European Journal of Human Genetics (2015), 1–6
© 2015 Macmillan Publishers Limited All rights reserved 1018-4813/15
www.nature.com/ejhg

ARTICLE

A *de novo FOXP1* variant in a patient with autism, intellectual disability and severe speech and language impairment

Reymundo Lozano*[,1], Arianna Vino[2], Cristina Lozano[1], Simon E Fisher[2,3] and Pelagia Deriziotis*[,2]

FOXP1 (forkhead box protein P1) is a transcription factor involved in the development of several tissues, including the brain. An emerging phenotype of patients with protein-disrupting *FOXP1* variants includes global developmental delay, intellectual disability and mild to severe speech/language deficits. We report on a female child with a history of severe hypotonia, autism spectrum disorder and mild intellectual disability with severe speech/language impairment. Clinical exome sequencing identified a heterozygous *de novo FOXP1* variant c.1267_1268delGT (p.V423Hfs*37). Functional analyses using cellular models show that the variant disrupts multiple aspects of FOXP1 activity, including subcellular localization and transcriptional repression properties. Our findings highlight the importance of performing functional characterization to help uncover the biological significance of variants identified by genomics approaches, thereby providing insight into pathways underlying complex neurodevelopmental disorders. Moreover, our data support the hypothesis that *de novo* variants represent significant causal factors in severe sporadic disorders and extend the phenotype seen in individuals with *FOXP1* haploinsufficiency.
European Journal of Human Genetics advance online publication, 8 April 2015; doi:10.1038/ejhg.2015.66

INTRODUCTION

FOXP1 (forkhead box protein P1; OMIM 605515) belongs to the FOX gene family of transcription factor proteins, defined by the presence of a characteristic DNA-binding domain known as the forkhead box (FOX).[1] The mouse ortholog, *Foxp1*, is required for normal development of the heart, lung and esophagus[2,3] and acts as an accessory factor to Hox transcription factors in regulating the projection and connection of motor neurons to target muscles.[4,5] The importance of *Foxp1* in neurodevelopment is highlighted by studies in mice with a brain-specific *Foxp1* deletion.[6] These mutant mice show impaired neuronal development and present with autistic-like behaviors. In recent years, *FOXP1* variants have been reported in a number of patients with sporadic autism spectrum disorders (ASD), intellectual disability (ID), global developmental delay and moderate to severe speech delay, where expressive language is most severely affected (OMIM 613670).[7,8] The presence of speech and language impairments as characteristic of *FOXP1*-deficiency syndrome is interesting because the most similar gene to *FOXP1* is *FOXP2*, which is disrupted in a rare form of speech and language disorder (OMIM: gene 605317, disorder 602081).

FOXP1 variants identified in patients with ASD and/or ID include whole gene deletions,[7,9–12] translocations,[13] nonsense variants,[14] missense variants[15] and frameshift variants.[16] The identification of whole gene deletions suggests that the mechanism of pathogenicity is haploinsufficiency. The severity of the phenotype indicates that these mutations are not inherited and in all cases where parental DNA has been tested the variants have been found to occur *de novo*. Using next-generation sequencing, in this study we identified and characterized a *de novo FOXP1* variant in a female child with a history of severe hypotonia, ASD and mild ID with severe speech/language deficits.

MATERIALS AND METHODS

Clinical exome sequencing

Clinical exome sequencing of the proband and her unaffected parents was performed in the UCLA Clinical Genomics Center (see Lee *et al*[17] for a detailed description of the UCLA clinical exome sequencing pipeline). There was only one high-quality nonsynonymous *de novo* coding variant identified in the patient, affecting the *FOXP1* gene. This sequence variant had a quality score ≥ 500, no reads supporting the alternate allele in the parents and had never been observed in the general population (based on 1k Genomes Project, dbSNP135, NHLBI ESP). There were no clinically significant inherited homozygous, hemizygous, homoplasmic or compound heterozygous variants identified in the patient.

Cell culture and transfection

Epstein–Barr virus-transformed live lymphoblast cell lines were obtained from the father, mother, proband and one unaffected sibling. Cultures were grown in RPMI-1640 (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). HEK293 cells were grown in DMEM and SHSY5Y in DMEM-F12 (Invitrogen). Media were supplemented with 10% fetal bovine serum (Invitrogen). Transfections were performed using GeneJuice according to the manufacturer's instructions (Merck-Millipore, Amsterdam, The Netherlands).

