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A 785 kb deletion of 3p14.1p13, including the *FOXP1* gene, associated with speech delay, contractures, hypertonia and blepharophimosis

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Abstract

We report a child with a 785 kb deletion of the 3p14.1p13 region including the genes *FOXP1*, *EIF4E3*, *PROK2*, *GPR27* resulting in speech delay, contractures, hypertonia and blepharophimosis. *FOXP1* and *FOXP2* are transcription factors containing a polyglutamine tract and a forkhead DNA binding domain. They both play a role in the developing human foregut and brain [W. Shu, M.M. Lu, Y. Zhang, P. Tucker, D. Zhou, E.E. Morrissey, *Foxp2* and *Foxp1* cooperatively regulate lung and esophagus development, *Development* 134 (2007) 1991–2000, E. Spiteri, G. Konopka, G. Coppola, J. Bomar, M. Oldham, J. Ou, et al. Identification of the transcriptional targets of *FOXP2*, a gene linked to speech and language, in developing human brain, *Am. J. Hum. Genet.* 81 (2007) 1144–1157, S. Tamura, Y. Morikawa, H. Iwanishi, T. Hisaoka, E. Senba. Expression pattern of the winged-helix/forkhead transcription factor *Foxp1* in the developing central nervous system, *Gene Expr. Patterns.* 3 (2003) 193–197.]. Mutations in *FOXP2* are known to cause severe speech and language abnormalities [C.S.L. Lai, S.E. Fisher, J.A. Hurst, F. Vargha-Khadem, A.P. Monaco, A forkhead-domain gene is mutated in a severe speech and language disorder, *Nature* 413 (2001) 519–523.] in humans and animals. It has been suggested that overlap of *FOXP1* and *FOXP2* expression in the songbird and human brain may indicate that mutations in *FOXP1* would also result in speech and language abnormalities. The roles of *EIF4E3*, *PROK2* and *GPR27* are also evaluated.

Keywords

FOXP1; *FOXP2*; *EIF4E3*; *PROK2*; *GPR27*; Speech delay; Blepharophimosis; 3p14; 3p13; del(3)(p14.1p13)

1. Methods of detection

Microarray analysis using the Signature GenomicsWG BAC array with 4685 BAC clones detected an abnormality characterized by a *de novo* single copy loss of 3 BAC clones from the short arm of chromosome 3 at del(3)(p14.1p13) (~400 kb in size). The deleted clones at del(3)(p14.1p13) were RP11-766C13, RP11-120N20, and RP11-794B13. The nearest distal and proximal BACs that are not deleted are RP11-1005P22 (800 kb distal to the deleted region) and RP11-16C21 (4.9 Mb proximal to the deleted region) respectively. The deletion was confirmed by fluorescence *in situ* hybridization (FISH) analysis (Fig. 1).

Oligonucleotide array investigation by Signature Genomics refined the deletion to 795 kb between nucleotides 71,164,161 and 71,958,845 (Fig. 2). This corresponds to a deletion of 89% of the gene through the N-terminus of *FOXP1* and a complete deletion of *EIF4E3*, *PROK2* and *GPR27*. The *FOXP1* deletion results in a deletion of the important N-terminal DNA binding domain, likely rendering the transcription factor functionless and a target for lysosomal destruction. Parental FISH analyses were performed and neither parent was found to carry a deletion or other rearrangement of the 3p14.1p13 region. Thus, the deletion identified in the patient appears to be *de novo* in origin.

2. Clinical description

We present a case of a 23 month-old Caucasian boy who is the product of an uneventful 39-week pregnancy. He is the only child of a 28 year-old mother and a 28 year-old father. At birth, he was under the 5th percentile for his gestational age (3123 g) and was found to have contractures of his hands and feet (similar to that seen in Trisomy 18), blepharophimosis, and hypertonia. Since birth, he had intermittent muscle spasms throughout the day. Subsequent EEG and brain MRI studies were normal. He required a tonsillectomy and adenoidectomy at age 17 months for obstructive sleep apnea and feeding difficulties. At 10 months, the child rolled over, grasped toys placed in his hands, and babbled nonspecifically. He developed a pincher grasp at age 14 months and sat up without assistance at age 15 months. He pulled himself up to a stand and began cruising at age 18 months.

