ARTICLE

Clinical findings and a DNA methylation signature in kindreds with alterations in *ZNF711*

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ZNF711 is one of eleven zinc-finger genes on the X chromosome that have been associated with X-linked intellectual disability. This association is confirmed by the clinical findings in 20 new cases in addition to 11 cases previously reported. No consistent growth aberrations, craniofacial dysmorphology, malformations or neurologic findings are associated with alterations in *ZNF711*. The intellectual disability is typically mild and coexisting autism occurs in half of the cases. Carrier females show no manifestations. A *ZNF711*-specific methylation signature has been identified which can assist in identifying new cases and in confirming the pathogenicity of variants in the gene.

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INTRODUCTION

Zinc finger protein 6 or *ZNF6* (now *ZNF711*), was localized to Xq13.3–q21.2 using a yeast artificial chromosome contig and proposed as a candidate gene for X-linked intellectual disability (XLID) based on its high expression in the brain by Colleaux et al. [1]. May et al. had previously suggested that *ZNF6*/*ZNF711* was likely responsible for intellectual disability in patients with microdeletions in Xq21 encompassing the choroideremia (*CHM*) and deafness (*DFN3*) loci [2]. Alterations in the gene were found a decade later in two families as part of a large X-chromosome sequencing project using XLID pedigrees [3]. Two additional families designated nonsyndromic XLID were reported by van der Werf et al. which confirmed the association between XLID and variants in *ZNF711* [4].

Although ten other zinc-finger genes, *ZNF41*, *ZNF81*, *ZNF674*, *ZNF741* (aka *KLF8*), *ZNF897* (aka *ZFP92*), *ZC4H2*, *ZDHHC9*, *ZDHHC15*, *ZMYM3* (aka *ZNF261*), and *ZCCHC12* (aka *SIZN1*) have been implicated in XLID, the strength of the associations for seven of these genes has been challenged [5–7]. The clinical presentation in kindreds associated with variants in eight of the 11 zinc-finger genes on the X chromosome has been considered to be nonsyndromic without consistent growth, dysmorphic, neurologic or metabolic manifestations [7, 8]. *ZC4H2* variants are associated with Wieacker-Wolff syndrome and Miles-Carpenter syndrome [9, 10]. Variants in *ZDHHC9* have been associated with XLID-macrocephaly-marfanoid habitus [11, 12]. Variants in *ZMYM3* have been associated with XLID-aortic stenosis-hypospadias [13].

This paper provides the clinical details for the two kindreds, K8440 and K9336 with variants in *ZNF711* identified by Tarpey et al., adds a multigenerational kindred with a nonsense alteration and three isolated cases, two with nonsense alterations and one with a missense change [3]. The clinical findings in 31 affected males in the nine known kindreds confirm the nonsyndromic nature of intellectual disability associated with *ZNF711* alterations. Additionally, a novel DNA methylation signature for *ZNF711* has been observed in peripheral blood which will assist further in the diagnosis of patients and the resolution of variants of uncertain pathogenicity.

METHODS

The research protocol and consent were reviewed and approved by the Self Regional Healthcare Institutional Review Board (IRB). Consents were signed for publication of patient photographs by the parents or guardians. Kindreds with pedigrees suggesting X-linked inheritance were recruited worldwide for clinical delineation, linkage studies and gene identification [3, 7]. Molecular studies were performed at the Wellcome Trust Sanger Institute (Cambridge, UK), Greenwood Genetic Center (South Carolina, USA), Dunedin School of Medicine (Dunedin, NZ), Institute of Pathology and Genetics (Gosselies, BE), University of Paris (Paris, FR), Mugla Sitki Kocman University (Mugla, TR), and Amsterdam University Medical Center

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(Amsterdam, NL). Methylation profiling was conducted at the Verspeeten Clinical Genome Centre at London Health Sciences Centre (London, CA).

Sequencing

A single male affected with intellectual disability from 201 families (including kindreds K9336 and K8440) with at least two males affected in pedigrees consistent with X-linkage were included in the initial study cohort [3]. Sanger sequencing of 719 of the 824 known protein coding genes on the X chromosome (Vega database) was performed using ABI3730 sequencers after the patient DNA was amplified by PCR. Sequence variants were detected using autoCSA and alignment to the X chromosome sequence. The potential significance of sequence variants was evaluated by segregation studies in the families and by sequencing of 800 normal controls.

Standard laboratory procedures for DNA isolation and whole exome sequencing as detailed by Louie et al. were used for the study of kindreds 9638, 9712, and 9715 and by Larcher et al. for the study of kindred 9711 [14, 15].

