ORIGINAL ARTICLE

Setleis syndrome due to inheritance of the 1p36.22p36.21 duplication: evidence for lack of penetrance

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Setleis syndrome, focal facial dermal dysplasia type III (FFDD3, MIM #227260), is characterized by scar-like bitemporal lesions and other ocular and facial dysmorphic features. The syndrome results from recessive mutations in the *TWIST2* gene, encoding a basic helix-loop-helix transcription factor or *de novo* genomic duplication or triplication, which include 1.3 Mb at 1p36.22p36.21, or other yet undefined lesions, emphasizing the syndrome's genetic heterogeneity. Recently, three patients were reported with 1p36.22p36.21 duplications/triplication that had the characteristic FFDD3 features and developmental delay or intellectual disabilities. Here, we describe a male with this microduplication, and the typical FFDD3 phenotype, but normal intelligence. Notably, his duplication was inherited from his father who did not have any FFDD3 manifestations, indicating lack of penetrance of the 1p36.22p36.21 microduplication. These findings emphasize phenotypic heterogeneity of the 1p36.22p36.21 copy number variant and the importance of screening the parents of patients with the 1p36.22p36.21 copy number variant to determine whether the duplication/triplication is *de novo* or inherited, for informed reproductive and genetic counseling.

Journal of Human Genetics (2015) 60, 717–722; doi:10.1038/jhg.2015.103; published online 27 August 2015

INTRODUCTION

The focal facial dermal dysplasias (FFDDs) are a group of developmental disorders characterized by scar-like atrophic lesions on the temporal (FFDD1, 2 and 3) or peri-auricular (FFDD4) areas.^{1–4} Patients with FFDD3, known as Setleis syndrome (MIM #227260), manifest characteristic bitemporal scar-like lesions and other facial dysmorphic features, including a low frontal hairline, upslanting and sparse lateral eyebrows, multiple rows of upper eyelashes (distichiasis), paucity of lower eyelashes, periorbital puffiness, flattened nasal bridge, bulbous nasal tip, prominent upper lip, loose and wrinkled facial skin and a vertical chin groove and cheek dimple.^{1–4}

To date, >25 families with FFDD3 have been reported.^{2–7} Using positional cloning techniques, Tükel *et al.*³ identified two different homozygous nonsense mutations, c.193C>T (p.Gln65*) and c.355C>T (p.Gln119*), in the *TWIST2* gene in a large consanguineous Puerto Rican family and a consanguineous Arab family, respectively. Since the original report, three additional *TWIST2* mutations, c.168del (p.Ser57Alafs*45), c.91del (p.Arg31Glyfs*71) and c.326T>C (p.Leu109Pro), were identified in consanguineous Mexican Nahua, Indian and Turkish families, respectively.^{4–6} *TWIST2* is a member of the basic helix-loop-helix transcription factor family and regulates the

expression of target genes, which are involved in the craniofacial dermal and bone development of mammalian embryos.^{3,8,9} All five *TWIST2* mutations are predicted to prematurely truncate the encoded protein or change its tertiary structure. Therefore, the mutant *TWIST2* cannot bind to its target genes or cannot bind effectively.

Recently, three unrelated FFDD3 patients with normal *TWIST2* sequences were found to have a *de novo* 1.3 Mb duplication or triplication at chromosome 1p36.22p36.21 by array comparative genomic hybridization (aCGH).⁷ Notably, all three patients had marked developmental delay or intellectual disability. In addition, the patient with the *de novo* triplication had a more pronounced FFDD3 phenotype than those with the duplications, indicating a dosage effect. Here, we report the first FFDD3 patient with a 1p36.22p36.21 duplication inherited from his clinically unaffected father, indicating the lack of penetrance for this duplication. Moreover, the patient was intellectually normal, in marked contrast to the previously identified patients with the microduplication who were intellectually impaired. These findings have important implications for the phenotypic heterogeneity of the 1p36.22p36.21 copy number variant as well as for genetic and reproductive counseling in families of patients with FFDD3 owing to the 1p36.22p36.21 duplication or triplication.

