



Research paper

Mutations in two large pedigrees highlight the role of *ZNF711* in X-linked intellectual disability



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ABSTRACT

Intellectual disability (ID) affects approximately 1–2% of the general population and is characterized by impaired cognitive abilities. ID is both clinically as well as genetically heterogeneous, up to 2000 genes are estimated to be involved in the emergence of the disease with various clinical presentations. For many genes, only a few patients have been reported and causality of some genes has been questioned upon the discovery of apparent loss-of-function mutations in healthy controls. Description of additional patients strengthens the evidence for the involvement of a gene in the disease and can clarify the clinical phenotype associated with mutations in a particular gene.

Here, we present two large four-generation families with a total of 11 males affected with ID caused by mutations in *ZNF711*, thereby expanding the total number of families with ID and a *ZNF711* mutation to four. Patients with mutations in *ZNF711* all present with mild to moderate ID and poor speech accompanied by additional features in some patients, including autistic features and mild facial dysmorphisms, suggesting that *ZNF711* mutations cause non-syndromic ID.

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Abbreviations: ADID, autosomal dominant intellectual disability; ARID, autosomal recessive intellectual disability; CADD, Combined Annotation Dependent Depletion; cDNA, complementary deoxyribonucleic acid; DNA, deoxyribonucleic acid; ExAC, Exome Aggregation Consortium; GRCh37/hg19, human genome build 19; HEK293, human embryonic kidney cells 293; ICD-10, 10th revision of the International Statistical Classification of Diseases and Related Health Problems; ID, intellectual disability; IQ, intelligence quotient; MRX65, mental retardation, X-linked 65; (q)PCR, (quantitative) polymerase chain reaction; REVEL, rare exome variant ensemble learner; RNA, ribonucleic acid; SNP, single nucleotide polymorphism; STR, short tandem repeat; TCGA, The Cancer Genome Atlas; UTR, untranslated region; XLID, X-linked intellectual disability; *CAMKMT*, calmodulin-lysine N-methyltransferase; *CDCA7*, cell division cycle associated 7; *CHDH*, choline dehydrogenase; *CNN3*, calponin 3; *FYN*, *FYN* proto-oncogene, Src family tyrosine kinase; *HPRT1*, hypoxanthine phosphoribosyltransferase 1; *KDM5C*, lysine demethylase 5C; *LRRC4*, leucine rich repeat containing 4; *PHF8*, PHD finger protein 8; *PIEZO2*, piezo type mechanosensitive ion channel component 2; *PQBPI1*, polyglutamine binding protein 1; *PTBP1*, polypyrimidine tract binding protein 1; *SYP*, synaptophysin; *TAF6*, TATA-box binding protein associated factor 6; *THY1*, Thy-1 cell surface antigen; *YWHAZ*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta; *ZNF41*, zinc finger protein 41; *ZNF81*, zinc finger protein 81; *ZNF674*, zinc finger protein 674; *ZNF711*, zinc finger protein 711.

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1. Introduction

1.1. Intellectual disability

Intellectual disability (ID) is defined by an IQ below 70 and problems in adaptive functioning that manifest before the age of 18 (ICD-10) and is present in around 1–2% of the population (Leonard and Wen, 2002). ID is both clinically and genetically very heterogeneous, which complicates the establishment of a genetic diagnosis. Multiple genetic causes of ID have been identified to date, including large chromosomal abnormalities (aneuploidies, translocations), deletions and duplications encompassing single exons up to multiple genes and point mutations affecting a single gene. ID is often caused by de novo mutations in sporadic cases, but can also be inherited in autosomal dominant (ADID), autosomal recessive (ARID) and X-linked manner (XLID). Until the recent development of next-generation sequencing techniques, the hereditary forms of ID were studied most extensively in large families that enabled linkage-based gene mapping.

