

Common variation near *ROBO2* is associated with expressive vocabulary in infancy

Journal Information
Journal ID (nlm-ta): Nat Commun
Journal ID (iso-abbrev): Nat Commun
Title: Nature Communications
ISSN (electronic): 2041-1723
Publisher: Nature Pub. Group

Article/Issue Information
Date received: 15 January 2014
Date accepted: 28 July 2014
Publication date (electronic): 16 September 2014
Volume: 5
Electronic Location Identifier: 4831
Article Id (accession): PMC4175587
Article Id (pmcid): PMC4175587
Article Id (pmc-uid): 4175587
PubMed ID: 25226531
Publisher Item ID: ncomms5831
DOI: 10.1038/ncomms5831

Categories

Subject: Article

Common variation near *ROBO2* is associated with expressive vocabulary in infancy

Beate St Pourcain
Rolieke A.M. Cents
Andrew J.O. Whitehouse
Claire M.A. Haworth
Oliver S.P. Davis
Paul F. O'Reilly
Susan Roulstone
Yvonne Wren
Qi W. Ang
Fleur P. Velders
David M. Evans
John P. Kemp
Nicole M. Warrington
Laura Miller
Nicholas J. Timpson
Susan M. Ring
Frank C. Verhulst
Albert Hofman
Fernando Rivadeneira
Emma L. Meaburn
Thomas S. Price
Philip S. Dale
Demetris Pillas
Anneli Yliherva
Alina Rodriguez
Jean Golding
Vincent W.V. Jaddoe
Marjo-Riitta Jarvelin
Robert Plomin
Craig E. Pennell
Henning Tiemeier
George Davey Smith

1 Medical Research Council Integrative Epidemiology Unit, University of Bristol, Oakfield House, 15-23 Oakfield Grove, Bristol BS8 2BN, UK

2 School of Oral and Dental Sciences, University of Bristol, Lower Maudlin Street, Bristol BS1 2LY, UK

3 School of Experimental Psychology, University of Bristol, 12a Priory Road, Bristol BS8 1TU, UK

4 Generation R Study Group, Erasmus MC-University Medical Centre, Postbus 2040, 3000 CA Rotterdam, The Netherlands

5 Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC-University Medical Centre, Postbus 2060, 3000 CB Rotterdam, The Netherlands

6 Telethon Kids Institute, Centre for Child Health Research, University of Western Australia, 100 Roberts Road, Subiaco, Western Australia 6008, Australia

7 Department of Psychology, University of Warwick, Coventry CV4 7AL, UK

Copyright © 2014, Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.
Copyright 2014, Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.

License (open-access): This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

8 8Medical Research Council, Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, UK

9 9Department of Genetics, Evolution and Environment, UCL, UCL Genetics Institute, Darwin Building, Gower Street, London WC1E 6BT, UK

10 10Department of Epidemiology and Biostatistics, Medical Research Council (MRC) Public Health England (PHE) Centre for Environment and Health, School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK

11 11Bristol Speech and Language Therapy Research Unit, University of the West of England, Frenchay Hospital, Frenchay Park Road, BS16 1LE Bristol, UK

12 12School of Women's and Infants' Health, University of Western Australia, 374 Bagot Road, Subiaco, Western Australia 6008, Australia

13 13School of Social and Community Medicine, University of Bristol, Canynge Hall, 39 Whatley Road, Bristol BS8 2PS, UK

14 14University of Queensland Diamantina Institute, Translational Research Institute, University of Queensland, 37 Kent Street Woolloongabba, Queensland 4102, Australia

15 15Department of Epidemiology, Erasmus MC-University Medical Centre, Postbus 2040, 3000 CA Rotterdam, The Netherlands

16 16Department of Internal Medicine, Erasmus MC-University Medical Centre, Postbus 2040, 3000 CA Rotterdam, The Netherlands

17 17Department of Psychological Sciences, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK

18 18Institute for Translational Medicine and Therapeutics, University of Pennsylvania School of Medicine, 3400 Civic Center Boulevard, Building 421, Philadelphia, Pennsylvania 19104-5158, USA

19 19Department of Speech and Hearing Sciences, University of New Mexico, 1700 Lomas Boulevard NE Suite 1300, Albuquerque, New Mexico 87131, USA

20 20Faculty of Humanities, Logopedics, Child Language Research Center, University of Oulu, BOX 1000, Oulu 90014, Finland

21 21Mid Sweden University Department for Psychology/Mittuniversitetet Avdelningen för psykologi, 83125 Östersund, Sweden

22 22Department of Pediatrics, Erasmus MC-University Medical Centre, Postbus 2060, 3000 CB Rotterdam, The Netherlands

23 23Unit of Primary Care, Oulu University Hospital, Kajaanintie 50, PO Box 20, FI-90220, Oulu 90029, Finland

24 24Department of Children and Young People and Families, National Institute for Health and Welfare, Aapistie 1, Box 310, FI-90101 Oulu, Finland

25 25Institute of Health Sciences, University of Oulu, PO Box 5000, Oulu FI-90014, Finland

26 26Biocenter Oulu, University of Oulu, PO Box 5000, Aapistie 5A, OuluFI-90014, Finland

27 27These authors contributed equally to this work

Beate.StPourcain@bristol.ac.uk

Abstract

Twin studies suggest that expressive vocabulary at ~24 months is modestly heritable. However, the genes influencing this early linguistic phenotype are unknown. Here we conduct a genome-wide screen and follow-up study of expressive vocabulary in toddlers of European descent from up to four studies of the EARly Genetics and Lifecourse Epidemiology consortium, analysing an early (15–18 months, ‘one-word stage’, $N_{\text{Total}}=8,889$) and a later (24–30 months, ‘two-word stage’, $N_{\text{Total}}=10,819$) phase of language acquisition. For the early phase, one single-nucleotide polymorphism (rs7642482) at 3p12.3 near *ROBO2*, encoding a conserved axon-binding receptor, reaches the genome-wide significance level ($P=1.3 \times 10^{-8}$) in the combined sample. This association links language-related common genetic variation in the general population to a potential autism susceptibility locus and a linkage region for dyslexia, speech-sound disorder and reading. The contribution of common genetic influences is, although modest, supported by genome-wide complex trait analysis (meta-GCTA $h^2_{15-18\text{-months}}=0.13$, meta-GCTA $h^2_{24-30\text{-months}}=0.14$) and in concordance with additional twin analysis (5,733 pairs of European descent, $h^2_{24\text{-months}}=0.20$).

Abstract

The genetic basis of expressive vocabulary in children around 2 years old is poorly understood. Here, the authors show that a genetic variant near the *ROBO2* gene is associated with early language acquisition in the general population and highlight a potential genetic link between language-related common genetic variation and a linkage region for dyslexia, speech-sound disorder and reading.