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Received 13 May 2015; revised 17 July 2015; accepted 21 July 2015; published online 27 August 2015

MATERIALS AND METHODS

Clinical specimens

Peripheral blood (10–20 ml) was obtained by venipuncture from the proband and both parents. Genomic DNA was isolated for the analyses as described below. Informed consent was obtained for these studies including photography of the proband and both parents. His unaffected two sisters declined genetic testing and photography.

TWIST2 sequence analysis

The *TWIST2* gene was sequenced using genomic DNA as previously described.³ The two different transcript variants (NM_057179 and NM_001271893), each of which consist of two exons, were analyzed.

aCGH

aCGH was performed on a Custom180K CGH+SNP Microarray according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA, USA). The protocol was as previously described with the following modifications.¹⁰ Experimental and gender-matched reference DNAs, 1.5 µg each, (Promega, Madison, WI, USA) were digested with *AluI* and *RsaI* restriction endonucleases (Promega) and fluorescent-labeled with cyanine 5-dCTP (Cy-5; experimental) and cyanine 3-dCTP (Cy-3; reference). Labeled experimental and reference DNAs were purified, combined, denatured, pre-annealed and hybridized to the microarrays in a rotating oven (20 rpm) at 65 °C for 16 h.

Microsatellite analyses

PCR of the microsatellite markers, D2S406 (2p16.1), D7S1817 (7q31.1), D7S817 (7p14.3) and DXS981 (Xq13.1) was performed using a fluorescent-labeled forward primer and conventional reverse primer. PCR products were separated on the ABI PRISM 3130 XL Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). Alleles were analyzed using GeneMapper Software Version 4.0 (PE Applied Biosystems).

Fluorescence *in situ* hybridization analyses

RP11-90D15 at 1p36.21 (chr1:12 864 679–13 137 263 [hg19]) was used as a probe to detect the duplication (Empire Genomics, Buffalo, NY, USA), and a probe at 1q44 (chr1:243 700 001–249 250 621 [HG19]) was used as a control (Abbott Laboratories, Abbott Park, IL, USA). The procedure was performed as previously described.¹¹

RESULTS

Clinical description

The proband, a 21-year-old male, was the third child of non-consanguineous Caucasian parents. Pregnancy, labor and vaginal delivery were uncomplicated, and his growth, development and

intellect were normal. He graduated from high school with good grades and has an administrative position in a company. Examination revealed the characteristic bitemporal scar-like lesions of FFDD3 as well as a low frontal hairline, whorled hairs, upward slanting eyebrows, thin lateral eyebrows, periorbital puffiness, short palpebral fissures, distichiasis of his upper eyelashes, paucity of the lower eyelashes, bulbous nasal tip, the nasal septum below his alae, prominent lips and a horizontal chin furrow (Figure 1a). He did not have a pectus deformity, or cardiac or genitourinary abnormalities (Table 1). His parents and two sisters did not have any FFDD3 manifestation.

Molecular studies

For this study, both exons and the exon–intron boundaries of the *TWIST2* two transcript variants (NM_057179 and NM_001271893) were sequenced. The patient did not have a mutation in the coding region, although two common single-nucleotide polymorphisms were present in the 3'-untranslated region (rs60432037 and rs35076696). Note that the upstream regulatory, complete intronic and distal 5'- and 3'-untranslated regions were not sequenced. However, aCGH revealed a 3.36 Mb duplication of chromosome 1p36.22p36.21 (chr1:10 536 144–13 992 333 [hg19]) (Figure 2a). The proband's mother had a normal copy number at this locus, whereas his clinically unaffected father had the same 1p36.22p36.21 duplication (Figures 1b and 2a). FISH analysis using interphase peripheral leukocytes was performed to confirm the duplication as well as to investigate possible somatic mosaicism in the father. The duplication was present in every cell tested from both the patient and his father, but was not found in his mother (Figures 2b–d). Analyses of the microsatellite markers, D2S406, D7S1817, D7S817 and DXS981, for the patient and his parents indicated normal biparental inheritance (Figure 3).