1.2. X-linked intellectual disability

Historically, X-linked forms of intellectual disability have been studied most extensively, notably because X-linked pedigrees may be large and have multiple affected members throughout different generations. This is due to the presence of usually unaffected female carriers of the disorder, making these families eligible for linkage analysis. Large pedigrees with autosomal recessive forms are rarer and autosomal dominant mutations are predominantly de novo in origin. Nevertheless, the identification of disease causing genes in large XLID families is not straightforward. Tarpey et al. (2009) studied 208 XLID families by sequencing most coding exons on the X chromosome, leading to the identification of nine novel disease genes. Mutations in these genes, along with mutations in known disease genes on the X chromosome, likely resolved the genetic cause of disease in 25% of the studied families. Hu et al. (2016) studied 405 XLID families using X-exome resequencing and targeted sequencing of coding regions of known XLID genes and found evidence for seven novel disease genes (Hu et al., 2009; Hu et al., 2016). Targeted resequencing of 107 XLID genes in 50 patients that were negative for chromosomal aberrations, Fragile-X syndrome and had a family history suggestive for XLID, resulted in a diagnostic yield of 26% (Tzschach et al., 2015). Thus, despite these apparent successes in gene-identification, the majority of mutations in XLID genes have been identified in a limited number of families only. Interestingly, at least 1% of the genes on the X chromosome can be affected by loss-of-function mutations without noticeable phenotypic consequences (Tarpey et al., 2009). These observations inspired Piton et al. (2013) to revisit all genes on the X chromosome previously reported as causal for ID. Their evaluation was based on multiple criteria, including the presence of truncating mutations in a control cohort and the scarcity of ID families with mutations affecting a postulated XLID gene. In the conclusions of their report, they challenge the causality of no less than ten reported XLID genes, including the zinc-finger genes *ZNF41*, *ZNF81* and *ZNF674*. Taken together, these studies highlight the relevance of reporting novel mutations in potential XLID genes with limited support for causality.

1.3. ZNF711

One of the genes with limited evidence for causality is *ZNF711*, as hitherto only two truncating mutations in this gene have been reported in a single study (Tarpey et al., 2009). The exact function of *ZNF711* has not been clarified yet, but many of the Cys₂His₂ zinc finger proteins function as a transcription factor by binding to specific DNA sequences. *ZNF711* contains two Zfx/Zfy transcription activation regions and 12 C2H2-like domains. Zfx/Zfy transcription activation regions are often found N-terminal to multiple copies of a C2H2 Zinc finger and have been shown to activate transcription (Mardon et al., 1990). C2H2 domains are classical zinc fingers, in which two cysteine and two histidine residues coordinate a zinc ion (Bohm et al., 1997; Marco et al., 2003).

Here, we present two large families with X-linked intellectual disability, in which we identified mutations in *ZNF711*.

2. Patients and methods

2.1. Patients

Families were ascertained from Belgium and the Netherlands. DNA was isolated from peripheral blood using standard methods. This study was approved by the Ethics Committee of the University Hospital Antwerp, Belgium and the Medical Ethical Committee Arnhem-Nijmegen, the Netherlands and adhered to the Declaration of Helsinki (7th revision, 2013). Written informed consent was obtained from each individual.

2.2. Linkage analysis and genome-wide copy number analysis

Linkage analysis of family A had been performed previously using 17 markers (DXS1060, DXS8051, DXS987, DXS1226, DXS1214, DXS1068, DXS993, DXS991, DXS986, DXS990, DXS1106, DXS8055, DXS1001, DXS1047, DXS1227, DXS8043, DXS1073) on 27 individuals (AII.1, AII.2, AII.1, AII.2, AII.4, AII.6, AII.7, AII.8, AII.10, AII.11, AIII.3, AIII.5, AIII.6, AIII.9, AIII.12, AIII.14, AIII.15, AIII.16, AIII.18, AIII.19, AIII.20, AIII.21, AIII.22, AIV.9, AIV.10, AIV.12 and AIV.13). More recently, we obtained SNP array data using HumanCytoSNP-12 v2.1 beadchips scanned on an iScan system, following standard protocols as provided by the manufacturer (Illumina, San Diego, California, USA). X-linked recessive single point linkage analysis was performed using the MINX package of Merlin (v1.1.2) on SNP data of 12 family members (AII.2, AII.4, AII.8, AII.11, AIII.12, AIII.14, AIII.16, AIII.18, AIII.20, AIII.22, AIV.7 and AIV.13) (Abecasis et al., 2002). To define the parametric model, disease allele frequency was set to 0.0001 and penetrance to 0.0001 and 1.000 for male individuals carrying wild type or mutant alleles respectively. Analysis was performed on the X chromosome only and the intermarker distance used was 0.45 cM. In addition to the linkage analyses, we performed genome-wide copy number analysis on the same SNP array datasets, using the CNV-Webstore (Vandeweyer et al., 2011).

2.3. Exome sequencing

Exome sequencing was performed for individual AIV.13 using TruSeq DNA sample preparation (Illumina, San Diego, CA, USA) and SeqCap EZ Exome v3 enrichment (Roche, Basel, Switzerland) according to the standard protocols supplied by the manufacturers. Sequencing was performed on an Illumina HiSeq 1500 (Illumina, San Diego, CA, USA) using a 2 × 100 bp sequencing run. Data-analysis was done using an in-house pipeline as described before (Helsmoortel et al., 2015). Data filtering and annotation of variants in the linkage interval was performed with VariantDB (Vandeweyer et al., 2014b), using the settings as described before (Helsmoortel et al., 2015). A summary of the quality metrics for this sample has been included in supplementary Table 1.

