SPANDIDOS PUBLICATIONS

A novel IRF6 nonsense mutation (Y67X) in a German family with Van der Woude syndrome

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Abstract. Van der Woude syndrome (VWS) is the most common type of syndromic orofacial cleft, which accounts for ~2% of all cleft lip and palate cases. It is characterised by variable association of lower lip pits, cleft lip and cleft palate, and hypodontia. VWS arises as the result of mutations in the gene encoding interferon regulatory factor 6 (*IRF6*). The disorder is transmitted in an autosomal dominant manner, with high penetrance and variable expressivity. Very recently, mutations of the *IRF6* gene in exons 2-9 have been found in VWS patients, suggesting that this gene plays an important role in orofacial development. We report a novel mutation of the *IRF6* gene in a German family. Five out of the 12 persons affected were able to be investigated. The mutation produced a stop codon within exon 4 of the *IRF6* gene. All 5 patients were heterozygous for a base substitution c.201C>A changing the tyrosine codon at amino acid position 67 into a stop codon (p.Y67X) in exon 4. The premature stop codon was responsible for a truncated protein lacking parts of the DNA-binding domain and the complete Smad-interferon regulatory factor-binding domain probably essential for interactions with the Smad transcription factors.

Introduction

Clefts of the lip and palate are a craniofacial anomaly requiring complex multidisciplinary treatment. While no specific disease-causing gene mutations have been identified in non-syndromic clefting, a number of candidate genes have been isolated through both linkage and association studies (1).

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Van der Woude syndrome (VWS) is an autosomal dominant disorder with cleft lip and/or palate and lower lip pits. It has been reported to have a penetrance of 80% with variable expression (2). The disorder was first described in 1845 by Demarquay in a family and was later called van der Woude syndrome (VWS) (3). Cleft types (CLP and CP) occur in VWS in the same proportions as in the general non-VWS population, i.e. about twice as many cleft-bearing individuals have CLP as have CP. In VWS, we do not find the usually observed excess of females with CP and excess of males with CLP; the sex ratios are nearly equal (4). VWS accounts for ~2% of all cleft lip and palate cases (5).

The VWS locus was mapped to a 1.6-cM region in 1q32-q41 between D1S491 and D1S205, and a 4.4-Mb contig of YAC clones of this region was constructed (5). Previously, mutations have been found in the *IRF6* gene (interferon regulatory factor 6) in patients with VWS and popliteal pterygium syndrome (12). IRF6 belongs to a family of nine transcription factors sharing two protein domains; a highly conserved helix-turn-helix DNA-binding domain (amino acids 13-113) and a less conserved protein-binding domain (amino acids 226-394) called SMIR (Smad-interferon regulatory factor-binding domain) (6). The SMIR domain is required to form homodimers and heterodimers (7,8). The dimers then translocate to the nucleus where they associate with other transcription factors and then bind to their DNA targets (7). Because most of the missense mutations are localized within regions encoding these two domains, they must be critical for the function of *IRF6* (9).

Most IRFs regulate the expression of interferon-α and -ß after viral infection, but the function of IRF6 is unknown (9). Both the occurrence of CLP and CP within the same genealogy and a recurrence risk <40% for CP among descendants with VWS have suggested that the development of clefts in this syndrome is influenced by modifying genes at other loci (13). The aim of our study was to find the mutation in affected individuals in a family consisting of 24 members, 12 of them suffering from VWS.

Materials and methods

Participants. A German family (Fig. 1) with five first-degree relatives affected with CLP and one simplex case were