DISCUSSION

FFDDs are divided into four subtypes FFDD types 1, 2, 3 and 4. Bitemporal lesions ('forceps marks') are the sole abnormality in FFDD1 (Brauer Syndrome, MIM 136500), whereas additional manifestations occur in FFDD2 (Brauer–Setleis syndrome, MIM 614973) and FFDD3 (Setleis syndrome). The characteristic facial dysmorphic features are more pronounced in FFDD3.^{1,2,4} FFDD4 (MIM #614974) is characterized by preauricular skin lesions without other systemic manifestations.¹²

Figure 1 The facial features of the 21-year-old, male proband (a) and his unaffected father (b). Note the typical FFDD3 features including bitemporal scar-like lesions, distichiasis, paucity of lower eyelashes, puffy eyes, bulbous nose, horizontal chin crease and redundant skin in the proband (a), and the absence of these FFDD3 features in his father (b). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 The clinical characteristics of the subjects with 1p36.21 duplication

Subject	Our patient	Weaver <i>et al.</i> ⁷				ISCA ^a			DICIPHER ^a
		Patient 1	Patient 2	Patient 3	nssv1602095	nssv578510	nssv578509	259769	
Position [hg19]	chr1:10 536 144– 13 992 333	chr1:10 649 383– 12 920 040	chr1:11 696 993– 13 188 029	chr1:10 774 848– 14 030 771	chr1:6 614 945– 16 382 506	chr1:6 913 573– 17 685 411	chr1:6 712 399– 12 784 811	chr1:712 577– 25 120 526	
Bands	1p36.22p36.21 3.46 Mb	1p36.22p36.21 2.27 Mb	1p36.22p36.21 1.32 Mb	1p36.22p36.21 3.26 Mb	1p36.31p36.13 9.77 Mb	1p36.31p36.13 10.77 Mb	1p36.31p36.21 6.07 Mb	1p36.33p36.11 24.41 Mb	
Copy number	Duplication	Duplication	Triplication	Duplication	Duplication	Duplication	Duplication	Duplication	
Inheritance	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	Unknown	Unknown	<i>De novo</i>	<i>De novo</i>	
Clinical	Paternal								
manifestation									
Bitemporal scarring	+	+	+	+	?	?	?	?	
Low frontal hairline	+	+	?	+	?	?	?	?	
Sparse hair/abnormal whorl	–	–	?	–	?	?	?	?	
Thinned lateral eyebrows	+	+	+	+	?	?	?	?	
Distichiasis upper lashes	+	?	?	+	?	?	?	?	
Paucity of lower lashes	+	+	?	–	?	?	?	?	
Low set/dysplastic ears	–	–	?	–	?	?	?	+	
Flattened nasal bridge	–	–	?	–	?	?	?	?	
Bulbous nasal tip	+	+	?	–	?	?	?	?	
Septum below alae nasi	+	–	?	+	?	?	?	?	
Prominent upper lip	+	+	+	+	?	?	?	?	
Skin dimples lateral to lips	–	–	–	+	?	?	?	?	
Redundant skin	–	–	–	+	?	?	?	?	
Horizontal chin furrow/vertical chin cleft	+	–	–	+	?	?	?	?	
Developmental/Intellectual Problems	–	+	+	+	?	+	+	+	
Problems		Autism, MR	DD, MR	DD, ADHD	Cleft palate, velopharyngeal insufficiency	DD	Autism, DD	Autism, ADHD, MR	
Others		Syndactyly,	Cardiac anomaly, mega-ureter, perianal fistula	Cutaneous schwannoma		Dysmorphism	Dysmorphism, cardiac anomaly, syndactyly	Brittle hair, hypospadias, tapered fingers	

Abbreviations: ADHD, attention deficit hyperactivity disorder; DD, developmental delay; MR, mental retardation.