Additional copy number analyses with higher resolution were performed based on the whole exome sequencing data. Starting from the BAM files from patient AIV.13 and one male control sample that was included in the same sequencing run and had similar coverage, the average per base coverage was calculated for each exon within the linked region using Bedtools (v2.17.0) (Quinlan and Hall, 2010). Exons with average coverage below 10× in either sample were excluded from the analysis. Log₂ scaled ratios were computed using R (v3.2.3) for 3883 exons. The presence of CNVs was checked in the linked region by either visual inspection or by a sliding window approach. For the sliding window, thresholds were set to three consecutive exons with a deviation from zero of at least 3 times the standard deviation (sd = 0.263).

2.4. Sanger confirmation

Confirmation of the *ZNF711* variants was performed using Sanger sequencing. Primer sequences and conditions for PCR are available upon request. PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing V2.0 Ready Reaction Kit and analyzed with the ABI PRISM 3730 or ABI3130XL Genetic Analyzer (Applied Biosystems, Foster City, USA). The identified variants have been entered into the *ZNF711* LOVD database (<http://databases.lovd.nl/shared/genes/ZNF711>) under individuals 00088189 (AIV.13) and 00088190 (BIV.1).

2.5. Genome-wide expression arrays

RNA was isolated from lymphoblastoid cell lines using Trizol Reagent (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol for individuals AIII.18 and BIII.6. Potential

residual DNA was depleted using the DNA-free DNA Removal Kit (ThermoFisher Scientific). RNA concentration and quality was assessed using both NanoDrop (ThermoFisher Scientific) and the Experion RNA Standard Sensitivity Analysis Kit (BioRad, Hercules, CA, USA). Sample preparation was done using the Illumina TotalPrep RNA Amplification Kit (ThermoFisher Scientific). Genome-wide expression was determined using HumanHT-12 v4 Expression BeadChips on an iScan system (Illumina, San Diego, CA, USA), according to the protocol supplied by the manufacturer. Data were analyzed in R (version 3.3.0), using the 'beadarray' and 'limma' packages. All data were normalized and probes with bad quality were filtered out. Genes were defined as differentially expressed when the adjusted p-value (FDR) was below 0.05 and the log Fold Change was smaller than -1.5 or larger than 1.5 . Lists of differentially expressed genes were compared with a list of genes causative for neurodevelopmental disorders (Grozeva et al., 2015; Vissers et al., 2016).

2.6. qPCR

RNA was isolated and assessed as described in Section 2.5 for individuals AIII.18, AIV.13, BII.8 and BIII.6. cDNA synthesis was performed with the SuperScript III First-Strand Synthesis System, using the random hexamer protocol supplied by the manufacturer (ThermoFisher Scientific). Primers were designed using our in-house qPCR primer design tool (Iqbal et al., 2013). Quantitative PCR was performed using the qPCR Mastermix Plus for SYBR Green I - no ROX (Eurogentec, Liège, Belgium) according to the manufacturer's protocol, on a Lightcycler480 (Roche, Basel, Switzerland). Data analysis was performed using qbase+ (Biogazelle, Ghent, Belgium). *HPRT1* and *YWHAZ* were used as reference genes for normalization of the data, stability of the reference genes was checked with the qbase+ software. The geNorm expression stability value of the reference genes ($M = 0.163$) and the coefficient of variation of the normalized reference gene relative quantities ($CV = 0.057$) are both well below threshold settings (0.5 and 0.2, respectively), indicating that the used reference genes are appropriate for normalization (Vandesompele et al., 2002; Hellemans et al., 2007).

2.7. ZNF711 variants in ExAC database

Variants in *ZNF711* were retrieved from the ExAC database (Exome Aggregation Consortium (ExAC), Cambridge, MA (URL: <http://exac.broadinstitute.org>) [accessed 07/2016]). Variants were annotated with CADD scores (Kircher et al., 2014) and REVEL scores (Ioannidis et al., 2016) after which the list was filtered for annotation (3' UTR, 5' UTR, intronic and synonymous mutations were not considered) and only variants with a CADD-Phred score > 20 were selected. Only variants found in hemizygous state were considered, as these could potentially evoke a phenotype in the males carrying them. ExAC datasets excluding the TCGA (The Cancer Genome Atlas) samples or the psychiatric cohort samples (53,105 and 45,376 samples remaining, respectively) were downloaded (ExAC database, release 0.3). Using ANNOVAR, variants present exclusively in the TCGA cohort or the psychiatric cohort were determined (Wang et al., 2010).