Methylation

DNA methylation data analysis included 25 individuals with *ZNF711* mutations, of which 15 and 10 were used as the training and testing sets, respectively (Table S1). Regarding the 15 training samples, they all have truncating nonsense or frameshift variants in *ZNF711* and amongst them sample K9336: IV-2 has an additional missense variant in *PKP2* gene but this variant is not considered as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines and results of the in silico prediction tool, PolyPhen-2 (Table S1). In terms of the 10 testing samples, six have nonsense variants, and amongst them three (K8440: III-1_replicate, K9336: III-2_replicate, and K9638: II-3_replicate) are replicates of the three training cases, and one (K9711: I-2) is an unaffected mother. The other four testing samples have missense variants of which two samples (K9107: III-2 and K9107: III-3) have an additional unknown variant in *CUL4B* gene. Additionally, seven control samples were also used. DNA samples extracted from peripheral blood were applied to Illumina Infinium methylationEPIC (EPIC) bead chip arrays following bisulfite conversion and methylation analysis was performed in accordance with the manufacturer's protocol. Sixty (case to control ratio of 1:4) age, sex, and array type-matched control samples were selected from the EpiSign Knowledge Database (EKD) using the MatchIt package as the control training set [16, 17].

Methylation levels calculated as the ratio of methylated signal intensity over the sum of methylated and unmethylated signal intensities, called the β-values, were converted to M-values using logit transformation in order to obtain homoscedasticity for linear regression modeling using the limma package [18]. The estimated blood cell proportions derived by the algorithm developed by Houseman et al. were added as confounding variables [19]. Subsequently, eBayes function was operated to moderate the created *p*-values.

The differentially methylated probes (DMPs) from the comparison between case and control groups were selected using the following steps. First, 1000 probes with the highest product of methylation difference means between the two groups and the negative of the logarithm of multiple-testing corrected *p*-values derived from the linear modeling by Benjamini–Hochberg (BH) method were selected. Then, a receiver's

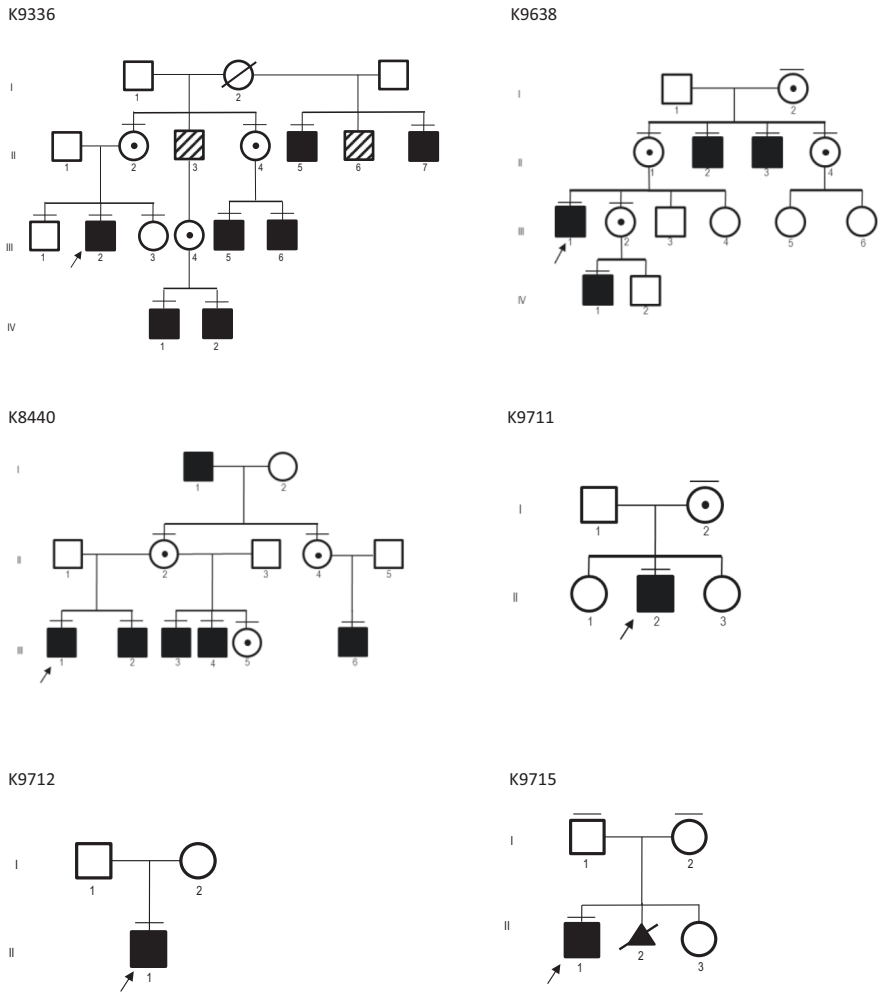

Fig. 1 Pedigrees of K9336, K9638, K8440, K9711, K9712, and K9715. Affected individuals are indicated with solid figures, carrier females by circumpunct, males assumed to be affected but not available for evaluation by hatched squares, individuals examined and tested by a bar over the figure, and probands indicated with an arrow.

