, et al. (2011) DCDC2, KIAA0319 and CMIP are associated with reading-related traits. Biol Psychiatry 70: 237–245.

Scerri TS, Paracchini S, Morris A, MacPhie IL, Talcott J, et al. (2010) Identification of candidate genes for dyslexia susceptibility on chromosome 18. PLoS ONE 5: e13712.

Schulte-Korne G, Grimm T, Nothen MM, Muller-Myhsok B, Cichon S, et al. (1998) Evidence for linkage of spelling disability to chromosome 15. Am J Hum Genet 63: 279–282.

Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, et al. (2006a) Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. Am J Hum Genet 78: 52–62.

Schumacher J, Hoffmann P, Schmal C, Schulte-Korne G, Nothen MM (2007) Genetics of dyslexia: the evolving landscape. J Med Genet 44: 289–297.

Schumacher J, Konig IR, Plume E, Propping P, Warnke A, et al. (2006b) Linkage analyses of chromosomal region 18p11-q12 in dyslexia. J Neural Transm 113: 417–423.

Schumacher J, Konig IR, Schroder T, Duell M, Plume E, et al. (2008) Further evidence for a susceptibility locus contributing to reading disability on chromosome 15q15-q21. Psychiatr Genet 18: 137–142.

Seshadri S, DeStefano AL, Au R, Massaro JM, Beiser AS, et al. (2007) Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham Study. BMC Med Genet 8 Suppl 1: S15.

Shaywitz SE, Escobar MD, Shaywitz BA, Fletcher JM, Makuch R (1992) Evidence that dyslexia may represent the lower tail of a normal distribution of reading ability. N Engl J Med 326: 145–150.

Smith SD, Kimberling WJ, Pennington BF, Lubs HA (1983) Specific reading disability: identification of an inherited form through linkage analysis. Science 219: 1345–1347.

Szalkowski CE, Fiondella CF, Truong DT, Rosen GD, LoTurco JJ, et al. (2013) The effects of Kiaa0319 knockdown on cortical and subcortical anatomy in male rats.

Int J Dev Neurosci 31: 116–122.

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, et al. (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci USA 100: 11553–11558.

Tammimies K, Vitezic M, Matsson H, Le Guyader S, Burglin TR, et al. (2013) Molecular Networks of DYX1C1 Gene Show Connection to Neuronal Migration Genes and Cytoskeletal Proteins. Biol Psychiatry 73: 583–590.

Tapia-Paez I, Tammimies K, Massinen S, Roy AL, Kere J (2008) The complex of TFII-I, PARP1, and SFPQ proteins regulates the DYX1C1 gene implicated in neuronal migration and dyslexia. FASEB J 22: 3001–3009.

Tarkar A, Loges NT, Slagle CE, Francis R, Dougherty GW, et al. (2013) DYX1C1 is required for axonemal dynein assembly and ciliary motility. Nat Genet 45: 995–1003.

Tran C, Gagnon F, Wigg KG, Feng Y, Gomez L, et al. (2013) A family-based association analysis and meta-analysis of the reading disabilities candidate gene DYX1C1. Am J Med Genet B Neuropsychiatr Genet 162: 146–156.

van Leeuwen T, Been P, van Herten M, Zwarts F, Maassen B, et al. (2008) Two-month-old infants at risk for dyslexia do not discriminate /bAk/ from /dAk/: A brain-mapping study. Journal of Neurolinguistics 21: 333 – 348, ¡ce:title¿Use of Electrophysiological Measures in Reading Research¡/ce:title¿.

van Zuijen TL, Plakas A, Maassen BA, Been P, Maurits NM, et al. (2012) Temporal auditory processing at 17 months of age is associated with preliterate language comprehension and later word reading fluency: An ERP study. Neurosci Lett 528: 31–35.

Veerappa AM, Saldanha M, Padakannaya P, Ramachandra NB (2013) Family-based genome-wide copy number scan identifies five new genes of dyslexia involved in dendritic spinal plasticity. J Hum Genet 58: 539–547.

Velayos-Baeza A, Levecque C, Kobayashi K, Holloway ZG, Monaco AP (2010) The dyslexia-associated KIAA0319 protein undergoes proteolytic processing with gamma-secretase-independent intramembrane cleavage. J Biol Chem 285: 40148–40162.

Velayos-Baeza A, Toma C, da Roza S, Paracchini S, Monaco AP (2007) Alternative splicing in the dyslexia-associated gene KIAA0319. Mamm Genome 18: 627–634.

Velayos-Baeza A, Toma C, Paracchini S, Monaco AP (2008) The dyslexia-associated gene KIAA0319 encodes highly N- and O-glycosylated plasma membrane and secreted isoforms. Hum Mol Genet 17: 859–871.

Venkatesh SK, Siddaiah A, Padakannaya P, Ramachandra NB (2013) Analysis of genetic variants of dyslexia candidate genes KIAA0319 and DCDC2 in Indian population. J Hum Genet 58: 531–538.

Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, et al. (2008) A functional genetic link between distinct developmental language disorders. N Engl J Med 359: 2337–2345.

Wang Y, Paramasivam M, Thomas A, Bai J, Kaminen-Ahola N, et al. (2006) DYX1C1 functions in neuronal migration in developing neocortex. Neuroscience 143: 515–522.

Wang Y, Yin X, Rosen G, Gabel L, Guadiana SM, et al. (2011) Dcdc2 knockout mice display exacerbated developmental disruptions following knockdown of doublecortin. Neuroscience 190: 398–408.

Whitehouse AJ, Bishop DV, Ang QW, Pennell CE, Fisher SE (2011) CNTNAP2 variants affect early language development in the general population. Genes Brain Behav 10: 451–456.

Wigg KG, Couto JM, Feng Y, Anderson B, Cate-Carter TD, et al. (2004) Support for EKN1 as the susceptibility locus for dyslexia on 15q21. Mol Psychiatry 9: 1111–1121.

Wilcke A, Ligges C, Burkhardt J, Alexander M, Wolf C, et al. (2012) Imaging genetics of FOXP2 in dyslexia. Eur J Hum Genet 20: 224–229.

Wilcke A, Weissfuss J, Kirsten H, Wolfram G, Boltze J, et al. (2009) The role of gene DCDC2 in German dyslexics. Ann Dyslexia 59: 1–11.

Zhong R, Yang B, Tang H, Zou L, Song R, et al. (2013) Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol 47: 435–442.

Zou L, Chen W, Shao S, Sun Z, Zhong R, et al. (2012) Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 159B: 970–976.

Zuo PX, Wu HR, Li ZC, Cao XD, Pang LJ, et al. (2012) Association of polymorphisms in the DCDC2 gene with developmental dyslexia in the Han Chinese. Chin Med J 125: 622–625.

3

ASSOCIATION ANALYSIS OF DYSLEXIA CANDIDATE GENES IN A DUTCH LONGITUDINAL SAMPLE

Dyslexia is a common specific learning disability with a substantive genetic component. Several candidate genes have been proposed to be implicated in dyslexia susceptibility, such as *DYX1C1*, *ROBO1*, *KIAA0319*, and *DCDC2*. Associations with variants in these genes have also been reported with a variety of psychometric measures tapping into the underlying processes that might be impaired in dyslexic people. In this study, we first conducted a literature review to select single nucleotide polymorphisms (SNPs) in dyslexia candidate genes that had been repeatedly implicated across studies. We then assessed the SNPs for association in the richly phenotyped longitudinal dataset from the Dutch Dyslexia Program. We tested for association with several quantitative traits, including word and nonword reading fluency, rapid naming, phoneme deletion, and nonword repetition. In this, we took advantage of the longitudinal nature of the sample to examine, if associations were stable across four developmental time-points (from 7 to 12 years). Two SNPs in the *KIAA0319* gene were found to be nominally associated with rapid naming, and these associations were stable across different ages. Genetic association analysis with complex cognitive traits can be enriched through the use of longitudinal information on trait development.

Keywords: association study, dyslexia, candidate genes, longitudinal

This chapter has been submitted as:

Carrion-Castillo, A., Maassen, B., Franke, B., Heister, A., Naber, M., van der Leij, A., Francks, C., & Fisher, S. E. Association analysis of dyslexia candidate genes in a Dutch longitudinal sample (under review).

3.1 INTRODUCTION

Reading ability is a complex behavioural trait. It is known that several cognitive processes are involved in the acquisition of this skill (Pennington, 2006). For example, successful reading of a novel word depends on phonological awareness, the ability to explicitly reflect on the internal sound structure of words, as well as phonological decoding, the ability to match phonetic units to their written equivalents (graphemes). The language in which reading is learned also plays an important role in the type of strategies learners use (Pennington, 2006). In spite of the essential role reading plays in many human societies nowadays, about 5-7% of the population have trouble in acquiring reading skills and may be diagnosed with dyslexia (Shaywitz et al., 1990).

It is well known that a substantial amount of the variance in reading ability is explained by inherited factors: genetic variance explains about 30-70% of the total variation in reading skills (Olson et al., 2014). However, we still know very little about the specific genetic underpinnings of this trait, since the genetic variants that have been identified so far can only explain a very small fraction of the heritability estimates. Nevertheless, some dyslexia candidate loci have been identified through linkage and candidate gene association studies, leading to proposal of several potential susceptibility genes, including the axon guidance receptor *ROBO1* in chromosome 3p12.3 (Hannula-Jouppi et al., 2005), *DYX1C1* in chromosome 15q21.3 (Nopola-Hemmi et al., 2000), and the genes *KIAA0319* and *DCDC2* in chromosome 6p22.3 (Francks et al., 2004; Cope et al., 2005).

The candidate genes have been studied in relation to dyslexia affection status and/or other reading-related traits in multiple studies. Some of the associations have supporting evidence from independent samples, in line with the hypothesis that they play a role in shaping the biology underlying the cognitive processes on which reading relies. Several of the candidate genes have been implicated in neuronal migration by *in utero* gene knockdown methods (Adler et al., 2013), which is intriguing given a possible association between disrupted cortical architecture and dyslexia (Giraud and Ramus, 2012).

Despite this, the evidence supporting the relevance of specific genetic variants that have been proposed so far remains inconclusive: some studies have been unable to replicate previous findings; in some other cases the associations were found with an opposite direction of effect (i.e. the risk allele of the original study was found to be protective in other studies). For example, the allele T of rs6935076, a SNP in

the *KIAA0319* gene, was originally reported to be associated with dyslexia affection status (Harold et al., 2006), and the same allele (T) was found to be associated with poorer performance on a language-standardized test and reading comprehension in a different sample (Rice et al., 2009). Nonetheless, multiple successive studies reported associations with the opposite direction of effect (i.e. risk allele= C) (Couto et al., 2010; Newbury et al., 2011; Scerri et al., 2011), and others did not find any association between this SNP and reading measures (Schumacher et al., 2006; Brkanac et al., 2007).

It is often argued that this lack of consistency could be at least partially explained by the heterogeneity across studies (Schumacher et al., 2006; Brkanac et al., 2007; Newbury et al., 2011), which occurs at various levels: from the study design (e.g. sample size, language), to trait characterization (e.g. ascertainment criteria, age at assesment), or the genetic background of the population that is being studied. It is also likely that some of the associations reflect false positive findings due to incomplete control of type-I error, a common challenge for genetic studies of complex traits.

An additional source of heterogeneity might come from variation in the developmental stage at which the diagnosis of dyslexia affection status or the quantitative trait measurement took place. In fact, it has been shown that there are changes in the relationship of reading-related traits (such as phonological awareness and rapid automized naming) with reading throughout development (de Jong and van der Leij, 2003; Wagner et al., 1997). There is evidence from other fields of human genetics that an age-varying effect could be one of the issues underlying the non-replication of some association studies (Lasky-Su et al., 2008; Franke et al., 2010). Despite the use of normative scores to compare across grades and efforts to account for the effects of age on reading ability, it is possible that the variability of ages within and between datasets might have contributed to the inconsistency of results. Most of the studies reported so far on the genetics of reading ability have been cross-sectional, where association has been tested between the SNPs of interest and dyslexia status, or quantitative traits measured only at one developmental time-point per subject. Apart from potentially providing higher statistical power than cross-sectional studies of equivalent sample sizes, longitudinal studies also give the possibility to evaluate associations that change over time (Sitlani et al., 2015). Age-dependent effects of SNPs have been reported in studies related to expressive language and autism-like traits (St Pourcain et al., 2014a,b).

Learning to read involves many cognitive processes, which makes it difficult to disentangle whether deficits that are used to characterize people with dyslexia (e.g. lower phonological awareness) are the reason for the difficulty or the consequence of a reduced experience in reading (Goswami, 2015). The direction of this causality can be better studied by looking at longitudinal samples, because they enable a comparison of developmental trajectories (even starting prior to reading instruction) between children that are eventually categorized as dyslexic from the trajectories of those that are not. For instance, mismatch negativity (MMN), an electrophysiological measure linked to speech processing, has been found to be reduced in children (Neuhoff et al., 2012) and adults (Schulte-Korne et al., 2001) diagnosed with dyslexia, and a longitudinal study by van Zuijen et al. (2013) showed that MMN at 2-months predicted later reading fluency scores. This supports the view that reduced MMN can be considered an early precursor of dyslexia, rather than a consequence of it.

In one of the few longitudinal studies of the genetics of reading skills, Zhang et al. (2012) looked at the association of three SNPs in *DYX1C1* and orthographic skills in relation to children's development over time. They found that rs11629841 in *DYX1C1* is associated with children's orthographic judgments at ages 7 and 8, but less-so at age 6 years.

In the current study, we have tested association of some of the most consistent dyslexia candidate genetic variants in a very extensively characterised longitudinal sample that has not yet been studied for genetic effects. The Dutch Dyslexia Programme (DDP) cohort consists of children with and without familial risk for dyslexia that have been evaluated at multiple developmental time-points, using psychometric measures related to the development of reading ability. In addition to studying the genetic association with measures of reading ability, the richness of this dataset allowed us to look into specific endophenotypes known to be linked to reading ability, such as speed of processing and phonological awareness (de Jong and van der Leij, 2003; van der Leij et al., 2013; Landerl et al., 2013). Importantly, some of these measures were taken across multiple developmental time-points, allowing us to observe the developmental trajectories of specific traits within this group of children. With these data, two simultaneous questions could be asked about the association of a given genetic variant (SNP) and quantitative trait: 1) Does the SNP have an effect on the overall level of the trait? 2) Does the effect of the SNP change over time?

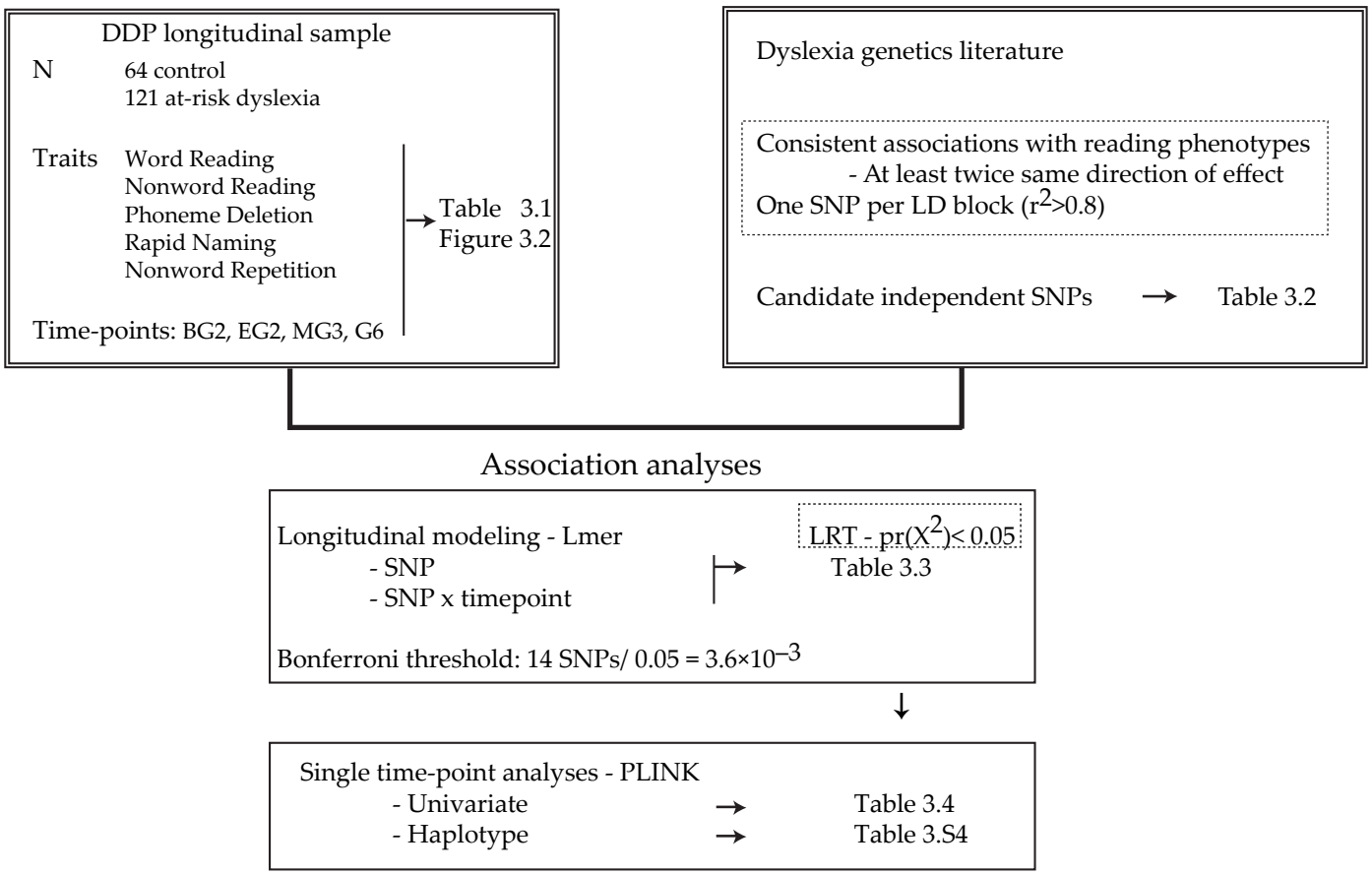


Figure 3.1: DDP longitudinal association study design.

3.2 MATERIAL AND METHODS

3.2.1 *Dataset*

The Dutch Dyslexia Program (DDP) dataset comprises children from families that were identified along two sets of diagnostic criteria. Some of the children were recruited based on family risk for dyslexia, that is, the child had at least one parent and another first degree relative with self-reported dyslexia, which was confirmed by tests measuring word and pseudoword reading fluency, as described by Koster et al (31) (N_{risk}=121). The remainder comprised control children without any family history of reading disability, according to the same criteria ($N_{control}$=64). All children had been followed from 0-12 years of age within the DDP longitudinal study. The present study focused on a number of reading- and language-related traits that had been measured at several developmental time-points over four years, as specified in Table 3.1. This study included 185 children with both behavioural measurements and available DNA (collected through Oragene saliva kits (DNA-Genotek, Ottawa, ON, Canada)), from 180 unrelated families. Therefore, most children were unrelated, but there were three sibships of 2 children, and one sibship of 3.

	Time points (N)	BG2 (168)	EG2 (180)	MG3 (180)	G6 (116)
	Average age (sd)	7.47 (0.41)	8.14 (0.37)	8.93 (0.36)	12.13 (0.40)
Trait	Description	Tests			
WRF	Word reading fluency	DMT	DMT	EMT	EMT
NWRF	Nonword reading fluency	-	Klepel	Klepel	Klepel
RAN_{dig}	Rapid naming of digits	RAN_{dig}	RAN_{dig}	RAN_{dig}	RAN_{dig}
PD	Phoneme Deletion	PD_1	PD_1,PD_2	PD_1,PD_2	PD_{AKT}
NWR	Nonword repetition	-	-	NRT	-

Table 3.1: Summary of the sample and longitudinal phenotypic measures available at different educational time-points. BG2: Beginning Grade 2, EG2: End Grade 2, MG3: Middle Grade 3, G6: Grade 6. Traits of interest are indicated, together with the name of the test that has been used to measure the trait in each time-point. DMT and EMT: word reading fluency tests, Klepel: nonword reading fluency test, PD1, PD2 and PDAKT: phoneme deletion tests. "-" indicates an absence of measurement at that time-point.

3.2.2 *Phenotypes*

A subset of measures available from the DDP project was selected to be tested in relation to dyslexia candidate gene variants (Table 3.1): word reading fluency (WRF), nonword reading fluency (NWRF), rapid naming (RAN), phoneme deletion (PD), and nonword repetition (NWR). Test reliabilities ranged from 0.73-0.97 (van Bergen et al., 2011). A detailed description of all traits measured in the DDP sample can be found in van van Bergen et al. (2011, 2012). Datapoints were excluded as outliers, if they deviated more than 3 standard deviations from the relevant trait mean within the developmental time-point.

Word reading fluency

Word reading fluency (WRF) was measured using standard Dutch reading tests that consist of reading aloud from a list as many words as possible within one minute. Two different tests were administered depending on the grade (see Table 3.1): the 'Drie Minuten Test' (DMT: three minute test: three lists, one minute each (Verhoeven, 1995)) and the 'Een Minuut Test' (EMT: one minute test, (EMT, (Brus and Voeten, 1972)). In both cases, the number of correctly read items per minute was taken as the outcome. These tests assess the reading accuracy as well as fluency.

Nonword reading fluency

Nonword reading fluency (NWRF) was measured using the 'Klepel' nonword reading test (van den Bos et al., 1994). Similarly to the word reading tests, a list of 50 nonwords must be read within a time limit, in this case two minutes. The outcome measure is the number of items read correctly.

Rapid naming

Serial rapid automatized naming (RAN_{dig}) (van den Bos, 2003) measures the speed of naming over-learned information. Children were asked to name 5 different digits, each occurring 10 times, as quickly as possible. The outcome measure is expressed as the number of digits named per second (i.e. 50 item/time in seconds).

Chr	Gene	Locus	SNP	Position (hg 19)	Identified by	Alleles Maj/Min	MAF	Risk	Associated phenotype*	Consistent associations	Inconsistent associations	Lack of associations
6	*DCDC2*	DYX2	rs793862	24207200	Schumacher et al. 2006	A/G	0.41	A	Dyslexia status	Deffenbacher et al. 2004; Wilcke et al. 2009; Scerri et al. 2011		Newbury et al. 2011; Cope et al. 2012
6	*DCDC2*	DYX2	rs807701	24273791	Schumacher et al. 2006	G/A	0.42	G	Dyslexia status	Wilcke et al. 2009; Scerri et al. 2011; Newbury et al. 2011	Cope et al. 2012	
6	*DCDC2*	DYX2	rs807724	24278869	Meng et al. 2005	T/C	0.24	C	Discriminant score	Wilcke et al. 2009; Scerri et al. 2011; Newbury et al. 2011	Cope et al. 2012	Brkanac et al. 2007
6	*KIAA0319*	DYX2	rs4504469	24588884	Francks et al. 2004	C/T	0.23	C	Single Word Reading	Cope et al. 2005; Harold et al. 2006; Paracchini et al. 2008; Rice et al. 2009; Newbury et al. 2011	Venkatesh et al. 2013	Schumacher et al. 2006; Luciano et al. 2007
6	*KIAA0319*	DYX2	rs761100	24632642	Harold et al. 2006	C/A	0.32	C	Single Word Reading	Rice et al. 2009	Newbury et al. 2011	
6	*KIAA0319*	DYX2	rs6935076	24644322	Cope et al. 2005	C/T	0.26	T	Dyslexia status	Rice et al. 2009; Darki et al. 2012	Couto et al. 2010; Newbury et al. 2011; Scerri et al. 2011	Schumacher et al. 2006; Brkanac et al. 2007

6	*KIAA0319*	DYX2	rs2038137	24645943	Francks et al. 2004	G/T	0.25	G	Single Word Reading	Cope et al. 2005; Harold et al. 2006; Paracchini et al. 2008	
6	*KIAA0319*	DYX2	rs9467247	24647219	Francks et al. 2004	C/A	0.33	A	Single Word Reading	Dennis et al. 2009	
6	*TTRAP*	DYX2	rs2143340	24659071	Francks et al. 2004	A/G	0.15	G	Single Word Reading	Paracchini et al. 2008; Newbury et al. 2011; Scerri et al. 2011	Luciano et al. 2007; Cope et al. 2012
7	*CNTNAP2*	-	rs759178	147575112	Vernes et al. 2008	C/A	0.40	C	Nonword repetition	Whitehouse et al. 2011	
7	*CNTNAP2*	-	rs17236239	147582305	Vernes et al. 2008	A/G	0.24	G	Nonword repetition	Whitehouse et al. 2011	
7	*CNTNAP2*	-	rs2710117	147601772	Vernes et al. 2008	A/T	0.37	A	Nonword repetition	Whitehouse et al. 2011	
15	*DYX1C1*	DYX1	rs3743204	55790310	Dahdouh et al. 2009	G/T	0.33	T	Short Term Memory	Bates et al. 2010	Darki et al. 2012
16	*CMIP*	SLI1	rs16955705	81673350	Newbury et al. 2009	A/C	0.36	C	Nonword repetition		Newbury et al. 2009; Scerri et al. 2011

Table 3.2: SNPs analysed in the current study. * Strongest signal (if several) on the first study reporting the association of the SNP with reading-related abilities. Risk: allele associated with lower scores/ affection status in the first study that reported the association. Consistent associations: significant association of the marker SNP with a reading-related trait with the same direction of effect. Inconsistent associations: significant association of the SNP marker with a reading-related trait with the opposite direction of effect. Lack of associations: tested the association of the SNP with a reading related trait but did not replicate the association.

Phoneme deletion

Phonological awareness was measured using a phoneme deletion task, in which a phoneme (always a consonant) had to be deleted from a nonword, resulting in another nonword (de Jong and van der Leij, 2003). There was no time limit for the completion of this task. The task was divided in two parts (PD_1 and PD_2), which differed in the type of tested nonwords. In the first part, nine monosyllabic and nine disyllabic nonwords were included. In the second part, the items were nine disyllabic nonwords, in which the phoneme to be deleted occurred twice. The outcome measure for each part was the proportion of correct items. We then calculated at each developmental time-point a composite score across parts, the proportion of correct items for all available parts (PD_{tot}).

A different phoneme deletion task was used in grade 6: PD_{AKT} (Amsterdam Phoneme Deletion Test) (van Bergen et al., 2015), which consisted of 12 items. The outcome measure was the proportion of correct items.

Nonword repetition

Nonword repetition (NWR) consisted of a test, in which children had to repeat a list of 27 nonwords that were presented to them auditorily. There was no time limit for the completion of this task. The outcome measure was the number of correctly repeated items.

3.2.3 *Genetic variants*

Fourteen candidate SNPs were tested for association in the DDP longitudinal sample. The choice of SNPs was based on a literature review at the time of designing the study (see Table 2). We first identified 18 SNPs that had been associated with reading-related traits (i.e. dyslexia affection status, reading fluency, nonword repetition, orthographic choice, spelling, phonological awareness, or discriminant score) at least twice in a consistent manner, i.e. with the same directions of allelic effect across studies. We then pruned the SNP list to reduce redundancy, based on linkage disequilibrium (LD), by selecting only one SNP per LD block ($r^2 > 0.8$) using SNAP (CEU population) (Johnson et al., 2008). As a result, four SNPs were excluded (rs2179515, rs2235676, rs3212236, rs9461045). The list of the 14 selected SNPs after the pruning is summarized in Table 3.2.

The DDP children and their parents (N=555) were directly genotyped in-house using KASP assays (LGC Ltd., Teddington, UK). We excluded 9 individuals with more than 3/14 missing genotypes (i.e. a missing genotype rate exceeding 20%) from analyses. Mendelian inconsistencies were flagged using PLINK v1.07 (Purcell et al., 2007) and, as a result, the genotypes for one SNP were excluded in one family. The total genotyping rate in the remaining individuals was 98.8%, with a missing rate $<5\%$ for all the SNPs. All SNPs were in Hardy-Weinberg equilibrium in the unrelated parents ($p>0.05$). Subsequent analysis was carried out using only child trait measurements and genotypes.

3.2.4 *Statistical analyses*

In this study, we conducted a number of tests to assess the association between 15 candidate SNPs and five quantitative traits related to reading ability, two of which were measured using two different instruments (see Table 3.1). Since these tests are not independent to each other (due to the correlation between phenotypes, see Figure 3.2) we do not correct our P-values for multiple testing for the traits. For the 15 SNPs tested, the application of a Bonferroni correction sets the threshold for significance at $p = 3.3 * 10^{-3}$, which we have applied despite the partial dependence of some of the SNPs as a result of linkage disequilibrium. We describe the trends of association across SNPs, traits and methods. The signals that are captured by multiple methodological approaches are highlighted as the most reliable results from the present study.

Phenotypic correlations

Pearson's correlations between traits, within developmental time-points, were computed using R statistical software (R, 2014). We also computed correlations for each trait across developmental time-points (Figure 3.S1).

Longitudinal modeling of SNP effects

To examine the longitudinal dimension of the genetic effects, a linear mixed model was fitted to each trait in R using the 'lme4' package (Bates et al., 2015). First, we fitted a null model for each trait, which consisted of a fixed-effect part and a random-effect part. All models contained the same fixed effect terms: age, developmental

time-point, sex, cohort (i.e. recruitment site), and group (risk and non-risk) (Table 3.S1). They also all contained a random effect for family intercept to account for the relatedness of some of the samples. The other random-effects varied per trait (see Table 3.S2), since the models were fitted depending on the number of repeated observations per subject that were available (i.e. same trait across time-points as indicated in Table 3.1).

For Klepel, RAN_{dig}, and PD_{tot}, three or more developmental time-points were available. Thus, we included a random effect for subject intercept and a slope for age per subject, to allow children to differ in their rates of development. For each of DMT and EMT (reading fluency measures), only two developmental time-points were available. Hence, it was not possible to include a random effect for slope, and we only included a random intercept per subject. When a trait had only been measured at one developmental time-point (i.e. NWR and PD_{AKT}), the time-point term was dropped, and the random effect part only contained the intercept for the family. The effect of a SNP on the overall level of a trait was then assessed by comparing the null model with a full model, in which SNP allele dosage was included as a fixed effect. The effect of a SNP on the trajectory was assessed by comparing the model including the SNP with a model that included the SNP and SNP x time-point interaction terms. A likelihood ratio test (LRT) between the nested models (see Equations in Supplementary information), was used to assess the significance of the term of interest (i.e. 'SNP' or 'time-point x SNP'). The significances of the estimates were calculated using Satterthwaite approximations to determine denominator degrees of freedom, in the package 'lmerTest' (Kuznetsova et al., 2015).

For the 14 SNPs tested, the application of Bonferroni correction would set a conservative threshold for significance at $p=3.6*10^{-3}$ (conservative because of the partial dependence of some of the SNPs as a result of linkage disequilibrium). Since the five traits were not independent of each other (due to substantial correlations between traits, see Figure 3.2), we did not consider a further correction of P-values for multiple testing across the five traits.

Single time-point analyses

SNPs that showed significant association in the longitudinal analysis (for SNP or time-point x SNP) were further explored by testing additive linear association at each separate developmental time-point using PLINK v1.07 (Purcell et al., 2007) (–qfam and permutations to correct for the sibship structure of a small minority of

families). For these analyses, we first adjusted the traits for covariate effects with a predictive linear model (separately for each developmental time-point). We considered age (centered by substracting the mean age) as a variable, and sex, cohort (i.e. recruitment site), and group (risk and non-risk) as factors for each trait at each time-point (see Table 3.S1). Although not all of these covariates were significant predictors of all traits, we kept them in order to be consistent in the way we analysed the different traits. Blom's transformation was used to rank-normalize residuals and attain normality within each time-point.

In order to assess whether trait-associations of several neighbouring SNPs were independent, we performed conditional association analysis using the –condition option in PLINK v1.07.

We also evaluated haplotypes for two SNPs in *KIAA0319* in relation to rapid naming using PLINK v1.07 (–hap-assoc).

3.3 RESULTS

The five traits were substantially inter-correlated within each developmental timepoint (Figure 3.2). Overall, the two reading fluency measures (word and nonword reading) were most highly correlated with each other (r=0.85), while the correlations of the other phenotypes were more moderate (r=0.11-0.62). The lowest correlations were seen between rapid naming and phoneme deletion (PD_{tot} and PD_{AKT}, r=0.22-0.32), and between rapid naming and nonword repetition (r=0.11). The correlation structure was largely stable across time points, although there was some variation, for example the correlation between reading fluency and rapid naming increased over developmental time.

The longitudinal assessments of the SNP effects and timepoint x SNP interactions are summarized in Table 3.3, showing associations for which Pr in likelihood ratio tests (see Table 3.S3 for full LRT tables). For the SNPs that showed significant effects (either main SNP effect or timepoint x SNP interaction effect), follow-up univariate association analyses per time-point are shown in Table 3.4. Five out of the 14 SNPs tested showed evidence of association with either rapid naming (rs761100, rs2038137), word reading fluency (rs6935076), or nonword repetition (rs17236239).

Specifically, rapid naming of digits was nominally associated with two neighbouring SNPs in *KIAA0319* (rs761100 $\chi^2(1) = 6.927$, p = 0.009; rs2038137 $\chi^2(1) = 6.496$, p = 0.011), the minor alleles being associated with lower scores, corresponding to slower

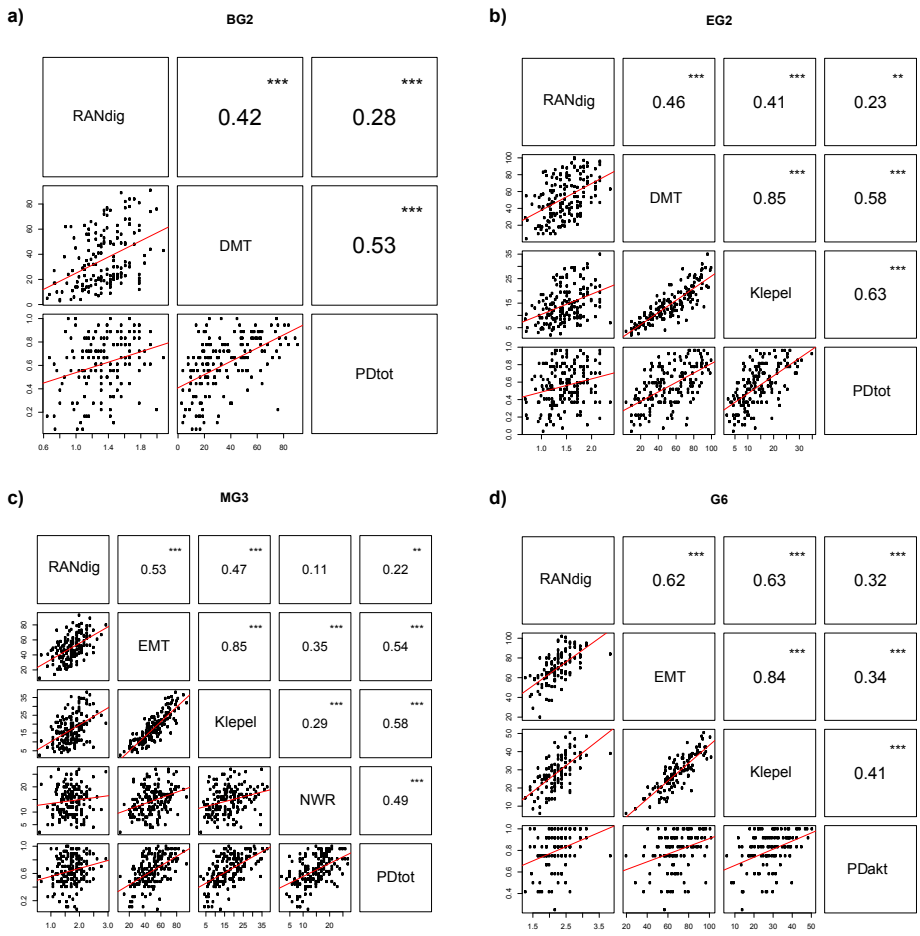


Figure 3.2: Correlation panel of phenotypes of interest per developmental time-point. The lower panel contains the scatter plot of the raw data for each pair of phenotypes, with a linear regression line. The values in the upper panel correspond to the Pearson's correlation coefficient, and the significance of the correlation. a) BG2: Beginning Grade 2; b) EG2: End Grade 2; c) MG3: Middle Grade 3; d) G6: Grade 6.

naming. These results were independent of developmental time-point, since the interactions between the SNPs and time-point were not significant. The single time-point analysis reflected the same signal, showing significant associations of these two SNPs at multiple developmental time-points (Table 4); the most significant was at beginning of grade 2 for rs761100 (p_{EG2} = 0.001) and at the middle of grade 3 for rs2038137 (p_{EG2} = 0.005). The directions of effect for both SNPs were the same across all developmental time-points, with the minor alleles yielding lower scores (Figure 3.3).

Rs761100 and rs2038137 are located 13 kb apart in the 5' untranslated region (UTR) of the *KIAA0319* gene, and they are in LD with each other (, for the 1000G CEU population) (Johnson et al., 2008). When both SNPs were modeled together as fixed effects for association with rapid naming, the second SNP was not a significant predictor. Similarly, the association was no longer significant when conditioning the test of one of the SNPs on the other (Table 3.S5). Haplotype analyses per developmental time-point indicated that the two minor alleles rs761100-A and rs2038137-T form the risk haplotype ($p \leq 0.01$ for all time-points except G6, Table 3.S5).

Rs6935076, another SNP in *KIAA0319*, was associated with word reading fluency (DMT: $\chi^2(1) = 3.568$, p = 0.059; EMT: $\chi^2(1) = 4.861$, p = 0.027). This association was confirmed in two of the developmental stages by the single time-point analysis (DMT: p_{EG2}= 0.042; EMT: p_{MG3}= 0.047). Although this SNP is located in between rs761100 and rs2038137, the two SNPs that were associated with rapid naming, it is in low LD with these (r^2= 0.21-0.28) and did not itself show any association with rapid naming (RAN_{dig}: $\chi^2(1) = 1.042$, p = 0.307).

The longitudinal analysis showed a developmental time-point-dependent association between the *CNTNAP2* variant rs759178 and nonword reading fluency ($\chi^2(2)$ = 7.131, p = 0.028) (Figure 3.4). Of note, there were no significant differences in nonword reading scores between the genotypes at the individual time-points themselves (Table 3.4).

We found that rs17236239, a second SNP in *CNTNAP2*, was associated with nonword repetition, both via linear regression in R ($\chi^2(1)$= 6.380, p= 0.012) and PLINK (p=0.025).

We did not find significant associations between phoneme deletion measures (PD_{tot} and PD_{AKT}) and any of the SNPs tested.

Trait	Model	Term	Time points BG2	EG2	MG3	G6	Nind	Nfam	Alleles Maj/Min	Risk	Estimate	SE	df	T	Pr(>T)
NWR	NWR	rs17236239			x		159	155	A/G	A	0.294	0.118	151.36	2.491	0.014
WRF	EMT	rs6935076			x	x	161	154	C/T	C	0.217	0.100	150.54	2.177	0.031
RAN	RAN_{dig}	rs2038137	x	x	x	x	167	163	G/T	T	-0.187	0.074	163.67	-2.526	0.012
RAN	RAN_{dig}	rs761100	x	x	x	x	165	161	C/A	A	-0.187	0.072	162.01	-2.610	0.010
NWRF	Klepel			x	x	x	164	160	C/A						
		rs759178									0.054	0.084	174.20	0.601	0.548
		MG3:rs759178									-0.083	0.037	180.30	-2.231	0.027
		G6:rs759178									0.024	0.072	129.50	0.325	0.745

Table 3.3: Nominally significant associations for the SNP fixed effect terms and timepoint*SNP interaction terms from the linear mixed models. The estimates for the SNP are for the centered dependent variables specified in the model. For the time-point*SNP interaction term, estimates for the centered dependent variables specified on the model are given for the marker per each level of the time-point (except for the baseline, i.e. EG2). The degrees of freedom (df) are estimated with Satterthwaite's approximation. Risk: allele associated with lower scores.

