Pediatrics and Neonatology (2019) 60, 218–220

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Short Communication

Novel mutations of *IRF6* gene in Taiwanese Van der Woude syndrome patients

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Received Jan 9, 2018; received in revised form Apr 9, 2018; accepted Apr 24, 2018
Available online 3 May 2018

1. Introduction

Orofacial clefts (OFC) are complex genetic traits that are often classified as syndromic or non-syndromic clefts. To date, over 500 types of syndromic clefts have been identified and listed in the Online Mendelian Inheritance in Man (OMIM) database (www.omim.org), and the underlying genetic factors for many of these have been identified. Van der Woude syndrome (VWS) (OMIM: 119300) is a dominantly inherited developmental disorder with high penetrance (96.7%) but variable expression.[1] VWS is characterized by pits and/or sinuses of the lower lip, cleft lip (CL), cleft lip and palate (CLP), or cleft palate only. Lip pits occur in approximately 88% of VWS patients and are the only visible defect in 64% of patients. A wide range of clefting has been reported, including submucous cleft palate, incomplete unilateral CL, bifid uvula, and complete bilateral CLP. The incidence of VWS is approximately 1/40,000 people worldwide, and it is one of the most common causes of syndromic clefts, accounting for 2% of all OFC cases.[2]

Interferon regulatory factor-6 gene (*IRF6*) (OMIM: 607199) belongs to a family of transcription factors that has two protein domains: a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain called Smad-interferon regulatory factor-binding domain (SMIR),[3] which is critical for protein dimer formation. Moreover, single nucleotide polymorphisms (SNPs) in *IRF6* have been associated with non-syndromic cleft palate in an ethnicity-specific manner, especially in Chinese–Taiwanese population. To date, more than 200 different mutations in *IRF6* have been identified in almost 70% of families with VWS in different ethnicities, including European, American, and Asian. These include missense, nonsense, frameshift, microdeletions, and splice-site mutations. Most of the mutations are non-randomly distributed, and functional enrichment analysis reveals that most of them are enriched in exon 4, which is in the highly conserved DNA-binding domain, and in exons 7 and 9, which are in the less conserved protein-binding domain.[1] Herein, we describe seven cases of VWS and further identify two novel mutations in *IRF6*.

2. Methods

2.1. Patients

This study was approved by the Chang Gung Medical Foundation Institutional Review Board (104-2128A3). Seven patients were referred to the Department of Pediatric

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https://doi.org/10.1016/j.pedneo.2018.04.008

Table 1 *IRF6* variants in 7 VWS patients.

Patient	Nucleotide change in coding sequence	Amino acid change	c.820G > A V274I rs2235371	Mutation Taster	PolyPhen2	Exon
VWS1	c.290A > G	Y97C	Heterozygous (V/I)	Disease causing	Probably damaging 1	4
VWS2	c.294T > G	D98E	Heterozygous (V/I)	Disease causing	Probably damaging 1	4
VWS3	c.1214T > C	M405T	Heterozygous (V/I)	Disease causing	Probably damaging 0.995	9
VWS4	—	—	Homozygous (I/I)	—	—	
VWS5	—	—	Homozygous (I/I)	—	—	
VWS6	—	—	Homozygous (I/I)	—	—	
VWS7	c.1138C > T	P380S	Homozygous (I/I)	Disease causing	Probably damaging 1	8

Genetics at Kaohsiung Chang Gung Memorial Hospital to be genetically tested for VWS. All patients were evaluated by referral pediatricians and plastic surgeons and were diagnosed with VWS. Diagnostic criteria to be considered for individuals affected with VWS included cleft palate or CL, and bilateral lower lip pits. The demographic data of these patients are summarized in Table S1.

2.2. Sequencing

After obtaining parental consent, blood DNA was extracted using a Qiagen DNA extraction kit. Polymerase chain reaction using primers designed to span all exon—intron boundaries (available as request). Exons 2—9 were amplified, and the amplification products were sequenced using an automatic sequencer with capillary electrophoresis (ABI Prism 3700). DNA sequences were aligned (NM_006147.2), and the identified variants were analyzed using *in silico* prediction programs, including Mutation Tasting (www.mutationtaster.org) and Polyphen2 (genetics.bwh.havard.edu/pph2). Genotypes of SNPs (rs2235371) were also determined in the sequences from all patients.

3. Results

Four of the seven recruited patients were carriers of one of four different heterozygous *IRF6* gene mutations (see Table 1 and Fig. S1). The disease-causing missense mutations were determined by *in silico* prediction, including two novel mutations (p.D98E in exon 4, p.P380S in exon 8). Two known missense mutations were also identified; p.M405T located in exon 9 was listed in the Human Gene Mutation Database (www.hgmd.org), while p.Y97C in exon 4 has recently been reported in a monozygotic twin pair with variable expression (Fig. S2).[4]

4. Discussion

In a large cohort of VWS patients reported by de Lima et al.,[1] 242 exonic mutations in *IRF6* were analyzed and revealed to be non-random, primarily occurring in exons 3, 4, 7, and 9, and accounting for 80% of all mutations. *IRF6* from the seven recruited VWS patients listed in Table 1 was sequenced, and four missense mutations located in exons 4, 8, and 9 were identified.

We identified two novel missense mutations (p.D98E, p.P380S) in the present study. One of the novel mutations (c.294T > G, p.D98E, found in patient VWS2) resulted in an amino acid change, which, according to *in silico* prediction programs (polyphen2 and MutationTaster), is likely to be pathogenic. Furthermore, a missense mutation in a nearby nucleotide resulting in a different amino acid change in the same locus (c.292G > C, p.D98H) has been reported by Kondo et al.[5] The mutation is located in the highly conserved DNA-binding domain and likely affects protein function. The other novel mutation (c.1138C > T, p.P380S) is located within the SMIR protein-binding domain and is predicted to be damaging *in silico*. Both amino acids are highly conserved in mammals, chickens, fishes, and frogs and are localized in the functional domains of *IRF6*, where they are likely to affect its physiological role.

Previous reports have suggested that the heterozygous V274I allele of SNP rs2235371 is negatively associated with non-syndromic cleft palate in an ethnicity-specific manner.[6] Heterozygous mutations (p.V274I) were identified in three of the seven VWS patients, and all three patients had concurrent missense mutations. In addition, three of the seven (42%) VWS patients were homozygous (V274I) for SNP rs2235371, which suggests that the allele's protective effect does not compensate for the deleterious consequence of the missense mutations. Furthermore, clinical phenotypes, especially nasal phonations and abnormal dental eruptions, tended to be more severely affected in *IRF6* mutation-positive than in *IRF6* mutation-negative VWS patients (Table S1).

In three of the recruited patients, no mutations in *IRF6* were found. It is important to note that the Sanger sequencing method used in our detection protocol has its limitations; only coding sequences and proximal intronic sequences were analyzed, but large-scale deletion, duplication, inversion, or recombinant mutations were undetected in the present study. Recently, a missense mutation in *GRHL3* (OMIM: 608317) was identified in a VWS patient who does not have an exonic mutation in *IRF6*.[7] It is possible that *GRHL3* is responsible for some of the *IRF6* mutation-negative VWS cases, and further study will be necessary to clarify this.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study was supported by Grant CMRPG8E0111, CMRPG8F0601 and CMRPG8F0831 from Chang Gung Memorial Hospital, Taiwan.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.04.008.