operating characteristic (ROC) curve analysis was performed and 500 probes with the highest area under the ROC curve (AUROC) were retained. Next, those probes with a pair-wise Pearson's correlation coefficients >0.75, within the case and control samples separately, were removed. Finally, those probes having a mean methylation difference >5% were selected and considered as the DNA methylation signature (episignature) for *ZNF711*-related XLID (also known as MRX97 or IDX97). In order to examine the robustness of this episignature in differentiating between case and control samples, unsupervised models, including hierarchical clustering heatmap and multidimensional scaling (MDS), were applied on the selected DMPs. Then, 15 rounds of cross-validation were performed on MDS plot from the 15 case samples, of which 14 samples were used as the training set and a single sample was used as the testing set. Using the selected DMPs, a binary support vector machine (SVM) with linear kernel and 10-fold cross validation (90% of the samples were used for training at each iteration) was constructed by the e1071 package as described previously [16]. The model provides a methylation variant pathogenicity (MVP) score for each sample, ranging between 0 and 1, indicating low and high similarity between the methylation profile of that sample and that of the identified episignature, respectively. In order to evaluate the specificity of the classifier, more than 1000 samples with other neurodevelopmental syndromes from EKD were added to the model [16].

Case reports

Pedigrees for each of the six kindreds are shown in Fig. 1. In each kindred, a brief clinical report on the proband is provided. The clinical manifestations of all affected individuals are given in Table 1 and available facial photographs in Fig. 2A–E.

K9336

Nine males in three generations of K9336 are presumed to be affected but only seven were available for clinical evaluation and laboratory testing (Figs. 1 and 2A, Table 1). Statural growth varied from the 15th centile to above the 97th centile. Most affected males had large head sizes and ears but no other consistent somatic manifestation. Cognitive function fell into the mild intellectual disability and low normal range.

III-2, the proband, was born at term weighing 4.3 kg. He met his motor milestones normally, but developed speech slowly. He performed poorly academically, but graduated from a home school program. The full scale I.Q. was 70 and adaptive function mildly-moderately impaired. At age 20 years, his height was at the 50th centile, weight 60th centile, and head circumference 90th centile (parents' head circumferences 70th–75th centiles). The face appeared long with deep-set eyes, downslanting palpebral fissures, small mouth, high narrow palate, and recessed chin. The testes were large (25 mL) and the hands greater than 97th centile in length.

K9638

Four males in three generations were affected in this New Zealand kindred (Figs. 1 and 2B, Table 1). The pregnancies, deliveries, and neonatal periods for the three older males were reported as normal. Specific developmental milestones are not known, but all 3 attended secondary schools and with educational support graduated from high school. Formal I.Q. testing was not performed. The 4 carriers identified in the family were considered normal without cognitive impairment or somatic abnormalities.

III-1, the proband, had dolichocephaly, light-colored irises, large ears and prognathia, giving an overall facial appearance resembling Fragile X syndrome (number of CGG repeats in *FMR1* was normal). At age 27 years, III-1 was healthy without medical problems other than intellectual disability. Growth parameters were normal except for large ears (>97th centile).

K8440

Five males in K8440, a Hispanic family from the United States were available for evaluation (Figs. 1 and 2C, Table 1). The maternal grandfather I-1, who carries the same nonsense mutation in *ZNF711* was not available for examination, but by history required special education in school, read at a second grade level and was considered to have the same cognitive skills as the 5 grandsons. He required several hospitalizations for psychiatric disturbances.

III-1, the proband, weighed 3.4 kg at term delivery. He walked at 18 months and said initial words at 24 months. He required special education throughout the school years. He appeared weak and exhibited facial weakness, which affected his speech.At his initial examination at age 7 years, his height was above the 97th centile, weight 75th centile, and head circumference 50th centile. He had 2 occipital hair whorls, deep-set eyes, malar hypoplasia, large ears (95th centile), narrow palate, long 5th fingers, hyperconvex nails, and slight hyperpigmentation in the axillary areas. He appeared weak pulling himself on furniture to the standing position. At age 16 years, the head circumference measured at 80th centile. The hands were thin with long fingers.