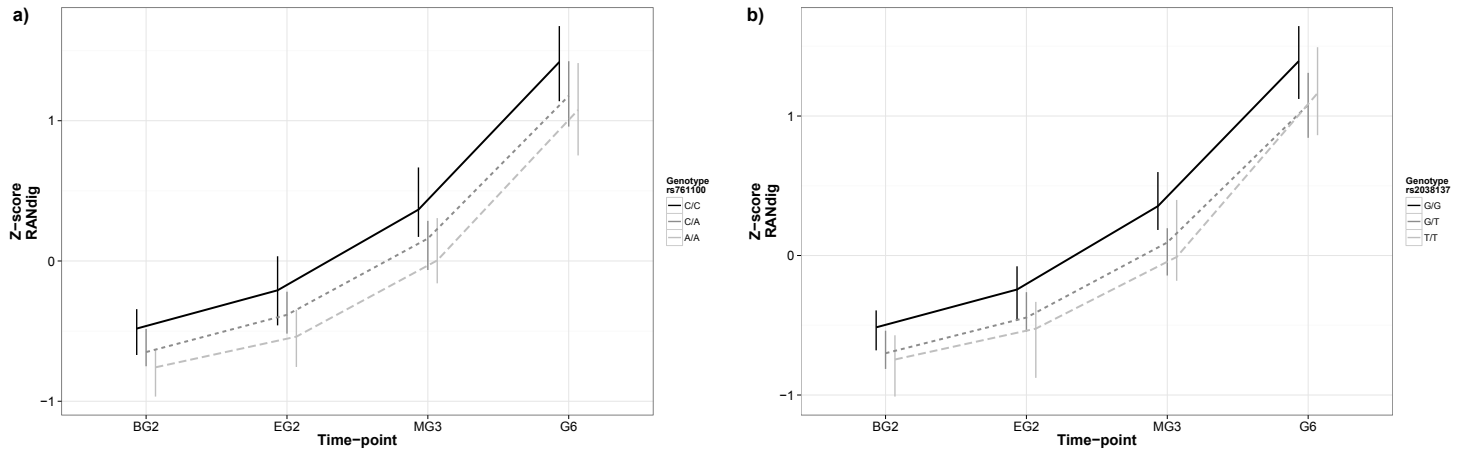


Figure 3.3: Z-standardized performance on rapid naming of digits (RAN_{dig}) per developmental time-point and genotypic groups of a) rs761100 and b) rs2038137. The lines represent the fitted residuals to the mixed linear model including the allelic state of the SNP as a variable, and the points and error bars are the mean and standard deviation of the mean per time-point.

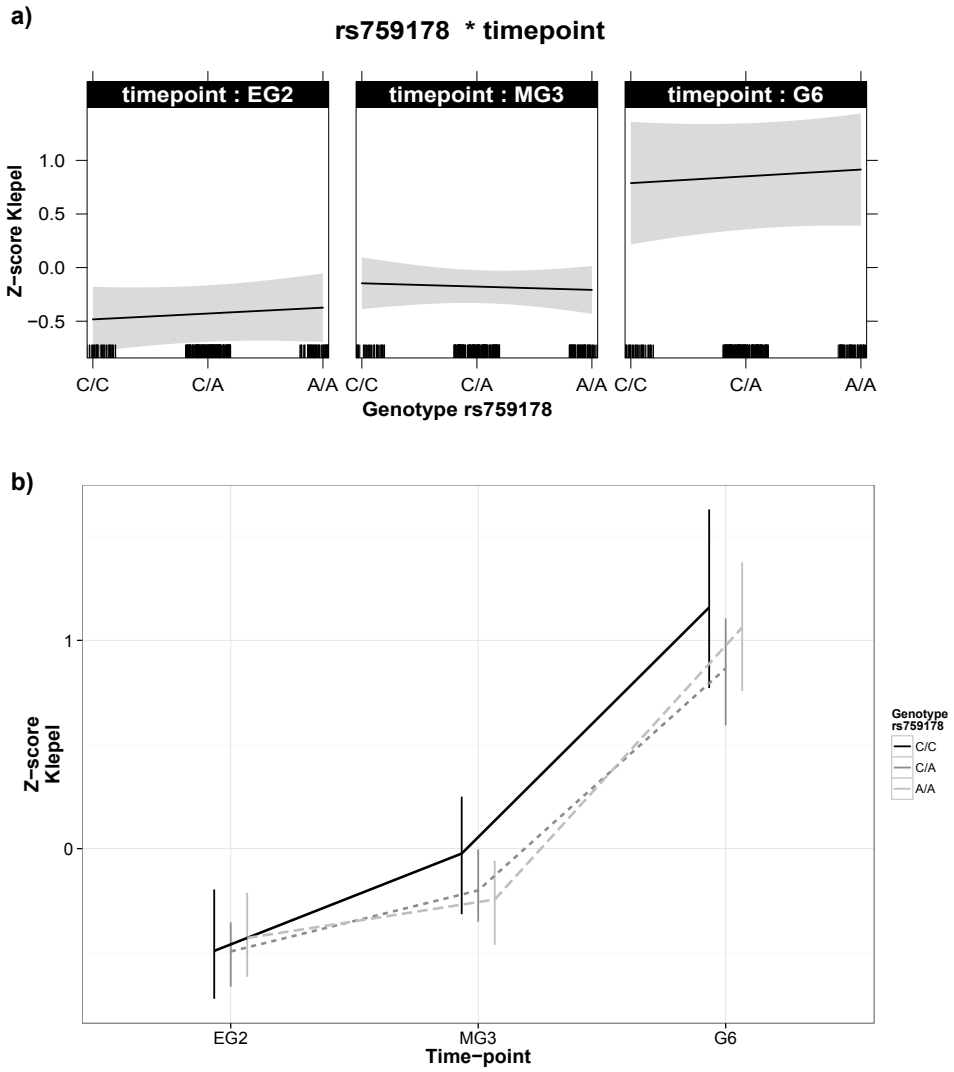


Figure 3.4: Z-standardised performance on nonword reading fluency per developmental time-point and genotypic groups of rs759178. a) Interaction plot of time-point x rs759178. b) Fitted residuals to the mixed linear model including the rs759178 and time-point x rs759178 as variables; the points and error bars are the mean and standard deviation of the mean per time-point.

	Time-points	BG2			EG2			MG3			G6		
	Test	N	β	EMP1	N	β	EMP1	N	β	EMP1	N	β	EMP1
Nonword Reading Fluency													
rs759178	Klepel				160	-0.162	0.167	160	0.011	0.929	110	-0.076	0.584
Word Reading Fluency													
rs6935076	DMT2/EMT	156	0.243	0.058	157	0.243	0.042	157	0.245	0.047	110	0.280	0.053
Rapid Naming													
rs761100	RAN_{dig}	158	-0.339	0.001	161	-0.294	0.009	162	-0.286	0.009	111	-0.256	0.062
rs2038137	RAN_{dig}	160	-0.302	0.006	163	-0.290	0.014	164	-0.307	0.005	112	-0.248	0.063
Nonword Repetition													
rs17236239	NRT							159	0.283	0.025			

Table 3.4: PLINK univariate association analyses per developmental time-point. N: number of individuals in the analysis. β: regression coefficient for the major allele. EMP1: Empirical P-values (10,000 permutations).

3.4 DISCUSSION

In this study, we performed candidate gene association analyses in a Dutch sample with longitudinal measures for several reading-related measures. This dataset consisted of a very richly phenotyped sample, in which genetic associations could be detected via intermediate measures related to the cognitive processes involved in reading (such as phoneme deletion and rapid naming) collected at multiple developmental time-points. Based on a literature search, we selected and genotyped 14 SNPs that had been associated with dyslexia and/or relevant quantitative traits, consistently in at least two separate studies, and found in the prominent candidate genes *DYX1C1*, *DCDC2*, *KIAA0319*, *CMIP*, and *CNTNAP2*. We modeled the data longitudinally to assess overall and developmental time-dependent effects of these SNPs on word and nonword reading fluency, nonword repetition, rapid naming, and phoneme deletion. A number of nominally significant associations were observed, and detailed single time-point analyses of these associations confirmed that most of them were consistent across developmental time-points. Below, we discuss the results across the different analyses, considering them in relation to the pool of existing data currently available in the field of dyslexia genetics.

The most significant association that we found in the DDP sample was between rapid naming and two SNPs, rs761100 and rs2038137, in the 5' UTR of the *KIAA0319* gene. The ability to rapidly name a limited set of well-known items is considered a measure of processing speed; it tackles the timing mechanisms necessary for the automaticity required in advanced stages of reading development (Pennington, 2006; van der Leij et al., 2013) and is one of the strongest predictors of reading ability in pre-literate children (van der Leij et al., 2013). Moreover, superior parental rapid naming proficiency has been shown to be a protective factor for children at familial risk for dyslexia in the DDP sample (van der Leij et al., 2013; van Bergen et al., 2012), suggesting that it is an important intergenerational precursor of reading (van Bergen et al., 2014). However, the effect of these SNPs on word reading fluency in our samples is at best only marginally significant (DMT, rs761100, $\chi^2(1) = 2.697$, $p = 0.100$). This could reflect some heterogeneity in reading strategies, since the overall variance in reading is explained by other factors in addition to rapid naming, and those other factors might not be affected by these SNPs. On the other hand, we also found that rs6935076, another SNP in the same region of *KIAA0319*, was associated

with word reading fluency in the DDP sample, although to a lesser extent and not at all developmental time-points, but was not associated with rapid naming.

Rapid naming has not often been investigated in previous genetic studies of reading ability; it has been included in only three linkage studies (Konig et al., 2011; Rubenstein et al., 2014; de Kovel et al., 2008) and a small number of recent association studies (Lim et al., 2014). One of these studies found evidence of linkage for the composite score of RAN colours/objects, on 6p21 (Konig et al., 2011). The region is close to the *DYX2* locus spanning *KIAA0319*, although the authors of the study argued that their results support the existence of an additional dyslexia susceptibility gene on 6p. The linkage was not found in two other studies that also included several rapid naming measures (Rubenstein et al., 2014; de Kovel et al., 2008). The lack of consistency across studies is a long-standing problem for the field, in part due to the heterogeneity across studies at various levels, such as study design, sample size, ascertainment scheme, and population (Fisher and DeFries, 2002).

A recent study that tested for association between rapid naming of digits and dyslexia candidate SNPs in a Chinese population, found that this trait was nominally associated with several SNPs in *KIAA0319* (Lim et al., 2014), including rs2038137 (p= 0.02454), one of the associated SNPs in the present study. This same SNP was also associated with scores on Chinese dictation and phonological awareness in the Chinese sample. However, the direction of effect of this SNP in the present study was not congruent with previous reports. We found the minor allele T to be associated with lower scores, but it was originally reported that the major allele G was associated with reduced performance on word reading, orthographic choice, and spelling (Francks et al., 2004), and this association has been observed in additional studies with this same direction of effect (i.e. risk allele=G) (Cope et al., 2005; Harold et al., 2006).

The other SNP that we found to be associated with rapid naming was rs761100, also in the *KIAA0319* gene. This SNP was first found associated with several quantitative traits including reading and spelling, and the risk allele was reported to be the major allele C (Harold et al., 2006), opposite to our direction of effect (risk allele=minor T). However, another study found that the minor allele was associated with reduced expressive language in a sample of children with specific language impairment (Newbury et al., 2011). This SNP was also included in a recent cross-linguistic meta-analysis across several European samples, and its minor allele T was nominally associated with lower spelling scores in the meta-analysis, although not

in any of the subsamples separately (Becker et al., 2014). This SNP was not included in the Chinese study that looked at rapid naming (Lim et al., 2014).

We observed association between nonword repetition and rs17236239, a *CNTNAP2* SNP that was selected based on its previous association with this trait. However, the DDP sample showed the opposite direction of effect to that previously reported (Vernes et al., 2008; Whitehouse et al., 2011).

We did not find association between any of the SNPs and phoneme deletion, which is a measure of phonological awareness that has been repeatedly associated with candidate SNPs in prior literature (Francks et al., 2004; Lim et al., 2014).

Another question that we asked concerned the stability of the associations across different developmental time-points. Overall, most of the associations that we found were time-independent. When looking at the single time-point analysis, we did observe that some of the association signals differed at distinct points of development, mainly becoming less significant at the latest time-point (G6, mean age=12.1 years). This drop in significance may well relate to the drop of sample size in the latest stages of the project, rather than indicating a decrease of the genetic effect on these traits as the age increases. Moreover, our time-span ranged only from age 7 to 12 years, and for some of the traits of interest the measurement instrument was not constant across developmental time-points (e.g. reading fluency with DMT and EMT), which broke the longitudinal analysis into two steps. These factors, together with our moderate sample size, might have reduced our chances of detecting developmentally sensitive genetic effects. Nevertheless, we did observe one suggestive finding in our longitudinal analysis: an interaction of rs759178, nonword reading fluency, and developmental time-point. This interaction involved a smaller difference across genotypes in the middle time-point (MG3, mean age=8.9 years) compared to the other earlier and later ages (Figure 3.4). Although the single time-point analysis did not show any significant difference between genotypes at any of the individual time-points, there was a trend (risk allele=C) at the end of grade 2 (EG2, mean age=8.14 years). This result is difficult to interpret biologically, but indicates how cross-sectional studies could miss associations that are present only at certain developmental stages. Longitudinal analysis of genetic effects in reading ability and related quantitative traits is a potentially powerful method that has been underexploited so far, and should be considered whenever this type of data is available, as in the DDP cohort.

Another strength of the DDP cohort is the richness of the assessment, involving several quantitative traits. Even when the effects that we observed were stable in

time, the availability of multiple reading- and language-related traits permitted a detailed understanding of the type of process that the genetic variation could be affecting.

The literature on candidate genetic variants for reading is difficult to interpret, as reflected by the summary in Table 3.2. Recent efforts have tried to integrate evidence across studies, to get insights into the relevance of these candidate SNPs for dyslexia. The NeuroDys consortium meta-analyzed association results for 19 SNPs (including 8 that we analyzed in the present study) across several European samples, but did not find any significant association after correcting for multiple comparisons (Becker et al., 2014). Some others have focused on just a handful of variants per study, and they have notably meta-analyzed results from case-control studies, concluding that many of the variants were not associated with an increase of dyslexia (Zou et al., 2012; Zhong et al., 2013; Tran et al., 2013), although a few were (e.g. rs4504469-T (Zou et al., 2012), rs807701- C (Zhong et al., 2013). These efforts have been highly constrained by heterogeneity across studies, as well as the limited availability of any given trait measurement across studies. One source of study heterogeneity is the orthography of the language (e.g. more transparent orthography in Dutch versus a more complex orthography in English). It is thought that the relationship between reading-related cognitive abilities and reading skills varies depending on the orthographic system. For example, it has been proposed that rapid naming might be an important predictor for reading in consistent, but not in complex, orthographies (Kirby et al., 2010). However, another study found that "the impact of phoneme deletion and RAN-digits was stronger in complex than in less complex orthographies" for predicting developmental dyslexia (Landerl et al., 2013). Despite the fact that the directionality of the relationship is not clear, it seems that the strategies children develop in order to master reading do differ across orthography types. Thus, it might be important to reconsider the available data on the genetic studies of reading, taking into account factors such as orthographic complexity.

Other recent investigations have tested the association of dyslexia status and/or reading-related measures with polymorphisms across the entire genome (Field et al., 2013; Luciano et al., 2013; Gialluisi et al., 2014). These genome-wide association screens have yielded some interesting new candidate genes, but no clearly significant results, probably because these studies have so far been performed using sample sizes that were not large enough to reliably detect effects explaining less than 1% of trait variance (which have to be expected based on experiences from other brain

disorders (Psychiatric Genomics Consortium, 2014)). Indeed, the main limitation of the present study is its moderate sample, which is not well powered to detect small effect sizes. However, the DDP dataset consists of a very well-characterized sample at the phenotypic level, and we have evaluated some of the most intensively studied candidate SNPs for dyslexia in a longitudinal dataset for the first time, while the previously reported effect sizes for many of these SNPs were large enough to be detected in comparatively sized datasets.

Future genetic studies of reading-related traits will probably depend on increasing power by meta-analysing many of the available samples, an approach that has proven successful for other complex traits. The present longitudinal study reminds us that there are also non-genetic dimensions that should be accounted for, including developmental time-point.

References

Adler WT, Platt MP, Mehlhorn AJ, Haight JL, Currier TA, et al. (2013) Position of neocortical neurons transfected at different gestational ages with shRNA targeted against candidate dyslexia susceptibility genes. PLoS ONE 8: e65179.

Bates D, Maechler M, Bolker B, Walker S (2015) lme4: Linear mixed-effects models using Eigen and S4. URL http://CRAN.R-project.org/package=lme4, r package version 1.1-8.

Bates TC, Lind PA, Luciano M, Montgomery GW, Martin NG, et al. (2010) Dyslexia and DYX1C1: deficits in reading and spelling associated with a missense mutation. Mol Psychiatry 15: 1190–1196.

Becker J, Czamara D, Scerri TS, Ramus F, Csepe V, et al. (2014) Genetic analysis of dyslexia candidate genes in the European cross-linguistic NeuroDys cohort. Eur J Hum Genet 22: 675–680.

Brkanac Z, Chapman NH, Matsushita MM, Chun L, Nielsen K, et al. (2007) Evaluation of candidate genes for DYX1 and DYX2 in families with dyslexia. Am J Med Genet B Neuropsychiatr Genet 144B: 556–560.

Brus BT, Voeten MJM (1972) Een-minuut-test [one-minute-test]. Swets & Zeitlinger .

Cope N, Eicher JD, Meng H, Gibson CJ, Hager K, et al. (2012) Variants in the DYX2 locus are associated with altered brain activation in reading-related brain regions in subjects with reading disability. Neuroimage 63: 148–156.

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, et al. (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76: 581–591.

Couto JM, Livne-Bar I, Huang K, Xu Z, Cate-Carter T, et al. (2010) Association of reading disabilities with regions marked by acetylated H3 histones in KIAA0319. Am J Med Genet B Neuropsychiatr Genet 153B: 447–462.

Dahdouh F, Anthoni H, Tapia-Paez I, Peyrard-Janvid M, Schulte-Korne G, et al. (2009) Further evidence for DYX1C1 as a susceptibility factor for dyslexia. Psychiatr Genet 19: 59–63.

Darki F, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T (2012) Three Dyslexia Susceptibility Genes, DYX1C1, DCDC2, and KIAA0319, Affect Temporo-Parietal White Matter Structure. Biol Psychiatry 72: 671–676.

de Jong P, van der Leij A (2003) Developmental changes in the manifestation of a phonological deficit in dyslexic children learning to read a regular orthography. Journal of Educational Psychology 95: 22–40.

de Kovel CG, Franke B, Hol FA, Lebrec JJ, Maassen B, et al. (2008) Confirmation of dyslexia susceptibility loci on chromosomes 1p and 2p, but not 6p in a Dutch sib-pair collection. Am J Med Genet B Neuropsychiatr Genet 147: 294–300.

Deffenbacher KE, Kenyon JB, Hoover DM, Olson RK, Pennington BF, et al. (2004) Refinement of the 6p21.3 quantitative trait locus influencing dyslexia: linkage and association analyses. Hum Genet 115: 128–138.

Dennis MY, Paracchini S, Scerri TS, Prokunina-Olsson L, Knight JC, et al. (2009) A common variant associated with dyslexia reduces expression of the KIAA0319 gene. PLoS Genet 5: e1000436.

Field LL, Shumansky K, Ryan J, Truong D, Swiergala E, et al. (2013) Dense-map genome scan for dyslexia supports loci at 4q13, 16p12, 17q22; suggests novel locus at 7q36. Genes Brain Behav 12: 56–69.

Fisher SE, DeFries JC (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. Nat Rev Neurosci 3: 767–780.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, et al. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75: 1046–1058.

Franke B, Vasquez AA, Johansson S, Hoogman M, Romanos J, et al. (2010) Multicenter analysis of the SLC6A3/DAT1 VNTR haplotype in persistent ADHD suggests differential involvement of the gene in childhood and persistent ADHD. Neuropsychopharmacology 35: 656–664.

Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, et al. (2014) Genome-wide screening for DNA variants associated with reading and language traits. Genes Brain Behav 13: 686–701.

Giraud AL, Ramus F (2012) Neurogenetics and auditory processing in developmental dyslexia. Curr Opin Neurobiol 23: 1–6.

Goswami U (2015) Sensory theories of developmental dyslexia: three challenges for research. Nat Rev Neurosci 16: 43–54.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Harold D, Paracchini S, Scerri T, Dennis M, Cope N, et al. (2006) Further evidence that the KIAA0319 gene confers susceptibility to developmental dyslexia. Mol Psychiatry 11: 1085–1091.

Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, et al. (2008) SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24: 2938–2939.

Kirby J, Georgiou G, Martinussen R, Parrila R (2010) Naming speed and reading: From prediction to instruction. Reading Research Quarterly 45: 341–362.

Konig IR, Schumacher J, Hoffmann P, Kleensang A, Ludwig KU, et al. (2011) Mapping for dyslexia and related cognitive trait loci provides strong evidence for further risk genes on chromosome 6p21. Am J Med Genet B Neuropsychiatr Genet 156B: 36–43.

Kuznetsova A, Bruun Brockhoff P, Haubo Bojesen Christensen R (2015) lmerTest: Tests in Linear Mixed Effects Models. URL http://CRAN.R-project.org/package=lmerTest, r package version 2.0-25.

Landerl K, Ramus F, Moll K, Lyytinen H, Leppanen PH, et al. (2013) Predictors of developmental dyslexia in European orthographies with varying complexity. J Child Psychol Psychiatry 54: 686–694.

Lasky-Su J, Lyon HN, Emilsson V, Heid IM, Molony C, et al. (2008) On the replication of genetic associations: timing can be everything! Am J Hum Genet 82: 849–858.

Lim CK, Wong AM, Ho CS, Waye MM (2014) A common haplotype of KIAA0319 contributes to the phonological awareness skill in Chinese children. Behav Brain Funct 10: 23.

Luciano M, Evans DM, Hansell NK, Medland SE, Montgomery GW, et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav 12: 645–652.

Luciano M, Lind PA, Duffy DL, Castles A, Wright MJ, et al. (2007) A haplotype spanning KIAA0319 and TTRAP is associated with normal variation in reading and spelling ability. Biol Psychiatry 62: 811–817.

Meng H, Smith SD, Hager K, Held M, Liu J, et al. (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci USA 102: 17053–17058.

Neuhoff N, Bruder J, Bartling J, Warnke A, Remschmidt H, et al. (2012) Evidence for the late MMN as a neurophysiological endophenotype for dyslexia. PLoS ONE 7: e34909.

Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, et al. (2011) Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behav Genet 41: 90–104.

Newbury DF, Winchester L, Addis L, Paracchini S, Buckingham LL, et al. (2009) CMIP and ATP2C2 modulate phonological short-term memory in language impairment. Am J Hum Genet 85: 264–272.

Nopola-Hemmi J, Taipale M, Haltia T, Lehesjoki AE, Voutilainen A, et al. (2000) Two translocations of chromosome 15q associated with dyslexia. J Med Genet 37: 771–775.

Olson RK, Keenan JM, Byrne B, Samuelsson S (2014) Why do Children Differ in Their Development of Reading and Related Skills? Sci Stud Read 18: 38–54.

Paracchini S, Steer CD, Buckingham LL, Morris AP, Ring S, et al. (2008) Association of the KIAA0319 dyslexia susceptibility gene with reading skills in the general population. Am J Psychiatry 165: 1576–1584.

Pennington BF (2006) From single to multiple deficit models of developmental disorders. Cognition 101: 385–413.

Psychiatric Genomics Consortium SWGot (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511: 421–427.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.

R CT (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org/.

Rice ML, Smith SD, Gayan J (2009) Convergent genetic linkage and associations to language, speech and reading measures in families of probands with Specific Language Impairment. J Neurodev Disord 1: 264–282.

Rubenstein KB, Raskind WH, Berninger VW, Matsushita MM, Wijsman EM (2014) Genome scan for cognitive trait loci of dyslexia: Rapid naming and rapid switching of letters, numbers, and colors. Am J Med Genet B Neuropsychiatr Genet 165B: 345–356.

Scerri TS, Morris AP, Buckingham LL, Newbury DF, Miller LL, et al. (2011) DCDC2, KIAA0319 and CMIP are associated with reading-related traits. Biol Psychiatry 70: 237–245.

Schulte-Korne G, Deimel W, Bartling J, Remschmidt H (2001) Speech perception deficit in dyslexic adults as measured by mismatch negativity (MMN). Int J Psy-

chophysiol 40: 77–87.

Schumacher J, Anthoni H, Dahdouh F, Konig IR, Hillmer AM, et al. (2006) Strong genetic evidence of DCDC2 as a susceptibility gene for dyslexia. Am J Hum Genet 78: 52–62.

Shaywitz SE, Shaywitz BA, Fletcher JM, Escobar MD (1990) Prevalence of reading disability in boys and girls. Results of the Connecticut Longitudinal Study. JAMA 264: 998–1002.

Sitlani CM, Rice KM, Lumley T, McKnight B, Cupples LA, et al. (2015) Generalized estimating equations for genome-wide association studies using longitudinal phenotype data. Stat Med 34: 118–130.

St Pourcain B, Cents RA, Whitehouse AJ, Haworth CM, Davis OS, et al. (2014a) Common variation near ROBO2 is associated with expressive vocabulary in infancy. Nat Commun 5: 4831.

St Pourcain B, Skuse DH, Mandy WP, Wang K, Hakonarson H, et al. (2014b) Variability in the common genetic architecture of social-communication spectrum phenotypes during childhood and adolescence. Mol Autism 5: 18.

Tran C, Gagnon F, Wigg KG, Feng Y, Gomez L, et al. (2013) A family-based association analysis and meta-analysis of the reading disabilities candidate gene DYX1C1. Am J Med Genet B Neuropsychiatr Genet 162: 146–156.

Tran C, Wigg KG, Zhang K, Cate-Carter TD, Kerr E, et al. (2014) Association of the ROBO1 gene with reading disabilities in a family-based analysis. Genes, Brain and Behavior 13: 430–438, URL http://dx.doi.org/10.1111/gbb.12126.

van Bergen E, Bishop D, van Zuijen T, de Jong PF (2015) How Does Parental Reading Influence Children's Reading? A Study of Cognitive Mediation. Scientific Studies of Reading 0: 1–15.

van Bergen E, de Jong PF, Plakas A, Maassen B, van der Leij A (2012) Child and parental literacy levels within families with a history of dyslexia. J Child Psychol Psychiatry 53: 28–36.

van Bergen E, de Jong PF, Regtvoort A, Oort F, van Otterloo S, et al. (2011) Dutch children at family risk of dyslexia: precursors, reading development, and parental effects. Dyslexia 17: 2–18.

van Bergen E, van der Leij A, de Jong PF (2014) The intergenerational multiple deficit model and the case of dyslexia. Front Hum Neurosci 8: 346.

van den Bos KP (2003) Serieel benoemen en woorden lezen [serial naming and word reading]. Rijksunversiteit Groningen .

van den Bos KP, Lutje Spelberg HC, Scheepstra AJM, de Vries JR (1994) De klepel: Een test voor de leesvaardigheid van pseudowoorden [the klepel: A test for the reading skills of pseudowords]. Swets & Zeitlinger .

van der Leij A, van Bergen E, van Zuijen T, de Jong P, Maurits N, et al. (2013) Precursors of developmental dyslexia: an overview of the longitudinal Dutch Dyslexia Programme study. Dyslexia 19: 191–213.

van Zuijen TL, Plakas A, Maassen BA, Maurits NM, van der Leij A (2013) Infant ERPs separate children at risk of dyslexia who become good readers from those who become poor readers. Dev Sci 16: 554–563.

Venkatesh SK, Siddaiah A, Padakannaya P, Ramachandra NB (2013) Analysis of genetic variants of dyslexia candidate genes KIAA0319 and DCDC2 in Indian population. J Hum Genet 58: 531–538.

Verhoeven L (1995) Drie-Minuten-Toets (DMT). Cito.

Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, et al. (2008) A functional genetic link between distinct developmental language disorders. N Engl J Med 359: 2337–2345.

Wagner RK, Torgesen JK, Rashotte CA, Hecht SA, Barker TA, et al. (1997) Changing relations between phonological processing abilities and word-level reading as children develop from beginning to skilled readers: A 5-year longitudinal study. Developmental Psychology 33: 468–479.

Whitehouse AJ, Bishop DV, Ang QW, Pennell CE, Fisher SE (2011) CNTNAP2 variants affect early language development in the general population. Genes Brain Behav 10: 451–456.

Wilcke A, Weissfuss J, Kirsten H, Wolfram G, Boltze J, et al. (2009) The role of gene DCDC2 in German dyslexics. Ann Dyslexia 59: 1–11.

Zhang Y, Li J, Tardif T, Burmeister M, Villafuerte SM, et al. (2012) Association of the DYX1C1 dyslexia susceptibility gene with orthography in the Chinese population. PLoS ONE 7: e42969.

Zhong R, Yang B, Tang H, Zou L, Song R, et al. (2013) Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol 47: 435–442.

Zou L, Chen W, Shao S, Sun Z, Zhong R, et al. (2012) Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 159B: 970–976.

SUPPLEMENTARY INFORMATION

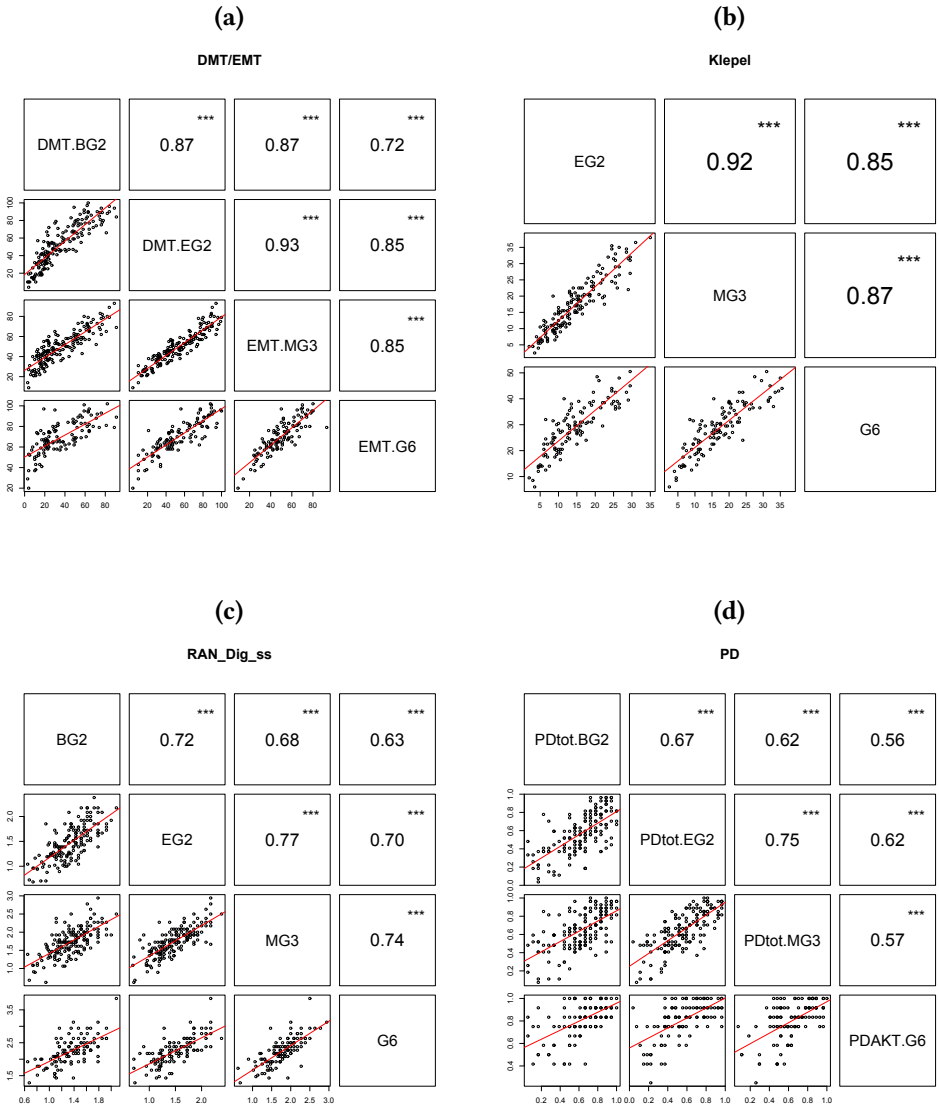


Figure 3.S1: Correlation panel of phenotypes of interest per phenotype. a Word reading fluency: DMT test in time-point BG2 and EG2, EMT test in time-point MG3 and G6; b Nonword reading fluency; c Rapid Naming Digits; d Phoneme deletion tests: PDtot and and PDakt.

Models

NRT and PD_{AKT}: 1 time-point

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3\text{timepoint} + \beta_4\text{sex} + \beta_5\text{cohort} + \beta_6\text{group} \quad (1)$$

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3\text{timepoint} + \beta_4\text{sex} + \beta_5\text{cohort} + \beta_6\text{group} + \beta_7\text{SNP}_\text{i} \quad (2)$$

DMT and EMT: 2 time-points

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} \quad (3)$$

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} + \beta_8\text{SNP}_\text{i} \quad (4)$$

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} + \beta_8\text{SNP}_\text{i} + \beta_9\text{timepoint} * \text{SNP}_\text{i} \quad (5)$$

Klepel, RAN_{Dig} and PD_{tot}: >3 time-points

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1 + \text{age.c}|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} \quad (6)$$

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1 + \text{age.c}|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} + \beta_8\text{SNP}_\text{i} \quad (7)$$

$$\text{Phenotype} = \beta_0 + \beta_1\text{age.c} + \beta_2(1|\text{fam}) + \beta_3(1 + \text{age.c}|\text{id}) + \beta_4\text{timepoint} + \beta_5\text{sex} + \beta_6\text{cohort} + \beta_7\text{group} + \beta_8\text{SNP}_\text{i} + \beta_9\text{timepoint} * \text{SNP}_\text{i} \quad (8)$$

Covariates

Covariate	Levels
Age	Continuous
Sex	Male, female
Cohort	Groningen, Nijmegen, Amsterdam
Group	Familial Risk, Non-familial risk

Table 3.S1: List of covariates that have been regressed out from the raw scores per time-point, and that have been included into the linear regression models.

Mixed effect models, null models

Time-points	Fixed terms				Random terms		Phenotype	Measure
1	age.c	sex	cohort	group	family		Nonword Repetition	NWR
							Phoneme Deletion	PD_{AKT}
2	age.c	sex	cohort	group	family	subject	Word Reading Fluency	DMT
							Word Reading Fluency	EMT
>3	age.c	sex	cohort	group	family	age.c|subject	Nonword Reading Fluency	Klepel
							Rapid Naming of Digits	RAN_{dig}
							Phoneme Deletion	PD_{tot}

Table 3.S2: Null models for each measurement, specifying the fixed effect and random terms for each trait, depending on the number of available measures per subject.

Likelihood ratio tests

	Formula	Df	AIC	BIC	logLik	deviance	χ^2	χ^2_{df}	Pr(χ^2)
H_0	Z EMT ~ age.c + (1|fam) + (1|id) + timep + sex + cohort + group	10	537.15	573.02	-258.57	517.15			
H_1	Z EMT ~ age.c + (1|fam) + (1|id) + timep + sex + cohort + group + rs6935076	11	534.29	573.75	-256.14	512.29	4.86	1	0.028
H_0	Z Klepel ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group + rs759178	14	576.43	633.29	-274.21	548.43			
H_1	Z Klepel ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group + rs759178 + timep*rs759178	16	573.30	638.28	-270.65	541.30	7.13	2	0.028
H_0	Z RAN_{dig} ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group	14	1009.63	1071.14	-490.81	981.63			
H_1	Z RAN_{dig} ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group + rs2038137	15	1005.13	1071.04	-487.57	975.13	6.50	1	0.011
H_0	Z RAN_{dig} ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group	14	997.56	1058.91	-484.78	969.56			
H_1	Z RAN_{dig} ~ age.c + (1|fam) + (1 + age.c|id) + timep + sex + cohort + group + rs761100	15	992.63	1058.36	-481.32	962.63	6.93	1	0.009
H_0	Z NWR ~ age.c +(1|fam) + sex + cohort + group	8	443.44	467.99	-213.72	427.44			
H_1	Z NWR ~ age.c +(1|fam) + sex + cohort + group + rs17236239	9	439.06	466.68	-210.53	421.06	6.38	1	0.01

Table 3.S3: Likelihood ratio test between nested mixed models for the SNPs and timepoint*SNP terms that are nominally significant.

Haplotype analyses

Time-points		BG2				EG2				MG3				G6			
Haplotype		N	β	R^2	P	N	β	R^2	P	N	β	R^2	P	N	β	R^2	P
rs2038137|rs761100	11	146	0.387	0.075	8.47e-04	149	0.312	0.046	0.008	150	0.304	0.045	9.47e-03	108	0.255	0.031	0.068
rs2038137|rs761100	12	146	-0.234	0.008	0.300	149	-0.005	3.38e-06	0.982	150	1.76e-03	4.16e-07	0.994	108	0.042	2.35e-04	0.875
rs2038137|rs761100	22	146	-0.337	0.054	0.005	149	-0.325	0.048	0.007	150	-0.316	0.047	8.05e-04	108	-0.265	0.034	0.057

Table 3.S4: Haplotype analyses of rs2038137-rs761100 for Rapid Naming Digits per time-point.

Conditional analyses

		BG2			EG2			MG3			G6		
SNP_{test}	SNP_{cov}	N	β	P	N	β	P	N	β	P	N	β	P
rs2038137	rs761100	158	-0.135	0.530	161	-0.228	0.288	162	-0.275	0.203	111	-0.186	0.489
rs761100	rs2038137	158	-0.230	0.264	161	-0.111	0.588	162	-0.063	0.762	111	-0.095	0.723

Table 3.S5: Conditional association of RAN digits with rs761100 and rs2038137 from PLINK.

Null model estimates, per phenotype

	Estimate	Std. Error	T value
(Intercept)	-0.51	0.13	-3.86
age_years.c	-0.06	0.13	-0.45
timepointEG2	0.71	0.10	7.22
sex2	0.18	0.14	1.27
cohort2	-0.21	0.15	-1.41
cohort3	-0.35	0.23	-1.52
group2	0.58	0.14	4.05

Table 3.S6: Estimates for the null model of DMT.

	Estimate	Std. Error	T value
(Intercept)	-0.50	0.19	-2.61
age_years.c	-0.01	0.12	-0.06
timepointG6	1.07	0.39	2.75
sex2	0.08	0.13	0.63
cohort2	-0.21	0.14	-1.54
cohort3	-0.31	0.22	-1.41
group2	0.45	0.13	3.37

Table 3.S7: Estimates for the null model of EMT.

	Estimate	Std. Error	T value
(Intercept)	-0.43	0.10	-4.19
age_years.c	-0.01	0.09	-0.14
timepointMG3	0.27	0.07	3.72
timepointG6	1.47	0.35	4.17
sex2	0.00	0.11	0.02
cohort2	-0.32	0.11	-2.84
cohort3	-0.36	0.18	-2.04
group2	0.41	0.11	3.71

Table 3.S8: Estimates for the null model of Klepel.

	Estimate	Std. Error	T value
(Intercept)	-0.16	0.30	-0.53
age_years.c	-0.07	0.22	-0.33
sex2	0.18	0.15	1.22
cohort2	-0.06	0.16	-0.40
cohort3	-0.05	0.23	-0.22
group2	0.58	0.16	3.63

Table 3.S9: Estimates for the null model of NWR.

	Estimate	Std. Error	T value
(Intercept)	-0.24	0.13	-1.88
age_years.c	-0.16	0.14	-1.08
timepointEG2	-0.15	0.12	-1.29
timepointMG3	0.34	0.22	1.55
sex2	0.28	0.13	2.07
cohort2	-0.17	0.14	-1.22
cohort3	-0.36	0.22	-1.62
group2	0.68	0.14	4.98

Table 3.S10: Estimates for the null model of PD_{tot}.

	Estimate	Std. Error	T value
(Intercept)	-0.63	0.10	-6.41
age_years.c	0.07	0.09	0.74
timepointEG2	0.21	0.08	2.75
timepointMG3	0.71	0.14	4.98
timepointG6	1.49	0.44	3.43
sex2	-0.05	0.10	-0.47
cohort2	-0.10	0.11	-0.96
cohort3	0.06	0.17	0.36
group2	0.12	0.10	1.12

Table 3.S11: Estimates for the null model of RAN_{dig}

Full model estimates

	Estimate	Std. Error	T value
(Intercept)	-0.90	0.27	-3.37
age_years.c	0.02	0.12	0.13
timepointG6	0.99	0.39	2.53
sex2	0.07	0.13	0.50
cohort2	-0.22	0.14	-1.59
cohort3	-0.28	0.22	-1.24
group2	0.46	0.14	3.40
rs6935076	0.22	0.10	2.18

Table 3.S12: Estimates for the full model of EMT rs6935076.

	Estimate	Std. Error	T value
(Intercept)	-0.55	0.22	-2.45
age_years.c	0.00	0.09	0.01
timepointMG3	0.44	0.11	4.20
timepointG6	1.37	0.38	3.59
sex2	0.00	0.11	0.02
cohort2	-0.32	0.11	-2.78
cohort3	-0.37	0.18	-2.00
group2	0.41	0.11	3.67
rs759178	0.05	0.08	0.60
timepointMG3:rs759178	-0.08	0.04	-2.23
timepointG6:rs759178	0.02	0.07	0.33

Table 3.S13: Estimates for the full model of Klepel timepoint*rs759178 interaction.

	Estimate	Std. Error	T value
(Intercept)	-0.52	0.34	-1.52
age_years.c	-0.13	0.22	-0.59
sex2	0.19	0.15	1.27
cohort2	-0.10	0.16	-0.61
cohort3	-0.19	0.23	-0.82
group2	0.53	0.16	3.25
rs17236239	0.29	0.12	2.49

Table 3.S14: Estimates for the full model of NWR rs17236239.