On examination at age 23 months, the child was babbling and making vowel sounds, but had not developed words. The child was small for his age with a height of 76 cm (below 2nd percentile) and weight of 11.5 kg (below 10th percentile). He was normocephalic with a head circumference of 48 cm (50th percentile). He had epicanthal folds and blepharophimosis (Fig. 3). Ophthalmologic evaluation revealed hypermetropia. His face was symmetric with prominent cheeks and small upturned alae nasi (Fig. 3). He had single transverse palmar creases bilaterally, persistent ulnar deviation of the proximal phalanges and persistent flexion of the distal phalanges with the 3rd and 5th fingers persistently overriding the 4th. The thumb was clasped against the flexed index finger. The child's hallux was cocked bilaterally with dorsiflexion of the proximal phalanx and plantar flexion of distal phalanx. There was also persistent flexion and medial deviation of the 2nd, 3rd and 4th toes. He exhibited increased tone in all of his limbs that became more severe distally. His reflexes are normal. His genital exam revealed a normal-sized penis and bilateral cryptorchidism. MRI imaging of the brain showed mild asymmetric enlargement of the ventricles and sulci, consistent with minor atrophy. The olfactory bulbs were visualized and there was no evidence of hypoplasia.

His clinical picture was initially thought to be consistent with Schwartz–Jampel syndrome type 1A. A subsequent skeletal survey was normal, and his neurophysiologic examination did not reveal the typical complex, repetitive discharges of Schwartz–Jampel syndrome. As a result, we elected not to perform *HSPG2* sequence analysis. Initial karyotype results were 46,XY. A bone survey revealed no evidence of skeletal dysplasia and MRI of the brain was normal. We were concerned that his clinical picture might represent blepharophimosis/ptosis/epicanthus inversus syndrome (BPES). FISH of the region containing the *FOXL2* gene was therefore performed and the results were normal. Sequence analysis of *FOXL2* was not performed.

3. Discussion

This is the first reported case of a patient with muscle contractures, hypertonia, blepharophimosis, speech delay, and developmental delay, who has a documented deletion of *FOXP1*. Deletions of *EIF4E3*, *PROK2* and *GPR27* were also present. There have been cases with large deletions of 3p reported in the literature that may also include deletions in

FOXP1, but to our knowledge, this is the first time it has ever been confirmed using modern cytogenetic techniques.

The forkhead-box family of genes, to which *FOXP1* belongs, consists of over 40 human genes; all of which encode 80–100 amino acids, which form a DNA binding winged helix or a forkhead domain. These genes code for a class of proteins that serve a wide array of roles including development, metabolism, immunology and cell cycle control [19]. The FOXP protein subfamily has four members — FOXP1, FOXP2, FOXP3, FOXP4 — and is characterized by a DNA binding dependent N-terminal transcriptional repression domain which encompasses both a zinc finger and a leucine zipper motif [9,13]. FOXP1 and FOXP2 are homologous structures that share a similar N-terminal domain. Both proteins are active in the developing human foregut and brain [14,16,18]. FOXP1 forms homodimers and heterodimers with FOXP2 and FOXP4 [9]. The FOXP transcription factors are made up of two subdomains. The first is a leucine zipper motif essential for binding and repression; the second is a co-repressor protein known as C-terminal binding protein 1 that represses transcription. FOXP4 only contains the first domain.

The *FOXP1* gene is located on chromosome 3p14.1 and is 628 kb in length. It codes for a protein with four isoforms; the most common is 677 amino acids in length. The FOXP1 protein is a key transcription factor which functions in tissue and cell-specific gene transcription during early development and adulthood. Recently, researchers have shown that FOXP1 plays a critical role in defining the columnar identity of motor neurons at each axial position, as well as organizing motor axon projections [12]. A disruption of this function may explain our clinical finding of arthrogryposis and hypertonia. Though we can only speculate, the limitations of movement in the finger joints of a case with a deletion of 3p11p14.1 described by Crispino et al. [1] may also represent arthrogryposis associated with a deletion in *FOXP1*. *FOXP1* has also been suggested as a potential tumor suppressor gene and likely plays a role in cardiac development. Mouse knockout models for homozygous mutations of the *Foxp1* gene have defects in cardiac morphogenesis and die at E14 [6]. To date, no reports of human mutations or deletions have been previously reported.