K9711

II-2 weighed 4.3 kg at term birth. He met motor and speech milestones at appropriate ages but exhibited poor balance, tiptoe ambulation, and orofacial dyspraxia. His verbal and performance ID scores were well above average (General Ability Index; 131). Behavioral problems (tantrums, emotional liability, anxiety, socialization difficulties) in childhood led to a diagnosis of Asperger syndrome. At age 12 years, his height was at the 75th centile, weight 70th centile, and head circumference 97th centile. His face was long and showed infraorbital circles, large philtrum, large central incisors, and full lips. The digits were long and the feet large with mild sandal gap (Fig. 1, Table 1). The mosaic carrier mother was considered normal without cognitive impairment and somatic anomalies.

K9712

II-1, the first child of non-consanguineous healthy parents, was born at 40 weeks of gestation with weight of 3.6 kg, length 50 cm, and occipito-frontal circumference 36 cm. The neonatal period was marked by gastroesophageal reflux disease, requiring treatment for the first year. Psychomotor development was delayed with sitting at 13 months and walking without assistance at age 22 months. He had speech delay and began to link words at age 3.5 years. He had autistic mannerisms, including stereotypies, but had not yet been formally assessed. At 3 years, his developmental quotient (Revised Brunet-Lézine scale) was 70. On his last clinical examination at 3.5 years of age, his weight was at the 90th centile, height at the 75th centile and head circumference at the 65th centile. Dysmorphic facial features included epicanthus, anteverted ear lobes and a thin upper lip (Figs. 1 and 2D, Table 1). Neurological examination was normal.

K9715

II-1 is a 3-year old male with global developmental delay who was born in the Netherlands as the first child of non-consanguineous healthy parents (Figs. 1 and 2E, Table 1). Pregnancy and delivery were uneventful. He was born at 38.5 weeks of gestation with weight of 3.985 kg. There were no feeding problems, but there was significant jaundice during breast feeding, which was solved by changing to bottle feeding. Psychomotor development was delayed with sitting at 9 to 10 months and walking without assistance at age 26 months. He experienced some difficulty in speech and articulation, more than in the understanding of words. He received physiotherapy and speech therapy. After receiving tympanic tube because of frequent ear infections, his speech did not improve significantly. He has no hearing problems. No diagnosis of autism was made. Furthermore, he is very empathic. Neurological examination was normal. In addition, he has had no seizures and the brain MRI showed no abnormalities. Physical examination after birth showed a small umbilical hernia at birth and a large tongue (which is now normal). His growth has been normal, height and head circumference were at +1.0 SD. He has creases in his ear lobes on the frontal side, an upturned nose and prominent nose bridge. His ears are a bit small and square shaped. Array CGH analysis and DNA methylation for Beckwith-Wiedemann syndrome showed no abnormalities.

Sequencing results

The location of the sequence alterations in *ZNF711* from the six kindreds reported here and the two kindreds from van der Werf et al. are shown in Fig. 3 [4]. Two of the samples from the initial study cohort had truncating mutations in *ZNF711*. In kindred 9336, a nonsense mutation (c.1543 C>T; p.Arg515*) segregated with cognitive impairment in males. Two carrier females had variable X-inactivation (69:31, 88:12). A 2 bp deletion (c.2127_2128 del; p.Cys709*) was identified in kindred 8440. The deletion segregated with cognitive impairment in males. Carrier females had variable X-inactivation (80:20, 68:32, 52:48). Nonsense variants were found in K9638 (c.2227 C>T, p.Arg743*), in K9711 (c.1555 G>T, p.Glu519*), and

Table 1. Clinical findings in families with *ZNF711* alterations.