	Estimate	Std. Error	T value
(Intercept)	-0.28	0.17	-1.68
age_years.c	0.10	0.09	1.05
timepointEG2	0.19	0.08	2.48
timepointMG3	0.67	0.14	4.69
timepointG6	1.36	0.43	3.13
sex2	-0.03	0.10	-0.30
cohort2	-0.14	0.11	-1.27
cohort3	0.05	0.17	0.27
group2	0.13	0.10	1.24
rs2038137	-0.19	0.07	-2.53

Table 3.S15: Estimates for the full model of RAN_{dig} rs2038137.

	Estimate	Std. Error	T value
(Intercept)	-0.23	0.18	-1.29
age_years.c	0.08	0.09	0.89
timepointEG2	0.20	0.08	2.58
timepointMG3	0.69	0.14	4.75
timepointG6	1.42	0.44	3.24
sex2	-0.05	0.10	-0.45
cohort2	-0.14	0.11	-1.30
cohort3	0.03	0.17	0.16
group2	0.12	0.10	1.14
rs761100	-0.19	0.07	-2.61

Table 3.S16: Estimates for the full model of RAN_{dig} rs761100.

4

EVALUATION OF RESULTS FROM GENOME-WIDE STUDIES OF LANGUAGE AND READING IN A NOVEL INDEPENDENT DATASET

Recent genome wide association scans (GWAS) for reading and language abilities have pin-pointed promising new candidate loci. However, the potential contributions of these loci remain to be validated. In the present study, we tested 17 of the most significantly associated single nucleotide polymorphisms (SNPs) from these GWAS studies ($p < 10^{-6}$ in the original studies) in a new independent population dataset from the Netherlands: known as FIOLA (Familial Influences On Literacy Abilities). This dataset comprised 483 children from 307 nuclear families, plus 505 adults (including parents of participating children), and provided adequate statistical power to detect the effects that were previously reported. The following measures of reading and language performance were collected: word reading fluency, non-word reading fluency, phonological awareness, and rapid automatized naming. Two SNPs (rs12636438, rs7187223) were associated with performance in multivariate and univariate testing, but these did not remain significant after correction for multiple testing. Another SNP (rs482700) was only nominally associated in the multivariate test. For the rest of the SNPs we did not find supportive evidence of association. The findings may reflect differences between our study and the previous investigations in respects such as the language of testing, the exact tests used, and the recruitment criteria. Alternatively, most of the prior reported associations may have been false positives. A larger scale GWAS meta-analysis than those previously performed will

This chapter has been published as:
Carrion-Castillo, A., van Bergen, E., Vino, A., van Zuijen, T., de Jong, P. F., Francks, C., & Fisher, S. E. (2016). Evaluation of results from genome-wide studies of language and reading in a novel independent dataset. Genes Brain Behav., 15(6), 531-541. doi:10.1111/gbb.12299

likely be required to obtain robust insights into the genomic architecture underlying reading and language.
Keywords: reading, language, association study, candidate SNPs

4.1 INTRODUCTION

It is well known that reading abilities have a genetic component, with reported heritability estimates ranging from 30% for a discriminant score of reading ability (DeFries et al., 1987) to 73% for word reading and 49% for reading comprehension in a recent meta-analysis (de Zeeuw et al., 2015). Until the last 2-3 years most research on the genetics of reading ability and disability (developmental dyslexia) was focused on candidate genes (e.g. *ROBO1, KIAA0319, DCDC2* and *DYX1C1*) that were often identified through linkage analysis followed by fine-mapping association studies. Several candidate associations were reported, and some of these have shown further supportive evidence in independent samples (reviewed in e.g. (Carrion-Castillo et al., 2013)). However, there is also a considerable lack of consistency across studies (Zhong et al., 2013; Zou et al., 2012; Tran et al., 2013; Becker et al., 2014), which has hindered efforts to precisely define the role of any specific variant in affecting the neurobiological basis of reading (Carrion-Castillo et al., 2013).

More recently, several genome-wide association scan (GWAS) studies have tried to identify common genetic variants that influence language and reading abilities, without prior hypotheses with regard to specific candidate genes or regions of the genome (Luciano et al., 2013; Harlaar et al., 2014; Gialluisi et al., 2014; Eicher et al., 2013; Field et al., 2013; Nudel et al., 2014). This new wave of research for the field queries the whole genome for association in a relatively unbiased manner, which is appropriate for phenotypes when the vast majority of the underlying genetic architecture is unknown. An important consideration for this approach is that, ideally, dataset sizes must be in the order of thousands of participants or more, in order to detect the small effect sizes that are expected for individual polymorphisms, and in the context of a high degree of statistical correction for multiple testing over millions of genetic variants (Visscher et al., 2012).

GWAS studies of reading and language performed so far have been based on a range of different designs and approaches. They have included cohorts ascertained through disorder (e.g. dyslexia or Specific Language Impairment (SLI)) or sampled from the general population. Such cohorts have been tested for association of SNPs with either a categorically-defined affection status or else with quantitative measures of performance for an array of reading/language-related skills. Here, we briefly summarize findings of the relevant GWAS reports published prior to October 2014, upon which we based the present study (see also Table 4.1).

Field et al. (2013) tested for association with a trait defined as 'phonological coding dyslexia' in a family-based sample (n=718, with 400 cases from 101 families), using the Transmission Disequilibrium Test (TDT) to screen $\sim$ 133,000 markers from the genome. A SNP within 5q35.1 (77kb downstream of *FGF18*) was associated with dyslexia status at a borderline-level of significance when considered against genome-wide multiple testing thresholds.

Eicher et al. (2013) performed case-control genome-wide association analyses with three different affection statuses for reading and language disorders defined from a general population sample of 4,291 children, the ALSPAC (Avon Longitudinal Study of Parents and their Children) cohort. Available quantitative traits were first used to define cases of reading disability (n=353), language impairment (n=163), as well as comorbid cases showing both reading and language deficits (n=174). Case-control analyses were then performed to evaluate associations of variants across the genome. Suggestive associations were found at several loci (3p24.4, 4q26, and within the *COL4A2* gene on 13q34), some of which could be tentatively linked to variation in brain white matter tracts in follow-up analysis of Diffusion Tensor Imaging (DTI) data in a separate sample of 332 healthy participants.

In another case-control GWAS study, Nudel et al. (2014) investigated a family-based sample recruited on the basis of probands with an SLI diagnosis; the SLI Consortium (SLIC) cohort, including 297 cases from 278 nuclear families. As well as using standard association analyses, the study tested for parent-of-origin effects and found two paternal effects; a significant association for a locus on 7p14.1, and a suggestive association within the *NOP9* gene on 14q11.2. For maternal parent-of-origin analyses they identified a suggestive association in the 5p13.1 region.

Moving away from case-control designs, Gialluisi et al. (2014) tested for pleiotropic effects of common genetic variants by carrying out GWAS meta-analysis of quantitative data from three different cohorts enriched for participants with reading/language disorders: the SLIC families, a UK-reading disability (UK-RD) dataset, and the Colorado Learning Disability Research Center (CLDRC) dataset (total n=1,862). The available quantitative traits variously included spelling, word reading, nonword reading, nonword repetition, phonological awareness, expressive and receptive language scores, and these were used to derive first principal components in each dataset. The GWAS meta-analysis identified two loci showing suggestive associations with the principal component, one within 7q32.1 (~10kb upstream of *FLNC*) and the other on 22q12.3 within the *RBFOX2* gene.

Several GWAS studies have focused on normal variation in reading and language in epidemiological cohorts. Luciano et al. (2013) meta-analysed GWAS results based on various quantitative traits in two general population cohorts, ALSPAC (maximum n=5,472) and the Brisbane Adolescent Twin Sample (BATS; n=1,177 from 538 families). They reported suggestive associations with a compound score of reading and spelling (19p13.3 within *DAZAP1*), word reading (1p13.1, 16q22.2 and 19p13.3) and nonword repetition (16q23.2, and 21q11.2 within *ABCC13*). A subsequent study tested for association with a composite score of receptive language measures at 12-years old in the UK Twins Early Development Study (TEDS) (Harlaar et al., 2014). Suggestive associations were identified within 2q31.2 in a primary discovery sample (n=2,329), but did not show any evidence of association in replication samples (total n= 2,639).

Finally, St Pourcain et al. (2014) focused on expressive vocabulary at an early age (15-18 months, n= 8,889) and later age (24-30 months, n=10,819) in four general population datasets: ALSPAC, TEDS, the Dutch Generation R cohort, and Western Australian Pregnancy Cohort (Raine) Study. One SNP within 3p12.3 (˜19kb downstream of the *ROBO2* gene) was significantly associated with early expressive vocabulary in GWAS meta-analysis. This SNP is roughly 1 megabase from the dyslexia susceptibility locus DYX5 (where the dyslexia candidate gene *ROBO1* lies; which is also a paralogue of *ROBO2*). Other SNPs were reported as suggestively associated in chromosomal bands 11p15.2, 12q15 and 19p13.3.

The sample sizes used for most of these studies of reading and language abilities are below what is optimal for GWAS of highly polygenic human traits, with the possible exception of two of the reports (Luciano et al., 2013; St Pourcain et al., 2014). Most of the reported top findings are statistically 'suggestive', and all remain to be validated in other independent samples, although they provide promising new candidate genes that could potentially play roles in the neurobiology of language and reading.

For the present study we have tested the most significantly associated SNPs from the above GWAS studies, targeting those that have shown association $p < 10^{-6}$ in the original reports. Through direct genotyping of the key SNPs, we attempted to find supportive evidence in a new Dutch general population sample in which there are several reading-related quantitative traits available, known as the FIOLA (Familial Influences On Literacy Abilities) dataset. While not all of the GWAS studies used datasets that are independent of one another, all of the top associations

were study-specific. The GWAS studies encompassed a broad range of phenotypic traits, including some measures, like expressive vocabulary (St Pourcain et al., 2014) and receptive language ability (Harlaar et al., 2014), which are not available in FIOLA. However, there is abundant evidence that reading and language performance measures have partly overlapping genetic contributions (Newbury et al., 2011; Luciano et al., 2013; Gialluisi et al., 2014). Several studies show evidence supporting the hypothesis that vocabulary size at different ages predicts later reading ability (van Bergen et al., 2014; Duff et al., 2015). A study of Dutch children with and without risk for dyslexia found that expressive vocabulary at 4.5 years is correlated (r=0.26) with reading scores at age 8 (van Bergen et al., 2014). Similarly, Duff et al. (2015) et al found that a latent variable comprising expressive and receptive vocabulary at 16-24 months predicts school-age (age range: 4-9 years) phonological awareness, reading accuracy and reading comprehension in a British sample (accounting for 4% of the variance in phonological awareness, 11% in reading accuracy and 18% in reading comprehension). Furthermore, these phenotypic relationships are supported by correlations at the genetic level as well, since twin studies have shown that genetic factors account for part of the correlation between language proficiency and later reading ability (Harlaar et al., 2008; Hayiou-Thomas et al., 2010).

Therefore, it was reasonable to use FIOLA to test all top associations from the GWAS studies, whether or not a given SNP had been originally reported to associate with a trait for which there was a matching measure available in the FIOLA dataset. Note that our goal was not strict replication, for which identical measures and recruitment strategies should be used across datasets. Rather, we aimed to test for supportive evidence that might help to validate any of the GWAS findings, and extend our understanding of them with respect to pleiotropy across measures of reading and language performance.

Reference	Design	Samples	Trait	Age (years)	Study Type	$Total_{max}$	$Cases_{max}$
Field et al. (2013)	GWAS	Dys-Canada	Phonological coding dyslexia	8-18	Case-Control	718	400
Eicher et al. (2013)	GWAS	ALSPAC	Dyslexia	7-9	Case-Control	4291	353
Eicher et al. (2013)	GWAS	ALSPAC	SLI	7-9	Case-Control	4291	163
Eicher et al. (2013)	GWAS	ALSPAC	Dyslexia/SLI	7-9	Case-Control	4291	174
Nudel et al. (2014)	GWAS: basic, maternal, paternal	SLIC	SLI	8-19	Case-Control	-	297
Gialluisi et al. (2014)	GWASMA	SLIC + UK-RD + CLDRC	Principal Component of Reading and Language	5-19; 5-31;8-19	Quantitative	1862	-
Luciano et al. (2013)	GWASMA	ALSPAC + BATS	Word Reading	8-9;12-25	Quantitative	6189	-
Luciano et al. (2013)	GWASMA	ALSPAC + BATS	Nonword Reading	8-9;12-25	Quantitative	6182	-
Luciano et al. (2013)	GWASMA	ALSPAC + BATS	Reading and spelling measure	8-9;12-25	Quantitative	6182	-
Luciano et al. (2013)	GWASMA	ALSPAC + BATS	Nonword Repetition	8-9;12-25	Quantitative	6583	-
Harlaar et al. (2014)	GWAS	TEDS	Receptive Language Composite Score	12	Quantitative	2329	-
St Pourcain et al. (2014)	GWASMA	ALSPAC + GenR	Expressive vocabulary CDI	1.25-1.5	Quantitative	8889	-
St Pourcain et al. (2014)	GWASMA	ALSPAC + GenR + Raine + TEDS	Expressive vocabulary CDI	2-2.5	Quantitative	10819	-

Table 4.1: Summary of genome wide association studies of language and reading traits published until October 2014, which provided the basis for selection of SNPs in the current study. Age range is specified per sample if it differs between the datasets included in each study.

4.2 METHODS

4.2.1 *Sample*

The Familial Influences On Literacy Abilities (FIOLA) project consists of a general population, family-based, Dutch sample that has been assessed with reading-related tests (van Bergen et al., 2015). Families that visited the Amsterdam Science Museum NEMO were invited to take part. This research is part of Science Live, the innovative research programme of Science Museum NEMO that enables scientists to carry out real, publishable, peer-reviewed research using NEMO visitors as volunteers. The museum offered quiet rooms for one-to-one testing. Ethical approval for this study was provided by the University of Amsterdam ethics committee, file number 2011-OWI-1882, and written informed consent of the participants (or their parents) was obtained. Two indicators of sample representativeness for the general population were evaluated: The level of education of the parents was 0.49 SD above the national average, and children's reading scores were 0.40 SD above the national average (van Bergen et al., 2015).

For the current study only individuals of European descent were included, in order to reduce the possible impact of genetic stratification (ancestry was assessed using an ethnicity questionnaire which queried as far back as the four grandparents and 60 individuals were excluded as a result). Other inclusion criteria were that participants had Dutch as their first language (no exclusion based on second language learning), and had attended Dutch primary education.

Our primary analysis was conducted on children, i.e. participants aged less than 200 months (16.6 years), because the previous GWAS studies have been carried out on children and teenagers (see Table 4.1), and genetic effects on reading and language performance may be partially age-dependent, targeting specific developmental stages (St Pourcain et al., 2014). In total, there were 483 children from 307 independent families, which comprised 149 singletons, 140 sibships for which two siblings were available, and 18 sibships for which 3 children were available. The minimum age was 6 years, Mean age=10.06 years, SD=1.97 years.

As a secondary step, we separately analysed all of the unrelated adults available (n=505, aged 33 to 68 years, Mean=43.27 years, SD=4.58 years), which included parents of the children (when available) plus 50 other unrelated individuals that had been tested at their homes using the same measures, including parallel form tests to

compute parallel form reliability. The inclusion of parents meant that this analysis was not entirely statistically independent from the analysis of children, but provided a useful complementary analysis available in this dataset. While programs do exist to analyze the child and parent information all together, we were unaware of an option that would do this in a multivariate context, to support simultaneous association analysis with multiple phenotypic measures (see below). Possible age-dependence of genetic effects on language-related traits also supports an approach of analyzing adults separately from children.

4.2.2 *Traits of interest*

The traits of interest for this study were reading fluency of words and nonwords, phonological awareness (PA), and serial rapid automatized naming (RAN). These are well-established indices of reading ability and related cognitive performance (Landerl et al., 2013; van der Leij et al., 2013). Phonological awareness and rapid naming are correlated with reading ability, and may each be linked to independent fractions of the variance in reading performance . Both have often been included in previous studies of the genetics of dyslexia and reading (e.g. (Rubenstein et al., 2014; Francks et al., 2004)).

Word reading fluency was assessed using the One-Minute-Test (in Dutch, Een-Minuut-Test or EMT), (Brus and Voeten, 1972), and nonword reading fluency was assessed using the Klepel test (van den Bos et al., 1994). Participants were asked to correctly read as many (non)words as possible within one minute (word reading) or two minutes (nonword reading). To avoid a ceiling effect in adults, the original lists were extended, resulting in lists of 145 words or non-words (van Bergen et al., 2015).

PA was measured with a phoneme deletion task (van Bergen et al., 2015). For each test item a phoneme (always a consonant) had to be deleted from a nonword, resulting in another nonword. Accuracy and speed were combined into a fluency measure.

RAN was measured by naming a matrix of 50 digits as quickly as possible (van den Bos, 2003). The time to completion was transformed to the number of digits per second, to normalize the score distribution.

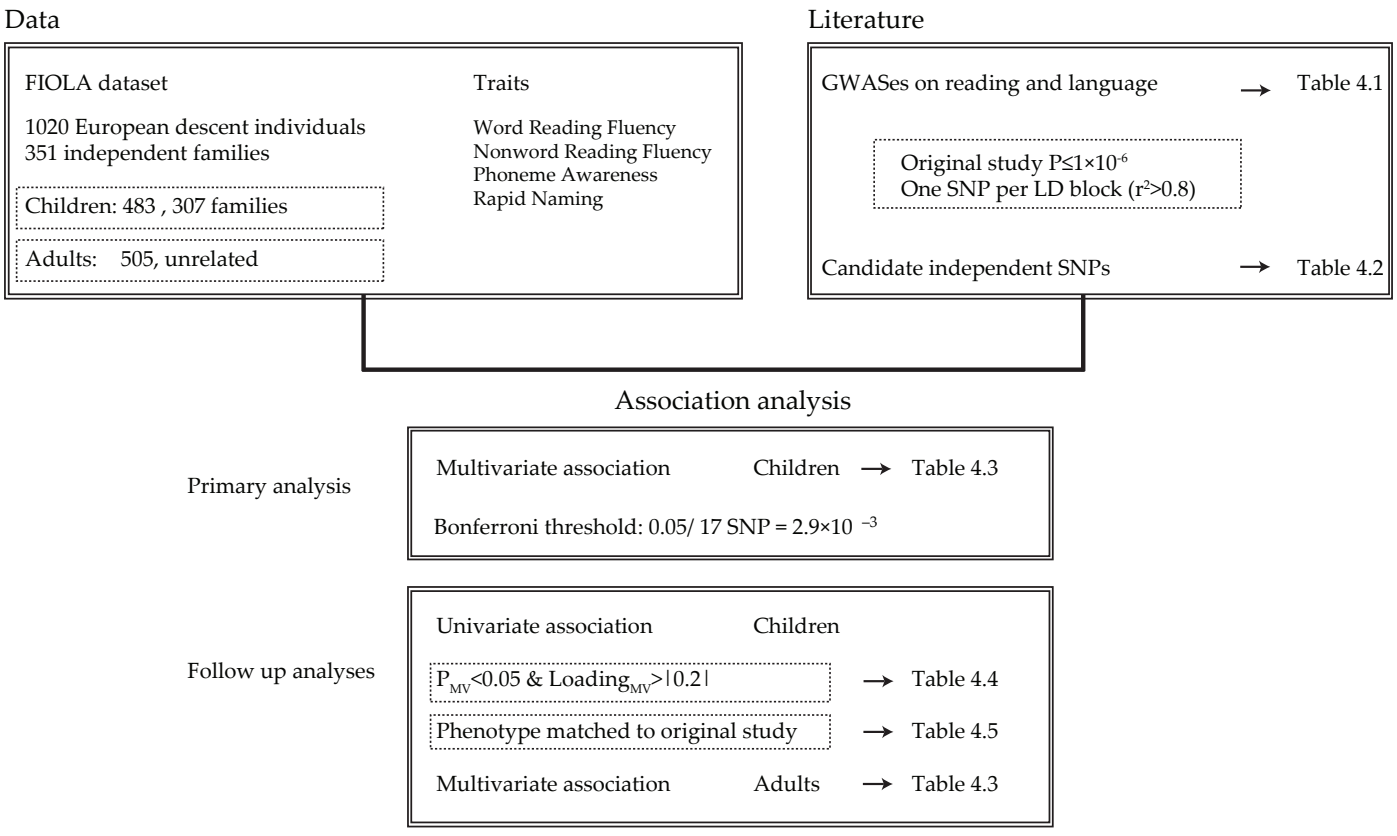


Figure 4.1: FIOLA genetics study design.

The reliability of all tasks was high. Reliability measures were available from manuals for children: parallel form reliabilities for word reading (0.76-0.96) and nonword reading (0.89-0.95) and split-form and test-retest reliabilities for RAN (0.78-0.92). Test-retest reliabilities were also estimated in a sample of 66 independent children for RAN (0.80) and PA (0.75-0.81) (van Bergen et al., 2015). A subset of 50 adults from the present sample were tested with parallel forms to estimate reliabilities for adults, which were 0.96 for word reading, 0.94 for nonword reading and 0.94 for RAN. Internal consistency for PA in this adult sample had a Cronbach's α of 0.71-0.89 (for accuracy and reaction time) (for details and full task descriptions see van Bergen et al. (2015)). In order to account for the linear and quadratic effects of age (in months), age and age^2 were regressed out from the scores of children (age $<$200 months), and the standardised residuals were used as trait scores. Scores for adults (age $>$200 months) were z-standardised to bring them on the same scale. Extreme values for each trait were identified as outliers when they were below/above 1.5 times the interquartile range from the 1st / 3rd quartiles. As a result 15 datapoints from the children's dataset were removed (word reading=3, nonword reading=1, PA=6, RAN=5) and 31 from the adults' (word reading =7, nonword reading=7, PA=8, RAN=9).

All of the phenotypic traits were normally distributed and significantly correlated with each other both for children and adults (see Supplementary information and van Bergen et al. (2015)). The correlations ranged from moderate ($r_{PA\text{-}RAN}$ for children 0.34 and for adults 0.39) to high ($r_{EMT\text{-}KL}$ for children 0.84 and for adults 0.68).

All of the phenotypic traits were normally distributed and significantly correlated with each other both for children and adults (see Supplementary information and van Bergen et al. (2015)). The correlations ranged from moderate (r_{PA-RAN} for children 0.34 and for adults 0.39) to high ((r_{EMT-KL} for children 0.84 and for adults 0.68).

4.2.3 *Power analysis*

Power estimates were performed using the Genetic Power Calculator (Purcell et al., 2003) , corresponding to univariate association analysis. Power was calculated for a range of type I error rates (alpha values) and QTL effect sizes, given two different experimental designs that were roughly comparable to analysis of either the children or the adults from the FIOLA dataset: 1) 240 nuclear families with sibship size of 2, with both parents genotyped, but with only offspring phenotyped, 2) 500 unrelated individuals.

4.2.4 *SNP selection and genotyping*

From the GWAS studies summarized in Table 4.1, and described in the Introduction, we selected all SNPs reported to be associated with reading or language traits with $p < 10^{-6}$. We then pruned the candidate variant list to reduce redundancy by selecting only one SNP per linkage disequilibrium (LD) block ($r^2 > 0.8$, CEU population) using SNAP (Johnson et al., 2008), which resulted in 20 SNPs as listed in Table 4.2. SNPs were directly genotyped using the KASP genotyping assay (LGC Ltd., Teddington, UK). Three of the SNPs failed at either the assay design or validation phase (rs59197085, rs5995177 and rs11176749), and were therefore excluded from the study. As a result, 17 SNPs were included in the present study (Table 4.2).

Mendelian inheritance errors were detected using pedstats (Wigginton and Abecasis, 2005). PLINK v1.07 (Purcell et al., 2007) was used to calculate missing genotype rates and to test for Hardy-Weinberg disequilibrium. 3 individuals (2 children, 1 adult) with more than 2/17 missing genotypes (missing genotype rate > 12%) were excluded. The total genotyping rate in the remaining individuals ($n_{children}$= 481, n_{adults}= 505) was 99.8%, with a missing genotype rate <5% for all the SNPs. All SNPs were in Hardy-Weinberg equilibrium (p>0.05).

Association analysis

We used multivariate association analysis as our primary means of testing for association of the reading and language traits with each SNP, using the '*mqfam*' option of PLINK-multivariate (Ferreira and Purcell, 2009). This approach was appropriate given the correlation structure of our traits of interest in both subsets of the dataset (children and adults, see Supplementary information), and it had the advantage that only one statistical test was performed per SNP in primary testing, thus reducing multiple testing (Bonferroni-adjusted significance threshold of $p=3*10^{-3}$ for 17 SNPs, α=0.05). The method finds the linear combination of a set of correlated measures which is most strongly associated with the given SNP genotypes. We performed the multivariate association analysis separately for children and parents. For children, 10,000 permutations were used in PLINK-multivariate to account for the family-based structure of the dataset (including children and parental genotypes when available), in order to obtain an empirical P value per SNP.

Reference	Phenotype	SNP	Risk	Pvalue	Effect	Chr	Position (bp)	MAF	Minor	Gene	Closest Gene
Luciano et al. (2013)	WordReading	rs4839516	C	3.62e-07	0.08(0.02)[b]	1	116197828	0.29	A	-	*MAB21L3*
Harlaar et al. (2014)	Receptive Lang	rs12474600	G	4.57e-07	-0.24(0.05)[b]	2	179020159	0.10	A	*CCDC141*	*SESTD1*
Eicher et al. (2013)	Case control	rs12636438	G	5.45e-07	1.81[a]	3	22021785	0.17	G	-	*ZNF385D-AS2*
St Pourcain et al. (2014)	Expressive early	rs7642482	G	1.3e-08	0.11(0.02)[b]	3	77668605	0.15	G	-	*ROBO2*
Eicher et al. (2013)	Case control	rs482700	G	1.4e-07	1.83[a]	4	115146334	0.28	G	-	*NDST4*
Nudel et al. (2014)	Maternal - SLI	rs10447141	G	1.16e-07	2.77[a]	5	39817065	0.29	T	-	*LINC00603*
Field et al. (2013)	Case control	rs9313548	T	6.2e-07	3.08[a]	5	171534296	0.45	C	-	*FGF18*
Nudel et al. (2014)	Paternal - SLI	rs7801303		3.51e-07	-	7	40878471	0.42	A	-	*LINC01450*
Gialluisi et al. (2014)	Principal Component	*rs59197085*	A	3.86e-07	0.001-0.033[a]	7	128820702	0.09	A	*CCDC136*	*FLNC*
Harlaar et al. (2014)	Receptive Lang	rs1326167	T	9.59e-07	0.17(0.04)[b]	10	90341269	0.25	C	-	*LOC101926942*
St Pourcain et al. (2014)	Expressive early	rs10734234	T	1.9e-07	-0.14(0.03)[b]	11	15444314	0.10	C	-	*LOC102724957*
St Pourcain et al. (2014)	Expressive early	*rs11176749*	T	7.2e-07	0.12(0.03)[b]	12	67459004	0.14	T	-	*LOC100507175*
Eicher et al. (2013)	Case control	rs9521789	C	7.59e-07	1.71[a]	13	110467273	0.41	C	*COL4A2*	*COL4A2-AS1*
Nudel et al. (2014)	Paternal - SLI	rs4280164	G	3.74e-08	3.87[a]	14	24302079	0.20	A	*C14orf21*	*NOP9*
Luciano et al. (2013)	WordReading	rs764255	T	1.8e-07	0.25(0.05)[b]	16	73679784	0.37	C	-	*LOC100506172*
Luciano et al. (2013)	NonWordRepetition	rs7187223	G	9.9e-08	-0.08(0.02)[b]	16	82424128	0.04	G	-	*CDH13*
St Pourcain et al. (2014)	Expressive early	rs1654584	G	3.4e-07	0.08(0.02)[b]	19	3970685	0.22	G	*DAPK3*	*EEF2*
Luciano et al. (2013)	NonWordRepetition	rs2192161	G	7.34e-08	0.20(0.04)[b]	21	14309673	0.07	A	-	*ABCC13*
Gialluisi et al. (2014)	Principal Component	*rs5995177*	A	5.01e-07	0.006-0.025[c]	22	35913505	0.07	A	*RBFOX2*	*RBFOX2*
Gialluisi et al. (2014)	Principal Component	rs12158565	G	7.57e-07	0.004-0.036[c]	22	35920795	0.10	G	*RBFOX2*	*RBFOX2*

Table 4.2: Candidate SNPs from the top hits of the recent GWAS literature on reading and language abilities, ordered by genomic coordinates. SNPs that failed genotyping assay design are marked in italics. SNP position is given for the human reference hg19. Effect sizes are given as: [a]the odds ratio (OR) for case-control studies and [b] β coefficient (SE) or [c] r^2 for the quantitative trait studies.

The multivariate analysis was followed by testing 'total' univariate association with PLINK 1.07, for 3 SNPs (see below) which showed multivariate $p < 0.05$ in the children, in order to explore the evidence for pleiotropy across measures. The 'total' test of association may potentially be affected by population stratification. Therefore a population stratification test was also performed using the *-ap* model in QTDT, which assesses the equivalence of the 'within-family' and 'between-family' mean allelic effects (Abecasis et al., 2000). Additionally, for two of the SNPs, the FIOLA dataset contained a particularly closely matched phenotypic measure to that which showed association in the original GWAS study (see Table 4.2), and therefore univariate association testing was performed for these SNPs using the closest matching measure, as a relatively direct attempt at replication.

The results from the univariate tests for selected SNPs (in Tables 4.4 and 4.5) were meta-analysed together with together to results from the original studies. This was implemented in the programme METAL (http://www.sph.umich.edu/csg/abecasis/Metal/index.html; (Willer et al., 2010)). We chose an approach that does not assume equivalence of allelic effect sizes between datasets, which was appropriate given the heterogeneity of study recruitment, assessment and trait definitions. Put briefly, the meta-analysis tested each SNP for a genetic effect, across the two contributing datasets, computing an overall z-score for that SNP determined by the P value, the direction of the allelic effect, and the sample size of each study involved in the meta-analysis. For the SNPs that had first been reported by studies with unequal numbers of cases and controls, we computed the effective sample size as recommended by Willer et al. (2010). ($N_{eff} = 4/(1/_{cases} + 1/N_{ctrls})$).

Finally, for three SNPs that had been associated with parent-of-origin SLI transmission to children in one GWAS study, we modelled the effects of maternally and paternally derived alleles separately in a parent-of-origin analysis using QTDT (Abecasis et al., 2000) with the *-ao –of* options.

4.3 RESULTS

4.3.1 *Power analysis*

Our study provided power of less than 60% to detect nominally significant results (α=0.05) for effects of 1%, for both the children and parent analysis. However, the effect sizes that were reported in the GWAS papers, for the SNPs we investigated here,

ranged from 0.5% to 12% (e.g. rs2192161 explained 3.9% of the variance in the original study (Luciano et al., 2013)). We had high power to detect effects that explain roughly 5% of the phenotypic variance (β>0.80 with α=0.003, i.e. Bonferroni correction for 17 SNPs) and moderate power to detect smaller effects (e.g. $\beta \approx 0.60$ for effects that explain 3% of the phenotypic variance, see Figure 4.2). Nevertheless, it is likely that the effect sizes in the GWAS studies where the associations were first reported are overestimates of any real effects, due to the winner's curse (Ioannidis, 2008), and hence our power to detect these effects may be only roughly 60%.

4.3.2 *Association analysis*

We performed multivariate association analysis first in the sample of children and then in adults, followed by univariate analysis for the SNPs that were nominally significant in the multivariate analysis for each sample.

Multivariate analysis in the children resulted in three nominally significant associations (rs12636438, p_{emp}=0.045; rs482700, p_{emp} =0.047 and rs7187223, p_{emp} =0.020) (see Table 4.3). The loadings were $>|0.2|$ for all four phenotypic measures in the multivariate models for all three SNPs, although for rs482700 word reading fluency had the opposite direction of effect to the rest of the measures. One of these markers, rs7187223, was the only SNP to show nominally significant association in the adults, with the highest loading for phoneme awareness ($loading_{PA}>|0.9|$, p=0.049, see Table 4.3). These associations did not survive statistical correction for multiple testing.

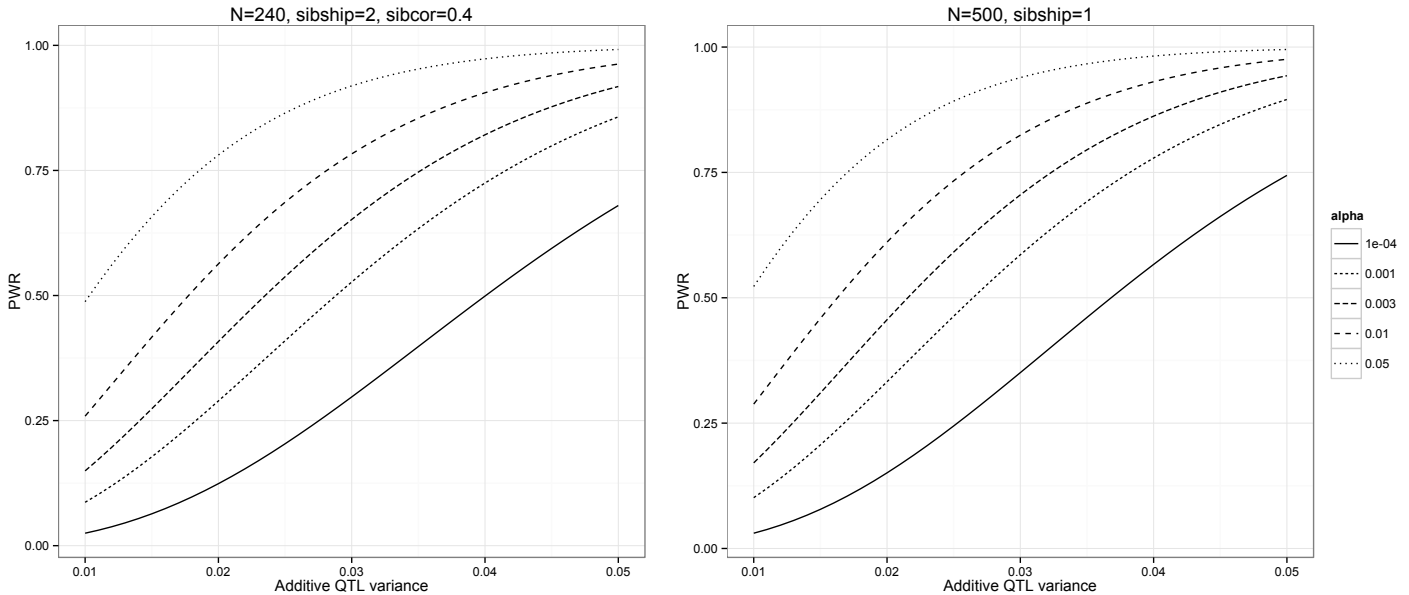


Figure 4.2: Power estimation. The x-axis is the additive proportion attributable to the Quantitative Trait Locus (range 0-5%). a) Children, family-based design, b) Adults, unrelated sample design.

Univariate analyses were performed for these three SNPs in the children, confirming that the putative effects of rs12636438 and rs7187223 encompassed several traits. However, there was no nominally significant univariate association with rs482700. The most significant associations with rs12636438 were with nonword reading fluency and rapid naming (p_{NWRF}= 0.01411 and p_{RAN}= 0.02376, see Figure S3). There was also a consistent but non-significant trend of association with word reading fluency. However, the direction of these effects in the FIOLA dataset was opposite to that previously reported: the allele associated with poorer performance was major allele (A) in our study, but the minor allele (G) was overrepresented in comorbid cases with LI and dyslexia in the original GWAS study of Eicher et al. (2013). This inconsistency in the direction of effect was reflected by the meta-analysed P values which were all higher than the originally reported one (Table 4.4). In the children, rs7187223 was nominally associated with rapid naming and there was a trend with the same direction of effect with phoneme awareness (p_{RAN}= 0.03897, p_{PA}= 0.03584). The minor allele G was associated with lower performance, which was consistent with the effect reported by Luciano et al. (2013), and reflected by meta-analysed P values $<$ 1.4e-08 for RAN and PA (Table 4.4). Because this SNP was nominally significant in the multivariate analysis of the adults, we tested association with univariate traits within this sample too, and found that it was also associated with phoneme awareness (p_{PA}= 0.0026). However, the result on the adults had the opposite direction of effect to that within the children, and also opposite to that previously reported for nonword repetition (Luciano et al., 2013) (see Figure S3). The tests for population stratification did not show significant differences of the between-family and within-family components of association for any SNP (all p$>$0.05), suggesting that population stratification was unlikely to be a substantial confounding factor in the analysis.

Word reading fluency in FIOLA is closely matched to the 'Word Reading' phenotype from Luciano et al. (2013), and hence we tested association of this trait within the children with the two SNPs (rs4839516 and rs764255) that were reported in Luciano et al. (2013). However, we did not find supportive evidence for associations of any of these SNPs with word reading fluency (see Table 4.5). Finally, because rs7801303, rs4280164 and rs10447141 had been reported to be associated with parent-of-origin-specific transmission to children with SLI (Nudel et al., 2014), we tested for such effects using QTDT for these SNPs and our traits of interest, but there were no significant parent-of-origin effects (p$>$0.1).

	Children						Adults				
SNP	Nfam	Nind	F	Loadings: WRF, NWRF, PA, RAN	P_{emp}	MAF	Nfam	Nind	F	Loadings: WRF, NWRF, PA, RAN	P
rs4839516	307	945	1.82		0.104	0.27	481	481	1.31		0.265
rs12474600	307	943	0.13		0.967	0.09	479	479	2.02		0.090
rs12636438	307	945	2.37	0.6044,0.847,0.2881,0.745	0.045	0.20	479	479	0.24		0.916
rs7642482	307	941	0.32		0.856	0.21	479	479	0.92		0.451
rs482700	307	946	2.31	-0.2751,0.21,0.3467,0.3788	0.047	0.26	481	481	0.75		0.560
rs10447141	307	945	1.61		0.144	0.33	481	481	1.28		0.278
rs9313548	307	942	0.42		0.759	0.48	480	480	0.74		0.564
rs7801303	307	945	2.18		0.052	0.44	480	480	0.41		0.800
rs1326167	307	940	0.75		0.507	0.24	481	481	1.94		0.102
rs10734234	307	944	2.01		0.097	0.11	481	481	0.84		0.501
rs9521789	307	944	1.19		0.291	0.40	481	481	0.21		0.935
rs4280164	307	943	0.36		0.822	0.21	481	481	0.27		0.896
rs764255	306	940	0.09		0.982	0.36	480	480	1.36		0.249
rs7187223	307	943	2.40	0.2892,0.2347,0.7195,0.6826	0.020	0.03	481	481	2.41	-0.4112,-0.5619,-0.9859,-0.2483	0.049
rs1654584	307	945	2.13		0.074	0.25	481	481	2.03		0.089
rs2192161	307	940	0.64		0.592	0.06	478	478	1.12		0.348
rs12158565	307	945	0.26		0.891	0.13	481	481	0.51		0.727

Table 4.3: PLINK multivariate association results (total test) from children and adults. P_{emp}: empirical P values (10,000 permutations). WRF: word reading fluency, NWRF: nonword reading fluency, PA: phonological awareness, RAN: rapid automatized naming. Loadings reflect the contribution of that trait to the multivariate test, and are only shown when $P<0.05$. Multiple testing correction for 17 SNPs sets the threshold to $2.9^{*}10^{-3}$.

SNP	Phenotype	N_{ind}	$Risk_{fiola}$	β	P_{emp}	P_{meta}	Effect
rs12636438	Word Reading Fluency	480	A	0.16	0.08	0.00732	-+
	Nonword Reading Fluency	481	A	0.22	0.01	0.02519	-+
	Phonological Awareness	470	A	0.07	0.39	0.001007	-+
	Rapid Naming	478	A	0.18	0.02	0.01759	-+
rs7187223	Word Reading Fluency	478	G	-0.18	0.36	7.298e-08	–
	Nonword Reading Fluency	478	G	-0.10	0.63	1.35e-07	–
	Phonological Awareness	467	G	-0.42	0.04	1.3e-08	–
	Rapid Naming	475	G	-0.41	0.04	1.338e-08	–
rs482700	Word Reading Fluency	480	G	-0.87	0.42	7.17e-06	–
	Nonword Reading Fluency	481	A	0.56	0.60	0.0002978	-+
	Phonological Awareness	470	A	1.01	0.33	0.000828	-+
	Rapid Naming	478	A	1.15	0.26	0.001237	-+

Table 4.4: PLINK univariate total association results for children. Empirical P values (10,000 permutations) shown. P_{meta}: meta-analyzed P value from the original report and present study. Effect: consistency of the direction of effect for the original and present studies.