3. Results

3.1. Clinical description patients family A

Family A is a four-generation family with five affected male individuals (Fig. 1). Affected individuals from family A are characterized by mild to moderate intellectual disability (5/5; Table 1), motor delay (4/5) and poor speech (4/5). Two of them present with autistic features, being hand flapping and avoiding eye contact. Craniofacial features include a long face (4/5), synophris (3/5) and relatively large ears (4/5).

3.1.1. Molecular diagnosis family A

Affected males of family A were tested negative for the Fragile-X Syndrome repeat expansion. Linkage testing with STR marker analysis revealed a linked region on chrX: 31260748-102732317 (GRCh37/hg19), delimited by STR markers DXS1214 and DXS1106. More recently, we performed linkage analysis using SNP markers on a subset of the family and confirmed the linkage interval. Copy number analysis did not reveal any deletions or duplications on chromosome X that could explain the phenotype of the patients. Higher resolution copy number analysis based on whole exome sequencing data confirmed the absence of exonic deletions and duplications in the linked region (Supplementary Fig. 1). Whole exome sequencing of individual AIV.13 resulted in five high quality variants within the linked region, of which three were known polymorphisms (rs2208592, rs144899652 and rs41297342). One variant (*SYP*, NM_003179.2:c.184G>C) was predicted to be a polymorphism by MutationTaster (Schwarz et al., 2014), with a CADD-Phred score of 8.4 (Kircher et al., 2014). We also found a one base pair deletion in *ZNF711* (NM_021998.4:c.2054del, p.(Phe685Serfs*7)), resulting in a frameshift with a premature stop codon (Fig. 2). This stop codon is located in the last exon of the gene and the resulting mRNA transcript is therefore predicted to escape from nonsense-mediated decay (Nagy and Maquat, 1998). The variant is predicted to be disease-causing by MutationTaster (Schwarz et al., 2014) and has a CADD-Phred score of 34 (Kircher et al., 2014). The variant has not been reported in ExAC. This frameshift variant fully segregated with the ID phenotype in the family (Fig. 1).

3.2. Clinical description patients family B

Family B has been described before as family MRX65 (Yntema et al., 1999). It is a family with six affected males in three generations (Fig. 1). They are characterized by mild to moderate intellectual disability (6/6; Table 1) and speech delay (6/6). Four out of five adults are obese. Craniofacial features include a broad face (6/6) and a prominent forehead (3/6).

3.2.1. Molecular diagnosis family B

Linkage analysis mapped the underlying disease gene to the pericentromeric region of chromosome X with flanking markers DXS573 (Xq11.23, chrX:49138851–49138993 (GRCh37/hg19)) and DXS990 (Xq21.32, chrX:93000597–93000819 (GRCh37/hg19)) (Yntema et al., 1999). Massively parallel sequencing of all exons on the X chromosome revealed a potential pathogenic variant in *ZNF711* (NM_021998.4:c.731T>C, p.Ile244Thr). This variant was identified in the family in a large screening study and is one of approximately 200 potentially damaging variants that were reported but not followed up in this study (Hu et al., 2016). The variant is predicted to be disease-causing by MutationTaster (Schwarz et al., 2014), has a CADD-Phred score of 26.2 and is not reported in ExAC. The c.731T>C variant is located in the previously determined linkage interval and fully segregates with the ID in the family (Figs. 1 and 2).

3.3. ZNF711 variants in ExAC database

A query on the ExAC database showed that no loss-of-function - being nonsense mutations or mutations disrupting a splice acceptor or donor site - or frameshift mutations are detected to date, suggesting that these type of mutations are very rare and likely detrimental. The ExAC database does contain 64 potentially damaging (CADD-Phred > 20) variants in *ZNF711*, including mainly missense variants but also one in-frame deletion and one mutation disrupting the start codon. None of these 64 variants were found in homozygous state, 29 of the variants have been detected in hemizygous state (Supplementary Table 2). Of these 29 variants, one variant was exclusively detected in the Cancer Genome Atlas (TCGA) cohort and three variants were detected solely in the psychiatric cohort.