	van der Werf et al. 2019		This paper						
Phenotype	**IDX65**	**IDX97**	**K9336**	**K9638**	**K8440**	**K9711**	**K9712**	**K9715**	**All**
ZNF711 variant	p.Ile244Thr	p.Phe685Serfs*7	p.Arg515*	p.Arg743*	p.709 fs*1	p.Glu519*	p.Arg721*	p.Cys706Arg	
Affected males	6	5	7	4	6	1	1	1	31/31
Low birth weight	NR	0/3	0/7	0/1	0/4	0/1	0/1	0/1	0/18
Short stature	2/6	1/5	0/7	0/1	0/5	0/1	0/1	0/1	2/27
Tall stature	0/6	0/5	2/7	0/1	2/5	0/1	0/1	1/1	5/27
Obesity	4/6	0/4	1/7	0/4	1/5	0/1	0/1	0/1	6/29
Macrocephaly	0/6	0/4	3/7	0/4	1/5	1/1	0/1	0/1	5/29
Dolichocephaly	NR	NR	0/7	4/4	0/5	0/1	NR	0/1	4/19
Broad face	6/6	1/5	0/7	0/4	3/4	0/1	0/1	0/1	10/29
Long face	0/6	4/5	2/7	3/4	1/4	1/1	0/1	0/1	11/29
Facial asymmetry	NR	NR	2/7	0/4	1/4	0/1	0/1	0/1	11/18***
Low anterior hairline	NR	NR	0/7	0/4	3/4	0/1	0/1	0/1	3/18
Synophrys	0/6	3/5	0/7	1/4	0/4	0/1	0/1	0/1	4/29
Hypertelorism	NR	NR	0/7	0/4	2/5	0/1	0/1	0/1	2/19
Deep set eyes	NR	NR	3/7	1/4	2/5	0/1	0/1	0/1	6/19
Downslanting palpebral fissures	NR	NR	3/7	1/4	1/4	0/1	0/1	0/1	5/18
Upslanting palpebral fissures	NR	NR	1/7	0/4	NR	0/1	0/1	0/1	1/14
Malar hypoplasia	NR	NR	5/7	0/1	3/5	0/1	0/1	0/1	8/16***
Midface hypoplasia/flattening	NR	NR	4/7	0/1	3/5	0/1	0/1	0/1	7/16
Broad nose	4/6	1/5	NR	0/4	NR	0/1	0/1	0/1	5/18
Narrow nose	NR	NR	2/7	2/4	NR	0/1	0/1	0/1	4/14
Prominent columella	NR	NR	1/7	NR	NR	0/1	0/1	0/1	1/10
Large ears	0/6	4/5	2/7	3/4	3/6	0/1	0/1	0/1	12/31
Narrow/high palate	NR	NR	2/7	NR	4/5	0/1	0/1	0/1	4/15
Prognathism	NR	NR	1/7	3/4	1/5	0/1	0/1	0/1	5/19
Seizures/abnormal EEG	NR	NR	1/7	0/4	2/5	0/1	0/1	0/1	3/19
Autism/autistic features	0/0	2/2	4/7	0/4	1/4	1/1	1/1	1/1	11/20***
Mild ID	3/6	3/5	7/7	4/4	5/5	0/1	1/1	0/1	23/30***
Moderate-severe ID	3/6	2/5	0/7	0/4	0/5	0/1	0/1	1/1	6/30
Acanthosis nigricans	NR	NR	0/7	0/4	3/5	0/1	0/1	0/1	3/19