SNP	Reference	$Phenotype_{ref}$	$Risk_{ref}$	$Phenotype_{fiola}$	N_{ind}	β	P_{emp}	P_{meta}	Effect
rs4839516	Luciano et al. (2013)	Word Reading	C	Word Reading Fluency	480	-0.104	0.188	4.812e-06	+-
rs764255	Luciano et al. (2013)	Word Reading	T	Word Reading Fluency	477	0.033	0.650	8.507e-07	-+

Table 4.5: PLINK univariate total association results for children, where SNPs and phenotypes matched those of the original report. P_{meta}: meta-analyzed P value from the original report and present study. Effect: consistency of the direction of effect for the original and present studies.

4.4 DISCUSSION

The aim of this study was to evaluate the most significant associations from a recent wave of the first GWAS studies of language and reading performance, by testing for their reported effects on reading-related quantitative traits in FIOLA, a new and independent Dutch dataset from the general population. We adopted an inclusive criterion for the selection of candidate SNPs, with the primary hypothesis that genetic effects might be pleiotropic and shared among several language and reading traits (Luciano et al., 2013; Gialluisi et al., 2014; Newbury et al., 2011). Seventeen candidate SNPs were selected, for which the FIOLA dataset provided sufficient statistical power to detect most of the previously reported effects, although it was underpowered to detect effects smaller than 1%. Three nominally significant associations were observed in the multivariate analysis of the children, and two of these (rs12636438, rs7187223) showed consistent signals in univariate analysis.

The association of rs12636438 loaded on all four traits, but more strongly on nonword reading fluency and rapid naming, with the major allele A being associated with lower performance in both cases. This SNP lies within the *ZNF385D* gene, which codes for a zinc finger protein that may act as a transcriptional regulator (Eicher et al., 2013). However, Eicher et al. (2013) found that the minor allele (G) of this SNP was overrepresented in comorbid cases of SLI and dyslexia, as well as associated with lower scores on a measure of receptive vocabulary (Picture Vocabulary Test) in a follow up sample. Thus, our findings show an opposite direction of effect to the original study. The incongruence of the allelic direction might be due to the different genetic backgrounds of the populations analysed. The haplotype structure in a region can differ between populations, changing the LD pattern between the tag SNP (where the association is detected) and the causal SNP that is driving the association, which is as yet unknown. Such factors could in principle lead to contrasting directions of effect of the same tag SNP in different studies when substantial population stratification is present (Lin et al., 2007), a phenomenon that has been suggested to potentially explain previous inconsistencies in the literature of the genetics of reading (Luciano et al., 2013; Gialluisi et al., 2014). It is difficult to evaluate this hypothesis without genome-wide information to assess the genetic homogeneity of all the samples (only directly genotyped SNP information is currently available for FIOLA). Despite the fact that only individuals of European descent were included in the analysis, different LD patterns between our sample and the ALSPAC sample

may account for the contrasting result for rs12636438. Alternatively, it might reflect type I error, representing a false positive. Eicher et al. (2013) also looked at the relationship of rs12636438 and white matter volumes and reported association with volumes of several DTI fiber tracts that are important for reading and language (the inferior longitudinal fasiculus, the inferior fronto-occipto fasiculus and the temporal superior longitudinal fasiculus). Of note, FIOLA did not involve any neuroimaging of participants, so we are unable to evaluate the reliability of those findings in the current study.

We found that rs7187223 was nominally associated with rapid naming and phonological awareness in the univariate analysis of children. The minor allele (G) was associated with lower performance in both cases, supporting the original association by Luciano et al. (2013) that reported that this allele was associated with lower nonword repetition scores. Rs7187223 was also the only SNP that was nominally significant for the multivariate analysis in the sample of unrelated adults. This effect loaded most strongly on phoneme awareness, which was also reflected by the univariate association with this trait. Surprisingly, the association in the adults had the opposite direction of effect to that on children, with the major allele (A) being associated with lower performance. Haplotype structure differences as discussed above cannot be responsible for this contrasting effect, since we are comparing two subsets of the same population. The main difference between the children and adult subsamples was obviously their age. There is some evidence suggesting that age-varying effects may also contribute to differences in allelic effects (Lasky-Su et al., 2008), and a longitudinal study found that a SNP in the *DYX1C1* dyslexia candidate gene is associated with children's orthographic judgments at ages 7 and 8, but not at age 6 (Zhang et al., 2012). Moreover, in the GWAS study of St Pourcain et al. (2014), an association of expressive vocabulary with rs7642482, near to *ROBO2* was also age-dependent (St Pourcain et al., 2014): it was genome-wide significant for an early developmental stage (15-18 months) but showed no evidence of association for a similar measure a few months later (24-30 months). Nevertheless, because language and reading acquisition are developmental processes, most of the studies that look at genetic effects on these traits tend to focus on datasets consisting of children (see Table 4.1), and there is very little known about the stability of genetic effects over time into adulthood. Alternatively, it is also possible that the opposite allelic effect between children and adults could be due to differences underlying the measured trait. Despite the fact that the same phoneme deletion task was used to measure PA in both samples, the

across-age invariance of this measurement has not been tested. Taken together, our results for rs7187223 do not provide compelling evidence to support the previously reported association.

Since one of the reading measures available for the FIOLA dataset (i.e. word reading fluency) was conceptually similar to some reported by Luciano et al. (2013), we specifically attempted to replicate their top hits with word reading (rs4839516 and rs764255), using univariate association tests. However, we did not replicate these associations. Similarly, we did not find any parental effect on the SNPs that had been reported to be associated with parent-of-origin transmission in relation to SLI (Nudel et al., 2014).

To our knowledge, this has been the first attempt to independently and systematically assess the results of recent GWAS reports for language and reading traits. We were only able to find limited support for three of the SNPs that had been previously associated with reading and language related traits in these GWAS studies. The associations with the other SNPs were not supported in our study. However, this does not translate into a complete rejection of these SNPs as potentially relevant, given the range of the studies that we have considered when selecting the SNPs, and the between-study heterogeneity of the measures even in the cases where similar phenotypes were available (e.g. word reading accuracy versus word reading fluency). The FIOLA dataset was ascertained in an unconventional manner through a public venue. This is an advantageous set-up to test large numbers of individuals and has the potential to contribute to the ascertainment of large cohorts in the future. However, the FIOLA dataset is not entirely representative of the Dutch population: it is biased towards a higher-than-average educational level for adults (0.49 SD above the average), and towards higher-than-average word reading scores for children (0.4 SD above national norms) (van Bergen et al., 2015). Moreover, there is a small but significant correlation of the spousal reading ability (0.16, p=0.019, (van Bergen et al., 2015)), which may indicate assortative mating. Environmental factors such as parental education and socio-economic status have been suggested to moderate genetic influences on reading disabilities, in terms of both heritability estimates and single SNP effects (Friend et al., 2008; Mascheretti et al., 2013). Hence, non-random sampling from the population, as is apparently the case for FIOLA, adds yet another potential source of heterogeneity across different datasets and studies. IQ, which correlates phenotypically and genetically with educational attainment, should also be considered (Davies et al., 2016). Several of the GWAS studies have adjusted

their reading-related measures for IQ prior to genetic association testing (Gialluisi et al., 2014; Luciano et al., 2013), and one showed that some association signals are sensitive to this manipulation (Gialluisi et al., 2014). In other words, some genetic effects may be more pleiotropic for reading-related cognition and IQ than others. The previously reported GWAS results remain to be further studied in other samples, and ideally meta-analysed across all available samples together, while accounting for the issues addressed in the present study.

References

Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66: 279–292.

Becker J, Czamara D, Scerri TS, Ramus F, Csepe V, et al. (2014) Genetic analysis of dyslexia candidate genes in the European cross-linguistic NeuroDys cohort. Eur J Hum Genet 22: 675–680.

Brus BT, Voeten MJM (1972) Een-minuut-test [one-minute-test]. Swets & Zeitlinger.

Carrion-Castillo A, Franke B, Fisher SE (2013) Molecular genetics of dyslexia: an overview. Dyslexia 19: 214–240.

Davies G, Marioni RE, Liewald DC, Hill WD, Hagenaars SP, et al. (2016) Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N=112 151). Mol Psychiatry .

de Zeeuw EL, de Geus EJ, Boomsma DI (2015) Meta-analysis of twin studies highlights the importance of genetic variation in primary school educational achievement. Trends in Neuroscience and Education pp. –, URL http://www.sciencedirect.com/science/article/pii/S2211949315000198.

DeFries JC, Fulker DW, LaBuda MC (1987) Evidence for a genetic aetiology in reading disability of twins. Nature 329: 537–539.

Duff FJ, Reen G, Plunkett K, Nation K (2015) Do infant vocabulary skills predict school-age language and literacy outcomes? J Child Psychol Psychiatry 56: 848–856.

Eicher JD, Powers NR, Miller LL, Akshoomoff N, Amaral DG, et al. (2013) Genome-wide association study of shared components of reading disability and language impairment. Genes Brain Behav 12: 792–801.

Ferreira MA, Purcell SM (2009) A multivariate test of association. Bioinformatics 25: 132–133.

Field LL, Shumansky K, Ryan J, Truong D, Swiergala E, et al. (2013) Dense-map genome scan for dyslexia supports loci at 4q13, 16p12, 17q22; suggests novel locus at 7q36. Genes Brain Behav 12: 56–69.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, et al. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75: 1046–1058.

Friend A, DeFries JC, Olson RK (2008) Parental education moderates genetic influences on reading disability. Psychol Sci 19: 1124–1130.

Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, et al. (2014) Genome-wide screening for DNA variants associated with reading and language traits. Genes Brain Behav 13: 686–701.

Harlaar N, Hayiou-Thomas ME, Dale PS, Plomin R (2008) Why do preschool language abilities correlate with later reading? A twin study. J Speech Lang Hear Res 51: 688–705.

Harlaar N, Meaburn EL, Hayiou-Thomas ME, Davis OS, Docherty S, et al. (2014) Genome-wide association study of receptive language ability of 12-year-olds. J Speech Lang Hear Res 57: 96–105.

Hayiou-Thomas ME, Harlaar N, Dale PS, Plomin R (2010) Preschool speech, language skills, and reading at 7, 9, and 10 years: etiology of the relationship. J Speech Lang Hear Res 53: 311–332.

Ioannidis JP (2008) Why most discovered true associations are inflated. Epidemiology 19: 640–648.

Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, et al. (2008) SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24: 2938–2939.

Landerl K, Ramus F, Moll K, Lyytinen H, Leppanen PH, et al. (2013) Predictors of developmental dyslexia in European orthographies with varying complexity. J Child Psychol Psychiatry 54: 686–694.

Lasky-Su J, Lyon HN, Emilsson V, Heid IM, Molony C, et al. (2008) On the replication of genetic associations: timing can be everything! Am J Hum Genet 82: 849–858.

Lin PI, Vance JM, Pericak-Vance MA, Martin ER (2007) No gene is an island: the flip-flop phenomenon. Am J Hum Genet 80: 531–538.

Luciano M, Evans DM, Hansell NK, Medland SE, Montgomery GW, et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav 12: 645–652.

Mascheretti S, Bureau A, Battaglia M, Simone D, Quadrelli E, et al. (2013) An assessment of gene-by-environment interactions in developmental dyslexia-related phenotypes. Genes Brain Behav 12: 47–55.

Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, et al. (2011) Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. Behav Genet 41: 90–104.

Nudel R, Simpson NH, Baird G, O'Hare A, Conti-Ramsden G, et al. (2014) Genome-wide association analyses of child genotype effects and parent-of-origin effects in

specific language impairment. Genes Brain Behav 13: 418–429.

Purcell S, Cherny SS, Sham PC (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19: 149–150.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.

Rubenstein KB, Raskind WH, Berninger VW, Matsushita MM, Wijsman EM (2014) Genome scan for cognitive trait loci of dyslexia: Rapid naming and rapid switching of letters, numbers, and colors. Am J Med Genet B Neuropsychiatr Genet 165B: 345–356.

St Pourcain B, Cents RA, Whitehouse AJ, Haworth CM, Davis OS, et al. (2014) Common variation near ROBO2 is associated with expressive vocabulary in infancy. Nat Commun 5: 4831.

Tran C, Gagnon F, Wigg KG, Feng Y, Gomez L, et al. (2013) A family-based association analysis and meta-analysis of the reading disabilities candidate gene DYX1C1. Am J Med Genet B Neuropsychiatr Genet 162: 146–156.

van Bergen E, Bishop D, van Zuijen T, de Jong PF (2015) How Does Parental Reading Influence Children's Reading? A Study of Cognitive Mediation. Scientific Studies of Reading 0: 1–15.

van Bergen E, van der Leij A, de Jong PF (2014) The intergenerational multiple deficit model and the case of dyslexia. Front Hum Neurosci 8: 346.

van den Bos KP (2003) Serieel benoemen en woorden lezen [serial naming and word reading]. Rijksunversiteit Groningen .

van den Bos KP, Lutje Spelberg HC, Scheepstra AJM, de Vries JR (1994) De klepel: Een test voor de leesvaardigheid van pseudowoorden [the klepel: A test for the reading skills of pseudowords]. Swets & Zeitlinger .

van der Leij A, van Bergen E, van Zuijen T, de Jong P, Maurits N, et al. (2013) Precursors of developmental dyslexia: an overview of the longitudinal Dutch Dyslexia Programme study. Dyslexia 19: 191–213.

Visscher PM, Brown MA, McCarthy MI, Yang J (2012) Five years of GWAS discovery. Am J Hum Genet 90: 7–24.

Wigginton JE, Abecasis GR (2005) PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 21: 3445–3447.

Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26: 2190–2191.

Zhang Y, Li J, Tardif T, Burmeister M, Villafuerte SM, et al. (2012) Association of the DYX1C1 dyslexia susceptibility gene with orthography in the Chinese population. PLoS ONE 7: e42969.

Zhong R, Yang B, Tang H, Zou L, Song R, et al. (2013) Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol 47: 435–442.

Zou L, Chen W, Shao S, Sun Z, Zhong R, et al. (2012) Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 159B: 970–976.

SUPPLEMENTARY INFORMATION

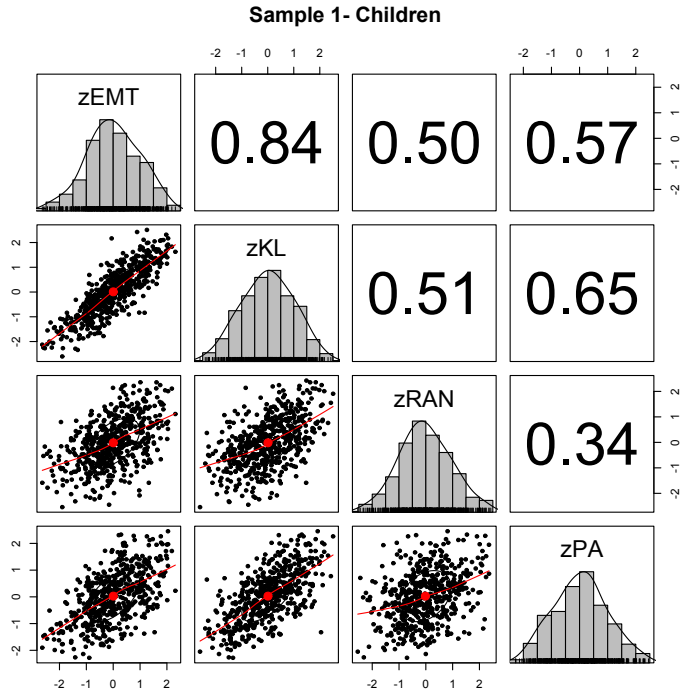


Figure 4.S1: Correlation panel of z-scores for the four phenotypes analysed in samples of children. The lower panel contains the scatter plot of the raw data for each pair of phenotypes. The values in the upper panel correspond to the Pearson's correlation coefficient, and the significance of the correlation. The diagonal shows the histogram of each of the scores. EMT= word reading fluency; Kl= nonword reading fluency; RAN=rapid automatized naming; PA=phonological awareness.

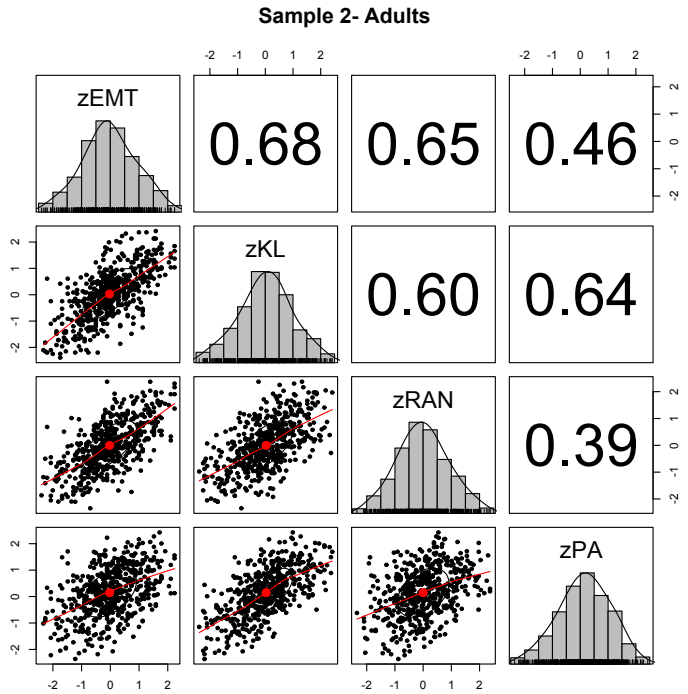


Figure 4.S2: Correlation panel of z-scores for the four phenotypes analysed in samples adults. The lower panel contains the scatter plot of the raw data for each pair of phenotypes. The values in the upper panel correspond to the Pearson's correlation coefficient, and the significance of the correlation. The diagonal shows the histogram of each of the scores. EMT= word reading fluency; Kl= nonword reading fluency; RAN=rapid automatized naming; PA=phonological awareness.

Children

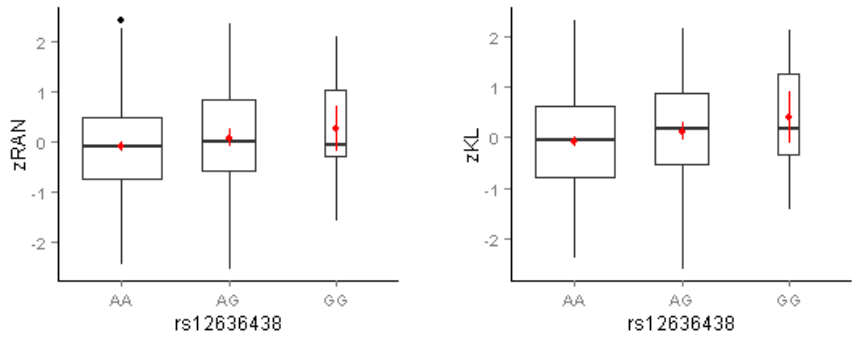
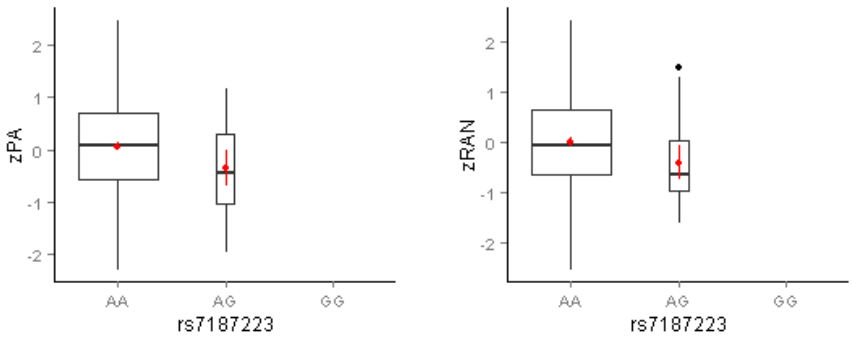


Figure 4.S3: Rapid naming (zRAN) and nonword reading fluency (zKL) scores in relation to rs12636438 genotypes for the children. The mean with the standard error per group shown in red.

Children



Adults

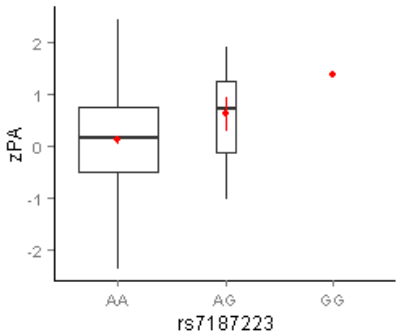


Figure 4.S4: Rapid naming (zRAN) and phoneme awareness (zPA) scores in relation to rs7187223 genotypes for children and adults. The mean with the standard error per group shown in red.

5

GENOME-WIDE SEQUENCING IN A LARGE FAMILY WITH DYSLEXIA

Dyslexia is one of the most common human neurodevelopmental disorders (5-10% prevalence), and it has a complex aetiology which is likely to involve contributions from both common and rare genetic variation. We analyzed a 30-member multigenerational family with dyslexia, with the goal of identifying a rare genetic variant that might have an individually substantial effect on this complex trait. We performed whole genome linkage analysis and sequenced the whole exomes or genomes of 15 members of the family (13 affected and 2 unaffected). No candidate variants were identified that might be causative for dyslexia in this family as perfectly penetrant Mendelian mutations. However, several rare variants were identified that may contribute to the trait in this family, including a nonsynonymous SNP within the gene *CACNA2D3*, a noncoding variant upstream of *RBMX*, and an intronic deletion within *AFF2*.

Keywords: mapping complex traits, family linkage analysis, whole exome sequencing, whole genome sequencing, dyslexia

5.1 INTRODUCTION

Dyslexia is one of the most common human neurodevelopmental disorders (5-10% prevalence), and it has a complex aetiology involving genetic and environmental factors. It is often considered to reflect the lower tail of the population distribution in reading ability (Shaywitz et al., 1992). Thus, much effort in dyslexia genetics research has been directed to find common genetic variants, with small and additive effects that contribute to susceptibility (Bishop, 2015). This "common disease-common variant" hypothesis has led to the identification of candidate genes, including *KIAA0319* and *DCDC2*, in which genetic variation is linked to reading performance in cohorts ascertained through poor reading performance (Francks et al., 2004; Cope et al., 2005; Meng et al., 2005), as well as in unselected populations (Paracchini et al., 2008; Scerri et al., 2011).

However, there are some unusual, extended families in which dyslexia is inherited in what resembles a Mendelian fashion, and with a much higher prevalence within the family than in the general population (Fagerheim et al., 1999; Nopola-Hemmi et al., 2001). In such families, one or a few genetic variants might have substantial penetrances, i.e. people carrying those variants would have relatively high probabilities of developing the trait. Such variants are expected to be rare in the general population, and because of this, not usually amenable to a standard genetic association study design, based on cohorts of unrelated people. Nevertheless, detecting highly penetrant, rare variants in family studies can pinpoint genes which have critically important biological roles for the trait of interest. Genes that are identified via highly penetrant and rare mutations can also be investigated further using common genetic variants in follow-up candidate gene association studies.

Family studies have already proven to be valuable for the identification of several dyslexia candidate genes. For example, *DYX1C1* (Taipale et al., 2003) was identified as a potential candidate because of a chromosomal rearrangement that co-segregated with dyslexia in multiple members of one family. A rare haplotype of *ROBO1* (Hannula-Jouppi et al., 2005) was found to co-segregate with dyslexia status in the majority of affected relatives of another large family, and the same gene was disrupted by a translocation in an unrelated case. Subsequently, common variants within these genes have been tested for association with reading-related quantitative traits (Dahdouh et al., 2009; Bates et al., 2010, 2011), as well as brain imaging endophenotypes (e.g. Darki et al. (2012)) in independent samples. The biological roles

of such genes have been further characterized in cellular (Lamminmaki et al., 2012; Tammimies et al., 2013) and animal models (Currier et al., 2011; Andrews et al., 2006), illustrating how identifying rare variants can shed light on molecular pathways involved in dyslexia. Linkage studies in multigenerational families have also identified other dyslexia candidate genomic regions, including DYX3 on chromosome 2p15-16 (Fagerheim et al., 1999) and DYX9 on Xq27.3 (de Kovel et al., 2004). However, specific genes or variants contributing to dyslexia within those genomic regions have not been identified. Indeed it remains unclear whether these specific genomic regions are responsible for the inheritance of dyslexia in these families.

The current study focuses on the family that led to the identification of DYX9 on Xq27.3, referred to hereafter as family 259 (de Kovel et al., 2004). The previous linkage study was based primarily on microsatellite markers, but also involved targeted sequencing of some individual candidate genes within Xq27.3 (*FMR1, TMEM257, DKFZp574M2010* and *SLITRK2*), which did not identify any protein-coding mutations that co-segregated with dyslexia (de Kovel et al., 2004). Another study that analysed twelve French families with dyslexia also found evidence for linkage within this genomic region, but again did not identify a mutation co-segregating with dyslexia by targeted sequencing of seven candidate genes (*FMR1, CXORF1, CXORF51, SLITRK2, FMR2, ASFMR1,* and *FMR1NB*) (Huc-Chabrolle et al., 2013).

Next generation sequencing (NGS) now offers the possibility to screen systematically and rapidly over the whole genome or whole exome (i.e. the protein-coding regions of the genome) for rare mutations affecting Mendelian traits. This approach has revolutionized genetic analysis of human Mendelian traits (Bamshad et al., 2011), identifying several disease-causing genes that could not be previously detected, such as *SETBP1* for Schinzel-Giedion syndrome (Hoischen et al., 2010), and *TGM6* for spinocerebellar ataxia (Wang et al., 2010). With respect to dyslexia, a two-base mutation within the *CEP63* gene, causing an amino acid substitution, was recently shown through this approach to co-segregate with the trait in an extended Swedish family (Einarsdottir et al., 2015). We have therefore re-visited family 259 (de Kovel et al., 2004) with NGS, and adopted an approach that combines linkage analysis with mutation analysis (Smith et al., 2011; Wijsman, 2012), with respect to three types of genetic variation: coding, noncoding, and structural.

5.2 MATERIAL AND METHODS

5.2.1 *Subjects and diagnostic criteria*

We studied a three generation family of thirty members (family 259, Figure 5.1), with a potentially rare Mendelian form of dyslexia. This pedigree was recruited as part of a multidisciplinary research effort into different aspects of dyslexia (the Dutch Dyslexia Programme: DDP (van der Leij and Maassen, 2013)), in which families were ascertained when at least two first degree relatives had a school history of reading problems, as described in de Kovel et al. (2004). Informed consent was obtained from all participants and the study was approved by the local ethics committee (CWOM) of the University Medical Centre Nijmegen under CWOM-nr 9811-025 (de Kovel et al., 2004).

Dyslexia was defined following the criteria of the Dutch Dyslexia Programme (van der Leij and Maassen, 2013), which are based on several quantitative measures described in detail by de Kovel et al. (2004). In short, people were defined as affected if they 1) performed below the 10th percentile on a word reading test, or 2) scored below the 10th percentile on a nonword reading test, or 3) scored below the 25th normative percentile on both word and nonword reading tests, or 4) had a word or nonword reading score that was >60 percentage points below their normalized score on a verbal competence test (discrepancy criterion). Following these criteria, 15 of the 30 subjects were identified as dyslexic.

5.2.2 *Genotyping with microsatellite markers*

This genotyping was performed previously and described by de Kovel et al. (2004). DNA samples were extracted from blood. 374 CA repeat markers (LMS-MD10 v2.5; Applied Biosystems, Foster City, CA, USA) were genotyped for 29 family members.

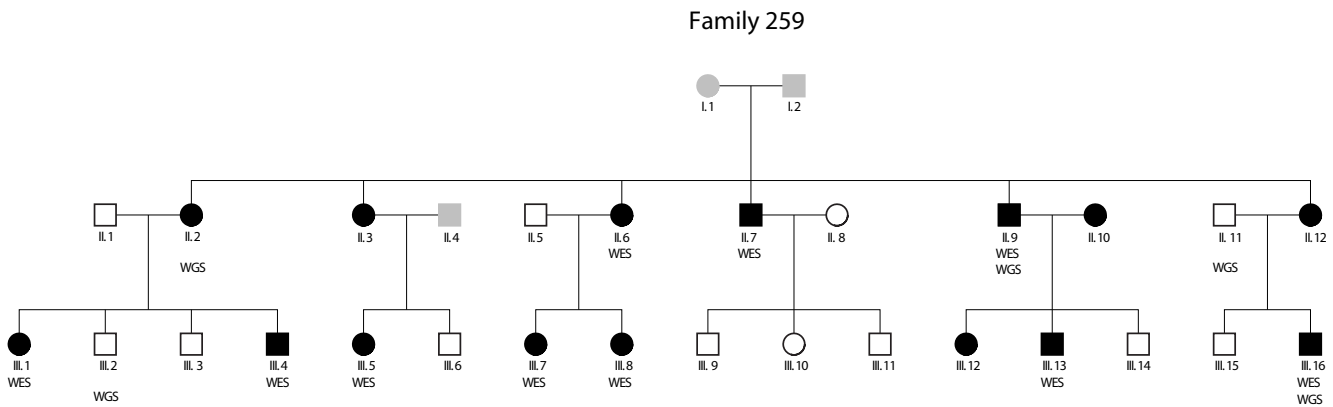


Figure 5.1: Pedigree of family 259. WES: individuals for which whole exome sequencing was performed. WGS: individuals for which whole genome sequencing was performed. Black symbols represent dyslexic individuals, white symbols represent unaffected individuals, and grey symbols represent individuals for which the phenotype was not known.

5.2.3 *Next generation sequencing*

A total of 13 members of the family were selected for either whole exome sequencing (WES) or whole genome sequencing (WGS). The sample selection and choices of sequencing method were motivated primarily by the individuals' quantitative trait scores (i.e. taking those most severely affected or obviously unaffected). It was constrained by cost and performed in phases (one phase of WES, one phase of WGS) due to practical considerations. WES was used for ten members and WGS for five members: two members had both WES and WGS, as indicated in Figure 5.1. This selection enabled us to sequence as many people as possible (within budget constraints) for the exonic regions, and to also thoroughly investigate non-exonic variation for a minority of samples. Furthermore, the two samples that were sequenced by both methods permitted a comparison between the two approaches, and helped to identify exonic variants that might have been missed by the targeted exome sequencing approach (Gilissen et al., 2014; Belkadi et al., 2015) (see Appendix 5.4 for a comparison of the exome coverage from WGS and WES data).

Whole exome sequencing

Genomic DNA samples collected from 10 affected family members (see Figure 5.1) were used for whole exome sequencing by the genomics research organization and service company 'BGI' (Hong Kong/Shenzhen) using Illumina's HiSeq 2000 technology (Illumina, 2016a). Exome capture was done with the Agilent SureSelect All Human Exon V4 (51 megabase) array (SureSelect, 2016) which targets over 80% of the coding exons. Sequencing was at 100 times average coverage depth with library insert size 150-200 base pairs (paired-end). Depending on the amount of available DNA, one or two libraries were constructed for each sample.

Whole genome sequencing

Genomic DNA samples collected from 5 family members (3 affected and 2 unaffected; see Figure 5.1) were used for whole genome sequencing by the genomics research organization and service company 'Novogene' (Hong Kong) using Illumina's HiSeq Xten technology (Illumina, 2016b). Sequencing was at 30 times average coverage depth with library insert size 2x 150 base pairs (paired-end).

5.2.4 *NGS data processing*

The WES and WGS data were processed following an identical pipeline if not specified otherwise below.

Alignment and pre-processing

Raw reads were cleaned by excluding adapter sequences, reads with low-quality bases for more than 50% of their lengths, and reads with unknown bases for more than 10% of their lengths. Clean reads, which comprised 94% of total reads for WES and 97% of toal reads for WGS, were mapped onto the human reference genome (hg19) using the software Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009). Bam files were sorted using SAMtools (Li et al., 2009) and PCR duplicate reads were marked using Picard (Picard, 2016).

For WES data, the mean sequence read length was 88 base pairs, and approximately 99.3% of the target exome was covered by at least a 10 times sequence read depth. For WGS data, the mean sequence read length was 150 base pairs, and approximately 97.5% of the genome was covered by at least a 10 times sequence read depth.

Re-alignment around indels (insertion/deletions) and base quality control recalibration was performed using the Genome Analysis Toolkit software (GATK v2.7.2 for WES, and v3.4 for WGS) (McKenna et al., 2010; DePristo et al., 2011).

Variant calling and annotation

Since more accurate variant calls can be achieved by including data from larger numbers of subjects simultaneously, we ran this process by pooling our data from family 259 together with additional subjects from different projects that had been sequenced with the identical protocols and in the same batches (i.e. 12 additional WES samples, and 56 additional WGS samples).

For the WES analysis, a target interval file was generated to define the exonic regions by using the UCSC Genome Browser (exons for Refseq genes $\pm$ 10bp, reference genome: hg19). Genetic variants were called using the HaplotypeCaller (HC) tool of GATK (McKenna et al., 2010; DePristo et al., 2011). For the WES, multisample calling was done using HC (GATK v2.7.2) to call variants jointly on all the available samples, using the default parameters with the exception of confidence thresholds that were

set to: stand_call_conf = 50.0, stand_emit_conf = 10.0. For the WGS, HC was run separately per sample using the '-ERC GVCF' mode, and then merged together using the GenotypeGVCFs tool, as recommended by the GATK best practices (GATK v3.4).

We performed Variant Quality Score Recalibration (VQSR) on the WGS data to exclude low quality variants (phred-scaled Qscore < 30) and to flag the rest into the sensitivity tier into which they fell (90, 99, 99.9 and 100). Variant quality filtering for the WES samples was based on hard filter cutoffs, i.e. QD < 2.0 || FS > 60.0 || MQ < 40.0 || HaplotypeScore > 13.0 || MappingQualityRankSum $<$ -12.5 || ReadPosRankSum $<$ -8.0).

WES and WGS variant calls were combined per subject using the 'CombineVariants' tool from GATK (v.3.4.) with the options '-genotypeMergeOptions UNIQUIFY'. This resulted in a total of 7,751,540 variant calls. Since a genetic variant can be represented in several different ways in a variant call format (vcf) file (e.g. the reference coordinate position can be 0 or 1), the representation of the variants in the combined vcf was unified using the variant tool set VT (Tan et al., 2015). By doing so, multiallelic variants (583,792) were decomposed into a biallelic representation (907,403 additional biallelic variants), and then normalized (425,861 variants normalized) using the VT decompose and normalize software tools (Tan et al., 2015). The variant calling of SNPs and indels predicted on average 46,366 (range: 43,065-55,188) variants per WES sample, and 4,755,537 (range: 4,701,300-4,807,194) per WGS sample. Variants were then annotated using Annovar (Wang et al., 2010) and Variant Effect Predictor (VEP v37) (McLaren et al., 2010).

Structural variant calling

WGS data was used to call structural variants (SVs), including deletions, duplications and inversions. SVs can be indicated from several different signals in WGS data, including read-pairs, split-reads, and read-depth. Different SV detection methods have different accuracies and resolutions for the various types of SV (Mohiyuddin et al., 2015). Hence, we called SVs using three complementary variant calling programs: CNVnator (Abyzov et al., 2011), BreakDancer (Chen et al., 2009) and Lumpy (Layer et al., 2014).

Overlapping calls from the three SV detectors were then combined within samples using MetaSV (Mohiyuddin et al., 2015), considering as high-confidence those SVs detected by at least two of the tools. The five WGS samples had on average 310,491

SV calls (range: 309,800-311,000), of which on average 1,875 (range: 1,850-1,907) had been called by at least two different callers.

SV calls across all samples were combined using the R package intanSV (Yao, 2016) by merging the calls that had been made in at least two samples with a reciprocal coordinate overlap larger than 10 %. There were a total of 23,383 overlapped calls: 20,315 deletions, 2,335 duplications, and 733 inversions.

The SV calls were compared against the Database of Genomic Variants (DGV; downloaded from UCSC genome browser hg19, February 2016) to annotate them as rare and common. Those that overlapped by $>$ 50% of their lengths with five or fewer variants in the DGV were considered rare. Those that did not overlap with any variant in the DGV were classed as novel.

We excluded SVs as being potentially causative for the phenotype when they were present in the unaffected individual II.11 (married into family 259; see Figure 5.1), or when they were common variants in the DGV database.

5.2.5 *Linkage analysis*

Exonic biallelic variants from WES which had rs identity numbers in the dbSNP database (Sherry et al., 2001), and minor allele frequency (MAF) >5%, were cleaned to remove Mendelian errors and unlikely recombination events using Merlin (Abecasis et al., 2002) and Pedcheck (O'Connell and Weeks, 1998). This yielded a total of 34,066 variants that were subject to linkage disequelibrium (LD) pruning (Variant Inflaction Factor=1) using PLINK (Purcell et al., 2007). LD was defined according to a set of 1303 Dutch subjects from Nijmegen's Brain Imaging Genetics (BIG) dataset (Franke et al., 2010; Guadalupe et al., 2014), and only variants genotyped or imputed in BIG could be selected for the pruning. This resulted in 12,129 independent (uncorrelated) variants. A genetic distance map was then created for the resulting variants using the cM map interpolator (based on the Human610 Quand cM/bP genetic map) tool within the SNP/Max system of BC Platforms (Platforms, 2015). Further filtering involved removing variants with low polymorphism information content (PIC) within the family ('minimum distance between markers' = 0.2 centiMorgans (cM), 'most informative marker selected within' = 0.1 cM) which resulted in 4,705 variants. Note that this set of high frequency polymorphisms merely provided a scaffold map that was suitable for multipoint linkage analysis, and it was not intended to contain the causative variant. The 4,705 variants were then combined with the 374 STR geno-

types into one genetic map. The combined genotype set was tested again for unlikely recombination events (Abecasis et al., 2002), and genotypes flagged as unlikely were removed. The final marker map was used to carry out an updated multipoint linkage analysis which was based on substantially more marker information than that previously used by de Kovel et al. (2004).

Parametric (LOD score) and non-parametric (NPL) multipoint linkage analyses were performed: Simwalk2 (Lange and Lange, 2004) was used for the autosomes, and Minx (Abecasis et al., 2002) for chromosome X. We found that the program Merlin was unable to analyze autosomes in this family due to its size, while Simwalk2 cannot analyze chromosome X. The autosomal parametric linkage analysis used a dominant inheritance model, with penetrances of 5%, 95% and 95% for wild-type, heterozygous and homozygous-mutation carriers respectively. The chromosome X parametric linkage used the same dominant model for females, and penetrances of 5% and 95% for wild-type and hemizygous male mutation carriers.

5.2.6 *Investigation of novel and rare variants*

Imputation

The availability of a scaffold genotype map for most of the family members also enabled the imputation of many rare variants without the need for genotyping them (Wijsman, 2012). All novel and rare variants from WES and WGS (i.e. having less than 1% frequency in the 1000 Genomes database) were subjected to pedigree-based imputation, in order to obtain genotype information on the family members that had not been sequenced. Inheritance vectors were sampled from a Markov chain Monte Carlo analysis of the multilocus marker data using gl_auto (Thompson, 2011) and these were used to impute genotype calls by GIGI (Cheung et al., 2013). The genotype probability threshold to call two alleles was set to 80%, and the threshold to call a single allele to 90%. This resulted in the imputation of 14,027 variants, which were imputed with a mean imputation rate across subjects of 77.3% for exonic variants, and 47.7% for non-exonic variants. When the imputation resulted in haploid calls (i.e. only one allele imputed), we assumed that the unknown allele was the reference allele, since only rare variants were being imputed. This was important since the downstream analysis software to evaluate cosegregation of variants with dyslexia in the family required diploid genotypes. Note that sex chromosomes were excluded from the imputation with GIGI (Cheung et al., 2013), since (to our knowledge) there

is currently no available software to impute genotypes on chromosome X, based on pedigree structure.

Single point linkage analysis

Single-point linkage analysis was conducted for all rare variants (i.e. novel in family 259 or MAF<0.01 in the 1000Genomes database) located within genomic regions with multipoint LOD> 1 or NPL >1, by using Pseudomarker2.0 (Hiekkalinna et al., 2011), whether or not these variants had been included in the map used for multipoint linkage analysis. Pseudomarker2.0 only performs parametric linkage analysis, and we specified a dominant model with the same parameters as used for multipoint analysis (noted above). We also performed a family-based test of allelic association (M_{QLS}) within this family, using the MQLS-XM package (Thornton and McPeek, 2007; Thornton et al., 2012), which does not assume a model. Imputed genotypes were used for these two analyses. When that was not possible (i.e. for variants on chromosome X, or when the imputation rate was zero), we used the available genotypes from the sequence data.