The number of distinct spoken words is a widely used measure of early language abilities, which manifests during infancy¹. Word comprehension (known as receptive language) in typically developing children starts at the age of about 6–9 months², and the spontaneous production of words (known as expressive language) emerges at about 10–15 months¹³. During the next months the accumulation of words is typically slow, but then followed by an increase in rate, often quite sharp, around 14–22 months of age (‘vocabulary spurt’)¹⁴. As development progresses, linguistic proficiency becomes more advanced, with two-word combinations (18–24 months of age)¹³ and more complex grammatical structures (24–36 months of age)¹³ arising, accompanied by the steady increase in vocabulary size. Expressive vocabulary is therefore considered to be a rapidly changing phenotype, especially between 12 and 24 months⁵, with zero size at birth, ~50 words at 15–18 months¹³, ~200 words at 18–30 months¹³, ~14,000 words at 6 years of age³⁴ and #50,000 words in high school graduates⁶⁷.

Twin analyses of cross-sectional data suggest that expressive vocabulary at ~24 months is modestly heritable ($h^2=0.16-0.38$)⁸⁹, and longitudinal twin analyses have reported an increase in heritability of language-related factors during development ($h^2=0.47-0.63$, #7 years of age)¹⁰. Large-scale investigations of common genetic variation underlying growth in language skills, however, are challenging owing to the complexity and varying nature of the phenotype. This is coupled with a change in psychological instruments, which are used to assess these abilities with progressing age. Current genome-wide association studies (GWASs) using cross-sectional data on language abilities in childhood

and adolescence have failed to identify robust signals of genome-wide association¹¹¹², and genes influencing earlier, less-complex linguistic phenotypes are currently unknown.

To attempt to understand genetic factors involved in language development during infancy and early childhood, we perform a GWAS and follow-up study of expressive vocabulary scores in independent children of European descent from the general population and analyse an early ('one-word stage') and a later ('two-word stage') phase of language acquisition. We report a novel locus near *ROBO2*, encoding a conserved axon-binding receptor, as associated with expressive vocabulary during the early 'one-word' phase at the genome-wide significance level, and provide heritability estimates for expressive vocabulary during infancy and early childhood.

Results

Genome-wide association analyses

We conducted two cross-sectional genome-wide screens corresponding to an early (15–18 months, $N_{\text{Total}}=8,889$) and a later (24–30 months, $N_{\text{Total}}=10,819$) phase of language acquisition, respectively, each adopting a two-stage design (Figs 1

Figure 1
Study design for the genome-wide screen of early expressive vocabulary.
 Expressive vocabulary between 15 and 18 months of age was assessed using different forms of the MacArthur Communicative Development Inventories (CDI). Detailed phenotype descriptions are given in Supplementary Data 1.

and 2

Figure 2
Study design for the genome-wide screen of later expressive vocabulary.
 Expressive vocabulary between 24 and 30 months of age was assessed with different forms of the MacArthur Communicative Development Inventories (CDI) and the Language Development Survey (LDS). Detailed phenotype descriptions are given in Supplementary Data 1.

; Supplementary Data 1). During these developmental phases, expressive vocabulary was captured with age-specific word lists (adaptations of the MacArthur Communicative Development Inventories (CDI)¹³¹⁴¹⁵¹⁶¹⁷ and the Language Development Survey (LDS)¹⁸, Methods). However, measures of expressive vocabulary were not normally distributed and differed in their symmetry (Supplementary Data 1; Supplementary Fig. 1), and association analysis was therefore carried out using rank-transformed scores (Methods). Within the discovery cohort, a total of 2,449,665 autosomal genotyped or imputed single-nucleotide polymorphisms (SNPs) were studied in 6,851 15-month-old and 6,299 24-month-old English-speaking toddlers, respectively. Genome-wide plots of the association signals are provided in Supplementary Figs 2 and 3. For the early phase, the strongest association signal was observed at rs7642482 on chromosome 3p12.3 near *ROBO2* ($P=9.5 \times 10^{-7}$, Supplementary Table 1) and for the late phase at rs11742977 on chromosome 5q22.1 within *CAMK4* ($P=3.5 \times 10^{-7}$, Supplementary Table 2). All independent variants from the discovery analysis (associated $P \leq 10^{-4}$, Supplementary Tables 1 and 2), including these SNPs, were taken forward to a follow-up study (Methods). This included 2,038 18-month-old Dutch-speaking children for the early phase and 4,520 24–30-month-old Dutch or English-speaking children for the later phase (Supplementary Data 1).

clusters, ENCODE histone modifications and chromatin state segmentation in umbilical vein endothelial cells (HUVEC), as well as Genomic Evolutionary Rate Profiling (GERP++) scores (lifted from hg19) are included.

) rs7642482 on chromosome 3p12.3 near *ROBO2* (the strongest signal in the discovery cohort) reached the genome-wide significance level ($P=1.3 \times 10^{#8}$), and the three other signals approached the suggestive level (rs10734234 on chromosome 11p15.2 near *INSC*, $P=1.9 \times 10^{#7}$; rs11176749 on chromosome 12q15 near *CAND1*; $P=7.2 \times 10^{#7}$ and rs1654584 on chromosome 19p13.3 within *DAPK3*; $P=3.4 \times 10^{#7}$).

Each of these four polymorphisms explained only a small proportion of the phenotypic variance (adjusted regression R^2 : for rs7642482=0.34–0.35%, rs10734234=0.27–0.35%, rs11176749=0.25–0.27% and rs1654584=0.22–0.49%) in both the discovery and the follow-up cohort, but together the four SNPs accounted for >1% of the variation in early expressive vocabulary scores (joint adjusted regression $R^2=1.10$ –1.45%). For the SNP reaching genome-wide significance, rs7642482, each increase in the minor G-allele was associated with lower expressive vocabulary, although, due to the rank-transformation, an interpretation of the magnitude of the genetic effect is not informative. An empirical estimate of the genetic effect in the discovery sample, suggested a decrease of 0.098 s.d. in expressive vocabulary scores (95% confidence interval: 0.058; 0.14) per increase in G-allele. We are aware, however, that this signal might be prone to the ‘winner’s curse’ (that is, an overestimation of the effect) and requires further replication within independent samples.