[1]Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, Department of Pediatrics, UC Davis Medical Center, Sacramento, CA, USA; [2]Language and Genetics Department, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands; [3]Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands
*Correspondence: Dr R Lozano, Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, Department of Pediatrics, UC Davis Medical Center, 2825 50th Street, Sacramento, CA 95817, USA. Tel: +1 916 703 0494; Fax: +1 916 703 0417; E-mail: reymundo.lozano@ucdmc.ucdavis.edu
or Dr P Deriziotis, Language and Genetics Department, Max Planck Institute for Psycholinguistics, Wundtlaan 1, Nijmegen 6525 XD, The Netherlands. Tel: +31 24 361 4305; Fax: +31 24 352 1213; E-mail: pelagia.deriziotis@mpi.nl
Received 30 October 2014; revised 5 February 2015; accepted 26 February 2015

RNA extraction and cDNA synthesis
Total RNA was extracted from immortalized lymphoblasts using the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands) and first-strand cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc., Foster City, CA, USA).

Nonsense-mediated decay
Nonsense-mediated decay (NMD) was assessed as previously described.[16] *FOXP1* cDNA was PCR-amplified using forward 5′-GTCTACAGAACCCAAA GCCGC-3′ and reverse 5′-GGTTCTGCGCAATATCTGCTG-3′ primers, followed by Sanger sequencing.

Quantitative RT-PCR
Quantitative RT-PCR was performed and analyzed as previously described,[18] using primers specific for the amplification of *FOXP1* c.1267_1268delGT (forward 5′-CGTCACCCAAGGCCCCTCTC-3′; reverse 5′-TTGCGTCGGAAG TAAGCAAAC-3′), followed by Sanger sequencing.

DNA expression constructs and site-directed mutagenesis
Wildtype (WT) *FOXP1* and *FOXP2* were PCR amplified and cloned into pCR2.1-TOPO (Invitrogen).[19] The p.V423Hfs*37 FOXP1 variant was generated using the pCR2.1-TOPO.FOXP1 as template with the QuikChange II Site-Directed mutagenesis kit (Agilent Technologies, Wilmington, DE, USA) and forward 5′-TGGTGGTTGTGATGAGAGGGGCCTTGGG-3′ and reverse 5′-CCCAAGGCCCCTCTCATCACAACCACCA-3′ primers. FOXP cDNAs were subcloned using *Bam*HI/*Xba*I restriction sites into pcDNA4HisMax, a modified pmCherry-C1 vector (Clontech, Saint-Germain-en-Laye, France), as well as pLuc and pYFP expression vectors.[20] All constructs were verified by Sanger sequencing.

SDS-PAGE and western blotting
HEK293 cells were transfected with equimolar concentrations of FOXP1 expression plasmids and cultured for 48 h. Cell lysis, SDS-PAGE and western blotting were performed as previously described.[19] Membranes were probed with Clontech mouse anti-EGFP (for pYFP constructs) overnight at 4 °C, followed by incubation with HRP-conjugated goat anti-mouse IgG for 45 min at room temperature (Bio-Rad, Veenendaal, The Netherlands). Blots were stripped and reprobed with Sigma anti-β-actin antibody to confirm equal protein loading.

Fluorescence microscopy
HEK293 and SHSY5Y cells were fixed on coverslips as previously described.[19] YFP and mCherry fusion proteins were visualized by direct fluorescence, and nuclei were visualized with Hoescht 33342 (Invitrogen). Fluorescence images were obtained using an Axiovert A-1 fluorescence microscope with ZEN Image Software (Zeiss, Oberkochen, Germany).

Luciferase reporter assays
Luciferase assays were carried out as previously described.[19]

Bioluminescence resonance energy transfer (BRET) assays
BRET assays were performed as previously described.[19,20]

Statistical significance
The statistical significance of the luciferase reporter assays was analyzed using a one-way analysis of variance and Tukey's *post hoc* test.

RESULTS
Clinical phenotype
The patient is a 14-year-old female, first-born child of nonconsanguineous parents. Her mother was 30 and her father was 32 years old at the time of her birth. She was born via C-section for oligohydramnios and preeclampsia and was noted to have a two-vessel umbilical cord and severe hypotonia at birth. During her first year she had severe gross motor delays; she started to crawl at 12 months and walk at 20 months. The patient also had speech delays, with first words at 20 months. At 3 years of age, she was diagnosed with expressive and receptive communication deficits.