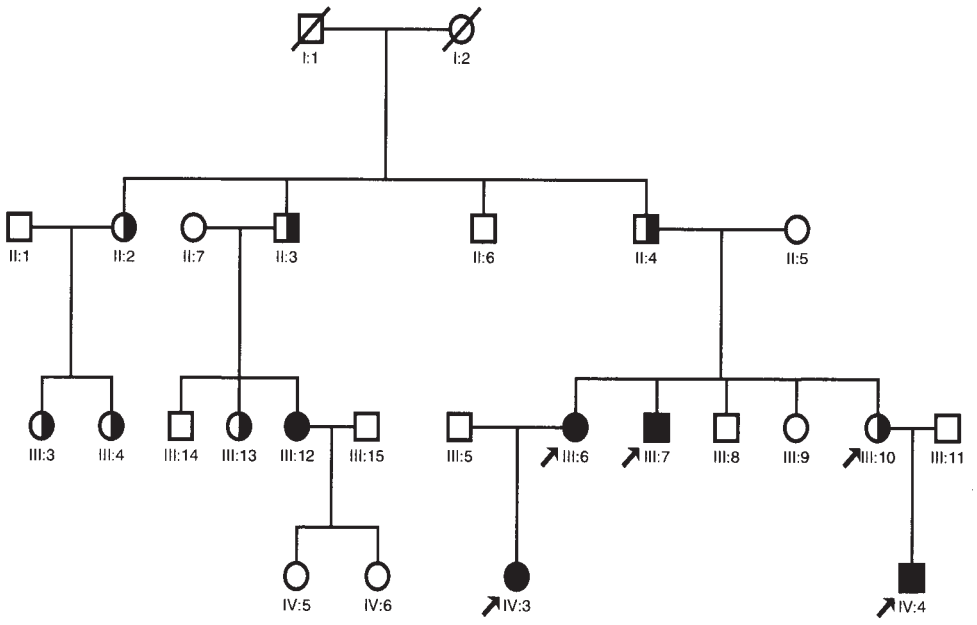

Figure 1. This pedigree shows the 12 family members (□, male; ○, female) with Van der Woude syndrome. The five who underwent DNA testing are shown with an arrow. Four had unilateral or bilateral lip and palate clefts and are shown with a black symbol. Black/white symbols represent those with lower lip pits but no clefts.

investigated. After informed consent, a sample of buccal cells was obtained from all five family members (III:6, III:7, III:10, IV:3 and IV:4). At the same time, two cousins (III:12 and III:13) of the mother with an uncle (II:3) and an aunt (II:2) and a father (II:4) were found with Van der Woude syndrome, but no DNA could be obtained from them.

DNA sequencing. DNA was extracted using the Puregene DNA isolation kit (Gentra Systems) according to the manufacturer's instructions. Exons 2-9, containing the coding region of *IRF6*, were amplified with primers as previously published (16). Only two primers were modified: exon 2 forward (5'-GTATGGATAGCCAGCTGAGA-3') and exon 5 forward (5'-CTAGGACATTGAGGGTGTGT-3'). The PCR products were sequenced using the DTCS quick start kit (Beckman Coulter). Analysis of the sequences was performed in a CEQ 8000 genetic analysis system. The sequences were compared with GenBank No. NT_021877 using the CEQ 8000 software (Beckman Coulter).

Results

Patients. A nine-month-old baby girl (III:3; Fig. 1) with unilateral cleft lip and palate and bilateral lower lip fistulas consistent with Van der Woude syndrome was referred for paediatric phoniatric assessment. She also had a ventricular septal defect, which had closed spontaneously. Her mother also had Van der Woude syndrome with similar fistulas, but with the clefts confined to the right side. She had taken folic acid during the pregnancy, which was characterised by early contractions from the 21st week.

Within the family, a sister of the mother (III:10; Fig. 1) had an isolated lower lip pit, and a brother (III:7; Fig. 1) and a nephew (IV:4; Fig. 1) had bilateral lip and palate clefts with lower lip pits.

The simplex case was a 2-year-old boy with VWS (bilateral CLP and bilateral lower lip pits).

Mutation screening. We sequenced exons 2-9 including all coding exons of the *IRF6* gene as well as the flanking intronic regions in the simplex case and one member of the family (IV:4, Fig. 1). This approach led to the identification of one primarily non-described missense mutation, one nonsense mutation and two single nucleotide polymorphisms (SNP).

In the sporadic case a heterozygous missense mutation in exon 4 was identified. This mutation c.251G>A (p.R84H) had already been described (9). Furthermore, this patient carried a c.459G>T transition at codon 153 in exon 5 not leading to an amino acid change. This sequence variant has also been reported before (refSNP ID: rs2013162).