***Manifestation present in ≥50 percent.

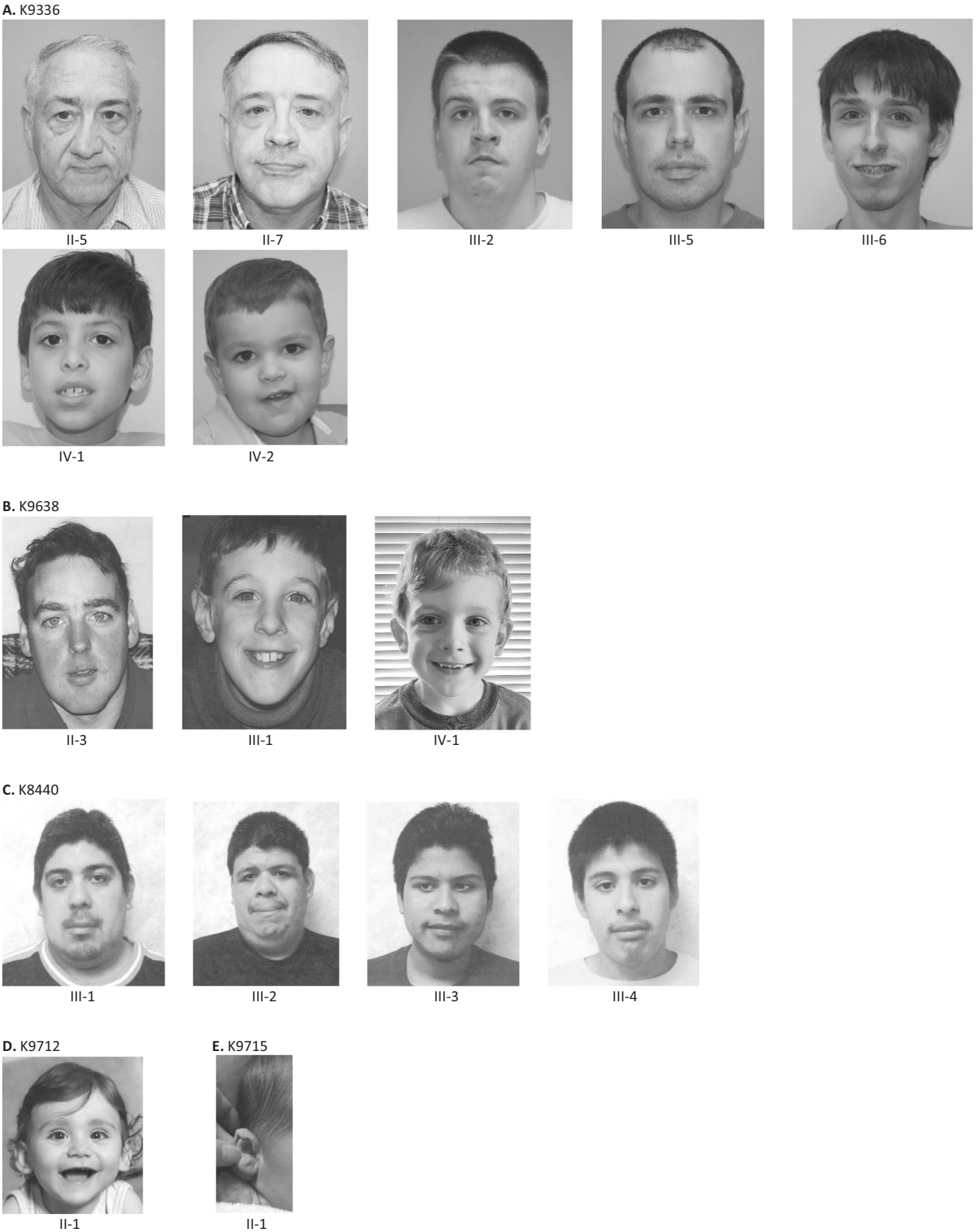

Fig. 2 Facial appearance of affected individuals from K9336, K9638, K8440, and K9712. A Affected males from K9336. Note the generous head size in all, variable palpebral fissure slant, deep-set eyes (III-2), thin nose and alae nasi (III-5 and III-6), and thin upper lip (II-5 and III-6). **B** Affected males from K9638. Note long face with downslanting palpebral fissures, narrow nasal bridge and prominent or pointed chin (II-3 and III-1). **C** Affected males from K8440. Note low anterior hairline, deep-set eyes, and full cheeks. **D** Affected male from K9712. Note upturned earlobes and thin upper lip. **E** Ear creases in II-1 from K9715.

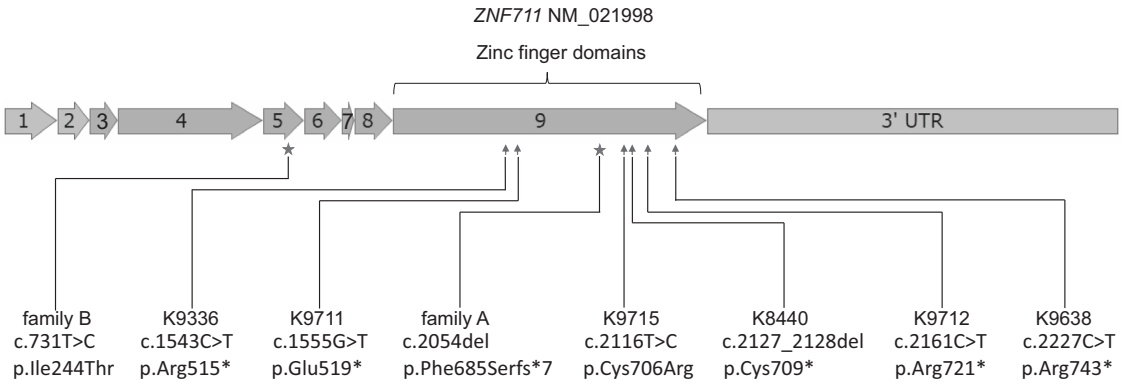

Fig. 3 Schematic diagram of the ZNF711 transcript NM_021998. Exons 1 and 2 are non-coding exons and indicated in gray arrows whereas coding exons 3–9 are in green. The red arrows indicate the corresponding DNA alterations identified reported in this study. The blue stars indicate the variants that has been previously reported by van der Werf et al. [4] in family A and family B. The nucleotide change in each family and kindred is indicated as well.