At present, only 50% of the patient's speech is coherent and she is not able to sustain a conversation. In particular, her speech lacks structure, has limited content and expansion and she repeats simple phrases (echolalia). Her preferred method of communication is via messaging through an electronic device. The patient performed poorly on language-skill evaluations: she scored 50 on the comprehensive assessment of spoken language (<0.1 percentile), 70 on the receptive one-word picture vocabulary test (2.0 percentile), 64 on the expressive one-word picture vocabulary test (1.0 percentile) and 78 on the Arizona articulation proficiency scale (3rd edition) (7.0 percentile). She has inconsistent production of 'j' and 'th', final consonant deletion with 'd', 't' and 'g', substitutes 'sh' for 'ch' and has difficulties with the vocalic 'r'. She continues to meet criteria for speech and language impairment as a discrepancy exists between her oral language skills in the areas of expressive and receptive language and her chronological age.

She is in eighth grade of special education and her cognitive evaluation this year showed a full-scale IQ of 54 (0.1 percentile), verbal comprehension index score of 59 (0.3 percentile), working memory index of 50 (<0.1 percentile) and processing speed index of 70 (2.0 percentile). She has obsessive behavior traits, restricted interests, repetitive and perseverative behaviors, likes routine and received the diagnosis of ASD. Furthermore, she has episodes of aggression and was diagnosed with attention deficit hyperactivity disorder at 7 years of age.

The patient is macrocephalic (+4 SD) and has underdeveloped ear lobes (Figure 1a). She also has mild ptosis, high palate, wide-spaced teeth and a hypotonic face. Her gait is abnormal with mild right foot dragging and a wide base. Her height is at 58th percentile (159 cm) and her weight is at 75th percentile (53.2 kg). Her adaptive physical education assessment indicated that she has difficulty with balance and overall low fitness scores. The rest of the physical examination was normal.

Investigations with normal results included genetic testing for muscular dystrophy, mitochondrial disorders, fragile X syndrome and SNP microarray. Electroencephalography and nerve conduction studies were normal. Magnetic resonance imaging, performed at 12 years of age, revealed multiple nonenhancing subcortical and deep white matter abnormalities and an incidental finding of venous angioma in the left frontal lobe.

De novo FOXP1 sequence variant identified by exome sequencing
In sporadic cases of ASD, gene-discovery approaches have recently focused on identifying *de novo* variants by employing whole-exome sequencing in parent–child trios. This strategy has been successful in uncovering a substantial fraction of population risk and identifying variation conferring relatively larger biological effects.[21] Therefore, to identify putative variants that may affect protein function, we performed clinical exome sequencing[17] on DNA from the proband and her unaffected parents. Using this approach we identified a heterozygous *de novo* two-base deletion in *FOXP1* present in the proband that was subsequently validated and confirmed as *de novo* by Sanger sequencing (Figure 1b). The *FOXP1* variant has been submitted to the NCBI ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/; ClinVar accession number SCV000189225).

This variant, c.1267_1268delGT (numbered from the first coding nucleotide in NM_032682.5), is located in exon 15 of *FOXP1*