Characterization of the lead association signals

rs7642482 is located ~19 kb 3# of *ROBO2* (OMIM: 602431), which encodes the human roundabout axon guidance receptor homologue 2 (*Drosophila*) gene. An *in silico* search for potentially functional effects using the University of California Santa Cruz Genome Browser19 provided no evidence that rs7642482 or proxy SNPs ($r^2>0.3$) relate to protein-coding variation within *ROBO2*. For this, we also confirmed the observed linkage disequilibrium structure within the discovery cohort through local imputation of chromosome 3 using the 1,000 Genomes reference panel (v3.20101123, Supplementary Fig. 4). The sequence at rs7642482 and the flanking genomic interval are, however, highly conserved (rs7642482 Genomic Evolutionary Rate Profiling (GERP)20 score=3.49; regional average GERP score near rs7642482 (derived from 100 bases surrounding rs7642482, GWAVA21)=3.06; average GERP score for coding sequences20 >2). Encyclopaedia of DNA elements (ENCODE)22 data indicate that in umbilical vein endothelial cells (HUVEC), rs7642482 overlaps with regulatory chromatin states, such as H3K27ac2324, which are predicted to be a strong enhancer25 (Fig. 3e). Additional searches using HaploReg (v2) (ref. 26)26 identified overlaps with further regulatory DNA features, such as DNase I hypersensitive sites and binding sites for transcription factors (Irx, Pou3f2_1). This suggests that variation at rs7642482 might be implicated within regulatory mechanisms in embryonic cell types, consistent with a peak of *ROBO2* expression in the human brain during the first trimester (Supplementary Fig. 5). There was no evidence for *cis* expression quantitative trait loci (eQTL) within ± 1 Mb of rs7642482 in postnatally derived cell types or adult brain tissue, based on searches of public eQTL databases (seeQTL)2728.

Since little is known about the genetic factors affecting language acquisition, the ‘suggestive’ signals at 11p15.2, 12q15 and 19p13.3 may also stimulate future research. rs10734234 resides within the vicinity of *INSC* (197 kb 3# of the gene), encoding an adaptor protein for cell polarity proteins (OMIM: 610668). rs11176749 is located near *CAND1* (144 kb 3# of the gene) encoding a F-box protein-exchange factor (OMIM: 607727), which regulates the ubiquitination of target proteins, and rs1654584 is an intronic SNP within

DAPK3 encoding the death-associated protein kinase 3, which plays a key role in apoptosis (OMIM: 603289).

Within a further step, we investigated whether the reported association signals are influenced by potential covariates, such as gestational age²⁹ and maternal education³⁰. These have been previously linked to late language emergence in infancy²⁹ and the total number of spoken words in early childhood³⁰, respectively. Studying up to 8,889 15–18-month-old children from the discovery and follow-up cohort, the association signal at rs7642482 increased when gestational age was adjusted for (adjusted $P_{\text{meta}}=4.0 \times 10^{\#9}$, 0.36–0.38% explained variance), while adjustment for maternal education did not affect the association (Supplementary Tables 5 and 6). For the remaining SNPs, there was little or no effect on the strength of the genetic association when these covariates were controlled for.

To explore whether the reported association signals influence linguistic skills other than early-phase expressive vocabulary, we also investigated a series of language-related measures during development. We observed no evidence for association between the four SNPs and first single-word utterances in 4,969 12-month-old Finnish children (Supplementary Data 1; Supplementary Table 7). However, this age pertains to a developmental stage where expressive vocabulary is very low, that is, the majority of children speak about one or two words, and pre-linguistic communication skills are still developing³¹. All early-phase signals were furthermore attenuated or even abolished when investigated for association with word-production scores during the later phase of language acquisition (24–30 months, Supplementary Fig. 6). This age band spans a phase where growth in linguistic proficiency may relate more to early grammar development including two-word combinations¹, than a vocabulary of single words. Overall, the phenotypic correlations between early and later expressive vocabulary scores were moderate within cohorts with multiple linguistic measures (0.48<##0.57, Supplementary Data 1), and evidence for genetic correlations, based on genome-wide complex trait analysis (GCTA)³²³³, was mixed (Avon Longitudinal Study of Parents and Children (ALSPAC): $r_g(\text{s.e.})=0.69(0.20)$, $P=0.02$), Generation R Study (GenR): $r_g(\text{s.e.})=\#0.32(0.97)$, $P=0.18$). There was also no association between the four reported SNPs and other language-related cognitive outcomes, including verbal intelligence scores, in middle childhood (8–10 years of age) when studying up to 5,540 children from the discovery cohort, apart from nominal associations with reading speed (rs7642482 $P=0.009$; rs1654584 $P=0.0035$; Supplementary Tables 8 and 9). Thus, the observed genetic associations, especially at rs7642482, are likely to be time-sensitive and specific to the early phase of language acquisition.

Twin analysis and GCTA

A twin study of 5,733 twin pairs of European descent, including a subset of children from the follow-up cohorts, supported the (modest) influence of additive genetic effects on variability in expressive vocabulary at ~24 months ($a^2(\text{s.e.})=0.20(0.008)$; Table 2

Table 2																											
Heritability of expressive vocabulary (15–30 months).																											
Sample	Age (m)	Measure	h^2 (s.e.)	LRT (df)	P	N	GCTA: early expressive vocabulary (15–18 months)																				
ALSPAC	15	Infant CDI	0.13 (0.05)	5.66 (1)	0.009	6,194																					
GenR	18	N-CDI-2A	0.19 (0.17)	1.23 (1)	0.10	1,828	Total	0.13 (0.05)	8,022																		
GCTA: later expressive vocabulary (24–30 months)																											
ALSPAC	24	Toddler CDI	0.17 (0.06)	8.09 (1)	0.002	5,739	Raine	24	LDS	<0.01 (0.34)	<0.01 (1)	0.50	866	TEDS	24	MCDI	<0.01 (0.15)	<0.01 (1)	0.50	1,720	GenR	30	LDS	0.11 (0.19)	0.33 (1)	0.30	1,641
Total			0.14 (0.05)			9,966																					
Sample Age (m) Measure a^2 (s.e.) N Twin analysis:																											
later expressive vocabulary (24 months)	TEDS	24	MCDI	0.20 (0.008)		5,733																					

Abbreviations: ALSPAC, Avon Longitudinal Study of Parents and Children; GCTA, genome-wide complex trait analysis; m, months; TEDS, Twins Early Development Study.

Expressive vocabulary was captured with different forms of the MacArthur Communicative Development Inventories (CDI: infant CDI, toddler CDI, N-CDI-2A and MCDI)1314151617 and the Language development Survey (LDS)18 (Supplementary Data 1).

*GCTA heritability based on rank-transformed expressive vocabulary scores adjusted for age, age-squared, sex and the most significant ancestry-informative principal components in each cohort.

†Sample number after exclusion of individuals with a relatedness of #2.5%.

‡Estimates were combined using fixed-effects inverse-variance meta-analysis (heterogeneity P value based on Cochran's Q -test based $P_{\text{het}}=0.72$).

§Additive genetic influence for rank-transformed expressive vocabulary scores adjusted for age, age-squared and sex, based on an ACE model (Supplementary Tables 10 and 11).

||Number of twin pairs.