Bold entries depict neurological problems.

^aDICIPHER (https://decipher.sanger.ac.uk/) and the International Standards for Cytogenomic Arrays (ISCA, www.iccg.org).

Figure 2 (a) Microarray analyses of the proband and his parents. Probes are ordered on the y axis according to physical mapping positions. The patient and his father have a 3.36 Mb duplication at 1p36.22p36.21, chr1:10 536 144–13 992 333 (UCSC Genome Browser, hg19), whereas his mother does not have. The duplication in our proband reported (proband) and 1p36 duplications or triplication in the three patients reported by Weaver *et al.*⁷ are represented schematically. The smallest region of overlap among the duplications and triplication is indicated by the red dotted lines, and the genes located in this region are indicated. (b–d) FISH images of the peripheral leukocytes in the interphase of the proband's mother (b) and father (c), and the patient (d). The probe at 1p36 is indicated in green, whereas the control probe at 1q44 is indicated in red. The duplication is noted both in the leukocytes of the patient (d) and his father (b), but not in the leukocytes of his mother (b). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Figure 3 Analysis of the four microsatellite markers, D2S406 (2p16.1), D7S1817 (7q31.1), D7S817 (7p14.3) and DXS981 (Xq13.1) identify the normal mother, affected proband and his unaffected father who has the 1p36 duplication. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

In the last few years, the genetic etiologies of FFDD3 and FFDD4 have been identified.^{3,12} In FFDD3, recessive *TWIST2* mutations were found in patients from five unrelated families, all having normal intelligence.^{3–6} In addition, *de novo* duplications or triplication of genomic region, 1p36.22p36.21, were found in three unrelated patients who had the typical FFDD3 manifestations and developmental delay

or intellectual disabilities.⁷ In patients with FFDD4, exome sequencing identified mutations in the *CYP26C1* gene, which encodes an enzyme involved in retinoic acid metabolism.¹²

The studies reported here further extend the genetic and phenotypic heterogeneity of FFDD3. In addition to the homozygous *TWIST2* mutations leading to the loss-of-function of transcription factor,

a microduplication or triplication at 1p36.22p36.21 was recently reported that alters the dosage and presumably the expression of genes located in the region. The three previously described FFDD3 patients with the *de novo* duplications or triplication were developmentally or intellectually impaired.⁷ By contrast, the patient reported here with the classic FFDD3 facial features was intellectually normal, as were the FFDD3 patients with *TWIST2* mutations (Table 1 and Figure 2).^{3,4,6} Of note, the proband's father had the same microduplication, but no FFDD3 clinical manifestations. This is the first description of the lack of penetrance for the FFDD3-causing microduplication.

Varying clinical manifestations have been reported for other well-known genomic duplications, including 1q21.1,¹³ 15q24,¹⁴ 16p11.2,¹⁵ 17q12¹¹ and 22q11.2.¹⁶ Some patients show the full spectrum of manifestations of the respective duplication syndrome, including developmental delay, intellectual disability, behavioral abnormalities, birth defects and dimorphisms, whereas other duplication carriers appear unaffected. This phenotypic heterogeneity has been noted in the family members with the above duplications, whereas the probands with these duplications typically had the full phenotype.^{13,14} This variable expressivity of individual clinical features or the complete lack of the manifestations for these duplications can be explained by the incomplete penetrance of these genomic duplications. Unknown genomic factors can modify the expression of these duplications, leading to the absence of the typical clinical features of the respective duplication. Somatic mosaicism also may contribute to the incomplete penetrance. However, no mosaic pattern was observed in the peripheral leukocytes of the FFDD3 proband's unaffected father described here. To rule out somatic mosaicism more clearly, other tissues should be tested. However, they were not available.