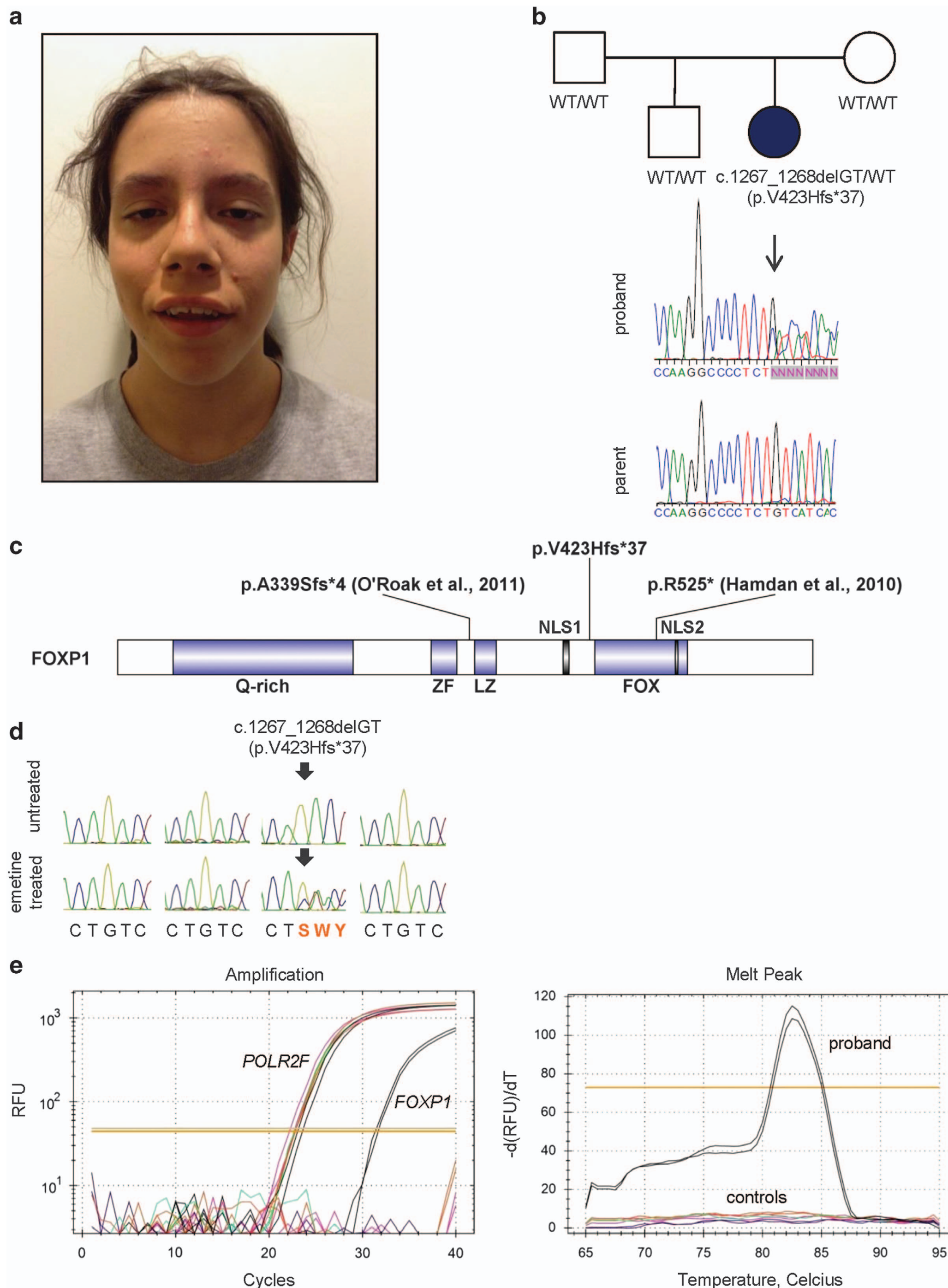

Figure 1 Identification of a *de novo FOXP1* variant in a patient with hypotonia, autism, mild ID and speech/language deficits. (**a**) Photograph of the patient at 14 years of age showing macrocephaly, mild ptosis, wide-spaced teeth and a hypotonic face. (**b**) Family pedigree showing Sanger traces of genomic DNA from the patient and one unaffected parent. The affected proband (shaded symbol) carries a heterozygous *de novo* two-base deletion (-GT relative to mRNA; c.1267_1268delGT) that introduces a frameshift and results in a premature stop codon. Site of deletion is indicated by black arrow. (**c**) Schematic representation of the FOXP1 protein indicating the change found in the patient. The FOXP1 protein has a glutamine-rich (Q-rich) region and zinc-finger (ZF), leucine zipper (LZ) and FOX DNA-binding domains, as well as two nuclear localization signals (NLS1 and NLS2). The change in *FOXP1* is predicted to yield a C-terminally truncated protein (p.V423Hfs*37) that lacks the FOX DNA-binding domain and NLS. Other truncated FOXP1 variants previously reported in ASD (p.A339Sfs*4) and ID (p.R525*) are also shown. (**d**) Sanger traces of *FOXP1* cDNA from immortalized lymphoblasts derived from the affected proband and controls (father, mother and unaffected sibling). Two sets of cultures were grown in parallel, one of which was treated with 100 μg/ml emetine for 7 h to inhibit NMD. In untreated cells, there is no evidence of the variant transcript, suggesting that the majority of these transcripts are degraded by NMD. Sanger traces from left to right: father, mother, proband, unaffected sibling. Arrows indicate site of deletion. (**e**) Quantitative RT-PCR amplification of variant *FOXP1* transcripts in cDNA derived from the proband and controls (father, mother and unaffected sibling) (left panel). Melting curve analysis was performed to assess the specificity of the amplification (right panel). Primers were designed to specifically detect the variant allele and amplification of c.1267_1268delGT was only seen in proband-derived cDNA (black lines). Results represent two technical replicates per condition. *POLR2F* was used as a control. RFU, raw fluorescence unit.