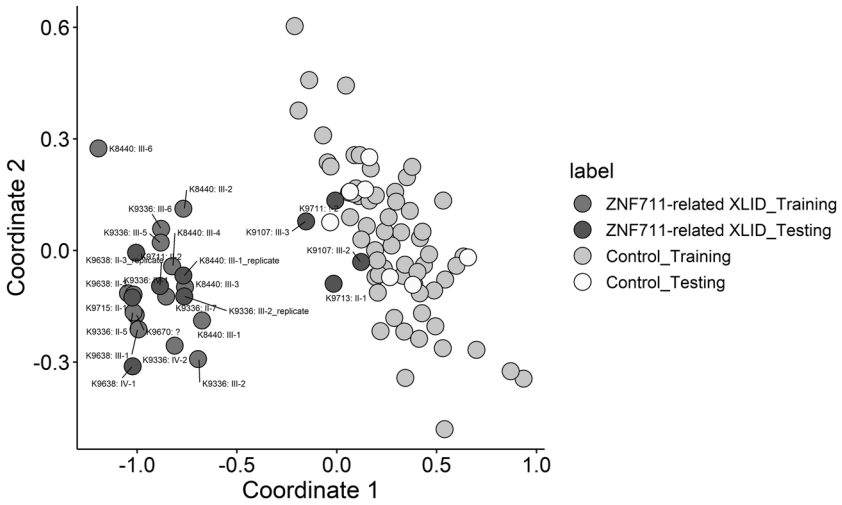

Fig. 4 Identification of a ZNF711-related XLID episignature using the selected probes illustrated in a multidimensional scaling (MDS) plot. Red and purple (color online) circles represent 14 ZNF711-related XLID training and 11 ZNF711-related XLID testing samples, respectively. Blue and yellow (color online) circles represent control training and testing samples, respectively.

in K9712 (c.2161 C>T, p.Arg721*), and a de novo missense variant was identified in K9715 (c.2116 T>C, p.Cys706Arg). The missense variant in K9715 is predicted to be deleterious (PolyPhen score 0.99, SIFT score 0).

Methylation results

Comparison between *ZNF711* training and control samples resulted in identification of 161 DMPs. The methylation levels at these 161 CpG sites were considered as the identifying episignature for *ZNF711*-related XLID. In order to assess the robustness of the episignature in differentiating between the case and control samples, hierarchical clustering (Fig. S1) and MDS analysis (Fig. 4) were performed, resulting in a complete segregation between these two groups. Among the 10 *ZNF711* testing samples, six and four were grouped with training and control samples, respectively (Figs. S1 and 4). Fifteen rounds of cross-validation on MDS plot were performed using different combinations of case samples ($n = 14$) as training set and single samples as testing set. In all steps, the testing samples were correctly clustered with the training samples further providing evidence of a robust common DNA methylation signature (Fig. S5). To increase the specificity of the classifier, an SVM was trained by comparing the 15 *ZNF711* training samples against *ZNF711* testing samples, controls, as well as 37 neurodevelopmental disorders and congenital anomalies with known episignatures present in the EKD [16]. A high MVP score was seen for 15 *ZNF711* training samples and six *ZNF711* testing samples along with much improved specificity relative to other EpiSign conditions (Fig. S2). Furthermore, two samples from patients with Sotos syndrome received MVP scores of 0.36 and 0.66: however, plotting them in MDS analysis (Fig. S3) and hierarchical clustering (Fig. S4), ruled out the existence of a similar episignature, showing their separation from *ZNF711* and control samples.

DISCUSSION

To date, 30 genes on the X-chromosome have been linked to nonsyndromic XLID, 102 genes with syndromic XLID, and 29 genes with both forms of XLID [7]. Alterations in eight zinc-finger genes on the X chromosome have been associated with nonsyndromic XLID [7]. Families with alterations in five of the zinc-finger genes have been assigned nonsyndromic XLID numbers: *ZNF41* in IDX89, *ZNF81* in IDX45, *ZDHHC15* in IDX91, *ZNF674* in IDX92, and *ZNF711* in IDX65 and IDX97 [4, 7, 20]. The female with a translocation that disrupted in *ZNF741*, and males with alterations in *ZCCHC12* and *ZNF897* also have nonsyndromic XLID but have not received IDX numbers [8, 21, 22]. *ZC4H2, ZMYM3*, and *ZDHCC9* variants have been associated with syndromic forms of XLID [9, 12, 13].

No consistent phenotype was observed among the 20 males in the six kindreds with *ZNF711* alterations reported herein or in the 11 individuals in the two previously reported kindreds (Table 1) [4]. Prenatal growth advanced normally and postnatal growth

showed no consistent aberration. Although one third of patients were described as having elongation of the face and large ears, there are no distinctive facial dysmorphology that would allow clinical recognition. Motor milestones were typically reached within an appropriate age range or only minimally delayed whereas acquisition of speech was delayed more frequently and to a greater degree. With the exception of the male with Asperger syndrome in K9711, the ultimate cognitive function was most commonly in the low normal or mildly impaired range. Autistic manifestations were observed in half of the individuals (Table 1). Female carriers showed no manifestations.

Nonsense, frame shift, and missense variants are included among *ZNF711* alterations (Table 1). Contiguous gene microdeletions and microduplications that include *ZNF711* have been previously reported [2, 23–26].