; Supplementary Tables 10 and 11, Methods) and was consistent with previous reports on a smaller sample⁹. Estimates from twin analysis and GCTA³², performed on the discovery sample, were furthermore in close concordance (ALSPAC GCTA $h^2(\text{s.e.})_{15\text{-months}}=0.13(0.05)$; GCTA $h^2(\text{s.e.})_{24\text{-months}}=0.17(0.06)$; Table 2). However, in the smaller-sized follow-up samples, GCTA heritability, especially for the later phase, was close to zero (Table 2), and is likely to reflect impaired power during the follow-up. Combining GCTA heritability estimates using meta-analysis techniques (Methods), provided similar estimates as observed for the discovery cohort alone (meta-GCTA $h^2(\text{s.e.})_{15\text{-}18\text{-months}}=0.13(0.05)$, meta-GCTA $h^2(\text{s.e.})_{24\text{-}30\text{ months}}=0.14(0.05)$).

Discussion

This study reports a genome-wide screen and follow-up study of expressive vocabulary scores in up to 10,819 toddlers of European origin investigating an early phase (15–18 months) and a later phase (24–30 months) of language acquisition. On the basis of the combined analysis of all available samples, our study identifies a novel locus near *ROBO2* as associated with expressive vocabulary during the early phase of language acquisition.

Robo receptors and their Slit ligands (secreted chemorepellent proteins) are highly conserved from fly to human³⁴³⁵ and play a key role in axon guidance and cell migration. In vertebrates, Robo2 is involved in midline commissural axon guidance³⁶, the proliferation of central nervous system progenitors³⁷, the spatial positioning of spiral ganglion neurons³⁸ and the assembly of the trigeminal ganglion³⁹, which is the sensory ganglion of the trigeminal nerve. The latter is particularly important for speech production in humans⁴⁰, as the trigeminal nerve provides motor supply to the muscles of mastication, which control the movement of the mandibles, and in addition the nerve transmits sensory information from the face. Thus, genetic variation at *ROBO2* may be linked to both speech production abilities and expressive vocabulary size within children of the general population.

Rare recurrent *ROBO2* deletions have been discovered in patients with autism spectrum disorder⁴¹, a severe childhood neuro-developmental condition where core symptoms include deficits in social communication⁴², and decreased *ROBO2* expression has been observed in the anterior cingulate cortex⁴³ and in lymphocytes of individuals with autism⁴⁴. Indeed, the 3p12-p13 region has been linked to dyslexia⁴⁵, and quantitative dyslexia traits⁴⁶, as well as quantitative speech-sound disorder traits and reading⁴⁷. The dyslexia linkage findings⁴⁵ have been related to a specific SNP haplotype within *ROBO1*⁴⁸, a neighbouring gene of *ROBO2*. In animal models, *Robo1* and *Robo2* are mostly co-expressed and it has been shown that both receptors function cooperatively, for example, with respect to the guidance of most forebrain projections⁴⁹. Thus, it is possible that variation within

both *ROBO1* and *ROBO2* might also contribute to the linkage signals within the reported regions, and our findings highlight *ROBO2* as a novel, not yet investigated candidate locus. Common polymorphisms within *ROBO1* have also been associated with reading disability⁵⁰ and with performance on tasks of non-word repetition⁵¹, which is related to phonological short-term memory deficits. However, none of these previously reported *ROBO1* variants (rs12495133, rs331142, rs4535189 and rs6803202)^{50,51} were associated with early word production scores within our study (Supplementary Table 12). Vice versa, we also found no association between rs7642482 (*ROBO2*) and language-related measures, including phonological memory and verbal intelligence in middle childhood, nor was there any association with expressive vocabulary during the later phase of language acquisition (24–30 months of age) or with very first single-word utterances at about 12 months of age. Instead, our findings suggest that the identified *ROBO2* signal is specific for an early developmental stage of language acquisition (15–18 months of age), which is characterized by a slow accumulation of single words, followed by an increase in rate that is sometimes related to a ‘vocabulary spurt’¹⁴. Both *in silico* analyses and the increase in signal after adjustment for gestational age support the hypothesis that expressive vocabulary during this phase may be affected by perinatal or early postnatal gene regulatory mechanisms. It is furthermore possible that the enhancer effect predicted within HUVEC also relates to a yet uncharacterized embryonic cell type, where expression changes are only detectable on the single-cell level. For example, during the trigeminal ganglion formation placode/neural crest cells travel as individual cells to the site of ganglion formation, and *Robo2* appears to be expressed in discrete, dispersed regions in the surface ectoderm³⁹. This is characteristic of cells, which are about to detach and migrate³⁹. Thus, it will require further molecular studies to characterize the biological mechanisms underlying the observed *ROBO2* association in more detail.

In line with previous findings⁸⁹, estimates from twin analysis and GCTA (based on large samples) suggest that the proportion of phenotypic variation in early expressive vocabulary, which is attributable to genetic factors, is modest. The concordance of twin and large-sample GCTA heritability estimates indicates, however, that most of this genetic variation is common and that there is little ‘missing heritability’. Thus, a large proportion of common genetic variation influencing early expressive vocabulary might be captured by current GWAS designs, given sufficient power.

To conclude, this study describes genome-wide association between rs7642482 near *ROBO2* and expressive vocabulary during an early phase of language acquisition where children typically communicate with single words only. The signal is specific to this developmental stage, strengthened after adjustment for gestational age, and links overall language-related common genetic variation in the general population to a potential autism susceptibility locus as well as a linkage region for dyslexia, speech-sound disorder and reading on chromosome 3p12-p13.

Methods

Phenotype selection and study design

Consistent with the developmental pattern of language acquisition, the analysis of children’s expressive vocabulary in infancy was divided between an early phase (15–18 months of age, Fig. 1) and a later phase (24–30 months of age, Fig. 2) and conducted using independent individuals of up to four population-based European studies with both quantitative expressive vocabulary scores and genotypes available (early phase: total $N=8,889$; later phase: total $N=10,819$).

Expressive vocabulary scores were measured with age-specific-defined word lists and either ascertained with adaptations of the MacArthur CDI1314151617 or the LDS18 and based

on parent-report. The CDIs were developed to assess the typical course and variability in communicative development in children of the normal population (8–30 months of age)¹³. The LDS was designed as a screening tool for the identification of language delay in 2-year-old children¹⁸. Both measures have sufficient internal consistency, test-retest reliability and validity^{18,52,53}.

Expressive vocabulary during the early phase was captured by an abbreviated version of the MacArthur CDI (Infant Version¹³, 8–16 months of age, Supplementary Data 1) within the discovery cohort (ALSPAC, $N=6,851$, Supplementary Fig. 1a). Note, the Infant CDI has recently become also known as CDI Words and Gestures⁵⁴. A Dutch adaptation of the short-form version of the MacArthur CDI (N-CDI 2A)¹⁴¹⁶ was used within the follow-up cohort (GenR, $N=2,038$). Scores in both cohorts comprised both expressive and receptive language aspects ('says and understands') and showed a positively skewed data distribution ($1.95 < \text{skewness} < 2.39$; Supplementary Data 1).