Filtering of variants as potentially causative for the phenotype

Variants were excluded if they were not present in any affected members, or if they were present in both unaffected members who had WGS data. Then, variants were excluded which fell outside genomic regions of interest as specified by the linkage analysis (i.e. LOD or NPL > 1), and restricted to rare variation by filtering out common variants (MAF > 1 % in 1000G and ExAC's European populations). We also filtered out variants that were present in < 60% of the dyslexic individuals, given the imputed genotypes. For the variants that had not been imputed, we filtered out variants that were present in < 60% of the affected individuals with sequence data (i.e. at least 7/13 affected for coding variants, at least 2/3 for noncoding variants). The filtered set of variants was then queried under two different hypotheses: First, we identified exonic variants, excluding those annotated as synonymous variants by both Annovar and VEP. Then, we identified non-coding variants that were predicted to be likely pathogenic , given either of the following annotation summary scores: $GWAVA_{unmatched} > 0.5$ (Ritchie et al., 2014), $CADD_{phred} > 15$ (Kircher et al., 2014). These two scores consider information on potential regulation of expression and evolutionary conservation, and each uses different algorithms (and assumptions) to evaluate the pathogenicity or functional importance of variants. Note that pre-

computed scores are only available for SNVs, so that indels could not be evaluated by these scores. We further assessed the putative functional role of these variants by checking available databases on regulatory regions of the genome (RegulomeDB (Boyle et al., 2012) and HaploReg (Ward and Kellis, 2012)).

Sanger sequencing

Four variants were validated by Sanger sequencing in all the family members (indicated in Table 5.1). The validation rate was 100%, and all genotypes were concordant with the imputed genotype calls (albeit that one of these variants was not imputed, and for the other three variants 83.3% of the alleles had been imputed).

5.3 RESULTS

5.3.1 *Multipoint linkage*

Our multipoint analysis confirmed and refined the previously reported linkage on chromosome Xq27.3 (de Kovel et al., 2004): in our current analysis, the maximum LOD score was 3.19, and NPL score was 1.87 (Figure 5.S3), while the extent of the linked interval (LOD>3) was narrower (chrX:142795438-146312362, hg19) than that reported previously (STRs DXS1227-DXS8091,chrX:140892381-147603133, hg19). We also identified a region of linkage with NPL> 2 on chromosome 20q11.23 (peak NPL=2.51, LOD= 0.91), which had not been previously reported.

Additional linkages with LOD $>$ 1 or NPL $>$ 1 were present on all chromosomes except 16 (see Tables 5.S2 and 5.S1 for the exact definitions of all intervals with multipoint NPL $>$ 1 or LOD $>$1).

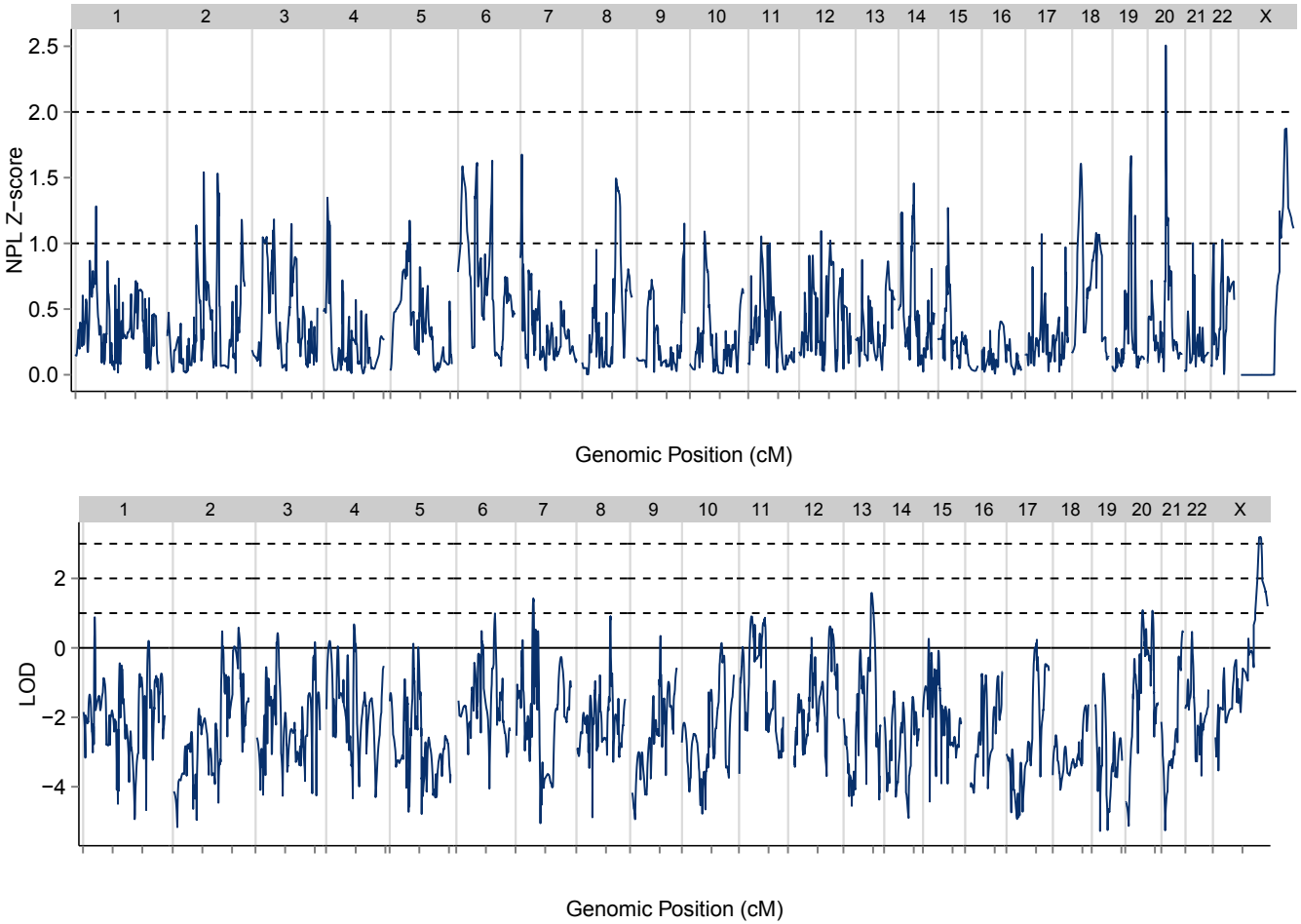


Figure 5.2: Nonparametric and parametric multipoint linkage analysis across the genome.

5.3.2 *Rare or novel variant*

After filtering (see above), there were 8 coding, non-synonymous variants that were present in at least 60% of the affected individuals with available genotype data, as summarized in Table 5.1. These variants fell within the following genes: *COL6A3, EOMES* (also known as *TBR2*), *CACNA2D3, LACE1, PTPRCAP, LAMA1, KIAA1468* and *DLL3.* However, the single point linkage scores for these variants were all below 1, and only the *CACNA2D3* variant had an MQLS pvalue <0.05.

After filtering, there were 105 non-coding variants within putatively linked genomic regions that were likely to be pathogenic or functional based on CADD or GWAVA calls. Among the non-coding variants within the regions of interest on chromosomes 20q and Xq (Table 5.2), there were six variants present in all three dyslexic individuals with WGS data, and in neither of the two controls. One was in the region upstream (49 bp from the 5'UTR) of the *RBMX* gene on Xq26.3. There is evidence that this variant alters transcription factor binding motifs (10 altered motifs), and is located within a region showing a DNase peak signal (53 tissues), as well as promoter histone marks (24 tissues) and evidence of having 31 bound proteins in ChIP-Seq (chromatin-immunoprecipitation-sequencing) experiments (Boyle et al., 2012; Ward and Kellis, 2012). These sources of information suggest that variation at this genomic location could have a regulatory effect on the expression of *RBMX*. This gene encodes an RNA-binding motif X-linked protein, that plays a role in tissue-specific regulation of pre- and post-transcriptional processes and is required for normal embryonic development (Tsend-Ayush et al., 2005). The other five variants were intergenic, with only limited evidence of effects on regulatory activity, although three of them (rs11697073, rs150856499 and rs192873740) are within evolutionarily constrained regions.

Chr	Position	Ref	Alt	cytoBand	$dbSNP_{138}$	Gene	Transcript	AA.change	MAF_{1000G}	MAF_{ExAC}	MQLS	LODsp	ImpRate	Affected	Unaffected	Sanger
2	238245107	G	A	2q37.3	rs150907698	*COL6A3*	NM_057166	p.T2272M	8.0e-04	7.6e-04	0.11	0.00	0.83	0.62	0.33	N
3	27763575	C	T	3p24.1	.	*EOMES*	NM_001278182	p.A71T	-	6.3e-05	0.09	0.31	0.88	0.71	0.14	N
3	54661890	A	G	3p14.3	rs35593475	*CACNA2D3*	NM_018398	p.N347S	2.0e-04	1.2e-04	0.03	0.22	0.78	0.77	0.25	Y
6	108843547	T	G	6q21	rs143107973	*LACE1*	NM_145315	p.F455L	6.0e-04	1.6e-03	0.39	0.28	0.75	0.79	0.33	Y
11	67203326	C	T	11q13.2	.	*PTPRCAP*	NM_005608	p.A167T	-	-	0.05	0.37	0.83	0.69	0.20	N
18	7007187	C	A	18p11.31	rs147345095	*LAMA1*	NM_005559	p.S1404I	-	4.0e-04	0.35	0.00	0.82	0.79	0.60	Y
18	59878477	T	G	18q21.33	rs75449685	*KIAA1468*	-	-	-	5.0e-03	-	-	0.00	0.54	0.00	N
19	39989989	C	A	19q13.2	.	*DLL3*	NM_016941	p.P76Q	-	8.7e-06	0.05	-	0.00	0.69	0.00	Y

Table 5.1: Rare, exonic, nonsynonymous variants present at least in 60% of the affected individuals. Ref: reference allele in hg19. Alt: alternative observed allele. AA.change: aminoacid change. LODsp: single point LOD score. ImpRate: Imputation rate of the variant. Affected, unaffected: frequency of variant carriers of each type in the family. If the ImpRate is not 0, the proportions of carriers are given for the imputed genotypes; otherwise, for the people with available data.

Chr	Position	Ref	Alt	cytoBand	$dbSNP_{138}$	Gene	Func	MAF_{1000G}	CADD	GWAVA	ImpRate	Affected	Unaffected
20	37729593	C	T	20q12	.	*DHX35,LOC339568*	intergenic	8.0e-04	16.48	-	0.32	0.67	0.50
20	37967464	C	G	20q12	rs11697073	*LOC339568,LINC01370*	intergenic	-	25.70	-	0.33	1.00	0.00
20	38583233	A	G	20q12	.	*LOC339568,LINC01370*	intergenic	-	15.67	-	0.37	1.00	0.00
20	40783158	G	T	20q12	.	*PTPRT*	intronic	-	20.40	-	0.32	0.67	0.50
20	42167234	C	T	20q13.12	rs190929877	*L3MBTL1*	intronic	6.0e-04	5.50	0.67	0.30	0.67	0.50
20	43495488	C	G	20q13.12	rs2903761	*RIMS4,YWHAB*	intergenic	-	15.62	-	0.63	1.00	0.67
20	43847789	T	C	20q13.12	rs139383729	*SEMG1,SEMG2*	intergenic	2.0e-04	2.31	0.55	0.52	0.67	0.00
20	44098189	C	T	20q13.12	rs118003431	*WFDC2*	upstream	4.8e-03	1.54	0.97	0.33	0.67	0.50
X	135962973	A	G	Xq26.3	rs187789123	*RBMX*	upstream	-	13.70	0.93	0.00	1.00	0.00
X	139025273	C	T	Xq27.1	rs150856499	*MIR505,CXorf66*	intergenic	7.9e-04	19.13	0.48	0.00	1.00	0.00
X	148102395	T	A	Xq28	rs192873740	*AFF2,IDS*	intergenic	7.9e-04	18.31	-	0.00	1.00	0.00
X	153604201	G	A	Xq28	rs186249683	*FLNA,EMD*	intergenic	2.4e-03	1.07	0.51	0.00	1.00	0.00

Table 5.2: Rare, noncoding variants that were predicted to be pathogenic within linked regions on chromosomes 20 and X. Affected, unaffected: frequency of variant carriers of each type in the family. If the ImpRate is not 0, the proportions of carriers are given for the imputed genotypes; otherwise, for the people with available WES data.

5.3.3 *Structural variant calls*

There were 70 SVs that fell within the genomic regions defined by the multipoint linkage analysis (NPL>1 or LOD>1). Of these, only four deletions cosegregated with dyslexia within the WGS samples (i.e. the three affected members had them, while the two unaffected did not). These four deletions were each only called by one out of the three callers, either by CNVnator or BreakDancer, and were therefore considered as uncertain calls. The potential deletion on chr20:40349033-40349733 falls within an intergenic region, $\sim$ 10Mb upstream of the closest gene (*CHD6*). The potential deletion on chrX:147785400-147786267 falls within the second intron of *AFF2*. The potential deletion on chr13:101663267-101664033 is 766bp long, within the non-coding RNA *NALCN-AS1*. The fourth deletion (chr14:21263100-21265900) is 2.8kb long and intergenic.

5.4 DISCUSSION

A combination of linkage analysis and NGS in families has proven to be a powerful approach to find causative variants for several monogenic diseases, as well as complex traits (Rosenthal et al., 2013; Norton et al., 2013). In the present study we adopted this strategy with the goal of identifying novel or rare variants affecting dyslexia in a multigenerational Dutch family (de Kovel et al., 2004).

By combining STR genotype data with common SNPs from WES, we were able to further characterize the haplotype that underlies a previously reported linkage on chromosome Xq27, as well as identifying other putatively linked genomic regions (including one with peak NPL=2.51 on 20q12). The linkage data provided a valuable filter to rank novel and rare variants as potentially causative for dyslexia in this family (Smith et al., 2011).

We used both parametric and nonparametric multipoint linkage analysis to define genomic regions of interest. The former approach models a particular inheritance pattern in the family (in this case dominant inheritance for the autosomes), while the latter measures an increase in identity-by-descent sharing in affected members without specifying a particular set of model parameters. It is likely that family 259 does not segregate a purely Mendelian form of dyslexia, as indicated by the fact that there is male-to-male transmission that cannot be explained by the X chromosome linkage (from individual II.9 to II.13, Figure 5.1), and that a relative marrying into the

family (individual II.4) was also diagnosed with dyslexia, which could be a source of genetic heterogeneity. Whenever the underlying inheritance model is misspecified, parametric linkage analysis can have less power than non-parametric analysis, and is therefore prone to false negative results for genomic intervals which may nonetheless harbour truly causative variants (Lange and Lange, 2004). Indeed, recent WES studies in families (Einarsdottir et al., 2015; Villanueva et al., 2015) have proposed possible causal variants that did not fall within obviously linked genomic regions (Svensson et al., 2011; Villanueva et al., 2011). However, when the model is specified correctly, then parametric analysis has the greatest power.

Within genomic regions showing a linkage score higher than one (NPL or LOD), there were eight rare coding variants after filtering. However, none of these had significant single-point linkage scores, and only one of them (within the *CACNA2D3* gene) was nominally significant in the MQLS association analysis. Thus, such variants are not likely to be strongly penetrant for dyslexia in family 259. This is not surprising given that our multipoint linkage threshold was quite mild in order to be inclusive (i.e. NPL or LOD$>$1), and that the two most strongly linked loci (on chromosomes 20q and Xq) did not contain any of these particular coding variants.

In spite of the imperfect segregation with dyslexia, it is possible that the coding variants identified could be contributing to the genetic background of the family by increasing the risk in an oligogenic manner. For instance, one of these variants was within the gene *LAMA1*, which lies in the dyslexia susceptibility locus DYX6 on chromosome 18 (Fisher et al., 2002; Scerri et al., 2010; Mueller et al., 2014) and was found to be linked to several reading-related quantitative traits in prior studies (e.g. word reading, phonological decoding and orthographic coding). Indeed, a SNP within *LAMA1* (rs17439829) was significantly associated with orthographic coding and reading ability in a sample of UK families, although this association was not replicated in an independent sample (Scerri et al., 2010). Another of the variants we identified is within the *CACNA2D3* gene, which is a strong candidate gene for autism spectrum disorder (Iossifov et al., 2012). This gene encodes a member of the alpha-2/delta subunit family ($\alpha 2\delta 3$), a protein in the voltage-dependent calcium channel complex. The $\alpha 2\delta 3$ subunit modulates the expression and function of voltage-gated calcium channels, and it has been found to be essential for the normal structure and function of specific classes of synapses in the mammalian auditory pathway, and suggested to be a candidate gene for auditory processing disorders (Pirone et al., 2014). A third nonsynonymous variant was within the gene *EOMES* (also known as

TBR2), which codes for a transcription factor that affects cortical development and has been linked to intellectual disability (Elsen et al., 2013).

Protein-altering DNA variation is the easiest to interpret, because we can often predict with relatively high confidence whether a given mutation will affect protein function. However, several of the genetic variants so far associated with dyslexia have been of a regulatory nature, affecting the expression of proximal genes (Dennis et al., 2009; Hannula-Jouppi et al., 2005). Hence, we also considered noncoding variants based on scores that integrate several types of annotation and provide a measure of potential functionality (CADD, GWAVA) (Kircher et al., 2014; Ritchie et al., 2014). One variant upstream of *RBMX* is in a potential regulatory region that is active in several tissues, including the brain. A 23 bp frameshift deletion within *RBMX* has been found recently in a family with intellectual disability, suggesting this gene as a novel candidate gene for X-linked intellectual disability syndrome (Shashi et al., 2015).

Another class of genetic variation is structural variation, such as the translocations that disrupted the genes *ROBO1* and *DYX1C1* (see Section 5.1) (Taipale et al., 2003; Hannula-Jouppi et al., 2005). We were able to identify several possible structural variants from the WGS data. Only four deletions co-segregated with dyslexia in the subset of samples with this type of data. One of them was a ∼800bp deletion that fell within an intron of *AFF2*, which was one of the candidate genes in Xq27 that had been scrutinized for coding mutations in the previous studies (de Kovel et al., 2004; Huc-Chabrolle et al., 2013). However, these variants were only called by one of the three SV callers that were used, and hence should be independently confirmed. In addition, the possible functional relevance of this type of small CNV in noncoding regions is difficult to predict.

It is possible, although somewhat unlikely, that our sequencing efforts may have missed a highly penetrant exomic variant, because the WES protocol that we used is known to result in less-than-100% coverage for detecting protein-coding DNA variation (Gnirke et al., 2009; SureSelect, 2016). However, our use of WGS on an additional five samples will have greatly reduced the chance that we missed a variant within the linked regions of the genome.

In summary, we used a combination of linkage analysis and NGS to screen for rare genetic variation that might underlie the inheritance of dyslexia in a large multigenerational family. No single variant stood out as a top candidate in this regard, which suggests that the pattern of inheritance in this family is more complex than

Mendelian, but we found several variants that could potentially be contributing to the phenotype. In order to establish a causal relationship between any of the variants found in this study and dyslexia, it would be important to evaluate the functional aspects of the variants in biological models, and/or to find convergent evidence for the relevance of any of these genes to reading ability in independent datasets. It remains possible that other large, extended families with elevated rates of dyslexia may be affected by rare, or unique, but relatively penetrant mutations. Therefore combined linkage and NGS analysis continues to hold the potential to identify key genes involved in the molecular underpinnings of dyslexia.

References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nature Genetics 30: 97–101.

Abyzov A, Urban AE, Snyder M, Gerstein M (2011) CNVnator: an approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. Genome Res 21: 974–984.

Andrews W, Liapi A, Plachez C, Camurri L, Zhang J, et al. (2006) Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain. Development 133: 2243–2252.

Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, et al. (2011) Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 12: 745–755.

Bates TC, Lind PA, Luciano M, Montgomery GW, Martin NG, et al. (2010) Dyslexia and DYX1C1: deficits in reading and spelling associated with a missense mutation. Mol Psychiatry 15: 1190–1196.

Bates TC, Luciano M, Medland SE, Montgomery GW, Wright MJ, et al. (2011) Genetic variance in a component of the language acquisition device: ROBO1 polymorphisms associated with phonological buffer deficits. Behav Genet 41: 50–57.

Belkadi A, Bolze A, Itan Y, Cobat A, Vincent QB, et al. (2015) Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proc Natl Acad Sci USA 112: 5473–5478.

Bishop DV (2015) The interface between genetics and psychology: lessons from developmental dyslexia. Proc Biol Sci 282.

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, et al. (2012) Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22: 1790–1797.

Chen K, Wallis JW, McLellan MD, Larson DE, Kalicki JM, et al. (2009) BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. Nat Methods 6: 677–681.

Cheung CY, Thompson EA, Wijsman EM (2013) GIGI: an approach to effective imputation of dense genotypes on large pedigrees. Am J Hum Genet 92: 504–516.

Cope N, Harold D, Hill G, Moskvina V, Stevenson J, et al. (2005) Strong evidence that KIAA0319 on chromosome 6p is a susceptibility gene for developmental dyslexia. Am J Hum Genet 76: 581–591.

Currier TA, Etchegaray MA, Haight JL, Galaburda AM, Rosen GD (2011) The effects of embryonic knockdown of the candidate dyslexia susceptibility gene homologue Dyx1c1 on the distribution of GABAergic neurons in the cerebral cortex. Neuroscience 172: 535–546.

Dahdouh F, Anthoni H, Tapia-Paez I, Peyrard-Janvid M, Schulte-Korne G, et al. (2009) Further evidence for DYX1C1 as a susceptibility factor for dyslexia. Psychiatr Genet 19: 59–63.

Darki F, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T (2012) Three Dyslexia Susceptibility Genes, DYX1C1, DCDC2, and KIAA0319, Affect Temporo-Parietal White Matter Structure. Biol Psychiatry 72: 671–676.

de Kovel CG, Hol FA, Heister JG, Willemen JJ, Sandkuijl LA, et al. (2004) Genome-wide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. J Med Genet 41: 652–657.

Dennis MY, Paracchini S, Scerri TS, Prokunina-Olsson L, Knight JC, et al. (2009) A common variant associated with dyslexia reduces expression of the KIAA0319 gene. PLoS Genet 5: e1000436.

DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, et al. (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43: 491–498.

Einarsdottir E, Svensson I, Darki F, Peyrard-Janvid M, Lindvall JM, et al. (2015) Mutation in CEP63 co-segregating with developmental dyslexia in a Swedish family. Hum Genet 134: 1239–1248.

Elsen GE, Hodge RD, Bedogni F, Daza RA, Nelson BR, et al. (2013) The protomap is propagated to cortical plate neurons through an Eomes-dependent intermediate map. Proc Natl Acad Sci USA 110: 4081–4086.

Fagerheim T, Raeymaekers P, T?nnessen FE, Pedersen M, Tranebjaerg L, et al. (1999) A new gene (DYX3) for dyslexia is located on chromosome 2. J Med Genet 36: 664–669.

Fisher SE, Francks C, Marlow AJ, MacPhie IL, Newbury DF, et al. (2002) Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. Nat Genet 30: 86–91.

Francks C, Paracchini S, Smith SD, Richardson AJ, Scerri TS, et al. (2004) A 77-kilobase region of chromosome 6p22.2 is associated with dyslexia in families from the United Kingdom and from the United States. Am J Hum Genet 75: 1046–1058.

Franke B, Vasquez AA, Veltman JA, Brunner HG, Rijpkema M, et al. (2010) Genetic variation in CACNA1C, a gene associated with bipolar disorder, influences brainstem rather than gray matter volume in healthy individuals. Biol Psychiatry 68: 586–588.

Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, et al. (2014) Genome sequencing identifies major causes of severe intellectual disability. Nature 511: 344–347.

Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, et al. (2009) Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 27: 182–189.

Guadalupe T, Zwiers MP, Teumer A, Wittfeld K, Vasquez AA, et al. (2014) Measurement and genetics of human subcortical and hippocampal asymmetries in large datasets. Hum Brain Mapp 35: 3277–3289.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Hiekkalinna T, Schaffer AA, Lambert B, Norrgrann P, Goring HH, et al. (2011) PSEUDOMARKER: a powerful program for joint linkage and/or linkage disequilibrium analysis on mixtures of singletons and related individuals. Hum Hered 71: 256–266.

Hoischen A, van Bon BW, Gilissen C, Arts P, van Lier B, et al. (2010) De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. Nat Genet 42: 483–485.

Huc-Chabrolle M, Charon C, Guilmatre A, Vourc'h P, Tripi G, et al. (2013) Xq27 FRAXA Locus is a Strong Candidate for Dyslexia: Evidence from a Genome-Wide Scan in French Families. Behav Genet 43: 132–140.

Illumina (2016a) HiSeq2000. URL http://support.illumina.com/sequencing/sequencing_instruments/hiseq_2000.html.

Illumina (2016b) Illumina X-ten. URL http://www.illumina.com/systems/hiseq-x-sequencing-system.html.

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, et al. (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74: 285–299.

Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, et al. (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46: 310–315.

Lamminmaki S, Massinen S, Nopola-Hemmi J, Kere J, Hari R (2012) Human ROBO1 regulates interaural interaction in auditory pathways. J Neurosci 32: 966–971.

Lange EM, Lange K (2004) Powerful allele sharing statistics for nonparametric linkage analysis. Hum Hered 57: 49–58.

Layer RM, Chiang C, Quinlan AR, Hall IM (2014) LUMPY: a probabilistic framework for structural variant discovery. Genome Biol 15: R84.

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078–2079.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297–1303.

McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, et al. (2010) Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics 26: 2069–2070.

Meng H, Smith SD, Hager K, Held M, Liu J, et al. (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci USA 102: 17053–17058.

Mohiyuddin M, Mu JC, Li J, Bani Asadi N, Gerstein MB, et al. (2015) MetaSV: an accurate and integrative structural-variant caller for next generation sequencing. Bioinformatics 31: 2741–2744.

Mueller B, Ahnert P, Burkhardt J, Brauer J, Czepezauer I, et al. (2014) Genetic risk variants for dyslexia on chromosome 18 in a German cohort. Genes Brain Behav 13: 350–356.

Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, et al. (2001) A dominant gene for developmental dyslexia on chromosome 3. J Med Genet 38: 658–664.

Norton N, Li D, Rampersaud E, Morales A, Martin ER, et al. (2013) Exome sequencing and genome-wide linkage analysis in 17 families illustrate the complex contribution of TTN truncating variants to dilated cardiomyopathy. Circ Cardiovasc Genet 6: 144–153.

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63: 259–266.

Paracchini S, Steer CD, Buckingham LL, Morris AP, Ring S, et al. (2008) Association of the KIAA0319 dyslexia susceptibility gene with reading skills in the general population. Am J Psychiatry 165: 1576–1584.

Picard (2016) Picard. URL https://broadinstitute.github.io/picard/.

Pirone A, Kurt S, Zuccotti A, Ruttiger L, Pilz P, et al. (2014) $\alpha 2\delta 3$ is essential for normal structure and function of auditory nerve synapses and is a novel candidate for auditory processing disorders is essential for normal structure and function of auditory nerve synapses and is a novel candidate for auditory processing disorders. J Neurosci 34: 434–445.

Platforms B (2015) BC Platforms. URL http://bcplatforms.com/.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.

Ritchie GR, Dunham I, Zeggini E, Flicek P (2014) Functional annotation of noncoding sequence variants. Nat Methods 11: 294–296.

Rosenthal EA, Ranchalis J, Crosslin DR, Burt A, Brunzell JD, et al. (2013) Joint linkage and association analysis with exome sequence data implicates SLC25A40 in hypertriglyceridemia. Am J Hum Genet 93: 1035–1045.

Scerri TS, Morris AP, Buckingham LL, Newbury DF, Miller LL, et al. (2011) DCDC2, KIAA0319 and CMIP are associated with reading-related traits. Biol Psychiatry 70: 237–245.

Scerri TS, Paracchini S, Morris A, MacPhie IL, Talcott J, et al. (2010) Identification of candidate genes for dyslexia susceptibility on chromosome 18. PLoS ONE 5: e13712.

Shashi V, Xie P, Schoch K, Goldstein DB, Howard TD, et al. (2015) The RBMX gene as a candidate for the Shashi X-linked intellectual disability syndrome. Clin Genet 88: 386–390.

Shaywitz SE, Escobar MD, Shaywitz BA, Fletcher JM, Makuch R (1992) Evidence that dyslexia may represent the lower tail of a normal distribution of reading ability. N Engl J Med 326: 145–150.

Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, et al. (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29: 308–311.

Smith KR, Bromhead CJ, Hildebrand MS, Shearer AE, Lockhart PJ, et al. (2011) Reducing the exome search space for mendelian diseases using genetic linkage analysis of exome genotypes. Genome Biol 12: R85.

SureSelect (2016) SureSelect Performance. URL http://www.genomics.agilent.com/article.jsp?pageId=3051.

Svensson I, Nilsson S, Wahlstrom J, Jernas M, Carlsson LM, et al. (2011) Familial dyslexia in a large Swedish family: a whole genome linkage scan. Behav Genet 41: 43–49.

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, et al. (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci USA 100: 11553–11558.

Tammimies K, Vitezic M, Matsson H, Le Guyader S, Burglin TR, et al. (2013) Molecular Networks of DYX1C1 Gene Show Connection to Neuronal Migration Genes and Cytoskeletal Proteins. Biol Psychiatry 73: 583–590.

Tan A, Abecasis GR, Kang HM (2015) Unified representation of genetic variants. Bioinformatics 31: 2202–2204.

Thiele H, Nurnberg P (2005) HaploPainter: a tool for drawing pedigrees with complex haplotypes. Bioinformatics 21: 1730–1732.

Thompson E (2011) The structure of genetic linkage data: from LIPED to 1M SNPs. Hum Hered 71: 86–96.

Thornton T, McPeek MS (2007) Case-control association testing with related individuals: a more powerful quasi-likelihood score test. Am J Hum Genet 81: 321–337.

Thornton T, Zhang Q, Cai X, Ober C, McPeek MS (2012) XM: association testing on the X-chromosome in case-control samples with related individuals. Genet Epidemiol 36: 438–450.

Tsend-Ayush E, O'Sullivan LA, Grutzner FS, Onnebo SM, Lewis RS, et al. (2005) RBMX gene is essential for brain development in zebrafish. Dev Dyn 234: 682–688.

van der Leij A, Maassen B (2013) Dutch Dyslexia Programme. Dyslexia 19: 189–190.

Villanueva P, Newbury DF, Jara L, De Barbieri Z, Mirza G, et al. (2011) Genome-wide analysis of genetic susceptibility to language impairment in an isolated Chilean population. Eur J Hum Genet 19: 687–695.

Villanueva P, Nudel R, Hoischen A, Fernández MA, Simpson NH, et al. (2015) Exome Sequencing in an Admixed Isolated Population Indicates ¡italic¿NFXL1¡/italic¿ Variants Confer a Risk for Specific Language Impairment. PLoS Genet 11: e1004925, URL http://dx.doi.org/10.1371%2Fjournal.pgen.1004925.

Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38: e164.

Ward LD, Kellis M (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40: D930–934.

Wijsman EM (2012) The role of large pedigrees in an era of high-throughput sequencing. Hum Genet 131: 1555–1563.

Yao W (2016) intansv: Integrative analysis of structural variations. R package version 1.6.2.

Supplementary information

Exome coverage: WES vs WGS

Two of the affected samples in family 259 were sequenced twice, once by WES and once by WGS. We compared the two sequencing strategies by comparing the average and cumulative read depth for the exome, as defined in two different ways: 1) by the exome capture that was used for the WES (Agilent 51M), and 2) by the definition of the exonic regions from the UCSC genome browser (hg19).

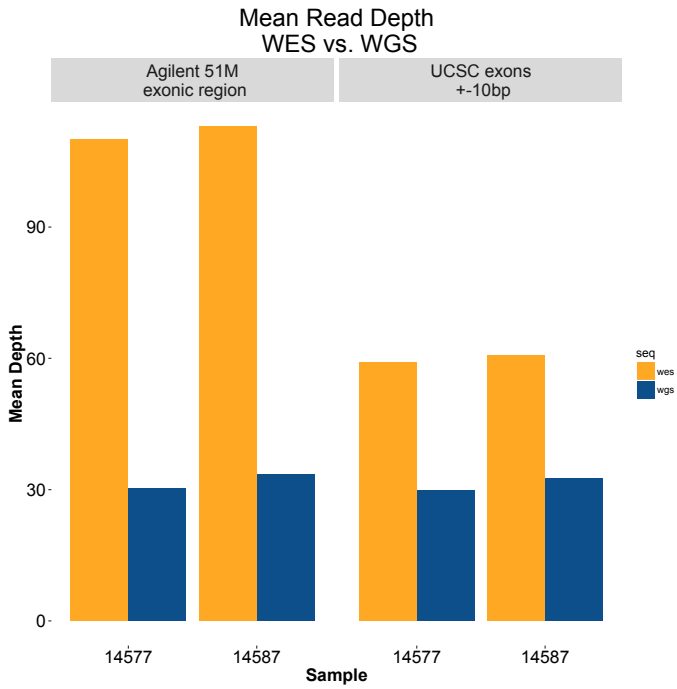


Figure 5.S1: Mean read depth comparison for the two samples that were used for WGS and WES. Two definitions of exonic regions were considered: 1) exonic regions targeted by the WES capture protocol (Agilent 51M) and 2) exons defined according to UCSC gene definitions.

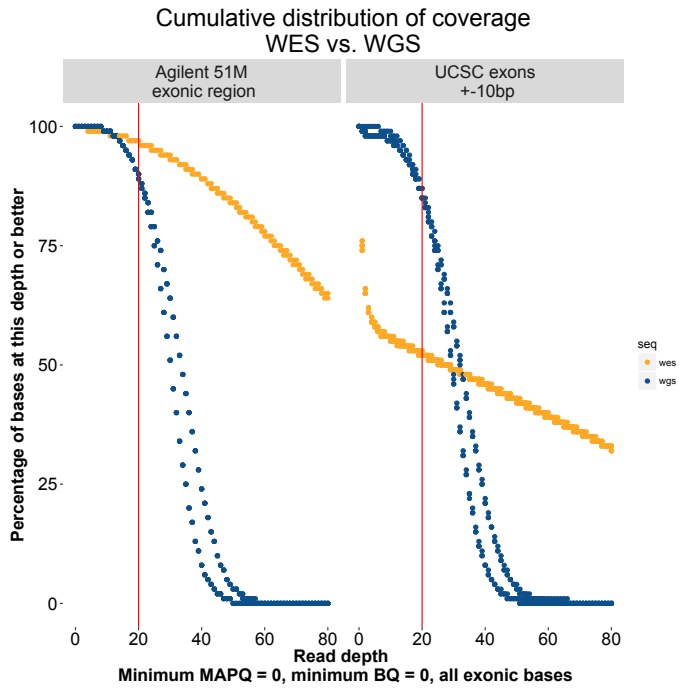


Figure 5.S2: Cumulative distribution of coverage for the two samples that were used for WGS and WES. Two definitions of exonic regions were considered: 1) exonic regions targeted by the WES capture protocol (Agilent 51M) and 2) exons defined according to UCSC gene definitions. MAPQ: mapping quality. BQ: base quality.

Multipoint linkage analysis, genomic regions of interest

Interval	Chr	Start	End	LOD_{max}	Software
1	7	37298800	40899967	1.42	Simwalk2
2	13	98829217	106119446	1.58	Simwalk2
3	20	34502107	36758778	1.09	Simwalk2
4	20	54941124	55072472	1.07	Simwalk2
5	X	138633280	152823728	3.19	Merlin

Table 5.S1: Regions of interest as defined by LOD > 1 using parametric linkage analysis. Coordinates are given for the genome reference hg19. LOD_{max} indicates the maximum LOD score within each interval.

Interval	Chr	Start	End	NPL_{max}	Software
1	1	44401384	47078637	1.28	Simwalk2
2	2	75113657	79254261	1.13	Simwalk2
3	2	111907691	116447277	1.54	Simwalk2
4	2	163128824	171070912	1.53	Simwalk2
5	2	237374309	240066417	1.38	Simwalk2
6	3	16640075	28381887	1.06	Simwalk2
7	3	52188361	53213657	1.09	Simwalk2
8	3	53700550	56627048	1.18	Simwalk2
9	3	126137558	128755814	1.14	Simwalk2
10	4	5838513	7435194	1.35	Simwalk2
11	4	7655855	9785134	1.17	Simwalk2
12	5	33998883	35644621	1.00	Simwalk2
13	5	38884071	44809162	1.17	Simwalk2
14	6	2955802	16145325	1.59	Simwalk2
15	6	36922684	43112267	1.61	Simwalk2
16	6	102503317	111283592	1.63	Simwalk2
17	7	2257612	5257573	1.67	Simwalk2
18	8	104153137	125498547	1.49	Simwalk2
19	9	138377853	139397707	1.15	Simwalk2
20	10	25701341	29581440	1.09	Simwalk2
21	11	25100229	27362359	1.05	Simwalk2
22	11	66626234	68030015	1.00	Simwalk2
23	12	58162739	60169265	1.09	Simwalk2
24	12	92539344	93258665	1.02	Simwalk2
25	14	21215728	21623648	1.24	Simwalk2
26	14	22687415	23549319	1.11	Simwalk2
27	14	51716188	57948380	1.46	Simwalk2
28	15	35273620	38794566	1.27	Simwalk2
29	17	27835138	28511978	1.07	Simwalk2
30	18	6046717	11492931	1.61	Simwalk2
31	18	52258548	55143766	1.08	Simwalk2
32	18	56940307	63489378	1.07	Simwalk2
33	19	37677748	43519362	1.67	Simwalk2
34	19	52825235	53270460	1.21	Simwalk2
35	20	37383640	44238741	2.51	Simwalk2
36	21	31692377	32201822	1.00	Simwalk2
37	22	34022284	35660875	1.02	Simwalk2
38	X	134713855	151869765	1.87	Merlin

Table 5.S2: Regions of interest as defined by NPL > 1. Coordinates are given for the genome reference hg19. NPL_{max} indicates the maximum NPL score within each interval.

Haplotype analysis of chromosome Xq

Haplotypes were generated for chromosome X. Haplotypes were created in the haplotype analysis tool Minx (Abecasis et al., 2002) and visualized using Haplopainter (Thiele and Nurnberg, 2005).

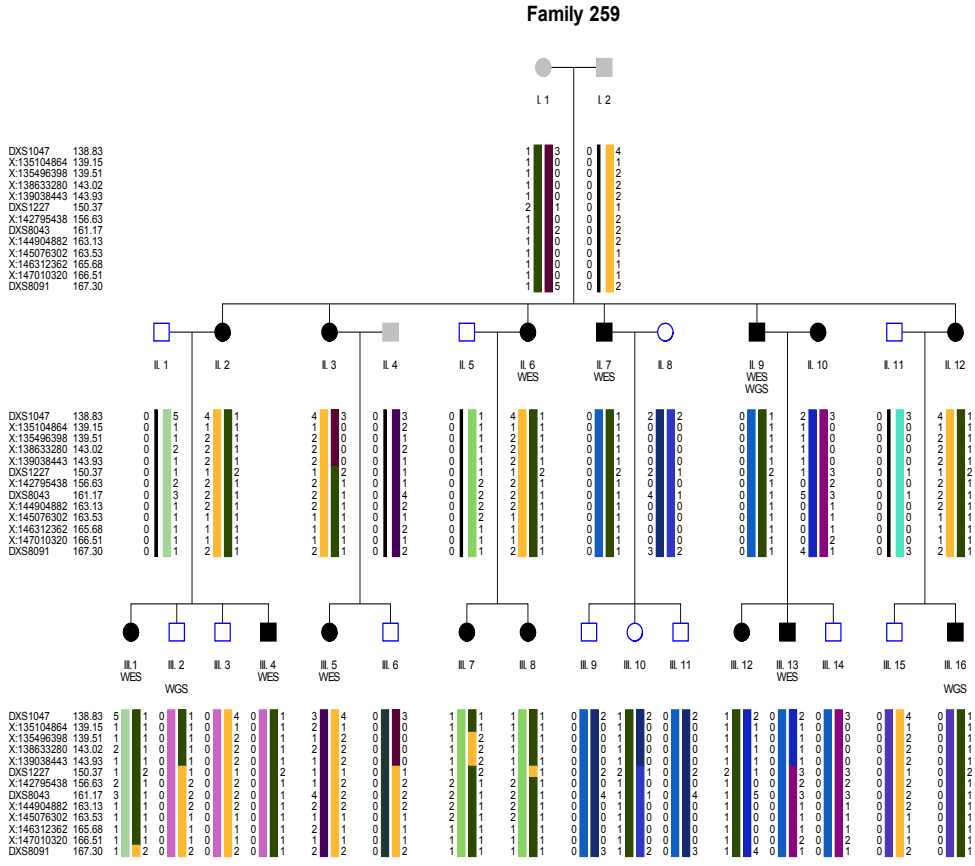


Figure 5.S3: Haplotype visualization of the linkage peak on chomosome Xq (DXS1047 to DXS8091). The risk haplotype is coloured in dark green.

6

NONCODING MUTATIONS IN *SEMA3C* CO-SEGREGATE WITH DEVELOPMENTAL DYSLEXIA IN A DUTCH FAMILY

Dyslexia is a common developmental disorder. It has high heritability estimates, and its genetic underpinnings are thought to be complex, involving common and rare genetic variation. Multigenerational families segregating apparent monogenic forms of language-related disorders can provide useful entrypoints into biological pathways. In the present study, we performed a genome-wide linkage scan in a previously unpublished extended family in which dyslexia seems to be transmitted with an autosomal dominant pattern of inheritance. We identified a locus on chromosome 7q21.11 showing evidence of linkage to the disorder (LOD=2.82). Whole genome sequencing of key individuals enabled the assessment of coding, noncoding and structural variation in the family. Two rare intronic SNPs within the *SEMA3C* gene, which were predicted to have functional effects, cosegregated with dyslexia and the risk haplotype on 7q21.11. *SEMA3C* encodes a secreted protein that acts as a guidance cue in several processes, including cortical neuronal migration and cellular polarization.