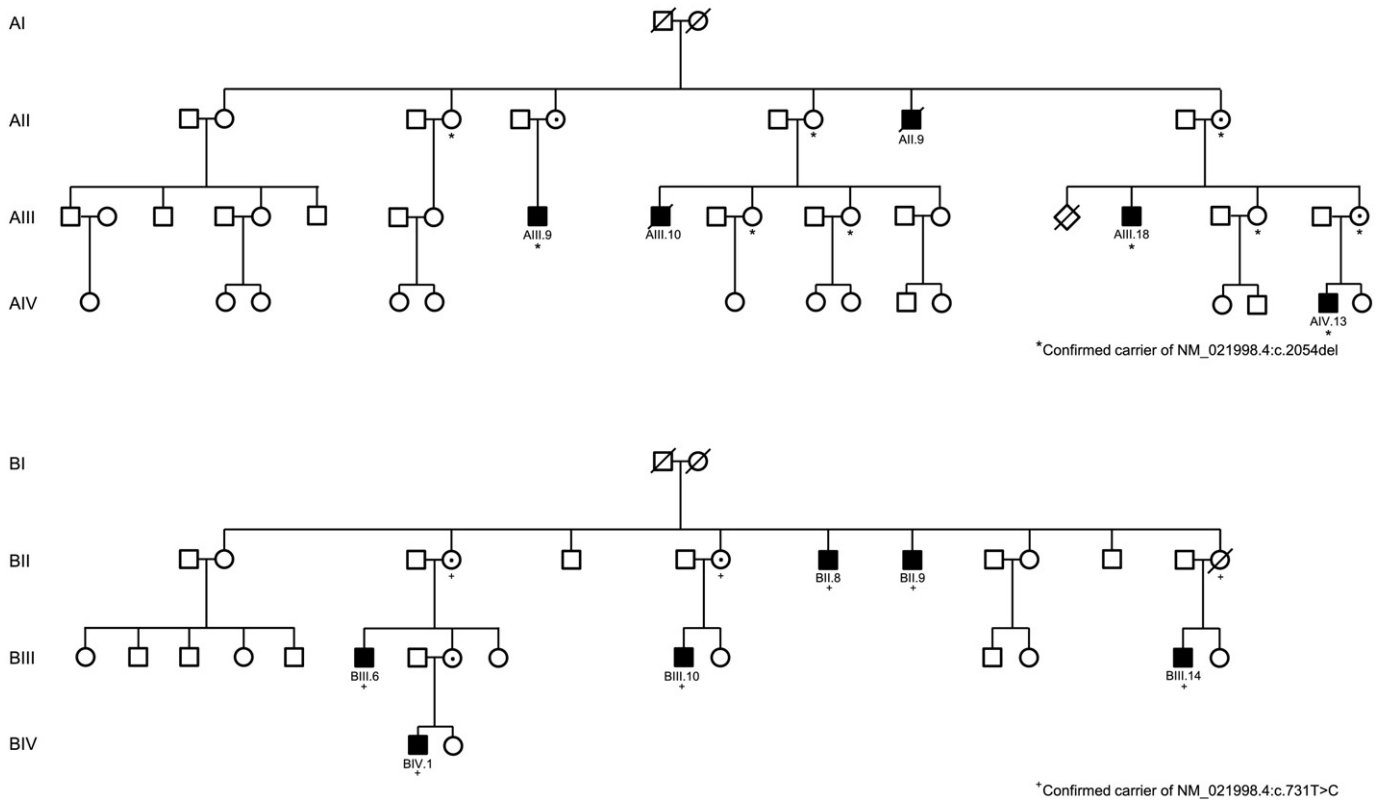


Fig. 1. Pedigrees of the studied families. Upper panel: pedigree family A. Lower panel: pedigree family B.

3.4. Expression analysis

We hypothesize that *ZNF711* may act as a transcription factor and influences the expression levels of (several) target genes, based on its functional domains. Genome-wide expression profiles were determined for two patients (AIII.18 and BIII.6) and compared to the expression profiles of seven male controls to detect differential expression. Analysis of the results revealed 19 differentially expressed genes when comparing the two patients with all the controls. To validate these results, we used qPCR to compare the expression of these 19

genes in two patients from each family (AIII.18, AIV.13, BII.8 and BIII.6) with four controls. Expression levels of three genes appeared to be too low to reliably quantify with qPCR, suggesting that these signals on the expression array originated from noise. The differential expression remained significant ($p < 0.05$, *t*-test) for five genes (*CHDH*, *CNN3*, *FYN*, *PIEZO2* and *THY1*) across all four patients (Table 2).

Following our genome-wide expression study, we investigated the expression levels of a few genes in more detail based on literature showing that *ZNF711* and the histone demethylase PHF8 interact directly at the promoter region of several target genes (Kleine-Kohlbrecher et al.,

Table 1
Clinical overview of the patients.