All 5 patients with the familial context were heterozygous for a base substitution c.201C>A changing the tyrosine codon at amino acid position 67 into a stop codon (p.Y67X) in exon 4. This mutation has not been reported before. One of our patients (IV:4; Fig. 1) also carried the c.459G>T transition in a homozygous state and moreover, we found a homozygous change from C to G in intron 6 (refSNP ID: rs11811534). In all other affected family members we analyzed only exon 4. We found that all of them carried the p.Y67X disease causing a mutation in a heterozygous state.

Discussion

Development of the lip and palate involves a complex series of events that are frequently disturbed resulting in the congenital anomalies, cleft lip and cleft palate. VWS arises as the result of mutations in the gene encoding interferon regulatory factor 6 (*IRF6*). To provide insights into the role of *IRF6* during embryogenesis, expression of this molecule during mouse and chick facial development was investigated

ll known mutations in the *IRF6* gene.

Mutation	nt change	aa change	Exon	Syndrome	Reference
Frameshift	-48A>T	5'UTR to M	2	VWS	Kondo *et al* (9)
Frameshift	3G>A	M1I	3	VWS	(9)
Missense	5C>T	A2V	3	VWS	(9)
Missense	16C>T	R6C	3	VWS	Wang *et al* (12)
Frameshift	17insC	R6fs	3	VWS	(9)
Missense	25C>T	R9W	3	VWS	Matsuzawa *et al* (14)
Missense	47T>C	A16V	3	VWS	Ghassibé *et al* (15)
Frameshift	49del CAGGTGGATAGTGGCC	Q17fs	3	VWS	(9)
Missense	52G>A	V18M	3	VWS	(9)
Missense	53T>C	V18A	3	VWS	(9)
Missense	65T>C	L22P	3	(PPS)	(15)
Nonsense	69A>C	Y23X	3	VWS	(9)
Nonsense	74G>C	G25A	3	VWS	Kim *et al* (16)
Missense	115C>G	P39A	3	VWS	(9)
Missense	134G>A	R45Q	3	VWS	Kayano *et al* (17)
Missense	178T>G	W60G	4	PPS	(9)
Missense	182C>G	A61G	4	VWS	(9)
Missense	191T>C	T64I	4	VWS	(15)
Missense	197A>G	K66T	4	PPS	(9)
Nonsense	201C>A	Y67X	4	VWS	Brosch *et al*/present study
Nonsense	202C>T	Q68X	4	VWS	(9)
Missense	208G>C	G70R	4	VWS	(9)
Missense	226C>T	P76S	4	VWS	(9)
Missense	244C>A	Q82K	4	PPS	(9)
Missense	250C>T	R84C	4	PPS	(9)
Missense	250C>G	R84G	4	VWS	Item *et al* (18)
Missense	251G>A	R84H	4	PPS/VWS	(9)
Missense	262A>C	N88H	4	VWS	(9)
Missense	265A>G	K89E	4	PPS	(9)
Missense	268A>G	S90G	4	VWS	(9)
Nonsense	274G>T	E92X	4	VWS	(9)
Missense	292G>C	D98H	4	VWS	(9)
Missense	298A>G	T100A	4	VWS	(15)
Missense	331T>C	Y111H	4	VWS	Ye *et al* (19)
Nonsense	352C>T	Q118X	4	VWS	(9)
Frameshift	399delC	P133fs	5	VWS	(16)
Frameshift	466insC	H156fs	5	VWS	(9)
Frameshift		F165fs	6	VWS	(19)
Nonsense	558C>A	C186X	6	VWS	(9)
Nonsense	576G>A	W192X	6	VWS	(9)
Frameshift	634insCCAC	S212fs	6	VWS	(9)
Nonsense	650G>A	W217X	6	VWS	Gatta *et al* (11)
Frameshift	657delCTCTCTCCCinsTA	S219fs	6	VWS	(9)
Frameshift	744delCTGCC	G248fs	7	VWS	(9)
Missense	749G>A	R250Q	7	VWS	(9)
Missense	755T>C	L251P	7	VWS	(15)
Nonsense	759T>A	Y253X	7	VWS	(9)
Missense	775C>T	P258S	7	VWS	(15)
Frameshift	795delC	L265fs	7	VWS	(9)
Missense	818A>G	Q273R	7	VWS	(9)
Frameshift	842delA	H281fs	7	VWS	(9)
Deletion	870delCACTAGCAAGCTGCTGGACinsA	FTSKLLD290L	7	VWS	(9)
Missense	881T>C	L294P	7	VWS	(9)

Table I. Continued.