Keywords: mapping complex traits, family linkage analysis, whole genome sequencing, dyslexia

6.1 INTRODUCTION

Dyslexia is a prevalent human neurodevelopmental disability, characterized by a difficulty learning to read despite conventional instruction, adequate educational opportunities and IQ, and a lack of sensory impairments (Shaywitz et al., 1992). It shows familial clustering and is moderately heritable, with heritability estimates that range from 0.3 to 0.8 (Peterson and Pennington, 2015). Studies thus far indicate a complex multifactorial aetiology involving genetic and environmental factors (Bishop, 2015).

Multiple genetic variants are likely to act as risk factors contributing to the liability to dyslexia. Until recent years, much of the research on the molecular basis of dyslexia focused on a handful of candidate genes (e.g. *ROBO1, KIAA0319, DCDC2*, and *DYX1C1*) that were identified through linkage analysis in families, and then followed up via fine-mapping of association with common variants within those genes (see Carrion-Castillo et al. (2013) for a review). In the last 2-3 years, genome wide association scan (GWAS) studies have tried to identify other common genetic variants affecting reading ability by querying the whole genome for association in a relatively unbiased manner (Luciano et al., 2013; Field et al., 2013; Gialluisi et al., 2014; Eicher et al., 2013). These GWAS efforts have yielded some promising new candidate genes, but so far no association reached genome-wide statistical significance. The "common-disease common variant" hypothesis assumes that multiple, relatively frequent variants in the population, each with a small effect, account for most of the genetic susceptibility to common disorders (Schork et al., 2009), and this has been the main focus of the field. Nevertheless, there are some unusual extended families in which dyslexia is more prevalent than in the general population, and in which it may follow a roughly Mendelian inheritance pattern (Fagerheim et al., 1999; Nopola-Hemmi et al., 2001; de Kovel et al., 2004). It is possible that one, or a few, genetic variants with substantial penetrances could explain dyslexia within these families, as was the case for a translocation that disrupted the gene *DYX1C1* and co-segregated with dyslexia in one family (Taipale et al., 2003).

Next generation sequencing (NGS) has recently revolutionized the genetic analysis of human disorders by enabling the systematic and rapid screen of common and rare mutations in the whole genome or whole exome (i.e. the protein-coding regions of the genome). This has led to the discovery of several disease-causing genes underlying previously unsolved Mendelian disorders (Bamshad et al., 2011), such as *SETBP1* for Schinzel-Giedion syndrome (Hoischen et al., 2010), and *TGM6*

for spinocerebellar ataxia (Wang et al., 2010). Moreover, this technology has also been used to explore the extent to which rare alleles can explain the heritability of complex diseases and health-related traits. For instance, through family-based approaches for detecting *de novo* mutations, NGS has identified new genes involved in the genetic architecture of complex and heterogenic diseases such as autism (Iossifov et al., 2014) and intellectual disability (Gilissen et al., 2014).

The use of NGS technology enables the inspection of almost the entire genome or exome, while unusual families with recurrent dyslexia provide the possibility to rank variants as putatively causal for the trait, according to how well they cosegregate with the phenotype and their likely functional consequences at the molecular level. For example, a recent study that performed whole exome sequencing (WES) in an extended Swedish family found that a two-base mutation (chr3:123264558-9, hg19), which resulted in an amino acid change (p.R229L) within the *CEP63* gene, co-segregated with dyslexia: six out of eight of the dyslexic people available for genotyping were carriers of the risk variant (Einarsdottir et al., 2015).

In the current study, we have adopted a similar strategy to that in Chapter 5, i.e. combining linkage analysis and whole genome sequencing (WGS). Evidence from linkage analysis was used to reduce the number of putatively causal variants arising from WGS, with the aim of identifying rare variants with high penetrance in this family. We focused on three types of genetic variation that could potentially contribute to explain the phenotype in this family: coding, noncoding, and structural. Identification of such genetic variants promises to yield new clues on genes and pathways that are important for the development of normal reading abilities, while common variants in the same genes may also be relevant for more typical forms of dyslexia.

6.2 MATERIAL AND METHODS

6.2.1 *Sample*

We studied a three generation family of thirty members (referred to hereafter as family 352, Figure 6.1), with a potentially rare Mendelian form of dyslexia. The inheritance pattern was consistent with autosomal dominant transmission, with all three generations presenting dyslexic people, roughly half of the family members affected (n=14), similar numbers of affected males (n=8) and females (n=6), and three instances of male-to-male transmission. This family, which has not been previously

described in the literature, was recruited as part of a multidisciplinary research effort into different aspects of dyslexia (the Dutch Dyslexia Programme) (van der Leij and Maassen, 2013), in which families were ascertained when at least two first degree relatives had a school history of reading problems (de Kovel et al., 2004; van der Leij and Maassen, 2013). Informed consent was obtained from all participants and the study was approved by the ethics committee (CWOM) of the University Medical Centre Nijmegen under CWOM-nr 9811-025.

6.2.2 *Phenotypic measures and diagnostic criteria*

The subjects were administered a battery of tests in single sessions, as briefly described below.

Word and nonword reading fluency

Word reading fluency was assessed using the One-Minute-Test (in Dutch, Een-Minuut-Test or EMT), (Brus and Voeten, 1972), while nonword reading fluency was assessed using the Klepel test (van den Bos et al., 1994). Participants were asked to correctly read as many items as possible within one minute (word reading) or two minutes (nonword reading).

Verbal competence

Verbal competence was assessed as part of the Dutch version of the Wechsler Adult Intelligence Test (Uterwijk, 2000), which tests the ability of the subject to express him/herself verbally. The subject is offered two words and is asked to describe as concisely as possible the similarities between them. Examples (in English) are car-aeroplane or courage-cowardice.

Diagnostic criteria

Dyslexia was defined following the criteria of the Dutch Dyslexia Programme, which is based on several reading-related quantitative measures (van der Leij and Maassen, 2013). People were defined as affected if they 1) performed below the 10th percentile on a word reading test, or 2) scored below the 10th percentile on a nonword reading test, or 3) scored below the 25th normative percentile on both word and nonword reading tests, or 4) had a word or nonword reading score that was $>$60 percentage

points below their normalized score on a verbal competence test (discrepancy criterion). Following these criteria, 14 out of 29 tested family members were identified as dyslexic.

6.2.3 *Genotyping*

Twenty-six samples for which DNA was available, including 13 dyslexics, 11 non-dyslexics and two people with unknown phenotype from family 352 (i.e. all except I.2, II.7, III.9 and III.11 in Figure 6.1) were genotyped using the Illumina Infinium OmniExpressExome-8 BeadChip (Illumina, 2016a) by the genomics service company 'Eurofins' (Germany).

6.2.4 *Genomewide linkage analysis*

A total number of 7,338 SNPs, with a minimum distance between SNPs of 0.25 centimorgans (cM) and an average heterozygosity of 49.7%, were selected for multipoint linkage analysis using LinkDataGen (Bahlo and Bromhead, 2009), which also filtered out Mendelian inheritance errors and removed non-polymorphic SNPs within the family. Given the presence of three male-to-male transmissions in the family, which are not compatible with a X-linked inheritance pattern, chromosome X was not analyzed.

Multipoint parametric linkage analysis was performed using a dominant inheritance model, assuming a disorder allele frequency of 1% and penetrances of 5%, 99% and 99% for wild-type, heterozygous and homozygous-mutation carriers respectively. We also performed an exploratory follow-up analaysis under a less penetrant dominant model (with penetrances of 5%, 90% and 90%).

Given the uncertainty in defining a parametric model for this trait *a priori,* we also conducted nonparametric (NPL) multipoint linkage analysis, which does not rely on assumptions regarding monogenic inheritance, estimates of penetrance levels and phenocopy rates (Fisher and DeFries, 2002). The NPL tests only for an increase in Identity-by-Descent (IBD) allele sharing in affected individuals, without specifying a parametric model.

Both multipoint linkage analyses were performed using the Morgan (v3.2) programs lm_bayes (parametric) and lm_ibdtests (nonparametric) (Thompson, 2011), with 30000 Monte Carlo iterations for the autosomes.

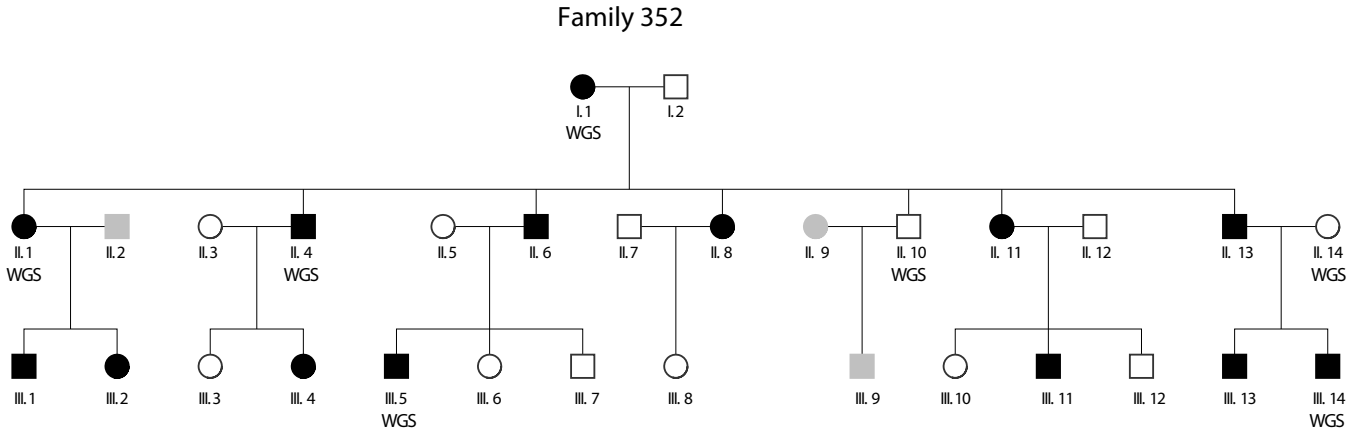


Figure 6.1: Pedigree of family 352. Black symbols represent individuals diagnosed with dyslexia, white symbols represent non-dyslexic individuals, and grey symbols are individuals for which the phenotype was not known. WGS: whole genome sequenced as part of this study.

Estimation of Genome-Wide Significant Values

Genome-wide significant linkage scores were calculated by simulations, using the SNPs that were included in the real linkage analysis. Permutations were performed using gene-dropping simulations, as implemented in Morgan. 1000 replicates were simulated and each replicate was analyzed with the same parametric model as the real data using lm_bayes. The significance for each LOD score was assessed by: 1) counting the number of replicates (n) in which the maximum LOD score exceeded the highest observed LOD score and 2) calculating the p value as $(n+1)/1001$. The threshold for genome-wide significant linkage was taken to be the highest LOD score of the 1000 replicates.

Haplotype analysis

Haplotypes were generated for the genomic regions that showed multipoint LOD $>$ 1. Haplotypes were created in the haplotype analysis tool simwalk2snp (Lange and Lange, 2004) and visualized using Haplopainter (Thiele and Nurnberg, 2005).

6.2.5 *Whole Genome Sequencing*

Genomic DNA samples collected from 7 members (5 affected and 2 unaffected, see Figure 6.1) of family 352 were used for whole genome sequencing by the genomics research organization and service company 'Novogene' (Hong Kong) using Illumina's HiSeq Xten technology (Illumina, 2016b). The sample selection was motivated by the individual's quantitative trait scores (i.e. taking those most severely affected or obviously unaffected), and it was constrained by cost. Sequencing was at 30 times average coverage depth with library insert size 2x 150 base pairs (paired-end).

6.2.6 *WGS data processing*

The sequencing and data preparation was done in the same batch and following the same pipeline as the samples in family 259 (Chaper 5). The pipeline proceeded as follows.

Alignment and pre-processing

Raw reads were cleaned by excluding adapter sequences, reads with low-quality bases for more than 50% of their lengths, and reads with unknown bases for more than 10% of their lengths. Clean reads comprised 97% of total reads, and were mapped onto the human reference genome (hg19) using the software Burrows-Wheeler Aligner (BWA) (Li and Durbin, 2009). Bam files were sorted using SAMtools (Li et al., 2009) and PCR duplicate reads were marked using Picard (Picard, 2016). The mean sequence read length was 150 base pairs, and approximately 97.5% of the genome was covered by at least a 10 times sequence read depth.

Re-alignment around indels (insertion/deletions) and base quality control recalibration was performed using the Genome analysis toolkit software (GATK v3.4) (McKenna et al., 2010; DePristo et al., 2011).

Variant calling and annotation

Since more accurate variant calls can be achieved by including data from larger numbers of subjects simultaneously, we ran this process by pooling our data from family 352 together with 54 additional samples from different projects that had been sequenced with the identical protocols and in the same batches.

Genetic variants were called using the HaplotypeCaller (HC) tool of GATK (McKenna et al., 2010; DePristo et al., 2011).. HC was run separately per sample using the '-ERC GVCF' mode, and then merged together using the GenotypeGVCFs tool, as recommended by the GATK best practices (GATK v3.4). We performed Variant Quality Score Recalibration (VQSR) to exclude the low quality variants (phred-scaled Qscore < 30) and to flag the rest into the sensitivity tier they fell into (90, 99, 99.9 and 100). The variant calling of SNPs and indels identified on average 4,523,372 per sample (range: 4,455,342-4,581,631), for a total of 14,980,001 different variants. These variants were then annotated using Annovar (Wang et al., 2010) and Variant Effect Predictor (VEP v37) (McLaren et al., 2010).

6.2.7 *Investigation of novel and rare variants*

Imputation

In order to obtain genotype information on the family members that had not been sequenced, all novel and rare variants from WGS (i.e. having less than 1% frequency

in the 1000 Genomes database) were subjected to pedigree-based imputation. Inheritance vectors were sampled from a Markov chain Monte Carlo analysis of the multilocus marker data using gl_auto (Thompson, 2011) and these were used to impute genotype calls by GIGI (Cheung et al., 2013). The genotype probability threshold to call two alleles was set to 80%, and the threshold to call a single allele to 90%. This resulted in the imputation of 306,597 variants, with a mean imputation rate of 51.58%. When the imputation resulted in haploid calls (i.e. only one allele imputed), we assumed that the unknown allele was the reference allele, since only rare variants were being imputed (the downstream analysis software to evaluate cosegregation of variants with dyslexia in the family required diploid genotypes). Sex chromosomes were excluded from the imputation with GIGI (Cheung et al., 2013), since (to our knowledge) there is currently no available software to impute genotypes on chromosome X, based on pedigree structure.

Single-point linkage analysis

Single-point linkage analysis was conducted for all rare variants (i.e. novel in family 352 or MAF$<$0.01 in the 1000Genomes database) located within genomic regions with multipoint LOD$>$ 1 or NPL $>$1, by using Pseudomarker2.0 (Hiekkalinna et al., 2011), regardless of whether they had been included in the marker map used for multipoint linkage analysis. We specified the same dominant model as used for the multipoint analysis (above). We also performed a family-based test of allelic association (M) within this family, using the MQLS-XM package (Thornton and McPeek, 2007; Thornton et al., 2012) which does not assume a model. Imputed genotypes were used for these two analyses. When that was not possible (i.e. when the imputation rate was zero), we used the available genotypes from the sequence data.

Filtering of variants as potentially causative for the phenotype

Variants were excluded if they were not present in any affected members, or if they were present in both unaffected members who had WGS data. Then, variants were excluded which fell outside genomic regions of interest as specified by the linkage analysis (i.e. LOD or NPL $>$ 1). These relatively low thresholds were chosen in order not to exclude potentially causative variants which might have shown imperfect co-segregation with dyslexia in the family. We then excluded common variants by filtering out any with reported MAF $>$ 1 % in 1000G and ExAC's European populations. Subsequently, we retained only those variants that were present in more than

60% of the dyslexic individuals. When available, imputed genotypes were used for this filtering step. For the variants that had not been imputed, we included variants that were present in more than 60% of the affected individuals with sequence data (i.e. at least 3/5 affected). The filtered set of variants was then queried under two different hypotheses:

First, we identified exonic variants, excluding those annotated as synonymous variants by both Annovar and VEP. Then, we identified non-coding variants that were predicted to be likely pathogenic, given either of the following annotation summary scores: GWAVA > 0.5 (Ritchie et al., 2014), CADD > 15 (Kircher et al., 2014). These two scores consider information on potential regulation of expression and evolutionary conservation, and each use different algorithms (and assumptions) to evaluate the pathogenicity or functional importance of variants. Note that precomputed scores are only available for single nucleotide variants (SNVs) and some indels, but not all indels could not be evaluated by these scores. Unscored variants (786/3182) for which neither CADD nor GWAVA metrics were available were filtered out. We further assessed the putative functional role of these variants by checking available databases on regulatory regions of the genome (RegulomeDB (Boyle et al., 2012) and HaploReg (Ward and Kellis, 2012)).

6.2.8 *Structural variant (SV) calling*

Several signals in WGS data (e.g. read-pairs, split-reads, and read-depth) can indicate the presence of SVs (Mohiyuddin et al., 2015). Genotyping intensity data from SNP arays can also be used to call copy number variants (CNVs). Hence, different types of SVs were called using the available data.

WGS

WGS data were used to call SVs, including deletions, duplications and inversions. SVs were called using three variant calling programs: CNVnator (Abyzov et al., 2011), BreakDancer (Chen et al., 2009) and Lumpy (Layer et al., 2014). Overlapping calls from the three SV detectors were then combined within samples using MetaSV (Mohiyuddin et al., 2015), considering as high-confidence those SVs detected by at least two of the tools. The seven WGS samples had on average 311,500 SV calls (range: 310,900-311,900), of which on average 1,915 (range: 1,855-1,949) had been called by at least two different callers.

SV calls across all samples were combined using the R package intanSV (Yao, 2016) by merging the calls that had been made in at least two samples with a reciprocal coordinate overlap larger than 10 %. There were a total of 26,799 overlapped calls, 22,954 deletions, 3,105 duplications, and 740 inversions.

The SV calls were compared against the Database of Genomic Variants (DGV, release July 2015; downloaded from UCSC genome browser hg19, February 2016) to annotate them as rare and common. Those that overlapped by $>$ 50% of their lengths with five or fewer CNV events in the DGV were considered rare, while if they overlapped by $>$ 50% of their lengths with more than five CNV events in the DGV they were considered common. Those that did not overlap with any CNV in the DGV were classed as novel.

We excluded SVs as being potentially causative for the phenotype when they were present in the unaffected individual II.14 (married into family 352), or when they were common variants (as defined above) in the DGV database.

SVs from SNP microarrays

PennCNV (Wang et al., 2007) was used to detect Copy Number Variants (CNVs) from the signal intensity data. This program uses the normalized intensity data (Log R Ratio, and B Allele Frequencies) for SNP and CNV probes to call putative CNVs using a Hidden Markov Model (HMM). For this analysis, we used default HMM parameters, as well as the PFB (Population Frequency of B allele) and GC (CG content model) files provided with the program (hhall.hg18.pfb, hhall.hg18.gcmodel), since our sample was too small to directly estimate these parameters from it. We used the joint calling option in order to take advantage of the family structure. Since only a trio-structure can be specified, a separate trio was defined for each non-founder sample. As a result, there were 1,947 CNV calls, and each sample had on average 92.7 calls (range: 31-211). We filtered out common CNVs (as defined for the DGV, see above) and CNVs that fell outside of the linked genomic regions (multipoint LOD $>$ 1 or multipoint NPL $>$ 1).

6.3 RESULTS

6.3.1 *Multipoint linkage*

Multipoint analysis identified two regions of linkage with LOD > 1 (Figure 6.2) on chromosomes 7 and 14 under the dominant parametric model. The maximum LOD score was 2.82 on 7q21.11 (chr7:80197286-83403157, hg19), in a region that encompasses several genes (*CD36, SEMA3C, LOC100128317, HGF, CACNA2D1, LOC101927356, PCLO, SEMA3E*). The linked region on 14q12 (chr14:29132877-29995029, hg19) had a maximum LOD score of 1.45. The empirical threshold for genome-wide significant linkage (occurring in 5% of genome-scans by chance in the permutation analyses) was estimated to be 3.44, and the empirical P value for the maximum observed LOD was P(LODmax=2.82)=0.109. Haplotypes of 7q21.11 showed that the linked region was shared among all affected family members except for two, and no unaffected members (Figure 6.4). The multipoint parametric model with lower penetrances it did not result in any genome-wide significant linkages and it yielded a similar peak at 7q21.11 (LOD_{max}=2.42).

Additional linkages with NPL > 1 were present on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 13, with a maximum NPL score of 1.27 (see Figure 6.S1 and Table 6.S1 for the exact definitions of all intervals with multipoint NPL > 1).

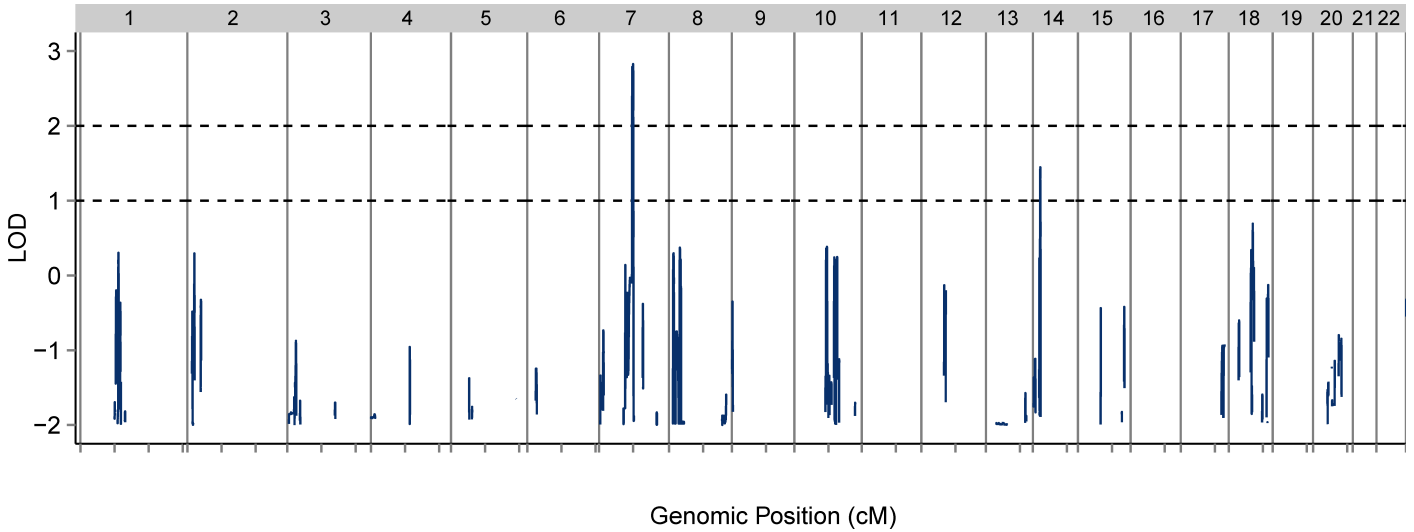


Figure 6.2: Parametric multipoint linkage analysis across the genome. Chromosomes are represented along the X axis (see top) in numerical ascending order from left to right, and from their p to q arms. The Y axis shows the multipoint linkage LOD score.

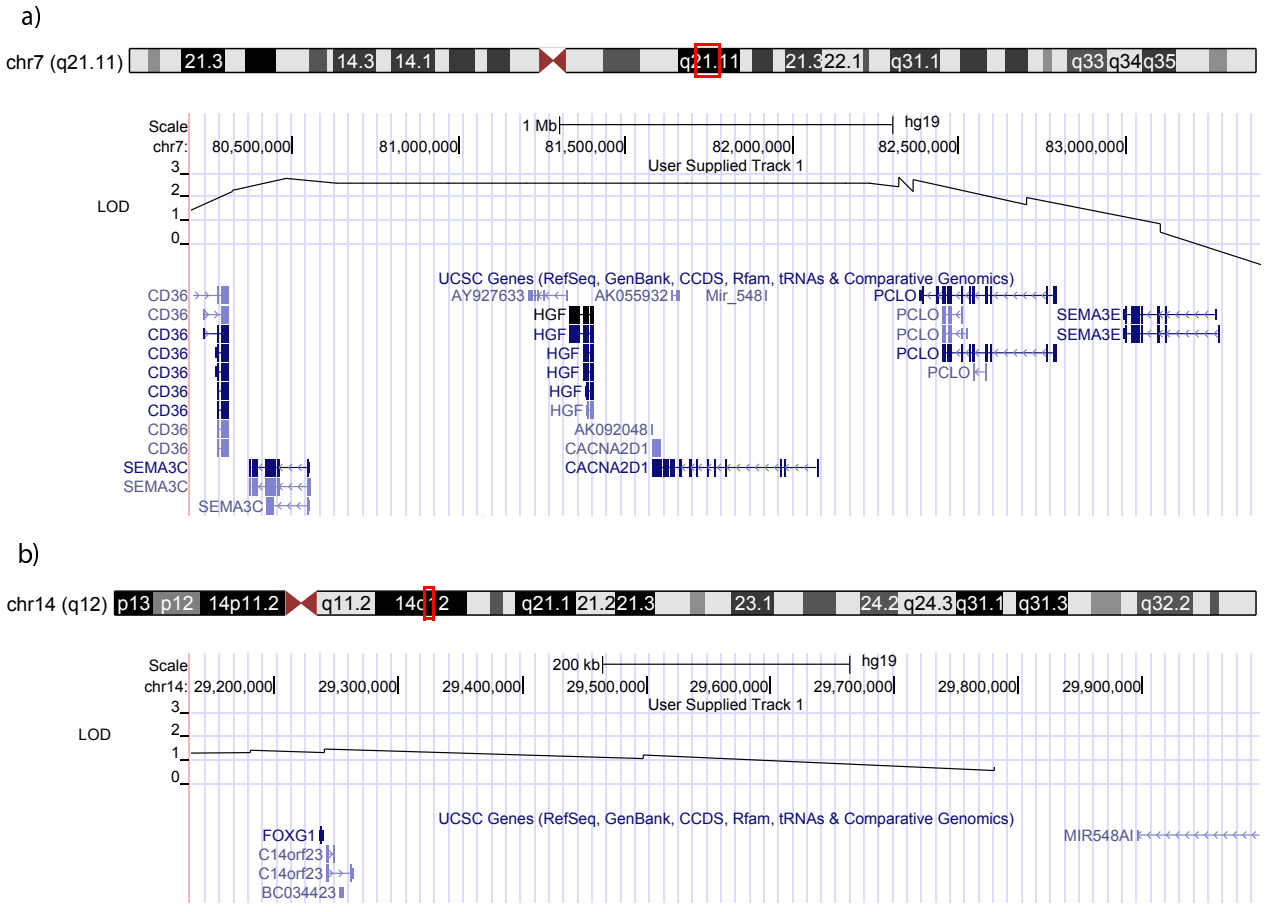


Figure 6.3: Genomic regions of interest as defined by LOD > 1 (parametric linkage analysis). Multipoint linkage score (upper track) shown over the UCSC genes (lower track) in a) chromosome 7q21.1 and b) chromosome 14q12.

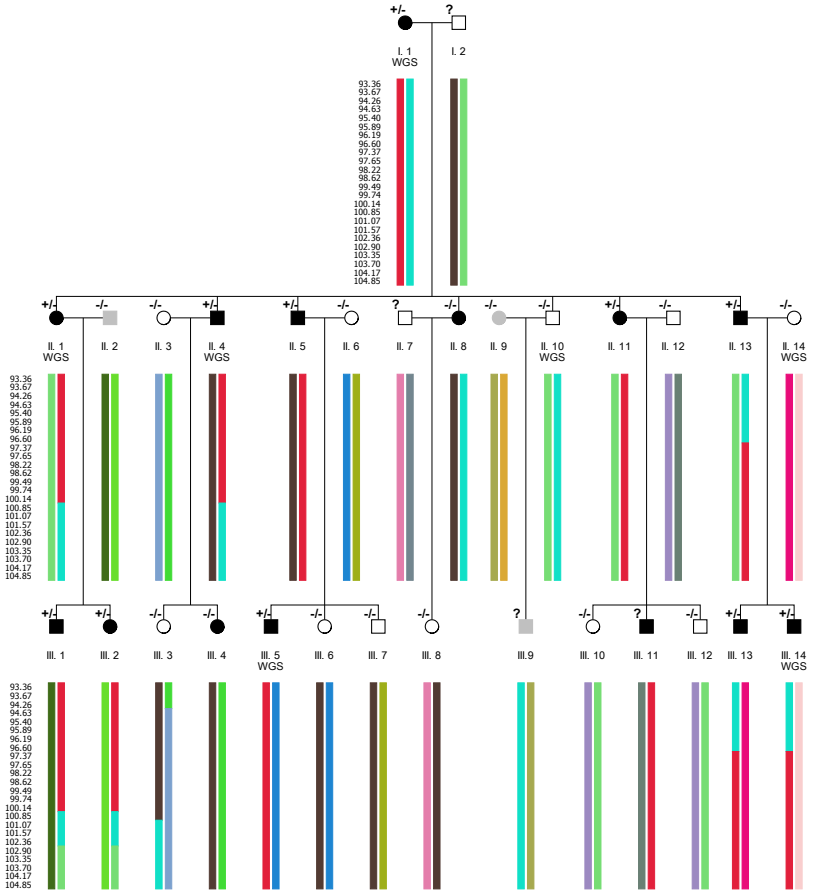


Figure 6.4: Haplotype visualization of the linked genomic region on chromosome 7q21.11. Carrier statuses for the rare variants rs144517871 (Sanger sequenced) and rs143835534 (imputed) are indicated above each individual as: wild type: -/-, heterozygote carrier: +/-, unknown: ?. The putative 'risk haplotype' is shown in red.

6.3.2 *Rare or novel SNPs and indels*

Coding variants

After filtering (see above), there were two coding, rare or novel, non synonymous variants that were present in at least 60% of the affected individuals with available genotype data, as summarized in Table 6.1. These variants fell within the genes *RP1L1* (chromsome 8p23.1) and *COL4A2* (chromosome 13.q34). Neither gene is located within either of the two most strongly linked regions that arose from multipoint parametric analysis. *RP1L1* codes for the retinitis pigmentosa like-1 gene, which has been associated with occult macular dystrophy (OMIM 608581). *COL4A2* encodes a subunit of type IV collagen, which is a major structural component of basement membranes. Although the *RP1L1* and *COL4A2* SNVs from family 352 are rare or new in the general population, they were relatively frequent in unaffected individuals within the family, as reflected by low single-point linkage scores and high MQLS pvalues (Table 6.1).

Non-coding or synonymous variants

After filtering there were 65 non-coding or synonymous variants within putatively linked genomic regions that were likely to be pathogenic or functional based on CADD or GWAVA calls, 9 of which were present in more than 60% of the affected people (Table 6.2). Most of them were also relatively frequent in the unaffected people within the family. However, there were two SNVs (rs144517871 and rs143835534) within the most strongly linked region on chromosome 7q21.11, located within the first intron of the *SEMA3C* gene. The imputed genotypes predicted that both would cosegregate with dyslexia within the family, except for two putative phenocopies: II.8 and III.4. This was confirmed by Sanger sequencing for rs144517871 (see Figure 6.4 for cosegregation). The two SNVs are 184bp apart, and in perfect linkage disequilibrium (LD) (r^2=1). Each SNP yielded a single-point linkage LOD score of 1.82, and a MQLS association pvalue of 0.02. The frequencies of the observed variants were 0.0031 in the 1000G overall population and slightly higher (0.0099) in the European subsample, as well as in a representative Dutch population (rs144517871=0.010 and rs143835534=0.009) (Genome of the Netherlands, http://www.nlgenome.nl/search/) (Francioli et al., 2014). We plotted the relationships of rs144517871 with word and nonword reading fluency (Figure 6.6): this showed that risk-allele carriers for rs144517871 performed overall worse than non-carriers on both measures.

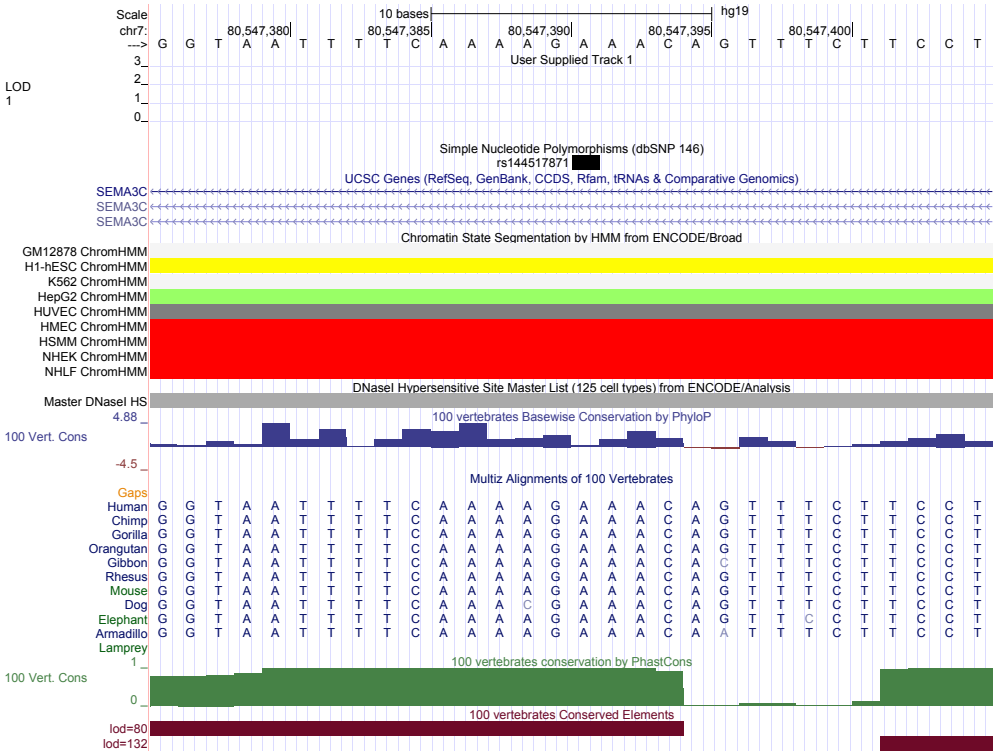


Figure 6.5: Detailed annotation of the genomic region around rs144517871 using the UCSC Genome Browser (hg19). Tracks are included for ENCODE digital DNaseI HS hypersensitivity clusters, ENCODE/Broad chromatin state segmentation by HMM in several cell lines, as well as 100 Vertebrate consevation scores (PhyloP, PhansCons, Conserved elements) and sequence alignment (Multiz Alignments of 100 Vertebrates).

The variant rs144517871 is predicted to be within a regulatory region according to the EnsemblRegulatory Build (Zerbino et al., 2015), and had high scores for functional prediction, CADD=26.3 and GWAVA=0.82. The variant has promoter and enhancer histone marks in several tissues (17 and 4 tissues respectively, Figure 6.5). It is predicted to alter several regulatory motifs (Haploreg: Pou2f2, Pou5f1,ZEB1 (Ward and Kellis, 2012); Regulome: Pou5f1 (Boyle et al., 2012)). It is also well conserved across mammals, with a GERP conservation score of 4.73. The other variant within the *SEMA3C* intron, rs143835534, had lower functional prediction scores, CADD=6.57 and GWAVA=0.74. It shares some of the histone marks with rs144517871, and also is predicted to alter some regulatory motifs, but it is not evolutionarily conserved (GERP=-1.28) (Figure 6.S2).

Chr	Position	Ref	Alt	cytoBand	$dbSNP_{138}$	Gene	Transcript	AA.change	MAF_{1000G}	MAF_{ExAC}	MQLS	LODsp	ImpRate	Affected	Unaffected
8	10465152	A	C	8p23.1	rs192863038	*RP1L1*	NM_178857	p.D2152E	-	-	0.33	0.01	0	0.6	0.5
13	111082914	A	T	13q34	rs201716258	*COL4A2*	NM_001846	p.H203L	2e-04	1.5e-04	0.11	0.00	0.48	0.80	0.33

Table 6.1: Rare, exonic, nonsynonymous variants present at least in 60% of the affected individuals in family 352 after filtering (see text). Ref: reference allele in hg19. Alt: alternative observed allele. AA.change: aminoacid change. LODsp: single point LOD score. ImpRate: Imputation rate of the variant. Affected, unaffected: frequency of variant carriers of each type in the family. If the ImpRate is not 0, the proportions of carriers are given for the imputed genotypes; otherwise, for the people with available data.

Chr	Position	Ref	Alt	cytoBand	$dbSNP_{138}$	Gene	Func	MAF_{1000G}	CADD	GWAVA	MQLS	LOSDsp	ImpRate	Affected	Unaffected
1	68296119	T	C	1p31.3	rs111907541	*GNG12*	intronic	1.8e-03	6.32	0.64	0.07	0.11	0.30	0.67	0.00
1	68300052	G	A	1p31.3	rs111472994	*GNG12-AS1*	ncRNA_intronic	2.0e-03	27.60	0.84	0.07	0.11	0.30	0.67	0.00
1	70507620	G	A	1p31.1	rs189591558	*LRRC7*	intronic	2.0e-04	8.55	0.51	0.23	0.06	0.30	0.83	0.50
2	5865630	G	A	2p25.2	rs145390307	*SOX11,LINC01105*	intergenic	1.6e-03	0.39	0.65	0.23	0.18	0.48	0.70	0.00
2	6776265	T	C	2p25.2	rs142428415	*LINC01246,MIR7515HG*	intergenic	2.8e-03	4.39	0.63	0.23	0.18	0.48	0.70	0.00
3	9419889	T	C	3p25.3	.	*THUMPD3*	intronic	-	19.05	-	0.23	0.00	0.48	0.70	0.33
7	80547391	A	C	7q21.11	rs144517871	*SEMA3C*	intronic	3.2e-03	26.30	0.82	0.02	1.84	0.52	0.90	0.00
7	80547575	T	C	7q21.11	rs143835534	*SEMA3C*	intronic	3.2e-03	6.57	0.74	0.02	1.84	0.52	0.90	0.00
7	93715698	G	-	7q21.3	-	-	-	-	15.19	-	0.32	0.31	0.52	0.70	0.25

Table 6.2: Rare, noncoding variants that are were predicted to be pathogenic within linked genomic regions. Affected, unaffected: frequency of variant carriers of each type in the family. If the ImpRate is not 0, the proportions of carriers are given for the imputed genotypes; otherwise, for the people with available WGS data.

6.3.3 *Structural and copy number variants*

There were 121 SV calls from the WGS data that fell within the linked regions with multipoint linkage scores of NPL>1 or LOD>1. Of these, 46 were present in > 60 % of the affected members (i.e. in at least 3/5 affected members) and only five cosegregated with dyslexia within the WGS samples (i.e. five affected members had them, and the two unaffected did not). These were four deletions and one duplication that were each called by only one out of the three callers (either CNVnator or BreakDancer) and were therefore considered as uncertain calls (see Table 6.S2). One of these (chr7:82725900-82727020) was a 1,120 bp deletion that fell within the most strongly linked region on 7q21.11, located in the third intron of the gene *PCLO*.

There were 12 CNV calls from the SNP array data that fell within regions with multipoint linkage scores of NPL>1 or LOD>1. Of these, most were only present in 1-3 affected members, and one was present in 8/14 of the affected members and only in 2/11 unaffected members. This CNV was a 5.58kb duplication on 7q21.11 (chr7:81070971-81076550), although some of the samples (3 affected and 1 unaffected) had a smaller call of 2kb encompassing only a subset of the region (chr7:81074519-81076550, see Table 6.S2). This duplication was located between the noncoding RNA *AY927633* (telomeric 12.89Kb) and the gene *SEMA3C* (51.91Kb centromeric).

6.4 DISCUSSION

In the present study we adopted a strategy of combining linkage analysis with WGS to identify genetic variants that contribute to dyslexia in an extended family.