	AII.9 ^a	AIII.9	AIII.10 ^a	AIII.18	AIV.13	BII.8	BII.9	BIII.6	BIII.10	BIII.14	BIV.1	Summary
Gender	M	M	M	M	M	M	M	M	M	M	M	
Age at last observation (y)	U	53	17	35	13	56	53	52	24	28	6.5	
Birth weight (SD)	U	+1	U	+2.5	+1	U	U	U	U	U	U	
Birth length (SD)	U	U	U	U	0	U	U	U	U	U	U	
Head circumference at birth (SD)	U	U	U	U	+1	U	U	U	U	U	U	
Weight at last observation (SD)	U	+0.5	0	+1	+0.5	+4	+5	+2.5	-1.33	+3	-2	
Height at last observation (SD)	-0.5	-3.25	-1.5	0	-1	-1.25	-2	-1	-3	0	-3.5	
Head circumference at last observation (SD)	-0.5	-0.5	U	+0.5	+1.25	+0.63	+1.25	0	+0.63	+1.5	-2.5	
Intellectual disability (+ mild ID; ++ moderate ID)	++	+	+	+	++	+	++	++	++	+	+	11/11
Speech delay	+	+	+	+	+	+	+	+	+	+	+	10/11
Motor delay	+	+	-	+	+	U	U	U	U	U	U	4/5
Autistic features	U	U	U	+	+	U	U	U	U	U	U	2/2
Constipation	+	+	-	+	+	U	U	U	U	U	U	4/5
Long face	+	+	+	+	-	-	-	-	-	-	-	4/11
Broad face	-	-	-	-	+	+	+	+	+	+	+	7/11
Synophris	-	+	-	+	+	-	-	-	-	-	-	3/11
Prominent forehead	-	-	-	-	-	+	+	+	-	-	-	3/11
Broad nasal base	-	-	-	-	-	-	+	-	+	-	-	2/11
Broad nasal tip	-	-	-	-	+	+	-	-	+	-	+	4/11
Large ears	+	+	+	+	-	-	-	-	-	-	-	4/11

M = male, y = year, SD = standard deviation, U = unknown.
^a Deceased.

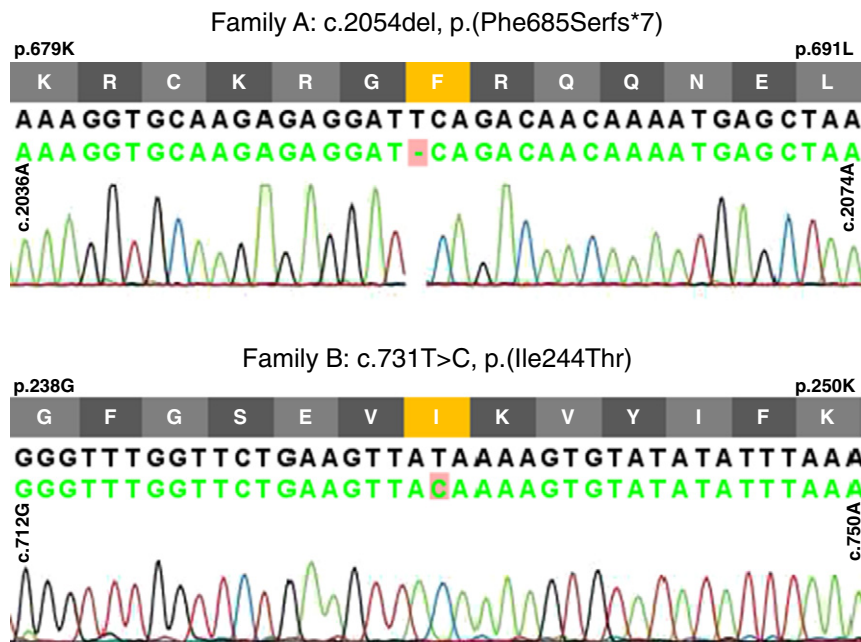


Fig. 2. Electropherograms of the mutations in *ZNF711*. Upper panel: mutation family A. Lower panel: mutation family B.

2010). Upon shRNA-mediated knockdown of *ZNF711* or *PHF8* in HEK293 cells, a total of eight genes were shown to be significantly underexpressed using qPCR (Kleine-Kohlbrecher et al., 2010). To determine whether mutations in *ZNF711* could have the same effect, we performed qPCR for these eight genes (*KDM5C*, *TAF6*, *CDCA7*, *CAMKMT*, *LRR4*, *PTBP1*, *ZNF41* and *PQBP1*) as well as *ZNF711* and *PHF8* for two patients from each family (AIII.18, AIV.13, BII.8 and BIII.6). *PHF8*, *CDCA7* and *CAMKMT* expression levels appeared to be too low in our lymphoblastoid cell lines to obtain reliable results and were therefore excluded from further analyses. Average expression per family was compared to expression of controls. Expression of *ZNF41* was significantly higher in both family A and family B when compared to controls (two-sample *t*-test, $p = 0.0025$ and 0.021 respectively). Expression of *KDM5C* was significantly lower in family B compared to controls ($p = 0.015$). All other tested genes were not differentially expressed in the patients ($p > 0.05$).