Vocabulary production during the later phase was measured with an abbreviated version of the MacArthur CDI (Toddler version, 16–30 months of age)¹³¹⁵ in the discovery cohort (ALSPAC, $N=6,299$, Supplementary Fig. 1b). Note, the Toddler CDI has recently become also known as CDI Words and Sentences⁵⁴. Within the follow-up cohorts, expressive vocabulary was either assessed with the LDS¹⁸ (GenR $N=1,812$; the Raine study $N=981$) or an adapted short form of the MacArthur CDI (MCDI)¹⁴¹⁷ (Twins Early Development Study, TEDS, $N=1,727$, independent individuals (one twin per pair), $N=5,733$ twin pairs (not all of them have genotype information available)). Later-phase expressive vocabulary scores measured expressive language only ('says') and were either symmetrically distributed or negatively skewed ($1.68 < \text{skewness} < 0.24$; Supplementary Data 1).

In total, three different languages were included in our analyses: English (three samples: ALSPAC; TEDS; Raine), Dutch (one sample: GenR) and Finnish (sensitivity analysis: Northern Finnish Birth Cohort (NFBC) 1966). The cross-cultural comparability of the CDI has been explored, and the measures in many languages, including Dutch and English, show minimal differences in vocabulary production scores in the early years⁵⁵. In addition, the standardization within each sample (see below) would have removed any minor differences between instruments.

Basic study characteristics, details on phenotype acquisition and psychological instruments as well as summary phenotype characteristics (including mean, s.d., kurtosis, skewness and age at measurement) are presented for each cohort and developmental phase in Supplementary Data 1.

For each participating study, ethical approval of the study was obtained by the local research ethics committee, and written informed consent was provided by all parents and legal guardians. Detailed information on sample-specific ethical approval and participant recruitment is provided in Supplementary Note 1.

Genotyping and imputation

Genotypes within each cohort were obtained using high-density SNP arrays (Supplementary Data 1). Cohort-specific genotyping information including genotyping platform, quality control (QC) for individuals and SNPs, the final sample size, the number of SNPs before and after imputation as well as the imputation procedures are detailed in Supplementary Data 1. Briefly, for individual sample QC, this included filtering according to call rate, heterozygosity and ethnic/other outliers, and for SNP QC (prior to imputation) filtering according to minor allele frequency, call rate and SNPs with deviations from Hardy–Weinberg equilibrium (detailed exclusion criteria are listed in Supplementary Data 1). Genotypes were subsequently imputed to HapMap CEU (phase II and/or III) and/or Wellcome Trust Controls (Supplementary Data 1). For sensitivity analysis, ALSPAC

genotypes on chromosome 3 were also locally imputed to 1,000 Genomes (v3.20101123, Supplementary Data 1).

Single-variant association analysis

Within each cohort, expressive vocabulary scores were adjusted for age, age-squared, sex and the most significant ancestry-informative principal components⁵⁶ and subsequently rank-transformed to normality to facilitate comparison of the data across studies and instruments. The association between SNP and the expressive vocabulary score was assessed within each cohort using linear regression of the rank-transformed expressive vocabulary score against allele dosage, assuming an additive genetic model.

In the discovery cohort, the genome-wide association analysis for each phase was carried out using MACH2QTL⁵⁷ using 2,449,665 imputed or genotyped SNPs. SNPs with a minor allele frequency of <0.01 and SNPs with poor imputation accuracy (MACH R^2 < 0.3) were excluded prior to the analysis, and all statistics were subjected to genomic control correction⁵⁸ (Supplementary Data 1). All independent SNPs from the early- and later-phase GWAS below the threshold of $P < 10^{-4}$ (85 and 50 SNPs, respectively) were selected for subsequent follow-up analysis in additional cohorts. Independent SNPs were identified by linkage disequilibrium-based clumping using PLINK⁵⁹ Proxy SNPs within ± 500 kb, linkage disequilibrium $r^2 > 0.3$ (Hapmap II CEU, Rel 22) were removed). All analyses within the follow-up samples were carried out *in silico* using MACH2QTL or SNPTEST⁶⁰ software (Supplementary Data 1). For the selected SNPs, estimates from the discovery (genomic-control corrected) and follow-up cohort(s) were combined using fixed-effects inverse-variance meta-analysis (R 'rmeta' package), while testing for overall heterogeneity using Cochran's Q -test. Signals below a genome-wide significance threshold of $P < 2.5 \times 10^{-8}$ (accounting for two GWAS analyses) were considered to represent robust evidence for association.

An empirical approach (Bootstrapping with 10,000 replicates) was selected to obtain meaningful genetic effects (basic 95% bootstrap confidence interval) of the reported SNPs in the discovery cohort. For this, we utilized a linear model of z -standardized expressive vocabulary scores against allele dosage, adjusted for age, age-squared, sex and the most significant ancestry-informative principal components. The local departmental server of the School of Social and Community Medicine at the University of Bristol was used for data exchange and storage.

Sensitivity analysis in ALSPAC using locally imputed genotypes on chromosome 3 (based on 1,000 Genomes) was performed as linear regression of the rank-transformed expressive vocabulary score against allele dosage, assuming an additive genetic model, using MACH2QTL (Supplementary Data 1).

Direct genotyping of reported SNPs

Reported SNPs with a medium imputation accuracy (MACH $R^2 < 0.8$) were re-genotyped in the discovery cohort (ALSPAC) to confirm the validity of the observed association signal (rs10734234, MACH $R^2 = 0.76$). Genotyping was undertaken by LGC Genomic Ltd (<http://www.lgcgenomics.com/>) using a form of competitive allele-specific PCR system (KASPar) for SNP analysis.

Variance explained

To estimate the variation in expressive vocabulary scores explained by each reported SNP and jointly by all reported SNPs together, we calculated the adjusted regression R^2 values from (i) univariate linear regression of the rank-transformed expressive vocabulary score (see above) against allele dosage and (ii) multivariate linear regression of the rank-

transformed expressive vocabulary score (see above) against the allele dosage from all reported SNPs. All analyses were performed using R, SPSS or STATA software.

Phenotypic characterization of association signals

To investigate whether there is an association between the first single-word utterances at ~12 months of age and the reported SNPs, we conducted an association analysis in the NFBC 1966. The number of spoken words in the NFBC 1966 (word-list free assessment, ‘words’ are undefined) were based on parental response to a questionnaire administered at 12 months of age (Supplementary Data 1). Given the scarcity of categories referring to three or more spoken words, word numbers were dichotomized into ‘1+ words’ (one or more words, 1) versus ‘no words’ (0). The association between early word-production scores and allele dosage of the reported SNPs was studied using logistic regression models, adjusted for sex and the most significant principal components (as exact age at measurement was not available) using SNPTEST.

Pre-school language deficits have been repeatedly associated with later problems in language development, especially reading skills⁶¹. To assess whether genetic effects affecting expressive language skills early in life also influence language competencies during later development, we investigated the association between reported SNP signals and a series of language-related cognitive measurements in the ALSPAC cohort (Supplementary Table 8). All outcomes were *z*-standardized prior to analysis. The association between the transformed outcome and SNP allele dosage was investigated using linear regression adjusted for sex, the most significant principal components and age (except for age-normalized intelligence quotient scores, Supplementary Table 9).