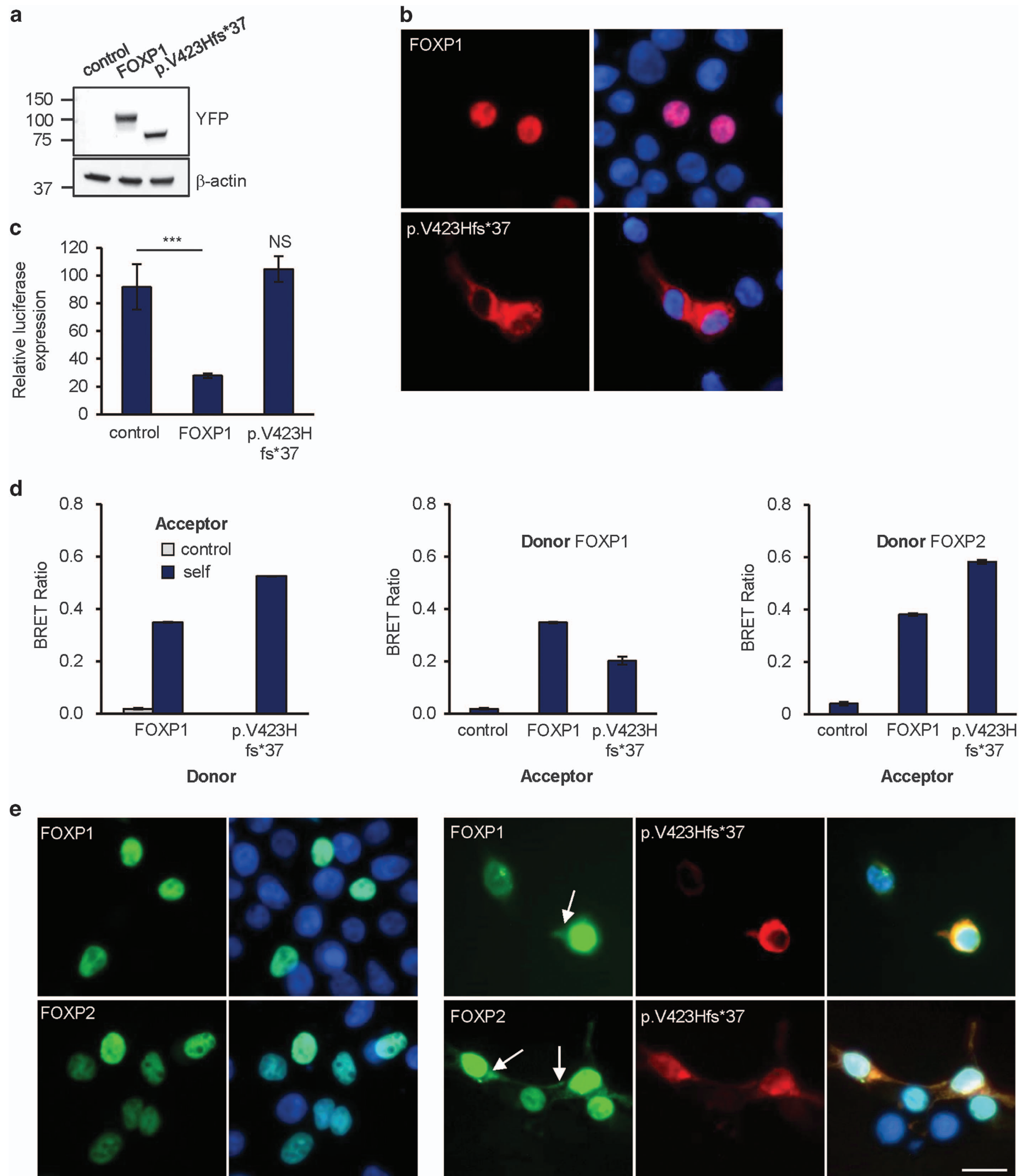

Figure 2 Functional characterization of the *de novo FOXP1* variant. (**a**) Immunoblot of FOXP1 proteins fused to YFP in transfected HEK293 cells demonstrates similar levels of expression between WT FOXP1 and the mutant protein. Control denotes cells transfected with empty pYFP vector. Equal protein loading was confirmed by stripping and reprobing with an anti-β-actin antibody. (**b**) Fluorescence imaging of HEK293 cells transfected with WT or mutant FOXP1 fused to mCherry (red). The p.V423Hfs*37 variant displays aberrant localization to the cytoplasm. Nuclei were stained with Hoescht 33342 (blue). Scale bar = 10 μm. Concordant results were seen in SHSY5Y cells, as shown in Supplementary Figure 3. (**c**) Luciferase reporter assays for transcriptional regulatory activity of FOXP1 variants in HEK293 cells. The FOXP1 p.V423Hfs*37 variant failed to repress expression of the luciferase reporter, indicating loss of transcriptional repressor activity. Values are expressed relative to the control (***$P<0.001$; NS, not significant). The mean ± SEM of three independent experiments performed in triplicate is shown. (**d**) BRET assays for interaction between the p.V423Hfs*37 variant and WT FOXP proteins in live HEK293 cells. Bars represent the corrected mean BRET ratios ± SEM of one experiment performed in triplicate. (**e**) Fluorescence images of HEK293 cells transfected with WT and mutant FOXPs. In the absence of the p.V423Hfs*37 variant (fused to mCherry; red), FOXP1 and FOXP2 are nuclear (fused to YFP; green) (left panel). When coexpressed with the p.V423Hfs*37 variant, FOXP1 and FOXP2 mislocalize to the cytoplasm (right panel). Nuclei were stained with Hoescht 33342 (blue). Scale bar = 10 μm. Concordant results were seen in SHSY5Y cells, as shown in Supplementary Figure 3.

(numbered from NG_028243.1). The change is predicted to introduce a frameshift into the encoded protein, resulting in premature termination and loss of the DNA-binding domain and nuclear localization signals (NLSs) (p.V423Hfs*37 according to HGVS recommendations) (Figure 1c). Two potentially causative heterozygous *de novo FOXP1* truncating variants have been reported previously in cases of ASD (c.1014_1015insA (p.A339Sfs*4)) and ID (c.1573C>T (p.R525*)) with speech and language deficits[14,16] (see Figure 1c for a comparison between the three truncated protein variants of FOXP1).

Because the variant introduces a stop codon before the final exon boundary in *FOXP1*, the mutant transcript may be subject to NMD. Therefore, we assessed the expression of *FOXP1* transcripts in proband-derived lymphoblasts. Under normal cell culture conditions, it was found that only the WT allele was expressed (Figure 1d). Following inhibition of NMD, expression of both the variant and WT alleles could be detected, indicating that variant transcripts are generally degraded by NMD (Figure 1d). To investigate whether any of the variant transcripts escape this process, we performed quantitative RT-PCR with primers specific for the variant *FOXP1* allele and found that c.1267_1268delGT FOXP1 is expressed in low levels in proband-derived lymphoblasts (Figure 1e and Supplementary Figure 1).

