Clinical Case Reports

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CASE REPORT

A case report of de novo missense *FOXP1* mutation in a non-Caucasian patient with global developmental delay and severe speech impairment

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Funding Information
This work was supported by the management expenses grants, University of Tsukuba.

Received: 21 May 2014; Revised: 26 July 2014; Accepted: 23 August 2014

Clinical Case Reports **2015; 3(2): 110–113**

doi: 10.1002/ccr3.167

Key Clinical Message

The FOXP protein family (FOXP1-4) is a group of transcription factors that play important roles in embryological, immunological, hematological, and speech and language development. Here, we report FOXP1 de novo mutation and severe speech delay in an individual belonging to a non-Caucasian population.

Keywords

Exome, FOXP protein family, Haploinsufficiencies, Japanese.

Introduction

The FOXP protein family (FOXP1-4) is a group of transcription factors that play an important role in embryological, immunological, hematological, and speech and language development [1]. Le Fevre et al. [2] reviewed a total of 10 patients with de novo mutations of *FOXP1* and showed that haploinsufficiencies of *FOXP1* are associated with global developmental delay/mental retardation with moderate/severe speech delay. Here, we report the case of a Japanese female patient with severe speech delay and the identification of a de novo *FOXP1* missense mutation by exome analysis.

Materials and Methods

Case report

The patient is a 22-year-old female and is the second-born child of nonconsanguineous Japanese parents. She has one healthy sister and no family history of mental retardation. She was born by normal vaginal delivery at 34 weeks gestation, and her birthweight was 2100 g (+0.1 SD). Her developmental delay was noted by a pediatrician when the patient was 1 year old. She started to walk alone at the age of 2. Development was globally delayed, especially in the area of speech and language acquisition. The patient's hearing and vision were normal, and no autistic features, developmental regression, or history of seizure were present. She began to menstruate at the age of 13. At the time of examination (22 years old), she displayed a short stature (141 cm, −3.2 SD, body weight 44.3 kg, −1.1 SD) and delayed speech (she was unable to speak), but her receptive language abilities were relatively developed as indicated by her understanding of relational concepts. She required assistance with routine daily activities, and hyperextension of her joints was observed. Chromosome analysis with G-banding showed a 46, XX karyotype. Her father's height is 171 cm and mother's height is 154 cm. Table 1 shows the neurodevelopmental features of the patient in comparison with data presented by Le Fevre et al. [2].

This study was approved by the Ethical Committee at the University of Tsukuba and was conducted according to the Principles of the Declaration of Helsinki. Informed consent was obtained from the parents.

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Table 1. Comparison of the neurodevelopmental features reported by Le Fevre et al. [2] and the patient in this study.

Symptoms	Le Fevre et al. [2]	Index case
De novo mutation	5/5	+
Low birthweight	1/3	+
FTT or small for age	2/6	−
Obesity	2/6	−
Prominent forehead	4/8	+
Down slanted palpebral fissures	3/8	−
Short nose with broad tip	5/8	+
Frontal hair upsweep	2/8	+
Prominent digit pads	2/8	−
Single palmar creases	2/8	−
Clinodactyly	2/8	−
Congenital malformation	4/9	−
Global delay	10/10	+
Regression	1/2	−
Intellectual delay	8/8	+
Gross motor delay	9/9	+
Speech and language delay	10/10	+
Expressive language more severely affected than receptive language	7/7	+
Articulation consonants	5/5	None
Poor grammar	4/4	None
Oro-motor dysfunction	3/7	−
Autistic feature	3/4	−
Autism	2/4	−
Behavioral problem	4/5	−
Tone	2/3	−
Reflexes	1/3	−
Seizures	2/7	−

Sequencing

Exome sequencing was performed, following the protocol described in the SureSelect Library prep kit (post-pool version 4; Agilent Technologies, Inc., Santa Clara, CA). The DNA library was subjected to emulsion PCR (SOLiD™ EZ Bead™ Emulsifier kit; Life Technologies, Carlsbad, CA) to generate clonal DNA fragments on beads, followed by bead enrichment (SOLiD™ EZ Bead™ Enrichment kit; Life Technologies). Enriched template beads were sequenced on a SOLiD 5500xl sequencer as single-end, 60-bp reads (Life Technologies). The SOLiD 5500xl output reads were aligned against the human genome reference sequence (hg19) using LifeScope version 2.5.1 (Life Technologies) to generate BAM files. Variant calling was performed following the Best Practices specified in the Genome Analysis Toolkit [3] (GATK, version 2.7.4), Picard (http://picard.sourceforge.net) and SAMtools [4], and only reads that mapped to a unique position in the reference genome were used.

A total of 68,391 variants were detected in the patient. We first filtered out the variants with low-quality values generated by GATK output, resulting in a new total of 62,200 variants. To distinguish potentially pathogenic variants from other variants, we filtered out variants in our in-house references (57 exome samples), public data from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/, version 137), and a 1000-genome database [5]. After this filtering step was applied, 393 variants remained. We then used ANNOVAR software to filter out synonymous variants and intronic variants because they are less likely to be pathogenic [6], which resulted in 84 remaining variants. We then used SIFT [7], Polyphen2 [8], LRT [9], or MutationTaster [10] software to predict the potential impact of an amino acid substitution on the function of human proteins, and we filtered out "benign" missense mutations as defined by the above-mentioned software. A total of 13 single-nucleotide variants and one frame-shift variant remained after this step (Table 2).