Mutation	nt change	aa change	Exon	Syndrome	Reference
Missense	889G>A	V297I	7	VWS	Kondo *et al* (9)
Missense	958A>G	K320E	7	VWS	(9)
Missense	961G>A	V321M	7	VWS	(9)
Missense	974G>A	G325E	7	VWS	(9)
Missense	1034T>C	L345P	7	VWS	(9)
Missense	1040G>T	C347F	7	VWS	(9)
Missense	1106T>C	F369S	8	VWS	(9)
Missense	1122C>G	C374W	8	VWS	(9)
Nonsense	1137G>A	W379X	8	VWS	Wang *et al* (12)
Missense	1162A>G	K388E	8	VWS	(9)
Nonsense	1177C>T	Q393X	8	PPS	(9)
Missense	1186C>T	P396S	9	VWS	Kayano *et al* (17)
Missense	1198C>T	R400W	9	VWS	(12)
Frameshift		S407fs	9	VWS	Ye *et al* (19)
Frameshift	1234delC	R412fs	9	VWS	Shotelersuk *et al* (20)
Nonsense	1234C>T	R412X	9	VWS	(9)
Missense	1288G>A	D430N	9	PPS	(9)
Frameshift	1381insC	P461fs	9	VWS	(9)

in the ectoderm covering the facial processes during their fusion to form the upper lip and primary palate (21). The results supported a role for *IRF6* during the fusion events that occur during development of the lip and palate.

IRF6 is a member of a family of transcription factors which is characterized by a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain called SMIR (Smad-interferon regulatory factor-binding domain).

Until recently, there were 70 known pathogenic mutations in the *IRF6* gene (9,14-21). Most of them are located in exons 4 and 7 encoding the conserved DNA-binding and SMIR domains (7,8). In this study, we described a sporadic case and a new family with autosomal dominantly inherited VWS. The sporadic patient carried the p.R84H mutation in exon 4 of the *IRF6* gene, leading to an amino acid change from arginine to histidine at amino acid position 84. The substitution was located in the well-conserved DNA-binding domain altering its three-dimensional structure and making it unable to bind DNA. This mutation was formerly described to cause popliteal pterygium syndrome (PPS) (9). In our patient, the same mutation was responsible for VWS. Our result confirms previous data that mutations in the *IRF6* gene can cause both VWS and PPS (9,15).

In addition, we found a novel mutation in the *IRF6* gene. All of the available affected family members (III:6, III:7, III:10, IV:3 and IV:4) carried the p.Y67X mutation in exon 4. This mutation changes a tyrosine codon into a stop codon at amino acid position 67. The premature stop codon is responsible for a truncated protein lacking parts of the well-conserved DNA-binding domain and the complete SMIR domain probably essential for interactions with the Smad transcription factors. This leads to a complete loss of function of the protein and confirms data indicating haplo-insufficiency as a cause of VWS (9,11,15). Notably, the mutation produced different phenotypes within our family. This phenomenon was reported before for different mutations (9,11,14,15), therefore this is a strong hint for at least one modifying gene (13).

We conclude that the present data confirm the association between *IRF6* and VWS proving the pathogenetic role of *IRF6* mutations in this syndrome and the important role of the *IRF6* gene in orofacial development. Furthermore, we report a novel nonsense mutation in the DNA-binding domain. We suggest that molecular analysis of VWS and PPS patients should be performed within the complete coding region of the *IRF6* gene by direct sequencing because mutations can be distributed over the whole gene.

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