Functional characterization of the *FOXP1* sequence variant in human cells

To examine the effects of variant transcripts escaping NMD, we performed functional characterization of the protein encoded by the mutated transcript. WT and variant forms of FOXP1 were expressed as YFP- or mCherry-fusion proteins in HEK293 cells. The p.V423Hfs*37 protein variant was expressed at a similar level to the WT protein as assessed by western blotting (Figure 2a). In contrast to FOXP1 that localized to the nucleus, the p.V423Hfs*37 variant fused to mCherry localized to the cytoplasm, consistent with loss of both nuclear localization signals (Figure 2b). Of note, when the p.V423Hfs*37 variant was fused to YFP, it formed aggregates in the cytoplasm (Supplementary Figure 2). Similar results were obtained when examining the subcellular localization of the FOXP1 variant in transfected human neuroblastoma SHSY5Y cells (Supplementary Figure 3). The lack of the FOX DNA-binding domain suggests that the FOXP1 variant is unlikely to retain DNA-binding capacity. Luciferase reporter assays demonstrated that the p.V423Hfs*37 variant is unable to repress transcription, consistent with loss of DNA-binding capacity (Figure 2c).

FOXP proteins are able to form homodimers and to heterodimerize with other FOXP proteins through a leucine zipper domain.[22] Of note, FOXP1 and FOXP2 show partly overlapping neural expression patterns[23,24] such that FOXP1/FOXP2 heterodimers could regulate downstream targets, including those involved in language development, such as *CNTNAP2*.[16,25] Therefore, we investigated the effect of the *de novo FOXP1* variant on the ability of the protein to homodimerize and to interact with WT FOXP1 and FOXP2 using the BRET assay. This method can monitor protein–protein interactions in live cells and has been successfully employed to study FOXP interactions.[19,20] The FOXP1 protein variant retains the leucine zipper domain required for homo- and heterodimerization with other FOXP proteins, and our results demonstrate that the p.V423Hfs*37 variant can interact with itself, and with WT FOXP1 and FOXP2 in transfected cells (Figure 2d). Strikingly, coexpression of the p.V423Hfs*37 variant with WT FOXP1 or FOXP2 resulted in colocalization of p.V423Hfs*37 and WT proteins in the cytoplasm, indicating that the FOXP1 variant could interfere with the function of the WT protein as a result of heterodimerization (Figure 2e and Supplementary Figure 3).