We performed direct sequencing to evaluate these 14 candidate mutations using DNA obtained from the patient and her parents. Among these, two mutations (*SPERT* and *GRP52*) were not confirmed by direct sequencing. Ten of the mutations existed in at least one of the healthy parents, suggesting that they are unlikely to be pathogenic. Two of the mutations, *FOXP1* and *PRKAA1*, were unique to the patient.

Discussion

It has been demonstrated that the use of next-generation sequencing techniques provides a high success rate in the diagnosis of unidentified genetic conditions. Need et al. performed exome sequencing on DNA from 12 patients with unexplained and apparent genetic conditions, which resulted in a diagnosis of a likely genetic origin of the condition in six of the 12 patients [11]. Exome sequencing has also been applied in autopsies of patients with sudden unexplained death and has been used to successfully identify mutations related to cardiac arrhythmia and cardiomyopathy [12]. Therefore, exome is becoming a powerful tool for the diagnosis of patients with unexplained conditions.

The FOXP protein family (FOXP1-4) is a group of transcription factors that play an important role in embryological, immunological, hematological, and speech and language development [1]. *FOXP2* was the first gene to be associated with severe speech disorders. It was identified using a three-generation pedigree in which a severe speech and language disorder was transmitted as an autosomal-dominant monogenic trait [13]. Subsequently, many de novo and familial cases of severe speech disorders associated with *FOXP2* mutations have been reported, and mutations in *FOXP2* are well known to cause developmental speech and language disorders [14].

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Table 2. Results of the exome sequence and confirmation by Sanger sequencing.

Gene	Chr	Position	Base change	Amino acid change (RefSeq Number)	De novo[1]
GPR52	1	174418259	C>T	T337I (NM_005684)	Not detected
MYOG	1	203055040	T>C	Y17C (NM_002479)	
IARS2	1	220315180	G>A	R817H (NM_018060)	
ITGA4	2	182374460	C>T	R591X (NM_000885)	
EML4	2	42556883	C>T	H770Y (NM_001145076)	
FOXP1	3	71102906	T>C	M1V (NM_001244813)	De novo
FHDC1	4	153896905	C>A	S821Y (NM_033393)	
PRKAA1	5	40765057	G>A	R369W (NM_006251)	De novo
SPERT	13	46287405	G>A	R82H (NM_152719)	Not detected
MCTP2	15	94899522	A>G	K388E (NM_001159643)	
TRPV3	17	3419802	C>T	R716Q (NM_001258205)	
ALOX12	17	6908595	G>A	R394H (NM_000697)	
USP36	17	76832441	G>A	P2L (NM_025090)	
COCH	14	31358916	AAG>A	COCH (NM_001135058)	

[1]Not detected; mutations detected in the exome data analysis but not confirmed by Sanger sequencing, de novo; mutations detected in the patient but not present in either parent.

Because *FOXP1* and *FOXP2* form heterodimers for transcriptional regulation, it has been suggested that they cooperate in common neurodevelopmental pathways through the coregulation of common targets [15]. Vernes et al. screened for mutations in *FOXP1* genes with a denaturing high-performance liquid chromatography method using DNA from 49 patients with developmental verbal dyspraxia. They found one missense mutation (P215A) in one patient, but P215A was also identified in an unaffected sibling of the patient [16], which suggests that P215A is unlikely to be pathogenic. Hamdan et al. identified two patients with de novo *FOXP1* mutations that caused haploinsufficiency and suggested that decreased expression of FOXP1 has a more global impact on brain development than does decreased expression of FOXP2 [17]. Subsequently, mutations in *FOXP1* have been reported to be associated with global developmental delay, intellectual disability, and speech defects [2, 17–21]. As noted by Le Fevre et al., the most consistent feature of a *FOXP1* mutation is global developmental delay with prominent speech delay, which was also observed in the present case study. To the best of our knowledge, this is the first report of a *FOXP1* de novo mutation in an individual with severe speech delay who belongs to a non-Caucasian population. All of the previously reported *FOXP1* mutations as well as the one in this study occurred de novo, suggesting that haploinsufficiency of *FOXP1* reduces fitness. In addition, some characteristic features of *FOXP1* mutations, such as speech delay, a prominent forehead, a short nose with a broad tip, and frontal hair upsweep, were concordant with the phenotype of the patient in this study.

PRKAA1 is a catalytic subunit of the 5′-prime-AMP-activated protein kinase (AMPK). AMPK is a cellular energy sensor conserved in all eukaryotic cells, and AMPK regulates the activities of a number of key metabolic enzymes through phosphorylation. Variations in *PRKAA1* have been reported to be associated with diabetes [22], cancer [23–25], coronary artery disease in type 2 diabetes [26], and open-angle glaucoma [27]. However, no reports have shown an association between *PRKAA1* mutations and developmental delay. Although mutations in *PRKAA1* or other genes may contribute to the patient's symptoms, it appears likely that the patient's severe speech delay may have been caused by a de novo missense mutation of the *FOXP1* gene based on results of previous studies. Next-generation sequencing techniques can provide information that is essential for the molecular diagnosis of patients with unexplained conditions.

Acknowledgments

The authors thank the patient and the parents for participating in the study. This work was supported by the management expenses grants, University of Tsukuba. Authors declare that there are no potential conflicts of interests.

Conflict of Interest

None declared.

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