In this study, the identified biomarkers of *ZNF711* could work as a functional test for patients with ambiguous genetic test findings. Amongst 10 testing samples, six and four were grouped with ZNF711-related XLID training and control cohorts (Fig. 4, S1, and S2), respectively. From the six samples grouped with training cases, three (K8440: III-1_replicate, K9336: III-2_replicate, and K9638: II-3_replicate) were replicates of the three training cases and as expected they were grouped with cases and of the other three, two have truncating nonsense variants (K9711: II-2 and K9638: IV-1) and one has missense variant (K9715: II-1) which based on ACMG guidelines and in silico prediction tools' results, including PolyPhen-2 and SIFT (Table S1), is classified as pathogenic so it was expected to see these three samples to be grouped with cases. In terms of the other four testing samples grouped with controls, one (K9711: I-2) is a carrier unaffected mother of K9711: II-2 and three has missense variants (K9107: III-2, K9107: III-3, and K9713: II-1) but based on both the ACMG guidelines and in silico prediction tools' results (Table S1), these variants are not pathogenic. Therefore, these four samples should be clustered with controls.

One functional consequence of genetic defects in patients with hereditary neurodevelopmental disorders can be the disruption of genomic DNA methylation [27]. DNA methylation is an epigenetic modification, resulting in changes in structural and chemical properties of the DNA, impacting molecular mechanisms including chromatin assembly and gene transcription. Our group and others have demonstrated that individuals among a growing number of rare disorders exhibit DNA methylation "episignatures" or "EpiSigns" as highly sensitive and specific DNA methylation biomarkers in the blood DNA of affected individuals [16, 28–31]. In this study, we also showed the robustness of *ZNF711* episignature in discriminating between case and controls, using unsupervised machine learning algorithms (MDS analysis in Fig. 4 and hierarchical heatmap clustering in Fig. S1), supervised machine learning algorithm (SVM classifier in Fig. S2) as well as cross-validation, where in all iterations one testing sample was grouped with case training samples. These genome-wide DNA methylation profiles currently include over 40 rare disorders being highly sensitive and specific for each disorder. As such, they represent effective biomarkers for the testing of patients with a broadening range of neurodevelopmental genetic conditions, as well as a reflex functional test for patients with ambiguous genetic test findings or clinical phenotypes [32]. Based on this technology, a genome-wide DNA methylation analysis test EpiSign has recently been adapted for the diagnosis of Mendelian disorders [33]. This study adds the DNA methylation EpiSign for the *ZNF711*-related gene defects, and as such provides a sensitive and specific biomarker for molecular diagnosis, as an alternative to DNA sequencing. In particular, the *ZNF711* episignature will be useful as a functional biomarker helping resolve cases with VUS as well as cases where DNA sequence variants may lie outside of coding regions. Further work focused on analysis of DNA methylation episignatures in other zinc finger genes will provide interesting insights in a potential overlap of these methylation defects and functional overlap in the molecular etiology of these disorders.

The presence of a DNA methylation signature in *ZNF711*, a transcriptional regulator, expands the evidence of DNA methylation signatures beyond strictly chromatinopathies, or Mendelian disorders of chromatin regulation. In our previous work, majority of the episignatures reported involved genes related to the functional pathways of DNA methylation regulation, chromatin assembly and histone modification regulatory mechanisms [16]. This highlights the existence of DNA methylation signatures in a broader range of Mendelian disorders which warrants further investigation. We have similarly demonstrated presence of episignatures associated with mutations in genes involved in chromatin remodeling that also act as transcription factors (e.g., *SRCAP*, *NSD1*) [16, 33, 34].

To date, *ZNF711* is the only X-linked zinc-finger gene with a specific episignature which can serve as a functional study to resolve the pathology of a variant of uncertain significance and as a primary diagnostic test for individuals with IDX. *ZNF711* joins 10 other XLID genes (*ATRX, BRWD3, FAM50A, KDM5C, KDM6A, PHF6, PQBP1, SMC1A, SMS, UBE2A*) in having a specific episignature [16].

DATA AVAILABILITY

The summarized, anonymized data for each subject in this study are described in the text. Publicly available DNA methylation datasets have been deposited in GEO, and include data referring to various developmental syndromes (e.g., Kabuki syndrome, Sotos syndrome, CHARGE syndrome, immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome, Williams-Beuren syndrome, Chr7q11.23 duplication syndrome, BAFopathies, Down syndrome), a large cohort of unresolved subjects with developmental delay/intellectual disability and congenital abnormalities, and also several large cohorts of DNA methylation data from the general population. Data in the EpiSign Knowledge Database, including methylation data from this *ZNF711* study cohort, are not available due to Research Ethics Board and institutional restrictions. EpiSign™ is proprietary software and is not publicly available.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

The research protocol and consent were reviewed and approved by the Self Regional Healthcare Institutional Review Board (IRB).

ADDITIONAL INFORMATION

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