DISCUSSION

Here we report a patient with a *de novo* 2-bp deletion in *FOXP1*. This change leads to degradation of variant transcripts and encodes a nonfunctional protein, indicating that the patient phenotype likely results from *FOXP1* haploinsufficiency. Furthermore, we demonstrate that a subset of variant *FOXP1* transcripts escapes NMD and sequesters WT FOXP1 and FOXP2 outside the nucleus, suggesting that, if translated, the p.V423Hfs*37 variant may also be exerting a dominant negative effect. In this case, the biological effects would be due to a combination of mechanisms, with the main pathological mechanism being haploinsufficiency. Future knockdown *FOXP1* studies (eg, using shRNA constructs) should be informative in assessing the impacts of *FOXP1* haploinsufficiency at the molecular level.

Our patient shares several phenotypic characteristics with other individuals who carry *FOXP1* variants that disrupt protein function, including autism, ID, severe language deficits and macrocephaly.[7] Together, these observations indicate that *FOXP1* haploinsufficiency results in a distinct syndrome that includes aspects of both ID and ASD, as well as other clinical features.[7] Targeted screens for *FOXP1* variants are therefore warranted in probands with clinical features consistent with the syndrome.

Even though speech and language were affected in our patient, she did not have childhood apraxia of speech (difficulty with coordinating the complex sequences of orofacial muscle movements required for speech), and this is the most prominent feature in cases with pathogenic *FOXP2* mutations.[26,27] In individuals with *FOXP2* variants, speech articulation problems are accompanied by impairments in multiple aspects of expressive and receptive language.[28,29] In our patient, expressive language was more severely affected than receptive language, in agreement with phenotypes seen in other individuals with *FOXP1* loss-of-function variants.[7,11,14] Thus far, *FOXP1* variants have not been observed to cause language disorder in the absence of more global cognitive dysfunction.[30] The observed differences between the phenotypes of *FOXP1* and *FOXP2* haploinsufficiency may reflect differences in neural expression patterns[23,24] and/or regulation of shared and nonshared downstream targets.

Our investigation provides additional evidence that a significant proportion of severe sporadic neurodevelopmental disorders may result from *de novo* variants. Furthermore, this study highlights the value of experimental characterization of variants detected through next-generation DNA sequencing in understanding the molecular and cellular networks underlying major neurodevelopmental disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the family for taking part in the research. This work was supported by the Max Planck Society, the National Institute on Aging of the National Institutes of Health (P30AG043097) and the NIH diversity supplement (HD036071). We also thank Dr Joshua Deignan, Hane Lee and the UCLA Clinical Genomics Centre for clinical exome sequencing and data interpretation, Dr Pedro Rodenas-Cuadrado and Paolo Devanna for technical advice and Dr Randi J Hagerman and Dr Sarah A Graham for useful discussions on the manuscript.