4. Discussion

ZNF711 has previously been linked to XLID (MRX97, OMIM #300803), based on mutations identified in a large study by Tarpey et al. (2009). Mutations in this gene appear to be very rare, as none were reported in any of the recent large ID/ASD gene-identification studies (de Ligt et al., 2012; Rauch et al., 2012; De Rubeis et al., 2014; Iossifov et al., 2014). Here, we report two mutations in *ZNF711* in 11 patients from two large families with an X-linked inheritance pattern of ID. Our study thus validates the

causality of *ZNF711* mutations and categorizes it as an XLID gene beyond doubt, since four families have now been reported (Fig. 3).

The five affected males of family A share mild to moderate ID, motor delay and poor speech. Some of the affected family members display autistic features in addition. The six affected males of family B display mild to moderate ID with non-consistent facial features, such as a rather broad face and a prominent forehead. Patients from the two families with truncating mutations in *ZNF711* described by Tarpey et al. displayed moderate intellectual disability without consistent additional features (Tarpey et al., 2009). Thus, mutations in *ZNF711* appear to cause non-syndromic intellectual disability. Patients have mild to moderate ID with occasional non-consistent comorbidities, including autistic features and poor speech.

The ExAC database does not contain any loss-of-function mutations (premature stop, frameshift or splice site mutations) in *ZNF711*, strengthening the hypothesis that deleterious mutations in *ZNF711* are causing disease. The database does encompass several missense variants and a start loss mutation. This latter mutation is reported only once, in a heterozygous female. A total of 29 potentially damaging mutations were present in hemizygous state, out of which three were exclusively found in the psychiatric subset of the ExAC cohort. We used CADD and REVEL, two scoring systems based on a combined analysis of different pathogenicity predictors, to evaluate these variants (Kircher et al., 2014; Ioannidis et al., 2016). We set the cut-offs at 20 for the CADD_Phred score (representing the top 1% of variants most likely pathogenic) and 0.45 for REVEL (the tipping point from which variants with a higher score are more likely to be pathogenic than neutral). Only three ExAC variants passed both criteria, as did the mutation found in family B (Supplementary Table 2 and Supplementary Fig. 2). Since the disease phenotype caused by mutations in *ZNF711* seems to be limited to ID with minor, non-consistent comorbidities, we cannot exclude that some of the potentially damaging variants in the control cohort might indeed cause (mild) ID in these individuals. In fact, variants in the Exome Sequencing Project (included in ExAC) that were predicted to be pathogenic have been identified in other known ID genes as well (Vandeweyer et al., 2014a).

In the study of Kleine-Kohlbrecher et al. (2010), multiple genes were found to be significantly underexpressed upon shRNA-mediated knockdown of *ZNF711*. In our patients, the expression of most of these genes did not deviate significantly from controls in lymphoblastoid cell lines.

Table 2
Differentially expressed genes in individuals with *ZNF711* mutations.

Gene	Encoded protein	Mean controls	Mean patients	Change in patients (%)	P-value
<i>CHDH</i>	Choline dehydrogenase	0.865	1.733	+100.3	0.027
<i>CNN3</i>	Calponin 3	0.795	1.978	+148.7	0.014
<i>FYN</i>	FYN proto-oncogene, Src family tyrosine kinase	1.498	0.435	-71.0	<0.001
<i>PIEZO2</i>	Piezo type mechanosensitive ion channel component 2	0.665	1.353	+103.4	0.018
<i>THY1</i>	Thy-1 cell surface antigen	0.748	1.943	+159.9	0.018
<i>ZNF41</i>	Zinc finger protein 41	1.026	1.461	+42.4	0.008

Group means are the mean expression levels measured with qPCR, after normalization. P-values are calculated using Welch two-sample *t*-tests.