The most strongly dyslexia-linked region, on chromosome 7q21.11, was identified by dominant parametric multipoint linkage analysis (LOD=2.82). Except for two affected members that were putative phenocopies, the risk haplotype cosegregated perfectly with dyslexia status in the family: it was found in heterozygote state in 12 members with dyslexia and absent from all those without dyslexia. The linked region encompassed several genes (*CD36, SEMA3C, LOC100128317, HGF, CACNA2D1, LOC101927356, PCLO, SEMA3E*). Within this region, there were two rare (MAF<0.01), noncoding single nucleotide variants (rs144517871 and rs143835534) predicted to have functional effects, located in the first intron of the gene *SEMA3C*, and in high LD with each other. These variants co-segregated perfectly with the risk haplotype. *In silico* characterization showed that rs144517871 affects an evolutionarily contrained

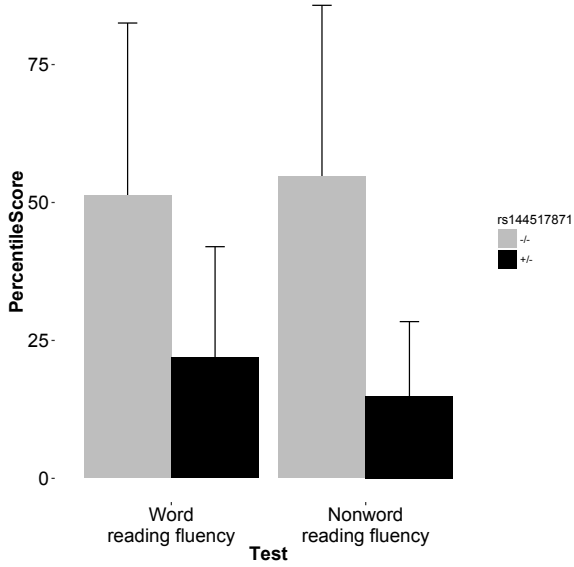


Figure 6.6: Mean percentile scores on word and non-word reading tests for carriers (black bars, n=11) and non-carriers (grey bars, n=15) of the rs144517871 risk allele (7q21 haplotype). Error bars show one standard deviation within this family.

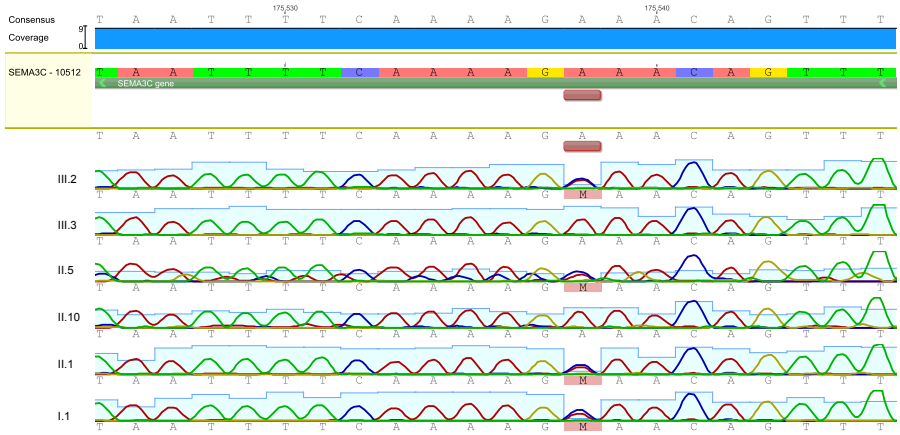


Figure 6.7: Sanger sequencing chromatograms for representative wile-type (A/A) and rs144517871 risk allele carrier (M=A/C) family members. Heterozygous genotypes: individuals III.2, II.5, II.1, I.1; homozygous individuals: III.3, II.10.

regulatory region in the first intron of *SEMA3C* (which is located between exons that are part of the 5'UTR). It is therefore possible that this variant has a *cis* regulatory effect on the expression of *SEMA3C*. Since dyslexia is a relatively subtle phenotype, genetic contributions to it need not have severe consequences at the molecular/cellular level. A regulatory mechanism that affects the amount of mRNA/protein, rather than a protein's function itself, is biologically plausible. In order to further explore this hypothesis, it will be necessary to perform functional molecular assays (e.g. luciferase assays), as was done in previous studies in which SNPs associated with dyslexia were shown to affect the expression of proximal genes (Dennis et al., 2009; Hannula-Jouppi et al., 2005).

SEMA3C encodes a class III semaphorin, which are secreted proteins that bind to plexin and play an important role in the regulation of developmental processes, including by providing guidance cues to migrating cortical neurons (Chen et al., 2008). In fact, *SEMA3C* has been shown to control cortical projection, neuron polarization and migration (Wiegreffe et al., 2015). Given its roles in brain development, *SEMA3C* represents a convincing candidate gene for dyslexia, a cognitive trait which has been associated with changes in cerebral cortical architecture (Giraud and Ramus, 2012). Of potential relevance, a previous linkage study of families with dyslexia and Rolandic epilepsy (Strug et al., 2012) reported a dyslexia-linked locus spanning the same location on 7q21.11 (multipoint LOD=3.08, microsatellite marker at linkage peak = D7S660). Since *SEMA3C* was the closest gene to the peak of linkage with dyslexia in the study by Strug et al. (2012), those authors screened the protein-coding and promoter regions of the *SEMA3C* gene for mutations in one of the families that contributed most to their linkage LOD score. However, they were unable to identify mutations that co-segregated with dyslexia, and suggested that either intronic regions or other genes could be responsible for the signal. In light of our data from the present study, it would be worth further investigating the families studied by (Strug et al., 2012) with a focus on potential regulatory variation affecting *SEMA3C*.

Given the scope for incomplete penetrance and phenocopy for dyslexia, we also considered other genomic regions that were linked to a lesser degree than 7q21.11 in family 359 (i.e. any regions with LOD or NPL scores>1): a locus on chromosome 14q12 from the parametric analysis, and several other loci on chromosomes 1-9 and 13 that were found linked in the nonparametric analysis. After filtering, there were two rare, coding, nonsynonymous variants within genomic regions showing multipoint linkage scores higher than 1 (NPL or LOD). However, neither of the two vari-

ants yielded significant single-point linkage scores or MQLS scores. This suggests that they are not likely to be strongly penetrant for dyslexia in this family. Nevertheless, it is interesting that one of them falls within the gene *COL4A2* on chromosome 13q34, since a common SNP in this gene was recently associated with comorbid reading disability and language impairment in a genome-wide association study (Eicher et al., 2013). However, this association was not replicated with reading-related quantitative traits in two other samples (Eicher et al. (2013) and see Chapter 4).

Non-synonymous variants in protein-coding regions can be used to infer changes in gene function in a relatively direct manner. Functional annotation of variants outside of protein-coding regions is more complex. In this study, we used summary scores (CADD and GWAVA) (Kircher et al., 2014; Ritchie et al., 2014) that integrate information from different sources at the DNA and protein levels (e.g. ENCODE annotation for DNaseI hypersensitivity, transcription factor binding sites and motifs, chemical similarity of amino acid substition) and genomic properties (including evolutionary conservation, GC content) to rank variants according to expected deleteriousness (CADD) or pathogenicity (GWAVA). The CADD metric measures deleteriousness by contrasting variants that survived natural selection (i.e. fixed in the human lineage) with simulated mutations (Kircher et al., 2014), in such a way that variants with higher scores are likely to have been selected against (given their annotation pattern). The GWAVA score uses similar sources of annotation, to discriminate between disease-causing (i.e. pathogenic) and control variants, and apply this information to weight variants across the genome (Ritchie et al., 2014). As dyslexia is not a pathogenic trait, we could not assume that causal variants in this family have necessarily been selected against. However, we took advantage of pathogenicity and deleteriousness metrics to rank the variants, as a proxy for affecting gene function. We are not aware of a method to predict biological effects of variants in a way that is entirely neutral with respect to selection, since comparative information on what changes are 'tolerated' are inherent to published approaches for both coding and noncoding variants (comparative information across proteins, across variants, or across species). Furthermore, a variant may cause a non-pathogenic trait in dominant form, but a disease in recessive form. Variants may also have pleiotropic effects, causing increased risk in relation to one pathogenic trait, and non-pathogenic modification of another trait. Thus, even for a non-pathogenic trait, the CADD and GWAVA metrics were useful tools to rank noncoding variants for possible functional relevance. Both tools predicted that rs144517871 (within *SEMA3C*) has functional effects.

Our SV analysis detected a putative intronic deletion of 1.12kb within the *PCLO* gene (also on 7q21.11), which was predicted to cosegregate with dyslexia (except for two putative phenocopies). However, the putatively deleted region did not contain any regulatory elements as annotated in ENCODE. Furthermore, this deletion fell within a highly repetitive region (containing a Short Interspersed Nuclear Element (SINE) and a simple TATAA repeat, as defined by Repeatmasker (Smit et al., 2013-2015) and was only called by one of the several algorithms used for identifying structural variants.

In summary, a combination of linkage analysis and WGS was used to identify novel or rare variants affecting dyslexia in an extended Dutch family. This strategy has proven to be a powerful approach to find causative variants for several monogenic and more complex traits (Rosenthal et al., 2013; Norton et al., 2013). We identified a region on chromosome 7q21.11 linked to dyslexia, which was concordant with a linkage reported in a previous study (Strug et al., 2012). There were no rare or novel exonic variants within this region, but two rare (MAF < 0.01) non-coding variants within the first intron of the gene *SEMA3C,* which were predicted to be functional, were found in all but two family members with dyslexia, while being absent from unaffected members). Thus, we propose *SEMA3C* as a candidate gene for dyslexia, and hypothesize that these intronic variants could have a *cis* regulatory effect on the expression levels of the gene. Further work will be required to assess the functional aspects of the variants in biological models, and to establish a causal relationship between these genetic variants and dyslexia.

References

Abyzov A, Urban AE, Snyder M, Gerstein M (2011) CNVnator: an approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. Genome Res 21: 974–984.

Bahlo M, Bromhead CJ (2009) Generating linkage mapping files from Affymetrix SNP chip data. Bioinformatics 25: 1961–1962.

Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, et al. (2011) Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 12: 745–755.

Bishop DV (2015) The interface between genetics and psychology: lessons from developmental dyslexia. Proc Biol Sci 282.

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, et al. (2012) Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22: 1790–1797.

Brus BT, Voeten MJM (1972) Een-minuut-test [one-minute-test]. Swets & Zeitlinger.

Carrion-Castillo A, Franke B, Fisher SE (2013) Molecular genetics of dyslexia: an overview. Dyslexia 19: 214–240.

Chen G, Sima J, Jin M, Wang KY, Xue XJ, et al. (2008) Semaphorin-3A guides radial migration of cortical neurons during development. Nat Neurosci 11: 36–44.

Chen WM, Manichaikul A, Rich SS (2009) A generalized family-based association test for dichotomous traits. Am J Hum Genet 85: 364–376.

Cheung CY, Thompson EA, Wijsman EM (2013) GIGI: an approach to effective imputation of dense genotypes on large pedigrees. Am J Hum Genet 92: 504–516.

de Kovel CG, Hol FA, Heister JG, Willemen JJ, Sandkuijl LA, et al. (2004) Genome-wide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. J Med Genet 41: 652–657.

Dennis MY, Paracchini S, Scerri TS, Prokunina-Olsson L, Knight JC, et al. (2009) A common variant associated with dyslexia reduces expression of the KIAA0319 gene. PLoS Genet 5: e1000436.

DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, et al. (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43: 491–498.

Eicher JD, Powers NR, Miller LL, Akshoomoff N, Amaral DG, et al. (2013) Genome-wide association study of shared components of reading disability and language impairment. Genes Brain Behav 12: 792–801.

Einarsdottir E, Svensson I, Darki F, Peyrard-Janvid M, Lindvall JM, et al. (2015) Mutation in CEP63 co-segregating with developmental dyslexia in a Swedish family. Hum Genet 134: 1239–1248.

Fagerheim T, Raeymaekers P, T?nnessen FE, Pedersen M, Tranebjaerg L, et al. (1999) A new gene (DYX3) for dyslexia is located on chromosome 2. J Med Genet 36: 664–669.

Field LL, Shumansky K, Ryan J, Truong D, Swiergala E, et al. (2013) Dense-map genome scan for dyslexia supports loci at 4q13, 16p12, 17q22; suggests novel locus at 7q36. Genes Brain Behav 12: 56–69.

Fisher SE, DeFries JC (2002) Developmental dyslexia: genetic dissection of a complex cognitive trait. Nat Rev Neurosci 3: 767–780.

Francioli LC, Menelaou A, Pulit SL, van Dijk F, Palamara PF, et al. (2014) Whole-genome sequence variation, population structure and demographic history of the Dutch population. Nat Genet 46: 818–825.

Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, et al. (2014) Genome-wide screening for DNA variants associated with reading and language traits. Genes Brain Behav 13: 686–701.

Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, et al. (2014) Genome sequencing identifies major causes of severe intellectual disability. Nature 511: 344–347.

Giraud AL, Ramus F (2012) Neurogenetics and auditory processing in developmental dyslexia. Curr Opin Neurobiol 23: 1–6.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Hiekkalinna T, Schaffer AA, Lambert B, Norrgrann P, Goring HH, et al. (2011) PSEUDOMARKER: a powerful program for joint linkage and/or linkage disequilibrium analysis on mixtures of singletons and related individuals. Hum Hered 71: 256–266.

Hoischen A, van Bon BW, Gilissen C, Arts P, van Lier B, et al. (2010) De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. Nat Genet 42: 483–485.

Illumina (2016a) Illumina Human OmniExpress-Exome. URL http://www.illumina.com/products/infinium_humanomniexpressexome_beadchip_kits.html.

Illumina (2016b) Illumina X-ten. URL http://www.illumina.com/systems/hiseq-x-sequencing-system.html.

Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, et al. (2014) The contribution of de novo coding mutations to autism spectrum disorder. Nature 515: 216–221.

Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, et al. (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46: 310–315.

Lange EM, Lange K (2004) Powerful allele sharing statistics for nonparametric linkage analysis. Hum Hered 57: 49–58.

Layer RM, Chiang C, Quinlan AR, Hall IM (2014) LUMPY: a probabilistic framework for structural variant discovery. Genome Biol 15: R84.

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078–2079.

Luciano M, Evans DM, Hansell NK, Medland SE, Montgomery GW, et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav 12: 645–652.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297–1303.

McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, et al. (2010) Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics 26: 2069–2070.

Mohiyuddin M, Mu JC, Li J, Bani Asadi N, Gerstein MB, et al. (2015) MetaSV: an accurate and integrative structural-variant caller for next generation sequencing. Bioinformatics 31: 2741–2744.

Nopola-Hemmi J, Myllyluoma B, Haltia T, Taipale M, Ollikainen V, et al. (2001) A dominant gene for developmental dyslexia on chromosome 3. J Med Genet 38: 658–664.

Norton N, Li D, Rampersaud E, Morales A, Martin ER, et al. (2013) Exome sequencing and genome-wide linkage analysis in 17 families illustrate the complex contribution of TTN truncating variants to dilated cardiomyopathy. Circ Cardiovasc Genet 6: 144–153.

Peterson RL, Pennington BF (2015) Developmental dyslexia. Annu Rev Clin Psychol 11: 283–307.

Picard (2016) Picard. URL https://broadinstitute.github.io/picard/.

Ritchie GR, Dunham I, Zeggini E, Flicek P (2014) Functional annotation of noncoding sequence variants. Nat Methods 11: 294–296.

Rosenthal EA, Ranchalis J, Crosslin DR, Burt A, Brunzell JD, et al. (2013) Joint linkage and association analysis with exome sequence data implicates SLC25A40 in hypertriglyceridemia. Am J Hum Genet 93: 1035–1045.

Schork NJ, Murray SS, Frazer KA, Topol EJ (2009) Common vs. rare allele hypotheses for complex diseases. Curr Opin Genet Dev 19: 212–219.

Shaywitz SE, Escobar MD, Shaywitz BA, Fletcher JM, Makuch R (1992) Evidence that dyslexia may represent the lower tail of a normal distribution of reading ability. N Engl J Med 326: 145–150.

Smit A, Hubley R, Green P (2013-2015) RepeatMasker Open-4.0. URL http://www.repeatmasker.org.

Strug LJ, Addis L, Chiang T, Baskurt Z, Li W, et al. (2012) The genetics of reading disability in an often excluded sample: novel loci suggested for reading disability in Rolandic epilepsy. PLoS ONE 7: e40696.

Taipale M, Kaminen N, Nopola-Hemmi J, Haltia T, Myllyluoma B, et al. (2003) A candidate gene for developmental dyslexia encodes a nuclear tetratricopeptide repeat domain protein dynamically regulated in brain. Proc Natl Acad Sci USA 100: 11553–11558.

Thiele H, Nurnberg P (2005) HaploPainter: a tool for drawing pedigrees with complex haplotypes. Bioinformatics 21: 1730–1732.

Thompson E (2011) The structure of genetic linkage data: from LIPED to 1M SNPs. Hum Hered 71: 86–96.

Thornton T, McPeek MS (2007) Case-control association testing with related individuals: a more powerful quasi-likelihood score test. Am J Hum Genet 81: 321–337.

Thornton T, Zhang Q, Cai X, Ober C, McPeek MS (2012) XM: association testing on the X-chromosome in case-control samples with related individuals. Genet Epidemiol 36: 438–450.

Uterwijk J (2000) WAIS-III Nederlandstalige Bewering. Technische Handleiding. Lisse: Swets and Zeitlinger.

van den Bos KP, Lutje Spelberg HC, Scheepstra AJM, de Vries JR (1994) De klepel: Een test voor de leesvaardigheid van pseudowoorden [the klepel: A test for the reading skills of pseudowords]. Swets & Zeitlinger .

van der Leij A, Maassen B (2013) Dutch Dyslexia Programme. Dyslexia 19: 189–190.

Wang K, Li M, Hadley D, Liu R, Glessner J, et al. (2007) PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res 17: 1665–1674.

Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38: e164.

Ward LD, Kellis M (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res 40: D930–934.

Wiegreffe C, Simon R, Peschkes K, Kling C, Strehle M, et al. (2015) Bcl11a (Ctip1) Controls Migration of Cortical Projection Neurons through Regulation of Sema3c. Neuron 87: 311–325.

Yao W (2016) intansv: Integrative analysis of structural variations. R package version 1.6.2.

Zerbino DR, Wilder SP, Johnson N, Juettemann T, Flicek PR (2015) The ensembl regulatory build. Genome Biol 16: 56.

Supplementary information

Nonparametric linkage analysis

Chr	Start	End	NPL_{max}
1	67231916	71001920	1.27
2	5241229	8102347	1.04
3	397958	1317869	1.27
3	9066156	9831970	1.04
4	97475233	98597993	1.27
5	172469848	172758076	1.27
6	10897488	11730612	1.27
7	52443808	53706347	1.27
7	62241041	66786374	1.27
7	93557588	94948841	1.27
8	3984856	21474049	1.27
9	290670	571192	1.27
13	110353253	111111777	1.27

Table 6.S1: Genomic regions of interest as defined by NPL > 1 (non-parametric linkage analysis). Coordinates are given for the genome reference hg19. NPL_{max} indicates the maximum NPL score within each interval.

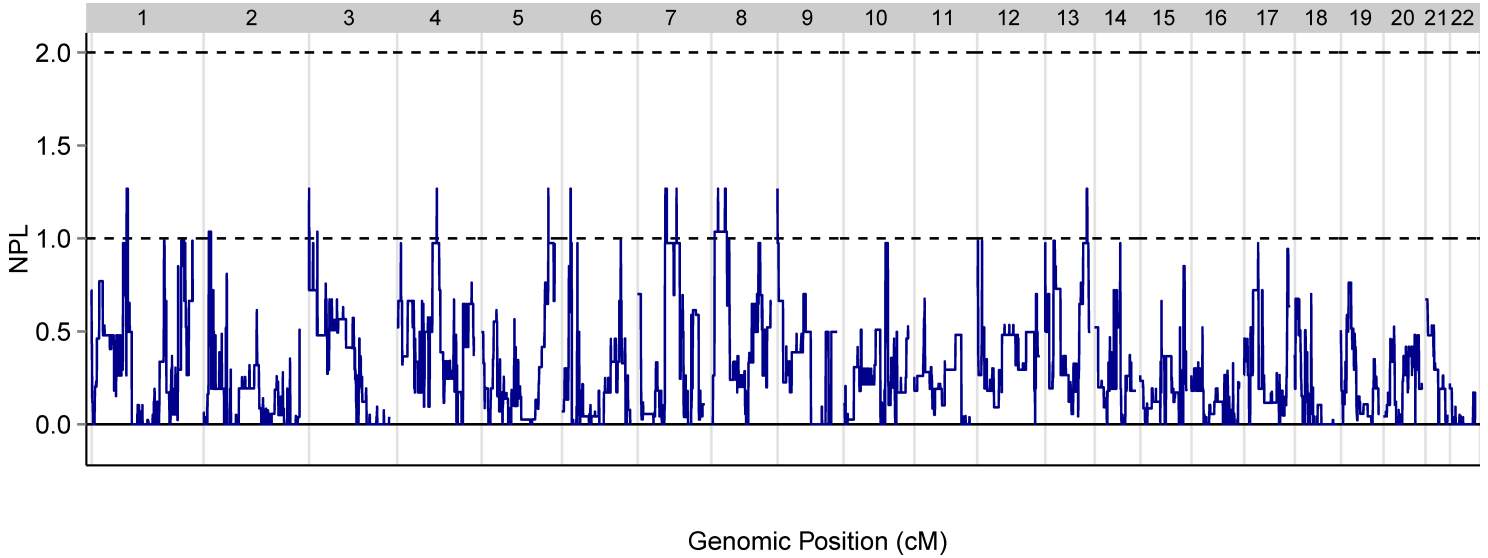


Figure 6.S1: Nonparametric multipoint linkage analysis across the genome. Chromosomes are represented along the X axis (see top) in numerical ascending order from left to right, and from their p to q arms. The Y axis shows the multipoint linkage LOD score.

Annotation of SEMA3C intron 1

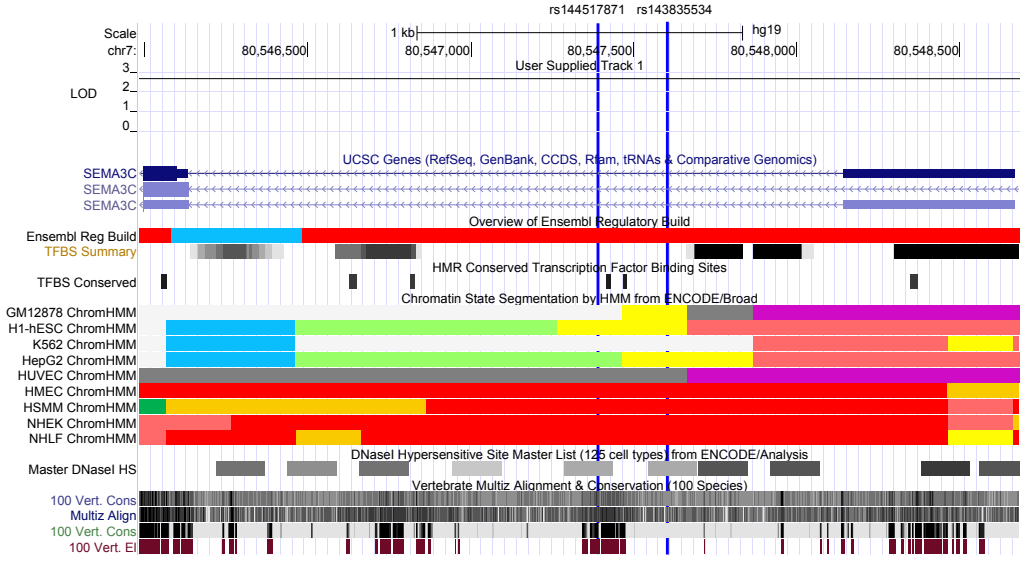


Figure 6.S2: Detailed annotation of the genomic region first intron of *SEMA3C* using the UCSC Genome Browser (hg19). The variants rs144517871 and rs143835534 are marked in blue. Tracks for ENCODE digital DNaseI HS hypersensitivity clusters, transcription factor binding sites, ENCODE/Broad chromatin state segmentation by HMM in several cell lines, as well as 100 Vertebrate consevation scores (PhyloP, PhansCons, Conserved elements) and sequence alignment (Multiz Alignments of 100 Vertebrates) are included.

Structural variation

Data	Chr	Start	End	Size	Type	Affected	Unaffected	CytoBand	Genes	Region
WGS	7	64968140	64970840	2700	dup	5	0	7q11.21	-	-
WGS	7	65679120	65680880	1760	del	5	0	7q11.21	*TPST1*	intron
PennCNV	7	81070971	81076550	5579	dup	3	1	7q21.11	-	-
PennCNV	7	81074519	81076550	2031	dup	5	1	7q21.11	-	-
WGS	7	82725900	82727020	1120	del	5	0	7q21.11	*PCLO*	intron
WGS	7	93831480	93832280	800	del	5	0	7q21.3	-	-
WGS	8	12274140	12276320	2180	del	5	0	8q23.1	*FAM66A, FAM90A25P, LOC100506990*	

Table 6.S2: Structural variants within linked genomic regions. Affected, unaffected: count of variant carriers of each type, among the family members with available data (WGS: 5 affected, 2 unaffected; SNP array: 14 affected, 11 unaffected). Type of SV call: del=deletion, dup=duplication.

7

DISCUSSION

Reading is a complex behavioural phenotype, and different types of genetic variants are expected to affect its variability. This thesis was aimed at clarifying the genetic underpinnings of individual differences in reading ability.

7.1 SUMMARY

A literature review in Chapter 2 provided a thorough overview of the known molecular basis of dyslexia. In particular, I evaluated statistical and biological evidence for the contributions to dyslexia and related traits of the most prominent candidate genes in the literature, including *DYX1C1, DCDC2, KIAA0319* and *ROBO1*. These genes have been implicated in dyslexia by several complementary lines of evidence: linkage studies have mapped possible dyslexia-susceptibility genomic regions; single nucleotide polymorphisms (SNPs) located within or neighbouring the genes have been associated with reading and related phenotypes; molecular studies have revealed gene-regulatory features of some of the associated SNPs, suggesting possible functional involvements; and animal models with altered gene functions have been found to have relevant phenotypes (e.g. heterotopias, abnormal migration of cortical neurons, ciliary motility defects). Moreover, some of the candidate genes and variants have recently been subjected to electrophysiological and functional or structural brain imaging studies, leading researchers to conclude that they are associated with brain regions and processes implicated in reading. In sum, these studies have provided support for the involvement of these genes in the neurobiology underlying reading ability. Yet, at the same time, the field contains contrasting results, with multiple non-replications and opposite directionality of allelic effects that hinder progress on the basis of the available data.

In Chapter 3, I targeted fourteen of the most consistently associated SNPs in the literature, which fell within the candidate genes *DYX1C1, KIAA0319, DCDC2, CNTNAP2* and *CMIP* and assessed them in relation to reading fluency, rapid naming, phoneme awareness and nonword repetition in the longitudinal dataset of the Dutch Dyslexia Programme (DDP). Evidence of association was found for 5 SNPs, but for most of these the directions of effect were not consistent with previous studies. Two SNPs within the 5'UTR of *KIAA0319* (rs2038137 and rs761100) were consistently associated with rapid naming across four developmental time-points (7-12 years). This study shows how developmental information can be incorporated into the study of genetic effects in reading.

In Chapter 4, I evaluated the most significant associations from the first genome-wide association scan (GWAS) studies of language and reading performance, by testing for their effects on reading-related quantitative traits in FIOLA, a new and independent dataset including children and adults from the general Dutch population. I selected seventeen SNPs to study (with association P values $<10^{-6}$ in the original GWAS), for which the FIOLA dataset provided sufficient statistical power to detect the previously reported effects. The primary association testing was performed within the subset of children in FIOLA. As a secondary analysis, I tested the same SNPs for association within the adults of this dataset. Three nominally significant associations were observed in the multivariate analysis of the children, and two of these (rs12636438, rs7187223) showed consistent signals in the univariate analysis as well. The association of rs12636438 had the opposite direction of effect to that previously reported by Eicher et al. (2013). The minor allele of rs7187223 was associated with lower performance on rapid naming and phoneme awareness in our sample of children, in a consistent manner to the association that Luciano et al. (2013) reported for nonword repetition. This SNP was the only nominally significant association within our adult sample, but there we found that the major allele was associated with lower phoneme awareness scores. In general, this study provided little support for variants previously implicated by GWAS, which suggests that such studies will need to be performed in future using much larger meta-datasets than those investigated so far.

In Chapter 5, I performed whole genome linkage analysis and sequenced the whole exomes or genomes of 15 members (13 affected) of a large extended family with dyslexia (de Kovel et al., 2004). No candidate variants were identified that might be causative for dyslexia as perfectly penetrant Mendelian mutations. Nonetheless,

several rare variants were identified that may contribute to dyslexia liability in this family, including a nonsynonymous SNP within the gene *CACNA2D3*, a noncoding variant upstream of *RBMX*, and an intronic deletion within *AFF2*. It is likely that an oligogenic or more complex genetic background underlies the inheritance pattern in this family.

In Chapter 6, I combined linkage analysis with whole genome sequencing (WGS) on another extended family with dyslexia. I identified a locus dominantly linked to dyslexia on chromosome 7q21.11. Two rare intronic single nucleotide variants in *SEMA3C* were found to co-segregate with the risk haplotype and almost perfectly with dyslexia within the family (i.e. 12 of the 14 dyslexic relatives were heterozygous for the risk variants, while none of the unaffected relatives were carriers). Both variants lie in a predicted regulatory region around the gene's promoter, and *in silico* characterization suggested that one of them (rs144517871) is a good candidate for *cis* regulation of *SEMA3C*.

In sum, these studies focused on specific aspects of the genetic basis underlying reading ability. Next, I will examine some broader implications for future research that have arisen from these and other studies. I will focus on a number of issues: the potential role of neuronal migration as a possible neurodevelopmental process affecting reading (dis)ability; the challenges faced to reconcile candidate gene association studies; the use of next generation sequencing technologies; and the utility of characterizing the phenotype at the brain level.

7.2 NEURONAL MIGRATION: A POTENTIALLY UNIFYING MECHANISM?

The functional impact of at least one of the variants identified in family 352 is likely to involve subtle gene regulation affecting the expression levels of *SEMA3C*. This gene encodes a class III semaphorin, a family of signalling molecules that play important roles in cerebral cortical layering by providing guidance cues to migrating neurons (Chen et al., 2008), in addition to other functions, such as tumor differentiation (Malik et al., 2016) or promoting endothelial-to-mesenchymal transition of cells in the developing heart (Plein et al., 2015). In the mouse brain, *Sema3c* is repressed by *Bcl11a* (Wiegreffe et al., 2015), which is an important transcription factor for cortical development. Of note, a *de novo* microdeletion on the dyslexia susceptibility locus 3 (DYX3) on chromosome 2p containing only the *BCL11A* gene was reported in a proband with severe speech sound disorder (Peter et al., 2014). Wiegr-

effe et al. (2015) found that homozygous mutant *Bcl11a* mice presented defects in neuronal morphology and neuronal migration, and that the mutant phenotype was rescued when knocking down *Sema3c*. Molecular assays, such as luciferase reporter gene assays, could be used to evaluate whether the putative functional variant(s) affect the expression of *SEMA3C* in cellular models. Another possibility to validate *SEMA3C* as a candidate gene for dyslexia is to test whether or not common variants within this gene are associated with reading-related phenotypes in independent datasets. It is possible that both rare and common variants in the same gene can be implicated in reading-related disorders, as in the case of *ROBO1*, where a rare haplotype co-segregated with dyslexia in an extended family (Hannula-Jouppi et al., 2005), and common SNPs were associated to nonword repetition in the general population (Bates et al., 2011). Convergent evidence for a role of *SEMA3C* influencing dyslexia would provide a new building block to understand the molecular landscape for reading ability. Indeed, several candidate genes for dyslexia have already been implicated in neuronal migration. Embryonic knockdown rat models of the homologue genes of *DYX1C1, DCDC2* and *KIAA0319* by *in utero* electroporation of plasmids encoding small hairpin RNA (shRNA) resulted in neuronal migration deficits (Adler et al., 2013; Peschansky et al., 2010; Szalkowski et al., 2013; Currier et al., 2011). However, off-target effects for shRNA technology have been reported (Baek et al., 2014) suggesting that caution is needed for the interpretation of the findings from the knockdown studies. Indeed, no such effect in neuronal migration was observed in *Dcdc2* knockout mice (Wang et al., 2011). *ROBO1* encodes an axonal guidance receptor that has roles in migration of interneurons, neuronal differentiation and synapse formation (Andrews et al., 2006, 2008). These animal models represent the severe end of the spectrum in the phenotypic variability that could be caused by genetic variations in these genes.

The fact that writing systems are a recent cultural development, together with the relatively high prevalence of dyslexia in current populations, suggests that subtle changes (at genetic, cognitive and brain levels) leading to differences in reading ability were unlikely to have involved overtly negative selection pressures in human history. Subtle changes could arise from (1) drastic genetic alterations of molecular components which are nonetheless compensated by partial redundancy, as in the case of *Dcx* and *Dcdc2*, where it was observed that *in utero* knockdowns of *Dcx* presented more developmental disruptions in *Dcdc2* mice than in control mice (Wang et al., 2011); or (2) subtle genetic alterations, as for candidate dyslexia SNPs that have

been reported to have regulatory effects on the expression levels of *KIAA0319* (Dennis et al., 2009) and *DCDC2* (Meng et al., 2005, 2011; Powers et al., 2013). Recent evidence suggests that *DYX1C1*, *DCDC2* and *KIAA0319* are also involved in the growth and function of cilia (Ivliev et al., 2012; Chandrasekar et al., 2013; Massinen et al., 2011; Tarkar et al., 2013) and it has been proposed that effects on cilia-dependent neuronal migration could result in subtle changes in neuronal positioning or connectivity (Kere, 2014).

Taken together, the evidence so far suggests that fine-tuning effects on cortical development (and the genetic factors that influence them) could lead to establishing suboptimal connections or lateralization patterns in the brain, which could in turn translate into reading difficulties.

7.3 REVISITING CANDIDATE GENES

My evaluation of prior candidate SNPs for reading-related traits did not yield clear support for their involvement (Chapter 3 and Chapter 4). In both studies, there were some SNPs showing evidence of association in the datasets that I used, but most of these signals were not in line with previously reported effects. This is a well-known issue in the field, and it is often argued that heterogeneity across studies at different levels might underlie this issue: study design parameters, population structure, ascertainment of probands, assessment of the traits, or diagnostic criteria (Paracchini et al., 2007). I also discussed the possibility that other factors, such as developmental stage, and language or orthography type, could be contributing to the heterogeneity. All of these factors are likely to be important, but an overarching problem for this literature is statistical power in the face of relatively limited sample sizes, and small effects that are inaccurately measured, leading both to false negative and false positive signals.

The genetic component of reading ability is complex and heterogeneous, and likely will require studies with large samples sizes to have adequate power to detect its smaller individual genetic contributions (Visscher et al., 2012). Recently, GWAS studies have taken a first step towards increasing sample sizes, moving from studies with a few hundreds to thousands of individuals (Luciano et al., 2013; Field et al., 2013; Eicher et al., 2013; Gialluisi et al., 2014). Each of these studies have reported new suggestively associated loci (as reviewed in Chapter 4), but the results did not yield any significant associations in the context of genome-wide multiple testing

and statistical correction. So far there has not been convergence of signals across these studies, despite some having used partially overlapping samples such as the ALSPAC dataset (Luciano et al., 2013; Eicher et al., 2013). This suggests that (1) the analysis is very sensitive to the characterization of the phenotypic traits, and (2) future studies will require even larger sample sizes, for which it will be necessary to meta-analyse results over multiple datasets, which has shown to be a powerful approach for other complex neuropsychiatric disorders, such as schizophrenia (Psychiatric Genomics Consortium, 2014). On the other hand, the phenotype itself is a moving target: the strategy that is used to decipher a word is influenced by several factors, including the transparency of the orthography (Landerl et al., 1997) and individual preferences for decoding strategies (McGeown et al., 2013). Hence, measures of reading ability can have different contributions to their variance across studies. Meta-analyses studies that tried to integrate the evidence for specific candidate SNPs from case-control or transmission disequilibrium test (TDT) studies (Zou et al., 2012; Zhong et al., 2013; Tran et al., 2013) reported evidence of association for just two of the considered SNPs (e.g. rs807701 in *DCDC2* (Zhong et al., 2013) and rs4504469 in *KIAA0319* (Zou et al., 2012)). The use of quantitative scores instead of categorical affection status may help in the face of heterogeneity of genetic effects. However, the quantitative measures used are not always assessed in a similar manner in different studies. For example, single word reading ability in English is normally assessed as the proportion of correctly read words (reading accuracy), which does not have a timing component, while other languages with shallow orthographies (a more direct spelling-sound correspondence) such as Dutch or German assess single word reading as the number of correctly read words per minute (reading fluency) (Becker et al., 2014). The different measurement instruments across languages are usually suited to differences in writing systems, and may be justified to avoid ceiling and floor effects, but are a source of heterogeneity which challenges a transparent comparison across studies. A recent study that adopted the same inclusion criteria for samples from eight European countries, and looked at word-reading and spelling performance as quantitative variables, was unable to find any significant association in meta-analysis of candidate dyslexia-genes (Becker et al., 2014).

By measuring skills such as phoneme awareness, rapid naming, and phonological short term memory, that may be endophenotypes with underlying roles in reading, more homogeneous results might be expected in genetic studies, because even if these processes impact differently on reading in different groups or populations

(Landerl et al., 2013; Caravolas et al., 2013), their genetic architectures may be less heterogeneous than reading ability itself. However, studies that have looked for genetic associations with these supposedly intermediate phenotypes have generally targeted different traits (depending on the available data) and focused on only a few candidate SNPs each (see Chapter 2 and Chapter 3). Comparing or meta-analysing genetic association results across different measures in different datasets is justified when pleiotropic/multivariate genetic effects are expected of the variants (also in Chapter 4). However, even if a variant can affect more than one trait, it is likely that the strength of the association will differ between these traits. Genome-wide meta-analyses of genetic association for reading-related endophenotypes have only just started to be performed (Luciano et al., 2013; Gialluisi et al., 2014).

Reading experience also influences these quantitative traits: for example it has been shown that phonological awareness is affected by literacy (Morais et al., 1979). Furthermore, a comparison between literate and illiterate people's brain activity responses during speech and visual processing tasks suggests that learning to read reorganizes the brain to an extent by enhancing responses at the visual cortices (e.g. in the visual word form area), by activating the left-lateralized language network upon the presentation of written sentences, and by enhancing activation of the regions involved in phonological coding (planum temporale) during speech perpection (Dehaene et al., 2010, 2015). Thus, literacy acquisition affects endophenotypes at the behavioural and brain level. The coming efforts for large sample GWAS meta-analysis studies should be careful to ensure an adequate handling of the phenotypic information, especially when samples contain children at different stages of learning to read. These studies will also yield evidence to re-evaluate the role of current dyslexia candidate genes in the literature.

7.4 UNRAVELING THE GENOME: PROMISES AND CHALLENGES

High throughput sequencing technologies have revolutionized genomic research, as virtually the whole genome or exome are now accessible for mutation screening. This provides the opportunity, from a single dataset, to simultaneously consider different classes of genetic variants: in terms of frequency (i.e. new or rare mutations in addition to common variants), as well as type (e.g. SNVs, indels and even structural variants). The family-based design that I adopted in Chapters 5 and 6 is a powerful approach to examine rare genetic variants contributing to dyslexia using NGS tech-

nology. The main assumption underlying this approach is that there are one or a few genetic variants that account for dyslexia within these families (the mono/oligogenic hypothesis), which is motivated by the apparent inheritance pattern and high recurrence of dyslexia in these unusual families.

In Chapter 5 I did not find any variant that could fully explain the dyslexic phenotype in family 259, in spite of the linked regions on chromosome Xq27.3 and on chromosome 20q11.23. It is possible that a complex genetic aetiology underlies the inheritance pattern in this family. Several rare and new mutations were identified within genes involved in potentially relevant biological pathways such as calcium regulation within neurons (*CACNA2D3*), cortical development (*EOMES*, also known as *TBR2*), or related to other disorders such as intellectual disability (*AFF2).* However, whether any of these mutations is causally related to dyslexia remains to be confirmed. Alternatively, a mutation with a large effect may have been missed. It is unlikely yet possible that such a 'causal' mutation was not detected by the sequencing. In order to reduce this possibility, WES and WGS were performed in several samples from the family. Another explanation could be that we were unable to interpret the biological relevance of a co-segregating variant that was present in the data. This would be more likely in the case of noncoding variants, since large-scale interpretation of this variation currently depends on automated annotations based on several sources of information, and experimental algorithms. Recent efforts have achieved the simplification of multiple sources of information (e.g. evolutionary conservation, effects on expression levels) into one or a few scores that can easily be used for variant ranking and filtering (Kircher et al., 2014; Ritchie et al., 2014). Nevertheless, these are dependent on currently available and imperfect knowledge. Each of these algorithms also makes different assumptions regarding how to define pathogenicity or functionality, and even when they aim to target the same aspect of variant functionality, they are not always congruent with each other (see Chapter 5 and Chapter 6). Furthermore, the evaluation of variants that are not present in other datasets (i.e. newly discovered mutations in a given study) is still a challenge for many noncoding variants.

Functional characterization of genetic variants in molecular assays remains the 'gold standard' way to evaluate both coding and noncoding variants. For instance, through a battery of molecular assays for *FOXP1 de novo* coding mutations that were thought to be causing intellectual disability, Sollis et al. (2016) were able to discern likely aetiological mutations from others of unknown significance. However, these

assays are time-consuming and it is not yet feasible to use them systematically to filter out or rank variants from sequencing studies. Furthermore, the exact type of molecular assays should be appropriate to assess the specific, potential biological function that each type of variant might have. Nevertheless, the NGS-based data presented here will need to be backed up by functional work, in order to translate the genomic findings into biological mechanisms.