To assess whether gestational age and maternal education influence the association between the reported signals and early expressive vocabulary scores, we (i) investigated the association between these potential covariates and the SNPs directly and (ii) adjusted the association between genotypes and language measures for potential covariate effects. Gestational age in the relevant cohorts was either estimated from medical records or obtained from midwife and hospital registries at birth (Supplementary Data 1), and measured in completed weeks of gestation. Information on maternal education was obtained from antenatal questionnaire data, and dichotomized into lower (1) and higher (0) maternal education (Supplementary Data 1). The association between gestational age and allele dosage for reported SNPs was investigated with linear regression models and adjusted for sex and the most significant principal components in each cohort. The link between maternal education and these SNPs was studied using logistic regression models adjusted for the most significant principal components in each cohort.

We furthermore created new transformations of expressive vocabulary scores, that is, the reported number of words were in addition to the previously described variables (see above) adjusted for gestational age and maternal education, respectively, before they were rank-transformed. Association analysis for reported SNPs was then carried out as described for discovery, follow-up and combined analysis before. All analyses were carried out using R, SPSS or STATA software.

GCTA

The proportion of additive phenotypic variation jointly explained by all genome-wide SNPs together (GCTA heritability) was estimated for all cohorts and analyses windows using GCTA³². In brief, using a sample of independent individuals, the method is based on the comparison of a matrix of pairwise genomic similarity with a matrix of pairwise phenotypic similarity using a random-effects mixed linear model³². Pertinent to this study, GCTA (Supplementary Data 1) was carried out using rank-transformed expressive vocabulary scores (previously adjusted for age, sex and the most significant ancestry-informative

principal components in each cohort, see above) and directly genotyped SNPs (ALSPAC, GenR, Raine) or most likely imputed genotypes (TEDS). GCTA estimates from different cohorts were combined using fixed-effects inverse-variance meta-analysis assuming symmetrically distributed s.e., while testing for overall heterogeneity using Cochran's Q -test.

The extent to which the same genes contribute to the observed phenotypic correlation between two variables can be furthermore estimated through genetic correlations⁶². For all cohorts with expressive vocabulary measures at two time points (ALSPAC and GenR), the genetic correlation (r_g) between the rank-transformed scores was estimated using bivariate GCTA analysis³³ (based on the genetic covariance between two traits).

Twin analysis

Twin analyses allow the estimation of the relative contributions of genes and environments to individual differences in measured traits. Twin intraclass correlations were calculated⁶³, providing an initial indication of the relative contributions of additive genetic (A), shared environmental (C) and non-shared environmental (E) factors. Additive genetic influence, also commonly known as heritability, is estimated as twice the difference between the identical and fraternal twin correlations. The contribution of the shared environment, which makes members of a family similar, is estimated as the difference between the identical twin correlation and heritability. Non-shared environments, that is, environments specific to individuals, are estimated by the difference between the identical twin correlation and 1, because they are the only source of variance making identical twins different. Estimates of the non-shared environment also include measurement error.

Maximum likelihood structural equation model-fitting analyses allow more complex analyses and formal tests of significance⁶⁴. Standard twin model-fitting analyses were conducted using Mx⁶⁵. The model fit is summarized by minus two times the log likelihood (#2LL). Differences in #2LL between models distributes as χ^2 , which provides a goodness of fit statistic. A change in χ^2 of 3.84 is significant for a 1 degree of freedom test. Model fit was compared between the full ACE model and the saturated model (where variances are not decomposed into genetic and environmental sources). Reduced models testing CE, AE and E models were compared with the full ACE model and the saturated model. A significant P value indicates a significantly worse fit.

Twin analysis was carried out on rank-transformed expressive vocabulary scores at 24 months (adjusted for age, age-squared and sex), which were assessed in 5,733 twin pairs (monozygotic twins $N=1,969$; dizygotic twins (male, female and opposite sex) $N=3,764$) from the TEDS⁶⁶.

The URLs for all utilized web pages are given in Supplementary Note 2.

Author contributions

B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., P.F.O'R., Q.W.A., F.P.V. and N.M.W. performed study-level data analysis. Study design was by B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., J.G., S.R., Y.W., H.T. and G.D.S. B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., P.F.O'R., S.R. and Y.W. wrote the paper. Data collection was by S.R., Y.W., L.M., F.C.V., P.S.D., A.Y., J.G., V.W.V.J., M.-R.J., R.P., C.E.P., H.T. and G.D.S. Genotyping was performed by B.S.P., O.S.P.D., D.M.E., J.P.K., N.M.W., S.M.R., F.R., E.L.M., T.S.P., D.P., V.W.V.J., M.-R.J., R.P., C.E.P. and G.D.S. B.S.P., R.A.M.C., A.J.O.W., C.M.A.H., O.S.P.D., P.F.O'R., S.R., Y.W., Q.W.A., F.P.V., D.M.E., J.P.K., N.W., L.M., N.J.T., S.M.R., F.C.V., A.H., F.R., E.L.M., T.S.P., P.S.D., D.P., A.Y., A.R., J.G., V.W.V.J., M.-R.J., R.P., C.E.P., H.T. and G.D.S. revised and reviewed the paper.

Additional information

How to cite this article: St Pourcain, B. *et al.* Common variation near *ROBO2* is associated with expressive vocabulary in infancy. *Nat. Commun.* 5:4831 doi: 10.1038/ncomms5831 (2014).

Supplementary Material

Supplementary Information

Supplementary Figures 1-6, Supplementary Tables 1-12, Supplementary Notes 1-3 and Supplementary References

Supplementary Data 1

Basic study characteristics of all cohorts contributing to discovery, follow-up and sensitivity analysis

Acknowledgements

Avon Longitudinal Study of Parents and Children (ALSPAC)

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council and the Wellcome Trust (Grant ref: 092731) and the University of Bristol provide core support for ALSPAC. ALSPAC GWAS data were generated by the Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corporation of America) using funding from 23andMe. This work was also supported by the Medical Research Council Integrative Epidemiology Unit (MC_UU_12013/1-9). D.M.E. is supported by a Medical Research Council New Investigator Award (MRC G0800582 to D.M.E.). J.P.K. is funded by a Wellcome Trust 4-year PhD studentship (WT083431MA). B.S.P. is supported by an Autism Speaks grant (7132). This publication is the work of the authors and they will serve as guarantors for the contents of this paper.