Three other cases have been described which may include a deletion of *FOXP1* [1,5,15]. Sichong et al. [15] described a case of a girl with a *de novo* deletion of the proximal segment of short arm of chromosome 3 (46,XX, del(3)(p11p14.2)). The child had growth delay, psychomotor delay, dysmorphic facies and hearing impairment. Crispino et al. [1] described an infant with a reciprocal translocation between chromosome 3 and 20 with an interstitial deletion of 3p 46XX,t(3;20)(p14.2;p12.2)del(3)(p11p14.1). This infant was small for gestational age (<3rd percentile), with dysmorphic facies, ulnar deviation of the wrists, transverse palmar creases and limitations of movement in the finger joints. A third case described by Hertz et al. [5] reports a male fetus with an interstitial deletion of the short arm of chromosome 3 with a karyotype of 46,XY,del(3)(p14.2p11). The fetus was described as having dysmorphic facies, ulnar deviation of the wrists, transverse palmar creases and a cardiac defect. Because these cases were only described by using classical cytogenetic techniques, the breakpoints were not well defined. As a result, it is unclear whether any of these cases truly represent deletions in *FOXP1*. Nevertheless, there are similarities in some of the clinical findings that might indicate a deletion of *FOXP1*. Conversely, the above cases represent large deletions that contain many genes, and may also represent deletions in other genes such as *EIF4E3*, *PROK2* and *GPR27* (see Table 1).

Deletions of *EIF4E3*, *PROK2* and *GPR27* were also present in our patient. *EIF4E3* interacts with the 5-prime mRNA cap and delivers mRNA to the ribosome [7]; mutations in this gene have not been linked to a human condition, however. *PROK2* was also deleted and heterozygous mutations of *PROK2* have been implicated in Kallmann syndrome type 4 [3]. As

cryptorchidism can be a sign of hypogonadotropic hypogonadism, testing is planned to rule this out in our patient. A previous patient was reported with a deletion of both *PROK2* and *GPR27* and other genes in the del(3)(p13p11) region [11]. This case was not diagnosed with Kallman syndrome, but did have an enlarged frontal subarachnoid space and hypospadias, as well as growth retardation, dysmorphic features, and language abnormalities. It is unclear what clinical findings we can attribute to a deletion of *GPR27*. *GPR27* is a highly conserved G-protein receptor, which is expressed in the central nervous system and genital organs. Its exact function and link to human pathogenesis is still unknown [10].

Although *FOXP1* mutations or deletions have eluded researchers, other genes in the *FOX* family have been implicated in disorders featuring blepharophimosis (*FOXL2*) [2] and speech delay (*FOXP2*) [8] (Table 1). The *FOXL2* protein is a transcription factor active in the early gonad prior to sex differentiation, the mature ovary, and the eyelid. Haploinsufficiency of the *FOXL2* gene results in blepharophimosis, epicanthus inversus, and low nasal bridge and amenorrhea or premature ovarian failure in females. Although the blepharophimosis *FOXL2* phenotype is similar to that seen in our patient with a *FOXP1* deletion, the timing and expression of these transcription factors do not share overlap or interact with the same proteins. *FOXL* is a class I *FOX* protein member with a wing helix DNA binding domain and a forkhead domain, whereas *FOXP* is a class II member with the wing helix domain, forkhead domain, and an additional zinc finger domain. The *FOXP2* protein is a transcription factor with many targets in the brain. In 1990, a family with autosomal dominant transmission of oral motor and speech dyspraxia caused by haploinsufficiency of *FOXP2* was discovered. The *FOXP2* arginine553histidine missense mutation of the forkhead domain is linked to speech and language abnormalities in humans [4]. The homologous region of *FOXP1* was completely deleted in our patient.