The minimal genomic region shared by the four FFDD3 patients with the 1p36.22p36.21 duplication or triplication was 1.3 Mb in size (chr1: 11 696 993–12 920 040 [hg19]) (Figure 2). Four other patients have been reported with increased copy numbers of this genomic region in two web-based databases, DECIPHER (<https://decipher.sanger.ac.uk/>) and the International Standards for Cytogenomic Arrays (ISCA, www.iccg.org) (Table 1). The major indication for aCGH studies of these patients was developmental delay or intellectual disability with dysmorphic features and other congenital anomalies. Unfortunately, the facial features were not described, so it is not known whether these patients had FFDD3. It is notable that the size of the duplicated region in these subjects ranged from 6.07 Mb to 24.41 Mb, which extended well beyond the 1.3 Mb shared region of the four FFDD patients. Importantly, developmental delay and/or intellectual disability were noted in six of the eight 1p36.22p36.21 duplication/triplication patients (three FFDD3 and three of the four patients from the public databases) (Table 1).⁷ Four also had other neurodevelopmental disorders, such as attention deficit hyperactivity disorder and autism. Additional congenital anomalies such as Chiari malformation, schwanomma, congenital heart defect, megaureter and brachydactyly were noted in several patients. By contrast, developmental delay/intellectual disability was rare in FFDD3 patients who did not have the 1p36.22p36.21 duplication/triplication, including those who had *TWIST2* mutations.⁴ Thus, neurodevelopmental assessments and systemic evaluations should be performed in patients with an overlapping 1p36.22p36.21 duplication/triplication.

Certain genomic regions including 1p36.2, 7q11.2, 17p11.2 and 22q11.2 are predisposed to reciprocal genomic duplications and deletions due to non-homologous allelic recombination between flanking low copy repeats.^{16–18} The 1p36 deletion syndrome is one of the most common subtelomeric deletions (chr1:10 001–12 840 259

[hg19]) with an estimated incidence of 1 in 5000–10 000 live births.^{19,20} Patients with the 1p36 deletion have distinct facial features: straight eyebrows, deep-set eyes, midface retrusion, depressed nasal bridge, long philtrum and pointed chin along with other congenital anomalies involving the heart, eyes and genitourinary tract, which are distinctly different from the phenotype of the four FFDD3 patients with the 1p36.22p36.21 duplication or triplication.^{7,19,20} Most patients with the 1p36 deletions have marked developmental delay and intellectual disability. Deletion sizes are quite variable, located from 1p36.33 to 1p36.31, and no common breakpoints are known.²⁰ In addition, only a few subjects have been reported in the ISCA and DECIPHER databases, who have interstitial deletions at 1p36.22p36.21 flanking the 1.3 Mb minimal overlapping region (chr1: 11 696 993–12 920 040), but there was insufficient information to assess their clinical manifestations except developmental delay. Based on available knowledge, the 1p36.22p36.21 genomic region does not appear to be markedly predisposed to genomic recombination. Thus, its duplication is a rare event manifesting a distinct clinical FFDD phenotype.

In summary, these studies have extended the genetic and phenotypic heterogeneity of FFDD3. Notably, the previously reported FFDD3 patients with *de novo* 1p36.22p36.21 duplications or a triplication had developmental delay or intellectual disabilities.⁷ By contrast, our patient with the 1p36.22p36.21 duplication and typical FFDD3 facial features had normal intelligence. Moreover, his 1p36.22p36.21 duplication was inherited from his unaffected father, indicating the lack of penetrance for this copy number variant. Therefore, we recommend that all first-degree relatives of 1p36.22p36.21 duplication/triplication patients have genetic testing for the microduplication and should be offered appropriate genetic and reproductive counseling.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to the family members for their participation in this study.

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