The Generation R Study (GenR)

We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam. We thank K. Estrada and C. Medina-Gomez for their support in the creation and analysis of imputed data. The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. The generation and management of GWAS genotype data for the Generation R Study were performed at the Genetic Laboratory of the Department of Internal Medicine at the Erasmus Medical Center. The Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw 10.000.1003). V.W.V.J. received an additional grant from the Netherlands Organization for Health Research and Development (ZonMw 90700303). H.T. received an additional grant from the Netherlands Organization for Scientific Research (VIDI 017.106.370). Additional support was provided to R.A.M.C. by a grant from the Sophia Foundation for scientific research (SSWO 547-2008).

Northern Finland Birth Cohort 1966 (NFBC 1966)

We thank the late Professor P. Rantakallio (launch of NFBC 1966 and 1986), Ms O. Tornwall and Ms M. Jussila (DNA biobanking). Financial support was received from the Academy of Finland (project grants 104781, 120315, 1114194 and Center of Excellence in Complex Disease Genetics), University Hospital Oulu, Biocenter, University of Oulu, Finland, NHLBI grant 5R01HL087679-02 through the STAMPEED

program (1RL1MH083268-01), ENGAGE project and grant agreement HEALTH-F4-2007-201413, the Medical Research Council (studentship grant G0500539, PrevMetSyn/Salve/MRC), the Wellcome Trust (project grant GR069224), UK. The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki.

The Twins Early Development Study (TEDS)

We are enormously grateful to the twins, parents and the twins' teachers who have supported the Twins Early Development Study (TEDS) for the past 18 years. The TEDS is supported by a program grant from the UK Medical Research Council (G0901245, and previously G0500079), with additional support from the US National Institutes of Health (HD044454, HD059215). We would like to thank the Wellcome Trust Case Control Consortium 2 (WTCCC2) consortium (Supplementary Note 3) for their help with genome-wide genotyping, which was made possible by grants from the WTCCC2 project (085475/B/08/Z, 085475/Z/08/Z). C.M.A.H. was supported by a research fellowship from the British Academy. O.S.P.D. was supported by a Sir Henry Wellcome Fellowship from the Wellcome Trust (WT088984). R.P. was supported by a research professorship from the UK Medical Research Council (G19/2) and a European Research Council Advanced Investigator Award (295366).

Western Australian Pregnancy Cohort study (Raine)

We are grateful to the Raine Foundation, to the Raine Study Families and to the Raine Study research staff. We gratefully acknowledge the assistance of the Western Australian Genetic Epidemiology Resource and the Western Australian DNA Bank (both National Health and Medical Research Council of Australia National Enabling Facilities). We also acknowledge the support of the Healthway Western Australia, the National Health and Medical Research Council of Australia (Grant 572613) and the Canadian Institutes of Health Research (Grant MOP 82893). We gratefully acknowledge the assistance of the Wind Over Water Foundation, the Telethon Institute for Child Health Research and the Raine Medical Research Foundation of the University of Western Australia. A.J.O.W. was supported by a Career Development Fellowship from the NHMRC (Grant number 1004065).

Wuerzburg University research collaboration

We thank T. Haaf, E. Schneider and N. El Hajj (Department of Human Genetics, University of Wuerzburg, Germany) for helpful discussions about the biological role of *ROBO2*.

Early Genetics and Lifecourse Epidemiology (EAGLE) consortium

This work was carried out in collaboration with the EAGLE consortium (<http://research.lunenfeld.ca/eagle/>).

References

- 1 L. Fenson *et al.* Variability in early communicative development. *Monogr. Soc. Res. Child. Dev.* 59, 1–185 (1994).7845413
- 2 E. Bergelson & D. Swingley At 6–9 months, human infants know the meanings of many common nouns. *Proc. Natl Acad. Sci. USA* 109, 3253–3258 (2012).22331874
- 3 E. Hoff in *Handbook Of Early Childhood Development* (eds. McCartney K., Phillips D. 233–251 Blackwell (2006).
- 4 E. V. Clark *First Language Acquisition* Cambridge Univ. Press (2010).
- 5 S. Reilly *et al.* The Early Language in Victoria Study (ELVS): a prospective, longitudinal study of communication skills and expressive skills and expressive vocabulary development at 8, 12 and 24 months. *Int. J. Speech Lang. Pathol.* 11, 344–357 (2009).
- 6 S. A. Kuczaj in *The Development of Language* ed. Barrett M. D. Psychology Press (1999).
- 7 S. Pinker *The Language Instinct* W. Morrow and Company (1994).
- 8 J. S. Reznick, R. Corley & J. Robinson *A Longitudinal Twin Study of Intelligence in the Second Year* University of Chicago Press (1997).

- 9 P. Dale *et al.* Genetic influence on language delay in two-year-old children. *Nat. Neurosci.* 1, 324–328 (1998).10195167
- 10 M. E. Hayiou-Thomas, P. S. Dale & R. Plomin The etiology of variation in language skills changes with development: a longitudinal twin study of language from 2 to 12 years. *Dev. Sci.* 15, 233–249 (2012).22356179
- 11 N. Harlaar *et al.* Genome-wide association study of receptive language ability of 12-year-olds. *J. Speech. Lang. Hear. Res.* 57, 96–105 (2014).24687471
- 12 M. Luciano *et al.* A genome-wide association study for reading and language abilities in two population cohorts. *Genes Brain Behav.* 12, 645–652 (2013).23738518
- 13 L. Fenson, P. Dale & S. Reznic Technical Manual for the MacArthur Communicative Development Inventories Developmental Psychology Laboratory (1991).
- 14 L. Fenson *et al.* Short-Form versions of the MacArthur Communicative Development Inventories. *Appl. Psycholinguist.* 21, 95–116 (2000).
- 15 J. S. Reznick & L. Goldsmith A multiple form word production checklist for assessing early language. *J. Child Lang.* 16, 91–100 (1989).2925817
- 16 I. Zink & M. Lejaegere N-CDIs: Korte Vormen, Aanpassingen en Hernormering van de MacArthur Short Form Vocabulary Checklists Acco (2003).
- 17 P. S. Dale, G. Dionne, T. C. Eley & R. Plomin Lexical and grammatical development: a behavioural genetic perspective. *J. Child. Lang.* 27, 619–642 (2000).11089341
- 18 L. Rescorla The Language Development Survey: a screening tool for delayed language in toddlers. *J. Speech Hear. Disord.* 54, 587–599 (1989).2811339
- 19 D. Karolchik, A. S. Hinrichs & W. J. Kent The UCSC genome browser. *Curr. Protoc. Bioinformatics* Chapter 1, Unit1.4 (2012).23255150
- 20 E. V. Davydov *et al.* Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput. Biol.* 6, e1001025 (2010).21152010
- 21 G. R. S. Ritchie, I. Dunham, E. Zeggini & P. Flicek Functional annotation of noncoding sequence variants. *Nat. Methods* 11, 294–296 (2014).24487584
- 22 The ENCODE project. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57–74 (2012).22955616
- 23 M. P. Creighton *et al.* Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl Acad. Sci. USA* 107, 21931–21936 (2010).21106759
- 24 N. D. Heintzman *et al.* Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 459, 108–112 (2009).19295514
- 25 J. Ernst *et al.* Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* 473, 43–49 (2011).21441907
- 26 L. D. Ward & M. Kellis HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* 40, D930–D934 (2012).22064851
- 27 K. Xia *et al.* seeQTL: a searchable database for human eQTLs. *Bioinformatics* 28, 451–452 (2012).22171328
- 28 A. J. Myers *et al.* A survey of genetic human cortical gene expression. *Nat. Genet.* 39, 1494–1499 (2007).17982457
- 29 S. R. Zubrick, C. L. Taylor, M. L. Rice & D. W. Slegers Late language emergence at 24 months: an epidemiological study of prevalence, predictors, and covariates. *J. Speech. Lang. Hear. Res.* 50, 1562–1592 (2007).18055773
- 30 C. A. Dollaghan *et al.* Maternal education and measures of early speech and language. *J. Speech. Lang. Hear. Res.* 42, 1432–1443 (1999).10599625