FOXP3 and *FOXP4* are also members of the *FOX* family of genes. *FOXP3* is located at Xp11.23 and serves as a master regulator of T cell development and function. Mutations in *FOXP3* have been linked to IPEX syndrome (immunodysregulation, polyendocrinopathy, and enteropathy, X-linked). *FOXP4* is located at 6p21.1. Although no human mutations in *FOXP4* have been found, expression has been seen in the developing rat forebrain along with *FOXP1* and *FOXP2* [17]. Heterozygous mice displayed no obvious defects, however, homozygous mice died at embryonic day 12.

The homology between the two genes, coupled with the observation that *FOXP1* and *FOXP2* form dimers, leads us to hypothesize that the clinical phenotype of the two conditions may overlap. Much like cases of *FOXP2* deletion, *FOXP1* mutations may also lead to speech delay. Our patient's clinical picture leads us to believe that mutations in *FOXP1* may result in disordered motor neuron development, resulting in hypertonia and contractures. As a result of our observations in this case, we feel that a deletion of *FOXP1* should be a part of any differential diagnosis when evaluating a patient with speech delay, developmental delay, blepharophimosis, and arthrogryposis.

References

- [1]. Crispino B, Cardoso H, Mimbacas A, Mendez V. Deletion of chromosome 3 and a 3;20 reciprocal translocation demonstrated by chromosome painting. *Am. J. Med. Genet* 1995;55:27–29. [PubMed: 7702091]
- [2]. De Baere E, Dixon MJ, Small KW, Jabs EW, Leroy BP, Devriendt K, et al. Spectrum of *FOXL2* gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype-phenotype correlation. *Hum. Mol. Genet* 2001;10(15):1591–1600. [PubMed: 11468277]
- [3]. Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLOS Genet* 2006;2(10):1648–1652.

- [4]. Enard W, Przeworski M, Fisher SE, Lai CSL, Wiebe V, Kitano T, et al. Molecular evolution of *FOXP2*, a gene involved in speech and language. *Nature* 2002;418:869–872. [PubMed: 12192408]
- [5]. Hertz JM, Coerdet W, Hahnemann N, Schwartz M. Interstitial deletion of the short arm of chromosome 3. Fetal pathology and exclusion of the gene for beta-galactosidase-1 (GLB-1) from 3(p11-p14.2). *Hum. Genet* 1988;79:389–391. [PubMed: 3137147]
- [6]. Hu H, Wang B, Borde M, Nardone J, Maika S, Allred L, et al. Foxp1 is an essential transcriptional regulator of B cell development. *Nat. Immunol* 2006;7(8):819–826. [PubMed: 16819554]
- [7]. Joshi B, Cameron A, Jagus R. Characterization of mammalian eIF4E-family members. *Eur. J. Biochem* 2004;271:2189–2203. [PubMed: 15153109]
- [8]. Lai CSL, 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. [PubMed: 11586359]
- [9]. Li S, Weindenfeld J, Morrissey 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(2):809–822. [PubMed: 14701752]
- [10]. Matsumoto M, Saito T, Takasaki J, Kamohara M, Sugimoto T, Kobayashi M, et al. An evolutionarily conserved G-protein coupled receptor family, SREB, expressed in the central nervous system. *Biochem. Biophys. Res. Commun* 2000;272:576–582. [PubMed: 10833454]
- [11]. Petek E, Windpassinger C, Simma B, Mueller T, Wagner K, Kroisel PM. Molecular characterisation of a 15 Mb constitutional de novo interstitial deletion of chromosome 3p in a boy with developmental delay and congenital anomalies. *J. Hum. Genet* 2003;48:283–287. [PubMed: 12836054]
- [12]. Roussou DL, Gaber ZB, Wellik D, Morrissey EE, Novitsch BG. Coordinated actions of the forkhead protein Foxp1 and Hox proteins in the columnar organization of spinal motor neurons. *Neuron* 2008;59:226–240. [PubMed: 18667151]
- [13]. Shu W, Yang H, Zhang L, Lu MM, Morrissey 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(29):27488–27497. [PubMed: 11358962]
- [14]. Shu W, Lu MM, Zhang Y, Tucker P, Zhou D, Morrissey EE. Foxp2 and Foxp1 cooperatively regulate lung and esophagus development. *Development* 2007;134:1991–2000. [PubMed: 17428829]
- [15]. Sichong Z, Bui TH, Castro I, Iselius L, Hakansson S, Lundmark KM. A girl with an interstitial deletion of the short arm of chromosome 3 studied with a high-resolution banding technique. *Hum. Genet* 1981;59:178–181. [PubMed: 7327578]
- [16]. Spiteri E, Konopka G, Coppola G, Bomar J, Oldham M, Ou J, et al. Identification of the transcriptional targets of *FOXP2*, a gene linked to speech and language, in developing human brain. *Am. J. Hum. Genet* 2007;81:1144–1157. [PubMed: 17999357]
- [17]. Takahashi K, Liu FC, Hirokawa K, Takahashi H. Expression of Foxp4 in the developing and adult rat forebrain. *J. Neurosci. Res* 2008;86:3106–3116. [PubMed: 18561326]
- [18]. Tamura S, Morikawa Y, Iwanishi H, Hisaoka T, Senba E. Expression pattern of the winged-helix/forkhead transcription factor Foxp1 in the developing central nervous system. *Gene Expr. Patterns* 2003;3:193–197. [PubMed: 12711548]
- [19]. Tuteja G, Kaestner KH. Snapshot: forkhead transcription factors II. *Cell* 2007;131:192–192e1. [PubMed: 17923097]