7.5 GENES, READING, AND THE BRAIN

Recently, brain-related endophenotypes have been studied in relation to the genetics of reading ability (Roeske et al., 2011; Pinel et al., 2012; Darki et al., 2012; Skeide et al., 2015). These measures (e.g. volumes of brain regions, white matter track integrity, brain activity as measured by BOLD signal, event related potentials) are thought to capture either the physical substrate for the realization of the cognitive processes that underlie reading, or the electrophysiological readouts of those processes. Accordingly, they should be influenced by genetic variants that affect reading ability. It is possible that some of these measures are stable across populations, as there seems to be a convergent network of brain areas that are activated in reading across several orthographic and writing systems (as measured by fMRI) (Rueckl et al., 2015), and a recent meta-analysis of functional neuroimaging studies comparing dyslexic and control readers in deep and shallow orthographies also found support for a consistent neurobiological pattern for dyslexia (Martin et al., 2016). However, the same studies also revealed small activation differences across languages for the brain areas implicated in reading (Rueckl et al., 2015), or showing differences between dyslexics and controls (Martin et al., 2016). Hence, the identification of brain endophenotypes that are even more stable would be helpful for future studies. Some fairly robust measures include: the auditory mismatch negativity, the size of the planum temporale, or the white matter integrity or morphology of the arcuate fasciculus nerve fibre tract. The auditory mismatch negativity (MMN) is an automatic brain response (event-related potential) to auditory discriminable stimuli (Naatanen, 2001) which differs between dyslexic and control adults (Schulte-Korne et al., 2001) and children (Neuhoff et al., 2012), and is also reduced in pre-literate, children who are at-risk for dyslexia (assessed on the basis of familial dyslexia), suggesting that this measure indexes susceptibility to reading difficulties rather than a consequence of them (van Zuijen et al., 2012). The planum temporale (PT) is a highly asymmetric region in the

temporal lobe, involved in auditory processing, that seems to have reversed asymmetry in dyslexic males (Galaburda et al., 1985; Altarelli et al., 2014). The asymmetry of the PT (i.e. left PT > right PT) has been described early in development (e.g. even in fetuses, (Chi et al., 1977))), suggesting that differences in this early asymmetric landmark could constrain reorganization of brain circuitry that occurs when learning to read (Altarelli et al., 2014; Dehaene et al., 2015). The arcuate fasciculus (AF) is a white matter tract connecting the frontal and temporal lobe regions involved in reading and language. Dyslexics presented reduced integrity (measured as fractional anisotropy) of the left AF when compared to controls (Vandermosten et al., 2012).

Several imaging genetic studies for reading ability have been performed so far, assessing a varied spectrum of endophenotypes including the AF (Skeide et al., 2015), MMN (Roeske et al., 2011), white and grey matter volumes (Darki et al., 2012) and task-related activation of regions of interest (Pinel et al., 2012). However, these studies generally used small sample sizes (from tens to a few hundreds), and in most cases were restricted to small numbers of SNPs within candidate genes.

A brain-oriented approach could help to elucidate the causal mechanisms from genes to behaviour. There are several possible causal directions. The most direct would go from genes to brain to behaviour, but more complex ones are also possible, as for example from genes to behaviour to brain. For instance, if a person is more highly motivated to indulge in reading behaviours for some genetically mediated reason, the reading behaviour could result in changes of reading-related structures in the brain. As a result, a genetic association could be observed between a SNP and brain structures, which would have been mediated by the behaviour. The establishment of the causality could be best accomplished through a research strategy that integrates data from brain imaging endophenotypes together with reading ability and related quantitative traits, ideally all in the same datasets. For this strategy, substantial sample sizes will be required, as the genetic effects on brain phenotypes are expected to be as small as the effects on behavioural traits (Hibar et al., 2015). Potentially, this approach should not only enable us to learn more about the genetic underpinnings of reading ability and its neurobiological substrates, but it may also help to inform us about cause-effect relationships between different psychometric and brain measures, for example through the use of mediation analysis.

7.6 CONCLUSION

Reading is a relatively recent human cultural trait (just a few thousand years old). Understanding how we are able to learn this highly specialized skill requires descriptions at multiple levels. On the one hand, well characterized reading and intermediate phenotypes are required (including both behavioural and brain measures). On the other hand, we need to identify genes that relate to the behavioural differences, and understand how they affect the development and function of the brain by looking at the cellular function of the proteins they encode and the biological pathways in which they are involved. In order to build a comprehensive account of the reading phenotype, it will be necessary to integrate the results from the different strategies and levels of description.

In this thesis, I approached the genetic basis of reading ability using complementary state-of-the-art strategies: by evaluating the role of common SNPs in known candidate genes, and by searching for rare, highly-penetrant genetic variants that could cause strongly familial forms of dyslexia. I related these investigations to knowledge about cognitive performance measures and molecular mechanisms. The findings from my thesis provide important new entry-points for future work bridging the gap between our genetic make-up and reading abilities.

REFERENCES

Adler WT, Platt MP, Mehlhorn AJ, Haight JL, Currier TA, et al. (2013) Position of neocortical neurons transfected at different gestational ages with shRNA targeted against candidate dyslexia susceptibility genes. PLoS ONE 8: e65179.

Altarelli I, Leroy F, Monzalvo K, Fluss J, Billard C, et al. (2014) Planum temporale asymmetry in developmental dyslexia: Revisiting an old question. Hum Brain Mapp 35: 5717–5735.

Andrews W, Barber M, Hernadez-Miranda LR, Xian J, Rakic S, et al. (2008) The role of Slit-Robo signaling in the generation, migration and morphological differentiation of cortical interneurons. Dev Biol 313: 648–658.

Andrews W, Liapi A, Plachez C, Camurri L, Zhang J, et al. (2006) Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain. Development 133: 2243–2252.

Baek ST, Kerjan G, Bielas SL, Lee JE, Fenstermaker AG, et al. (2014) Off-target effect of doublecortin family shRNA on neuronal migration associated with endogenous microRNA dysregulation. Neuron 82: 1255–1262.

Bates TC, Luciano M, Medland SE, Montgomery GW, Wright MJ, et al. (2011) Genetic variance in a component of the language acquisition device: ROBO1 polymorphisms associated with phonological buffer deficits. Behav Genet 41: 50–57.

Becker J, Czamara D, Scerri TS, Ramus F, Csepe V, et al. (2014) Genetic analysis of dyslexia candidate genes in the European cross-linguistic NeuroDys cohort. Eur J Hum Genet 22: 675–680.

Caravolas M, Lervag A, Defior S, Seidlova Malkova G, Hulme C (2013) Different patterns, but equivalent predictors, of growth in reading in consistent and inconsistent orthographies. Psychol Sci 24: 1398–1407.

Chandrasekar G, Vesterlund L, Hultenby K, Tapia-Paez I, Kere J (2013) The zebrafish orthologue of the dyslexia candidate gene DYX1C1 is essential for cilia growth and function. PLoS ONE 8: e63123.

Chen G, Sima J, Jin M, Wang KY, Xue XJ, et al. (2008) Semaphorin-3A guides radial migration of cortical neurons during development. Nat Neurosci 11: 36–44.

Chi JG, Dooling EC, Gilles FH (1977) Left-right asymmetries of the temporal speech areas of the human fetus. Arch Neurol 34: 346–348.

Currier TA, Etchegaray MA, Haight JL, Galaburda AM, Rosen GD (2011) The effects of embryonic knockdown of the candidate dyslexia susceptibility gene homologue

Dyx1c1 on the distribution of GABAergic neurons in the cerebral cortex. Neuroscience 172: 535–546.

Darki F, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T (2012) Three Dyslexia Susceptibility Genes, DYX1C1, DCDC2, and KIAA0319, Affect Temporo-Parietal White Matter Structure. Biol Psychiatry 72: 671–676.

de Kovel CG, Hol FA, Heister JG, Willemen JJ, Sandkuijl LA, et al. (2004) Genome-wide scan identifies susceptibility locus for dyslexia on Xq27 in an extended Dutch family. J Med Genet 41: 652–657.

Dehaene S, Cohen L, Morais J, Kolinsky R (2015) Illiterate to literate: behavioural and cerebral changes induced by reading acquisition. Nat Rev Neurosci 16: 234–244.

Dehaene S, Pegado F, Braga LW, Ventura P, Nunes Filho G, et al. (2010) How learning to read changes the cortical networks for vision and language. Science 330: 1359–1364.

Dennis MY, Paracchini S, Scerri TS, Prokunina-Olsson L, Knight JC, et al. (2009) A common variant associated with dyslexia reduces expression of the KIAA0319 gene. PLoS Genet 5: e1000436.

Eicher JD, Powers NR, Miller LL, Akshoomoff N, Amaral DG, et al. (2013) Genome-wide association study of shared components of reading disability and language impairment. Genes Brain Behav 12: 792–801.

Field LL, Shumansky K, Ryan J, Truong D, Swiergala E, et al. (2013) Dense-map genome scan for dyslexia supports loci at 4q13, 16p12, 17q22; suggests novel locus at 7q36. Genes Brain Behav 12: 56–69.

Galaburda AM, Sherman GF, Rosen GD, Aboitiz F, Geschwind N (1985) Developmental dyslexia: four consecutive patients with cortical anomalies. Ann Neurol 18: 222–233.

Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, et al. (2014) Genome-wide screening for DNA variants associated with reading and language traits. Genes Brain Behav 13: 686–701.

Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, et al. (2005) The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia. PLoS Genet 1: e50.

Hibar DP, Stein JL, Renteria ME, Arias-Vasquez A, Desrivieres S, et al. (2015) Common genetic variants influence human subcortical brain structures. Nature 520: 224–229.

Ivliev AE, 't Hoen PA, van Roon-Mom WM, Peters DJ, Sergeeva MG (2012) Exploring the transcriptome of ciliated cells using in silico dissection of human tissues. PLoS ONE 7: e35618.

Kere J (2014) The molecular genetics and neurobiology of developmental dyslexia as model of a complex phenotype. Biochem Biophys Res Commun 452: 236–243.

Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, et al. (2014) A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46: 310–315.

Landerl K, Ramus F, Moll K, Lyytinen H, Leppanen PH, et al. (2013) Predictors of developmental dyslexia in European orthographies with varying complexity. J Child Psychol Psychiatry 54: 686–694.

Landerl K, Wimmer H, Frith U (1997) The impact of orthographic consistency on dyslexia: a German-English comparison. Cognition 63: 315–334.

Luciano M, Evans DM, Hansell NK, Medland SE, Montgomery GW, et al. (2013) A genome-wide association study for reading and language abilities in two population cohorts. Genes Brain Behav 12: 645–652.

Malik MF, Satherley LK, Davies EL, Ye L, Jiang WG (2016) Expression of Semaphorin 3C in Breast Cancer and its Impact on Adhesion and Invasion of Breast Cancer Cells. Anticancer Res 36: 1281–1286.

Martin A, Kronbichler M, Richlan F (2016) Dyslexic brain activation abnormalities in deep and shallow orthographies: A meta-analysis of 28 functional neuroimaging studies. Hum Brain Mapp .

Massinen S, Hokkanen ME, Matsson H, Tammimies K, Tapia-Paez I, et al. (2011) Increased expression of the dyslexia candidate gene DCDC2 affects length and signaling of primary cilia in neurons. PLoS ONE 6: e20580.

McGeown SP, Medford E, Moxon G (2013) Individual differences in children's reading and spelling strategies and the skills supporting strategy use. Learning and Individual Differences 28: 75 – 81.

Meng H, Powers NR, Tang L, Cope NA, Zhang PX, et al. (2011) A dyslexia-associated variant in DCDC2 changes gene expression. Behav Genet 41: 58–66.

Meng H, Smith SD, Hager K, Held M, Liu J, et al. (2005) DCDC2 is associated with reading disability and modulates neuronal development in the brain. Proc Natl Acad Sci USA 102: 17053–17058.

Morais J, Cary L, Alegria J, Bertelson P (1979) Does awareness of speech as a sequence of phones arise spontaneously? Cognition 7: 323–331.

Naatanen R (2001) The perception of speech sounds by the human brain as reflected by the mismatch negativity (MMN) and its magnetic equivalent (MMNm). Psychophysiology 38: 1–21.

Neuhoff N, Bruder J, Bartling J, Warnke A, Remschmidt H, et al. (2012) Evidence for the late MMN as a neurophysiological endophenotype for dyslexia. PLoS ONE 7: e34909.

Paracchini S, Scerri T, Monaco AP (2007) The genetic lexicon of dyslexia. Annu Rev Genomics Hum Genet 8: 57–79.

Peschansky VJ, Burbridge TJ, Volz AJ, Fiondella C, Wissner-Gross Z, et al. (2010) The effect of variation in expression of the candidate dyslexia susceptibility gene homolog Kiaa0319 on neuronal migration and dendritic morphology in the rat. Cereb Cortex 20: 884–897.

Peter B, Matsushita M, Oda K, Raskind W (2014) De novo microdeletion of BCL11A is associated with severe speech sound disorder. Am J Med Genet A 164A: 2091–2096.

Pinel P, Fauchereau F, Moreno A, Barbot A, Lathrop M, et al. (2012) Genetic variants of FOXP2 and KIAA0319/TTRAP/THEM2 locus are associated with altered brain activation in distinct language-related regions. J Neurosci 32: 817–825.

Plein A, Calmont A, Fantin A, Denti L, Anderson NA, et al. (2015) Neural crest-derived SEMA3C activates endothelial NRP1 for cardiac outflow tract septation. J Clin Invest 125: 2661–2676.

Powers NR, Eicher JD, Butter F, Kong Y, Miller LL, et al. (2013) Alleles of a Polymorphic ETV6 Binding Site in DCDC2 Confer Risk of Reading and Language Impairment. Am J Hum Genet 93: 19–28.

Psychiatric Genomics Consortium SWGot (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511: 421–427.

Ritchie GR, Dunham I, Zeggini E, Flicek P (2014) Functional annotation of noncoding sequence variants. Nat Methods 11: 294–296.

Roeske D, Ludwig KU, Neuhoff N, Becker J, Bartling J, et al. (2011) First genome-wide association scan on neurophysiological endophenotypes points to trans-regulation effects on SLC2A3 in dyslexic children. Mol Psychiatry 16: 97–107.

Rueckl JG, Paz-Alonso PM, Molfese PJ, Kuo WJ, Bick A, et al. (2015) Universal brain signature of proficient reading: Evidence from four contrasting languages. Proc Natl Acad Sci USA 112: 15510–15515.

Schulte-Korne G, Deimel W, Bartling J, Remschmidt H (2001) Speech perception deficit in dyslexic adults as measured by mismatch negativity (MMN). Int J Psychophysiol 40: 77–87.

Skeide MA, Kirsten H, Kraft I, Schaadt G, Muller B, et al. (2015) Genetic dyslexia risk variant is related to neural connectivity patterns underlying phonological awareness in children. Neuroimage 118: 414–421.

Sollis E, Graham SA, Vino A, Froehlich H, Vreeburg M, et al. (2016) Identification and functional characterization of de novo FOXP1 variants provides novel insights into the etiology of neurodevelopmental disorder. Hum Mol Genet .

Szalkowski CE, Fiondella CF, Truong DT, Rosen GD, LoTurco JJ, et al. (2013) The effects of Kiaa0319 knockdown on cortical and subcortical anatomy in male rats. Int J Dev Neurosci 31: 116–122.

Tarkar A, Loges NT, Slagle CE, Francis R, Dougherty GW, et al. (2013) DYX1C1 is required for axonemal dynein assembly and ciliary motility. Nat Genet 45: 995–1003.

Tran C, Gagnon F, Wigg KG, Feng Y, Gomez L, et al. (2013) A family-based association analysis and meta-analysis of the reading disabilities candidate gene DYX1C1. Am J Med Genet B Neuropsychiatr Genet 162: 146–156.

van Zuijen TL, Plakas A, Maassen BA, Been P, Maurits NM, et al. (2012) Temporal auditory processing at 17 months of age is associated with preliterate language comprehension and later word reading fluency: An ERP study. Neurosci Lett 528: 31–35.

Vandermosten M, Boets B, Wouters J, Ghesquiere P (2012) A qualitative and quantitative review of diffusion tensor imaging studies in reading and dyslexia. Neurosci Biobehav Rev 36: 1532–1552.

Visscher PM, Brown MA, McCarthy MI, Yang J (2012) Five years of GWAS discovery. Am J Hum Genet 90: 7–24.

Wang Y, Yin X, Rosen G, Gabel L, Guadiana SM, et al. (2011) Dcdc2 knockout mice display exacerbated developmental disruptions following knockdown of doublecortin. Neuroscience 190: 398–408.

Wiegreffe C, Simon R, Peschkes K, Kling C, Strehle M, et al. (2015) Bcl11a (Ctip1) Controls Migration of Cortical Projection Neurons through Regulation of Sema3c. Neuron 87: 311–325.

Zhong R, Yang B, Tang H, Zou L, Song R, et al. (2013) Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol 47: 435–442.

Zou L, Chen W, Shao S, Sun Z, Zhong R, et al. (2012) Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 159B: 970–976.

NEDERLANDSE SAMENVATTING

Ik lees elke dag wel iets: een roman, de krant, emails en, als ik geluk heb, zelfs een handgeschreven brief. Ik lees ook op straat: de namen van treinstations, een paar zinnen van een advertentie, de namen van verschillende gebakjes bij de bakker. Lezen is een integraal onderdeel van mijn leven. Toch is lezen niet vanzelfsprekend: we moeten leren lezen en dit is niet voor iedereen even makkelijk. Net zoals sommige mensen langer zijn dan andere, lezen sommige mensen beter dan andere. Leesvaardigheid kan worden voorgesteld als een eigenschap met een continue distributie binnen de bevolking, net als bijvoorbeeld lichaamslengte. Mensen die onder een bepaalde drempelwaarde van leesvaardheidscores vallen, worden als dyslectisch beschouwd. Omdat lezen een complexe taak is, zijn er verschillende factoren (zowel genetische factoren als omgevingsfactoren) die de leesvaardigheid van mensen beïnvloeden. Het doel van dit proefschrift was om de genetische basis van individuele verschillen in leesvaardigheid te verhelderen.

Het literatuuroverzicht in Hoofdstuk 2 bood een gedegen overzicht van de bekende moleculaire basis van dyslexie. In het bijzonder heb ik statistisch en biologisch bewijs beoordeeld voor de bijdrage van de meest prominente kandidaatsgenen in de literatuur, namelijk *DYX1C1, DCDC2, KIAA0319* en *ROBO1* aan dyslexie en andere leesgerelateerde eigenschappen. Aan deze genen wordt betrokkenheid bij dyslexie toegeschreven in verschillende, aanvullende, bewijsvoeringen: ze vallen binnen regio's van het genoom die geassocieerd zijn met aanleg voor dyslexie; posities in het genoom die variatie in de bevolking bevatten (dat wil zeggen genetische varianten) binnen of aangrenzend aan deze genen zijn gerelateerd aan lezen en gerelateerde eigenschappen; moleculaire studies hebben onthuld dat sommige van deze genetische varianten betrokken zouden kunnen zijn bij genregulatie; en diermodellen met aangepaste genfunctionaliteit laten verschillen in relevante breineigenschappen zien (zoals abnormale migratie van corticale neuronen). Bovendien zijn sommige van deze varianten recent onderzocht met neuroimaging, die onderzoekers ertoe hebben geleid te concluderen dat zij geassocieerd zijn met hersengebieden die betrokken zijn bij het lezen. Alles bij elkaar hebben deze studies de betrokkenheid van deze genen in de neurobiologie die leesvaardigheid ondersteunt gestaafd. Maar tegelijkertijd bevat

het onderzoeksgebied contrasterende resultaten, met verscheidene non-replicaties en tegenovergestelde richting van effecten, die voortgang op basis van de bekende data belemmeren.

In de Hoofdstukken 3 en 4 heb ik de veelvoorkomende genetische variatie (dat wil zeggen vaak voorkomend in de bevolking) geëvalueerd waarvan wordt voorgesteld dat zij leesvaardigheid en dyslexie beïnvloeden. Van deze veelvoorkomende varianten verwacht men dat ze op een additieve manier als risicofactoren fungeren, waarbij ze elk een zeer kleine fractie van de variatie in leesvaardigheid verklaren.

In Hoofdstuk 3, heb ik me gericht op veertien van de meest consistent geassociëerde veelvoorkomende enkel-nucleotide polymorfieën (single nucleitide variants of SNPs; genetische varianten die een enkele positie in het genoom beïnvloeden) uit de literatuur, die binnen de kandidaatgenen *DYX1C1, KIAA0319, DCDC2, CNTNAP2* vielen. Ik heb deze SNPs beoordeeld in relatie tot verschillende leesgerelateerde eigenschappen (vloeiend lezen, snel woorden opzeggen, foneembewustzijn en niet-woord herhaling) in de longitudinale dataset van het Dutch Dyslexia Programme (DDP). Elk van deze eigenschappen meet verschillende processen die betrokken zijn bij het lezen. Er is bewijs gevonden voor de associatie van 5 SNPs met verschillende eigenschappen, maar voor de meeste van deze effecten was de directionaliteit niet overeenomstig met eerdere studies. Desalniettemin, waren twee SNPs binnen het KIAA0319 gen consistent geassociëerd met het snel opzeggen van worden over 4 ontwikkelingstijdpunten (7-12 jaar). Dit onderzoek laat zien hoe ontwikkelingsinformatie kan worden gebruikt bij het onderzoek naar genetische effecten op lezen.

In de afgelopen paar jaar heeft een golf van genoombrede associatie studies (genome-wide association studies of GWAS) geprobeerd om veelvoorkomende genetische varianten te vinden die taal- en leesvaardigheid beïnvloeden, zonder voorafgaande hypotheses met betrekking tot specifieke kandidaatsgenen of regio's van het genoom. Deze nieuwe onderzoeksgolf in het veld bestudeerd het gehele genoom voor associatie op een relatief onbevooroordeelde manier, wat geschikt is voor complexe eigenschappen waarvoor de overgrote meerderheid van de onderliggende genetische architectuur onbekend is. In Hoofdstuk 4 heb ik de meest significante associaties van de GWAS studies van taal en leesprestatie geëvalueerd door hen te testen op leesgerelateerde kwantitatieve eigenschappen in FIOLA, een nieuwe en onafhankelijke dataset van kinderen en volwassenen uit de Nederlandse bevolking. Ik heb zeventien SNPs geselecteerd om te bestuderen en heb hun effecten apart getest voor volwassenen en kinderen. In het algemeen bood dit onderzoek weinig onderbouwing voor de

varianten die eerder in GWAS zijn gevonden, wat suggereert dat zulke studies in de toekomst zullen moeten worden uitgevoerd met gebruikmaking van meta-datasets die veel groter zijn dan die die tot nu toe zijn gebruikt.

Zeldzame genetische variatie zou ook kunnen bijdragen tot leesvaardigheid. In Hoofdstuk 5 en 6 heb ik een gangbare benadering genomen om te zoeken naar zeldzame (niet veelvoorkomend in de bevolking) varianten die het erfelijkheidspatroon van dyslexie in twee multigenerationele families zouden kunnen verklaren. Deze families worden gekenmerkt door de relatief hoge prevalentie van dyslexie ($\sim$ 50% in vergelijking met $\sim$5-10% dat wordt bericht voor de bevolking). Hiervoor heb ik eerst de regio's van het genoom gedefiniëerd die hetzelfde erfelijkheidspatroon hebben als dat van dyslexie in ieder van de families, en heb ik deze informatie gecombineerd met de sequentie van een paar sleutelindividuen. Deze strategie biedt de mogelijkheid om het aantal kandidaatsvarianten dat dyslexie zou kunnen beïnvloeden terug te brengen.

In Hoofdstuk 5 werden geen kandidaatsvarianten geïdentificeerd die oorzakelijk zouden kunnen zijn voor dyslexie op de manier van dominante mutaties die een een-op-een overeenkomst hebben met dyslexie. Desalniettemin werd een aantal zeldzame varianten geïdentificeerd dat zou kunnen bijdragen aan dyslexiegevoeligheid in deze familie, waaronder varianten in de genen *CACNA2D3*, *RBMX* en *AFF2*. Het is dus waarschijnlijk dat er geen enkele mutatie op zichzelf verantwoordelijk is voor het voorkomen van dyslexie in deze familie, en dat een meer complexe genetische achtergrond aanwezig is.

In Hoofdstuk 6 heb ik een regio gevonden op chromosoom 7q21.11 dat gelinkt is aan dyslexie. Twee zeldzame enkel-nucleotide polymorfieën binnen het *SEMA3C* gen erfden over met deze regio van het genoom en erfden bovendien bijna perfect over met de dyslexie binnen de familie (12 van de 14 dyslectische familieleden waren dragers van de risicovariant, tegen geen enkele van de niet-dyslectische familieleden). Deze twee varianten zijn intronisch: ze bevinden zich binnen het gen maar beïnvloeden het eiwit niet. Beide varianten liggen binnen een mogelijke regulatorische regio en *in silico* karakterisatie van de regio suggereerde dat een van de varianten (rs144517871) een goede kandidaat is voor regulatie van *SEMA3C* . Dus stellen wij *SEMA3C* voor als een kandidaatsgen voor dyslexie en veronderstellen we dat deze intronische varianten een regulatorisch effect zouden kunnen hebben op de expressieniveaus van het gen.

Samenvattend heb ik in dit proefschrift de genetische basis van het lezen benaderd door gebruik te maken van complementaire state-of-the-art strategieën: door de rol van veelvoorkomende SNPs in bekende kandidaatsgenen te evalueren, en door te zoeken naar zeldzame, hoogpenetrante genetische varianten die sterk erfelijke varianten van dyslexie zouden kunnen veroorzaken. Ik heb deze onderzoeken gerelateerd aan kennis over cognitieve prestatieindicatoren en moleculaire mechanismen.

ACKNOWLEDGEMENTS

Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
(Antonio Machado, *Cantares*)

Wanderer, your footsteps are
the path, and nothing else;
wanderer, there is no path,
the path is made by walking.
(Trans. Betty Jean Craige)

This particular path has been a path of search, and as such a pleasure. I would like to express my gratitude to the many people that have accompanied me during these years.

I have navigated different seas, and despite sometimes feeling seasick, I ended up eager to explore new oceans. For this journey, I have to thank my supervisors Simon E. Fisher and Clyde Francks. For the opportunity, the resources and their guidance. For letting me explore, while providing a clear reference and framework. For pointing out incoherences, and forcing me to spell out and understand each choice, as well as its assumptions. For directing me (and the writing of the present thesis), even when I chose to be in the antipodes.

Next, I would like to thank my collaborators, who collected most of the data in these chapters, and provided useful insight and feedback to understand and analyze it better. To everyone from the Dutch Dyslexia Programme, and in particular Ben Maassen, Elsje van Bergen and Barbara Franke.

Thank you also to my reading committee - Anneke van Hollander, Juha Kere and Gerd Schulte-Körne - and all the corona members, for taking their time to evaluate this work.

I would also like to thank to everyone at the Radboud 'NGS multifactorial disease' sessions for providing a regular meeting point to discuss pipelines and workflows.

I am also deeply grateful to Martina Bernhard for her assistance before, during and after the duration of my PhD. Thanks also to everyone from the IMPRS office - Dirkje van der Aa, Els den Os and Kevin Lam - for the continuous support. Tobias van Valkenhoef, Herbert Baumann and Reiner Dirksmeyer, and everyone else in the TG -

thank you for your assistance and for ensuring that everything is running smoothly. I am also indebted to Floris de Vries for the Dutch summary, as well as for many insightful conversations.

So many times at the MPI, we have heard about the importance of language (for the 'human being/species'). As an individual too, having good conversations has been utterly important to me:

For multiple years, I had the chance to discuss about anything with Kate: how to get our science correct, how to correct the world, and last but not least, how to get the correct bread. With Amanda, the conversation continues. Thank you both for being great officemates. Sylvia showed me tiny bits of hugely useful code, and patiently listened while I elaborated on my confusion regarding analysis or results. I frequently knocked other doors in the corridor to share frustration or infinitesimal satisfaction. Thank you to everyone behind those doors: Alessandro, Tulya, Nicolas, Else, Carolien, Beate, Rick. Dan gets a special mention for always choosing the impossible but most interesting question. To the *functional* people: thank you for tolerating my intrusions into the wetlab, for sharing coffee and advice. Special thanks to Arianna, for rounds of genotyping, primer advice and general generous help. Other colleagues and friends at the MPI have helped me to widen the perspective, to zoom out from the specifics to the more general: Francisco, Harald, Sara I, Franziska, Carmen, Ely, Julija, Marisa, Sean, Julia, Markus, Floris and many others.

Sara, compañera de estreses y de estar tranquilas. Gracias por el trabajo y el no trabajo. Espero que nuestros caminos vuelvan a juntarse, i que contiuarem amb la xerrameca. Gaby, gracias por tantos capuchinos matutinos y tardíos, pero sobre todo, por los cotilleos. Tulio, gracias por ser mi mentor. Por escuchar mis razonamientos sin razón, y ayudarme a entender mejor a la ciencia y su embalaje. Ewelina, gracias por tu perseverancia, con tanto headstand me trasmites fuerza. Graciès a Joan pel català, els frisbies i els cocines! For the walks in the woods, the coffees, and everything else, thanks also to Britt (and Silas and Juniper).

Last but not least, thank you to so many others who have crossed and meaningfully shaped this road, be it in Nijmegen, Amsterdam, Paris, Barcelona, Donosti, or elsewhere.

Aita, ama, eta Irati, eskerrik asko kilometroak gora, kilometroak behera, egunerokotasun bat eta etxekotasun bat eskeintzeagatik. Mila esker Varuni, bidailagun izateagatik.

CURRICULUM VITAE

Amaia Carrión Castillo studied Biology at the University of Barcelona and received her degree in 2011. During her studies, she gained experience with molecular genetic research through several internships at the Genetics Departments of the University of Barcelona and Leicester University, as well as at the Institute of Molecular Pathology in Vienna. In 2011, she was introduced into the cognitive sciences during another internship at the Laboratoire de Sciences Cognitives et Psycholinguistiques (Paris). On that same year, she was awarded a 'la Caixa' scholarship for postgraduate studies to pursue her research master in Cognitive sciences at the École Normale Supérieure in Paris, completed in 2012. In her master thesis, she investigated the cerebral correlates of morphological and syntactic complexity in Basque. From 2012 to 2016 she carried out her PhD project at the Language and Genetics Department at the Max Planck Institute for Psycholinguistics (Nijmegen). Her research focused on the genetic underpinnings of reading ability by studying common and rare genetic variants that could affect its variability.

MPI SERIES IN PSYCHOLINGUISTICS

1. The electrophysiology of speaking: Investigations on the time course of semantic, syntactic, and phonological processing. *Miranda van Turennout*
2. The role of the syllable in speech production: Evidence from lexical statistics, metalinguistics, masked priming, and electromagnetic midsagittal articulography. *Niels O. Schiller*
3. Lexical access in the production of ellipsis and pronouns. *Bernadette M. Schmitt*
4. The open-/closed-class distinction in spoken-word recognition. *Alette Haveman*
5. The acquisition of phonetic categories in young infants: A self-organising artificial neural network approach. *Kay Behnke*
6. Gesture and speech production. *Jan-Peter de Ruiter*
7. Comparative intonational phonology: English and German. *Esther Grabe*
8. Finiteness in adult and child German. *Ingeborg Lasser*
9. Language input for word discovery. *Joost van de Weijer*
10. Inherent complement verbs revisited: Towards an understanding of argument structure in Ewe. *James Essegbey*
11. Producing past and plural inflections. *Dirk Janssen*
12. Valence and transitivity in Saliba: An Oceanic language of Papua New Guinea. *Anna Margetts*
13. From speech to words. *Arie van der Lugt*
14. Simple and complex verbs in Jaminjung: A study of event categorisation in an Australian language. *Eva Schultze-Berndt*

15. Interpreting indefinites: An experimental study of children's language comprehension. *Irene Krämer*
16. Language-specific listening: The case of phonetic sequences. *Andrea Weber*
17. Moving eyes and naming objects. *Femke van der Meulen*
18. Analogy in morphology: The selection of linking elements in Dutch compounds. *Andrea Krott*
19. Morphology in speech comprehension. *Kerstin Mauth*
20. Morphological families in the mental lexicon. *Nivja H. de Jong*
21. Fixed expressions and the production of idioms. *Simone A. Sprenger*
22. The grammatical coding of postural semantics in Goemai (a West Chadic language of Nigeria). *Birgit Hellwig*
23. Paradigmatic structures in morphological processing: Computational and cross-linguistic experimental studies. *Fermín Moscoso del Prado Martín*
24. Contextual influences on spoken-word processing: An electrophysiological approach. *Daniëlle van den Brink*
25. Perceptual relevance of prevoicing in Dutch. *Petra M. van Alphen*
26. Syllables in speech production: Effects of syllable preparation and syllable frequency. *Joana Cholin*
27. Producing complex spoken numerals for time and space. *Marjolein Meeuwissen*
28. Morphology in auditory lexical processing: Sensitivity to fine phonetic detail and insensitivity to suffix reduction. *Rachèl J. J. K. Kemps*
29. At the same time…: The expression of simultaneity in learner varieties. *Barbara Schmiedtová*
30. A grammar of Jalonke argument structure. *Friederike Lüpke*
31. Agrammatic comprehension: An electrophysiological approach. *Marlies Wassenaar*

32. The structure and use of shape-based noun classes in Miraña (North West Amazon). *Frank Seifart*

33. Prosodically-conditioned detail in the recognition of spoken words. *Anne Pier Salverda*

34. Phonetic and lexical processing in a second language. *Mirjam Broersma*

35. Retrieving semantic and syntactic word properties. *Oliver Müller*

36. Lexically-guided perceptual learning in speech processing. *Frank Eisner*

37. Sensitivity to detailed acoustic information in word recognition. *Keren B. Shatzman*

38. The relationship between spoken word production and comprehension. *Rebecca Özdemir*

39. Disfluency: Interrupting speech and gesture. *Mandana Seyfeddinipur*

40. The acquisition of phonological structure: Distinguishing contrastive from non-contrastive variation. *Christiane Dietrich*

41. Cognitive cladistics and the relativity of spatial cognition. *Daniel B.M. Haun*

42. The acquisition of auditory categories. *Martijn Goudbeek*

43. Affix reduction in spoken Dutch. *Mark Pluymaekers*

44. Continuous-speech segmentation at the beginning of language acquisition: Electrophysiological evidence. *Valesca Kooijman*

45. Space and iconicity in German Sign Language (DGS).*Pamela Perniss*

46. On the production of morphologically complex words with special attention to effects of frequency. *Heidrun Bien*

47. Crosslinguistic influence in first and second languages: Convergence in speech and gesture. *Amanda Brown*

48. The acquisition of verb compounding in Mandarin Chinese. *Jidong Chen*

49. Phoneme inventories and patterns of speech sound perception. *Anita Wagner*

50. Lexical processing of morphologically complex words: An information-theoretical perspective. *Victor Kuperman*
51. A grammar of Savosavo, a Papuan language of the Solomon Islands. *Claudia Wegener*
52. Prosodic structure in speech production and perception. *Claudia Kuzla*
53. The acquisition of finiteness by Turkish learners of German and Turkish learners of French: Investigating knowledge of forms and functions in production and comprehension. *Sarah Schimke*
54. Studies on intonation and information structure in child and adult German. *Laura de Ruiter*
55. Processing the fine temporal structure of spoken words. *Eva Reinisch*
56. Semantics and (ir)regular inflection in morphological processing. *Wieke Tabak*
57. Processing strongly reduced forms in casual speech. *Susanne Brouwer*
58. Ambiguous pronoun resolution in L1 and L2 German and Dutch. *Miriam Ellert*
59. Lexical interactions in non-native speech comprehension: Evidence from electro-encephalography, eye-tracking, and functional magnetic resonance imaging. *Ian FitzPatrick*
60. Processing casual speech in native and non-native language. *Annelie Tuinman*
61. Split intransitivity in Rotokas, a Papuan language of Bougainville. *Stuart Robinson*
62. Evidentiality and intersubjectivity in Yurakaré: An interactional account. *Sonja Gipper*
63. The influence of information structure on language comprehension: A neurocognitive perspective. *Lin Wang*
64. The meaning and use of ideophones in Siwu. *Mark Dingemanse*
65. The role of acoustic detail and context in the comprehension of reduced pronunciation variants. *Marco van de Ven*

66. Speech reduction in spontaneous French and Spanish. *Francisco Torreira*

67. The relevance of early word recognition: Insights from the infant brain. *Caroline Junge*

68. Adjusting to different speakers: Extrinsic normalization in vowel perception. *Matthias J. Sjerps*

69. Structuring language. Contributions to the neurocognition of syntax. *Katrien R. Segaert*

70. Infants' appreciation of others' mental states in prelinguistic communication: A second person approach to mindreading. *Birgit Knudsen*

71. Gaze behavior in face-to-face interaction. *Federico Rossano*

72. Sign-spatiality in Kata Kolok: how a village sign language of Bali inscribes its signing space. *Conny de Vos*

73. Who is talking? Behavioural and neural evidence for norm-based coding in voice identity learning. *Attila Andics*

74. Lexical processing of foreign-accented speech: Rapid and flexible adaptation. *Marijt Witteman*

75. The use of deictic versus representational gestures in infancy. *Daniel Puccini*

76. Territories of knowledge in Japanese conversation. *Kaoru Hayano*

77. Family and neighbourhood relations in the mental lexicon: A cross-language perspective. *Kimberley Mulder*

78. Contributions of executive control to individual differences in word production. *Zeshu Shao*

79. Hearing speech and seeing speech: Perceptual adjustments in auditory-visual processing. *Patrick van der Zande*

80. High pitches and thick voices: The role of language in space-pitch associations. *Sarah Dolscheid*

81. Seeing what's next: Processing and anticipating language referring to objects. *Joost Rommers*

82. Mental representation and processing of reduced words in casual speech. *Iris Hanique*

83. The many ways listeners adapt to reductions in casual speech. *Katja Poellmann*

84. Contrasting opposite polarity in Germanic and Romance languages: Verum Focus and affirmative particles in native speakers and advanced L2 learners. *Giuseppina Turco*

85. Morphological processing in younger and older people: Evidence for flexible dual-route access. *Jana Reifegerste*

86. Semantic and syntactic constraints on the production of subject-verb agreement. *Alma Veenstra*

87. The acquisition of morphophonological alternations across languages. *Helen Buckler*

88. The evolutionary dynamics of motion event encoding. *Annemarie Verkerk*

89. Rediscovering a forgotten language. *Jiyoun Choi*

90. The road to native listening: Language-general perception, language-specific input. *Sho Tsuji*

91. Infants'understanding of communication as participants and observers. *Gudmundur Bjarki Thorgrímsson*

92. Information structure in Avatime. *Saskia van Putten*

93. Switch reference in Whitesands. *Jeremy Hammond*

94. Machine learning for gesture recognition from videos. *Binyam Gebrekidan Gebre*

95. Acquisition of spatial language by signing and speaking children: a comparison of Turkish sign language (TID) and Turkish. *Beyza Sümer*

96. An ear for pitch: on the effects of experience and aptitude in processing pitch in language and music. *Salomi Savvatia Asaridou*

97. lncrementality and Flexibility in Sentence Production. *Maartje van de Velde*

98. Social learning dynamics in chimpanzees: Reflections on (nonhuman) animal culture. *Edwin van Leeuwen*

99. The request system in Italian interaction. *Giovanni Rossi*

100. Timing turns in conversation: A temporal preparation account. *Lilla Magyari*

101. Assessing birth language memory in young adoptees. *Wencui Zhou*

102. A social and neurobiological approach to pointing in speech and gesture. *David Peeters*

103. Investigating the genetic basis of reading and language skills. *Alessandro Gialluisi*

104. Conversation Electrified: The Electrophysiology of Spoken Speech Act Recognition. *Rósa Signý Gisladottir*

105. Modelling Multimodal Language Processing. *Alastair Smith*

106. Predicting language in different contexts: The nature and limits of mechanisms in anticipatory language processing. *Florian Hintz*

107. Situational variation in non-native communication. *Huib Kouwenhoven*

108. Sustained attention in language production. *Suzanne Jongman*

109. Acoustic reduction in spoken-word processing: Distributional, syntactic, morphosyntatic, and orthographic effects. *Malte Viebahn*

110. Nativeness, dominance, and the flexibility of listening to spoken language. *Laurence Bruggeman*

111. Semantic specificity of perception verbs in Maniq. *Ewelina Wnuk*

112. On the identification of FOXP2 gene enhancers and their role in brain development. *Martin Becker*

113. Events in language and thought: The case of serial verb constructions in Avatime. *Rebecca Defina*

114. Deciphering common and rare genetic effects on reading ability. *Amaia Carrión Castillo*