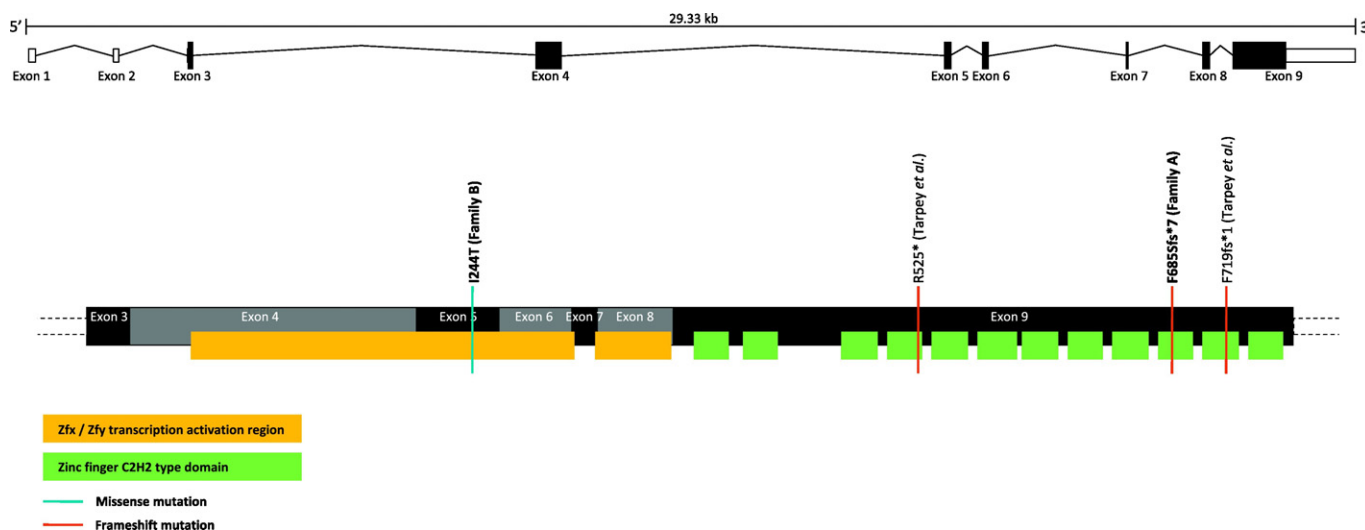


Fig. 3. Schematic overview of the *ZNF711* gene with the positions of disease-causing mutations.

Only *ZNF41* showed significant overexpression in the patients of both family A and B, whereas *KDM5C* was significantly underexpressed in family B, but not in family A. Thus, we did not replicate the results found in the study by Kleine-Kohlbrecher, which may be due to the fact that *ZNF711* expression levels in our patients are normal and hence have a less severe impact than functional null mutations. This may also explain the fact that no *ZNF711* premature termination codons resulting in nonsense-mediated mRNA decay have been reported thus far. Besides, the results of Kleine-Kohlbrecher et al. (2010) were obtained using HEK293 cells, whereas we tested expression in lymphoblastoid cell lines of patients. The discrepancy in obtained results between the two studies may therefore also be explained by the difference in tested cell line lineage.

Our genome-wide expression studies revealed only few differentially expressed genes in the patients compared to controls. Further validation experiments with qPCR including additional patients resulted in a total of five differentially expressed genes when comparing patients with mutations in *ZNF711* with controls. Combined with the specifically screened genes from the Kleine-Kohlbrecher et al. (2010) study, we detected six genes (*CHDH*, *CNN3*, *FYN*, *PIEZO2*, *THY1* and *ZNF41*) of which the expression is significantly altered in patients with *ZNF711* mutations. *CHDH* has recently been described as a potential vulnerability gene for bipolar disorder, amongst other evidence based on the fact that *CHDH* mRNA expression is consistently upregulated in the brains of bipolar disorder patients (Chang et al., 2016). In patients with *ZNF711* mutations, we also observed a significant increase in expression levels of *CHDH* when compared to controls. Similarly, we found an up-regulation of *CNN3* expression in the *ZNF711* patients. Significant up-regulation of Calponin-3 was also found in the temporal cortices and cerebrospinal fluid of epilepsy patients when compared to non-epileptic controls (Han et al., 2012). In *ZNF711* patients, the only significantly downregulated gene was *FYN*, encoding a nonreceptor protein tyrosine kinase expressed in neurons and glia (Bare et al., 1993). *Fyn* mutant mice display a reduction in long-term potentiation and impaired spatio-temporal learning, indicating a role for the *Fyn* protein in synaptic plasticity, which is a key process disturbed in patients with ID (Grant et al., 1992). Thus, aberrant expression levels of at least three out of six genes that were found deregulated by mutations in the putative transcription factor *ZNF711*, have been linked to phenotypes potentially related to the clinical presentation of patients carrying these mutations.

5. Conclusion

Here, we describe two mutations in *ZNF711* and unequivocally place *ZNF711* on the list of XLID genes. The causal mutation in family A is a

single basepair deletion, resulting in a frameshift with a premature stop codon. In family B a missense mutation with predicted deleterious effect causes the phenotype. These mutations complement existing evidence establishing the role of *ZNF711* in the emergence of ID. *ZNF711* mutations do not seem to elicit a clinically recognizable ID syndrome. Affected individuals have mild to moderate ID with poor speech and inconsistent additional common comorbidities, including autistic features and mild facial dysmorphologies. Mutations in *ZNF711* are accompanied by differential expression of six genes, that may be regulated directly or indirectly by *ZNF711*, which is predicted to be a transcription factor.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gene.2016.12.013>.

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