- 31 V. Reddi in *The Development of Language* ed. Barrett M. D. Psychology Press (1999).
- 32 J. Yang *et al.* Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet.* 42, 565–569 (2010).20562875
- 33 S. H. Lee, J. Yang, M. E. Goddard, P. M. Visscher & N. R. Wray Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. *Bioinformatics* 28, 2540–2542 (2012).22843982
- 34 M. Seeger, G. Tear, D. Ferres-Marco & C. S. Goodman Mutations affecting growth cone guidance in *Drosophila*: genes necessary for guidance toward or away from the midline. *Neuron* 10, 409–426 (1993).8461134
- 35 T. Kidd *et al.* Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* 92, 205–215 (1998).9458045
- 36 H. Long *et al.* Conserved roles for Slit and Robo proteins in midline commissural axon guidance. *Neuron* 42, 213–223 (2004).15091338
- 37 V. Borrell *et al.* Slit/Robo signaling modulates the proliferation of central nervous system progenitors. *Neuron* 76, 338–352 (2012).23083737
- 38 S. Wang *et al.* Slit/Robo signaling mediates spatial positioning of spiral ganglion neurons during development of cochlear innervation. *J. Neurosci.* 33, 12242–12254 (2013).23884932
- 39 C. E. Shiau, P. Y. Lwigale, R. M. Das, S. A. Wilson & M. Bronner-Fraser Robo2-Slit1 dependent cell-cell interactions mediate assembly of the trigeminal ganglion. *Nat. Neurosci.* 11, 269–276 (2008).18278043
- 40 A. J. Seikel, D. W. King & D. G. Drumright *Anatomy & Physiology for Speech, Language, and Hearing* Cengage Learning (2010).
- 41 A. Prasad *et al.* A discovery resource of rare copy number variations in individuals with autism spectrum disorder. *G3 (Bethesda)* 2, 1665–1685 (2012).23275889
- 42 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* American Psychiatric Association (1994).
- 43 S. Suda *et al.* Decreased expression of axon-guidance receptors in the anterior cingulate cortex in autism. *Mol. Autism.* 2, 14 (2011).21859478
- 44 A. Anitha *et al.* Genetic analyses of roundabout (ROBO) axon guidance receptors in autism. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 147B, 1019–1027 (2008).18270976
- 45 J. Nopola-Hemmi *et al.* A dominant gene for developmental dyslexia on chromosome 3. *J. Med. Genet.* 38, 658–664 (2001).11584043
- 46 S. E. Fisher *et al.* Independent genome-wide scans identify a chromosome 18 quantitative-trait locus influencing dyslexia. *Nat. Genet.* 30, 86–91 (2002).11743577
- 47 C. M. Stein *et al.* Pleiotropic effects of a chromosome 3 locus on speech-sound disorder and reading. *Am. J. Hum. Genet.* 74, 283–297 (2004).14740317
- 48 K. Hannula-Jouppi *et al.* The axon guidance receptor gene *ROBO1* is a candidate gene for developmental dyslexia. *PLoS Genet.* 1, (2005).
- 49 G. López-Bendito *et al.* Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain. *J. Neurosci.* 27, 3395–3407 (2007).17392456
- 50 C. Tran *et al.* Association of the *ROBO1* gene with reading disabilities in a family-based analysis: association of the *ROBO1* gene. *Genes Brain Behav.* 13, 430–438 (2014).24612512
- 51 T. C. Bates *et al.* Genetic variance in a component of the language acquisition device: *ROBO1* polymorphisms associated with phonological buffer deficits. *Behav. Genet.* 41, 50–57 (2011).20949370
- 52 L. Fenson & P. S. Dale *MacArthur Communicative Development Inventories: User’s Guide and Technical Manual* Singular Publishing Group (1993).

- 53 L. Rescorla & A. Alley Validation of the Language Development Survey (LDS): a parent report tool for identifying language delay in toddlers. *J. Speech. Lang. Hear. Res.* 44, 434–445 (2001).11324663
- 54 L. Fenson *et al.* The MacArthur-Bates Communicative Development Inventories User's Guide and Technical Manual Brookes Publishing Co (2006).
- 55 D. Bleses *et al.* Early vocabulary development in Danish and other languages: a CDI-based comparison. *J. Child. Lang.* 35, 619–650 (2008).18588717
- 56 A. L. Price *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* 38, 904–909 (2006).16862161
- 57 Y. Li, C. J. Willer, J. Ding, P. Scheet & G. R. Abecasis MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet. Epidemiol.* 34, 816–834 (2010).21058334
- 58 B. Devlin & K. Roeder Genomic control for association studies. *Biometrics* 55, 997–1004 (1999).11315092
- 59 S. Purcell *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575 (2007).17701901
- 60 J. Marchini, B. Howie, S. Myers, G. McVean & P. Donnelly A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* 39, 906–913 (2007).17572673
- 61 H. S. Scarborough in *Approaching Difficulties in Literacy Development: Assessment, Pedagogy and Programmes* SAGE (2009).
- 62 M. C. Neale & H. H. M. Maes *Methodology for Genetic Studies of Twins and Families* Kluwer Academic Publishers (2004).
- 63 P. E. Shrout & J. L. Fleiss Intraclass correlations: uses in assessing rater reliability. *Psychol. Bull.* 86, 420–428 (1979).18839484
- 64 F. V. Rijsdijk & P. C. Sham Analytic approaches to twin data using structural equation models. *Brief Bioinform.* 3, 119–133 (2002).12139432
- 65 M. Neale, S. Boker, G. Xie & H. Maes *Mx: Statistical Modeling* 7th edn Department of Psychiatry (2006).
- 66 C. M. A. Haworth, O. S. P. Davis & R. Plomin *Twins Early Development Study (TEDS): a genetically sensitive investigation of cognitive and behavioral development from childhood to young adulthood.* *Twin Res. Hum. Genet.* 16, 117–125 (2013).23110994