1 Shu W, Yang H, Zhang L, Lu MM, Morrisey EE: Characterization of a new subfamily of winged-helix/forkhead (Fox) genes that are expressed in the lung and act as transcriptional repressors. *J Biol Chem* 2001; **276**: 27488–27497.
2 Shu W, Lu MM, Zhang Y, Tucker PW, Zhou D, Morrisey EE: Foxp2 and Foxp1 cooperatively regulate lung and esophagus development. *Development* 2007; **134**: 1991–2000.
3 Wang B, Weidenfeld J, Lu MM *et al*: Foxp1 regulates cardiac outflow tract, endocardial cushion morphogenesis and myocyte proliferation and maturation. *Development* 2004; **131**: 4477–4487.
4 Rousso DL, Gaber ZB, Wellik D, Morrisey EE, Novitch BG: Coordinated actions of the forkhead protein Foxp1 and Hox proteins in the columnar organization of spinal motor neurons. *Neuron* 2008; **59**: 226–240.
5 Dasen JS, De Camilli A, Wang B, Tucker PW, Jessell TM: Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. *Cell* 2008; **134**: 304–316.
6 Bacon C, Schneider M, Le Magueresse C *et al*: Brain-specific Foxp1 deletion impairs neuronal development and causes autistic-like behaviour. *Mol Psychiatry* 2014; e-pub ahead of print 30 September 2014; doi:10.1038/mp.2014.116.
7 Le Fevre AK, Taylor S, Malek NH *et al*: FOXP1 mutations cause intellectual disability and a recognizable phenotype. *Am J Med Genet A* 2013; **161A**: 3166–3175.
8 Bacon C, Rappold GA: The distinct and overlapping phenotypic spectra of FOXP1 and FOXP2 in cognitive disorders. *Hum Genet* 2012; **131**: 1687–1698.
9 Pariani MJ, Spencer A, Graham JMJr., Rimoin DL: A 785kb deletion of 3p14.1p13, including the FOXP1 gene, associated with speech delay, contractures, hypertonia and blepharophimosis. *Eur J Med Genet* 2009; **52**: 123–127.
10 Carr CW, Moreno-De-Luca D, Parker C *et al*: Chiari I malformation, delayed gross motor skills, severe speech delay, and epileptiform discharges in a child with FOXP1 haploinsufficiency. *Eur J Hum Genet* 2010; **18**: 1216–1220.
11 Horn D, Kapeller J, Rivera-Brugues N *et al*: Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits. *Hum Mutat* 2010; **31**: E1851–E1860.
12 Palumbo O, D'Agruma L, Minenna AF *et al*: 3p14.1 de novo microdeletion involving the FOXP1 gene in an adult patient with autism, severe speech delay and deficit of motor coordination. *Gene* 2013; **516**: 107–113.
13 Talkowski ME, Rosenfeld JA, Blumenthal I *et al*: Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 2012; **149**: 525–537.
14 Hamdan FF, Daoud H, Rochefort D *et al*: De novo mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. *Am J Hum Genet* 2010; **87**: 671–678.
15 Srivastava S, Cohen JS, Vernon H *et al*: Clinical whole exome sequencing in child neurology practice. *Ann Neurol* 2014; **76**: 473–483.
16 O'Roak BJ, Deriziotis P, Lee C *et al*: Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet* 2011; **43**: 585–589.
17 Lee H, Deignan JL, Dorrani N *et al*: Clinical exome sequencing for genetic identification of rare mendelian disorders. *JAMA* 2014; **312**: 1880–1887.
18 Devanna P, Middelbeek J, Vernes SC: FOXP2 drives neuronal differentiation by interacting with retinoic acid signaling pathways. *Front Cell Neurosci* 2014; **8**: 305.
19 Deriziotis P, O'Roak BJ, Graham SA *et al*: De novo TBR1 mutations in sporadic autism disrupt protein functions. *Nat Commun* 2014; **5**: 4954.
20 Deriziotis P, Graham SA, Estruch SB, Fisher SE: Investigating protein-protein interactions in live cells using Bioluminescence Resonance. *J Vis Exp* 2014; e-pub ahead of print 26 May 2014; doi:10.3791/51438.
21 Buxbaum JD, Daly MJ, Devlin B, Lehner T, Roeder K, State MW: The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. *Neuron* 2012; **76**: 1052–1056.
22 Li S, Weidenfeld J, Morrisey EE: Transcriptional and DNA binding activity of the Foxp1/2/4 family is modulated by heterotypic and homotypic protein interactions. *Mol Cell Biol* 2004; **24**: 809–822.
23 Ferland RJ, Cherry TJ, Preware PO, Morrisey EE, Walsh CA: Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. *J Comp Neurol* 2003; **460**: 266–279.
24 Teramitsu I, Kudo LC, London SE, Geschwind DH, White SA: Parallel FoxP1 and FoxP2 expression in songbird and human brain predicts functional interaction. *J Neurosci* 2004; **24**: 3152–3163.
25 Vernes SC, Newbury DF, Abrahams BS *et al*: A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008; **359**: 2337–2345.
26 MacDermot KD, Bonora E, Sykes N *et al*: Identification of FOXP2 truncation as a novel cause of developmental speech and language deficits. *Am J Hum Genet* 2005; **76**: 1074–1080.
27 Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP: A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 2001; **413**: 519–523.
28 Watkins KE, Dronkers NF, Vargha-Khadem F: Behavioural analysis of an inherited speech and language disorder: comparison with acquired aphasia. *Brain* 2002; **125**: 452–464.
29 Fisher SE, Scharff C: FOXP2 as a molecular window into speech and language. *Trends Genet* 2009; **25**: 166–177.
30 Vernes SC, MacDermot KD, Monaco AP, Fisher SE: Assessing the impact of FOXP1 mutations on developmental verbal dyspraxia. *Eur J Hum Genet* 2009; **17**: 1354–1358.

Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)