Fig. 1.
A BAC clone from the deleted region (RP11-120N20) and the 3q subtelomere probe (RP11-23M2) confirmed the loss of the region covered by the RP11-120N20 probe. One signal was observed from the BAC clone and two signals were observed from the control probe.

Fig. 2.

(a) Oligonucleotide-based microarray analysis using a 105 k-feature whole-genome microarray with one probe every 10 kb in targeted regions showing a 795 kb deletion between the nucleotides 71,164,161 and 71,958,845; (b) region of 3p14.1p13 (70.5–73.0 Mb) with the genes and the deletion of the present patient and of the patient of Petek indicated (hatched bars). The distal breakpoint of Petek's patient is located between 71.6 and 71.9 Mb (dotted bar), the deletion extends into the centromeric region.

Fig. 3.

A) Facial characteristics of the child. His face is symmetric with prominent cheeks and small upturned alae. His most striking features are blepharophimosis and epicanthal folds. B) The child's hands show persistent ulnar deviation of the proximal phalanges and persistent flexion of the distal phalanges with the 3rd and 5th finger persistently overriding the 4th. The thumb is clasped against the flexed index finger. C) and D) The child's feet show that the hallux is cocked bilaterally with dorsiflexion of the proximal phalanx and plantar flexion of distal phalanx. There is also persistent flexion and medial deviation of the 2nd, 3rd and 4th toes.

Table 1

Phenotype comparison of our patient – del(3)(p14.1p13); case reports from Petek et al. [11] – del(3)(p13p11); Sichong et al. [15] – del(3)(p11p14.2); Crispino et al. [1] – 46XX,t(3;20)(p14.2;p12.2)del(3)(p11p14.1); FOXP2 deletion (speech–language disorder); *FOXL2* deletion (blepharophimosis, ptosis, and epicanthus inversus; BPES); *PROK2* deletion (Kallmann syndrome type 4); and *HSPG2* deletion (Schwartz–Jampel syndrome).

	FOXP1 deletion	Petek et al. [11]	Sichong et al. [15]	Crispino et al. [1]	FOXP2 deletion	FOXL2 deletion	PROK2 deletion	HSPG2 deletion
Growth retardation	X	X	X	X				
Blepharophimosis	X					X		X
Arthrogryposis	X			??				
Hypertonia	X							X
Unusual facies	X	X	X	X		X		X
Genital malformation	X (CRX)	X (HPS)				X (POF)	X	
Language abnormalities	X	X			X			
Skeletal abnormalities		X						X
Developmental delay	X	X			X			±
Abnormal imaging	X (glbl atropy)	X (lrg frnt Archnd spc)	X			±	X (hpsic olf blbs)	X
Hypogonadotropic hypogonadism							X	

CRX = cryptorchidism; HPS = hypospadias; POF = premature ovarian failure; lrg frnt Archnd spc = large frontal subarachnoid space; hpsic olf blbs = hypoplastic olfactory bulbs.