A novel mutation, 1234del(C), of the *IRF6* in a Thai family with Van der Woude syndrome

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Abstract. Van der Woude syndrome (VWS) is an autosomal dominant disorder and the most common cleft syndrome characterized by cleft lip and palate with lip pits. Very recently, mutations in the interferon regulatory factor 6 gene (*IRF6*) were identified to cause VWS in patients of northern European descent. We describe a Thai family with VWS. The proband, an 8-month-old boy, had bilateral complete cleft lip and palate, and two conical elevations with lip pits on his lower lip. Four other family members had various manifestations of the clefts and lower lip pits. Mutation analysis of the proband and his mother for the entire coding region of *IRF6* identified a novel mutation, 1234del(C), in its exon 9. The deletion is expected to result in some amino acid changes followed by truncation at amino acid 435. This observation supports that *IRF6* is the gene responsible for VWS across different populations and that haploinsufficiency of the gene disturbs development of the lip and palate.

Introduction

Van der Woude syndrome (VWS, OMIM 119300) is an autosomal dominant disorder characterized by cleft lip and palate with lip pits. It is the most common syndromic form of oral clefts (1). Most reported familial VWS cases have been linked to 1q32-q41 (2), but a second locus has been mapped to 1p34 (3). Very recently, mutations in the interferon regulatory factor 6 gene (*IRF6*) were demonstrated to cause VWS. So far, forty-six mutations in *IRF6* associated with VWS have been identified (4). Here we describe a Thai family with VWS with a novel mutation in exon 9 of *IRF6*.

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Materials and methods

Case report. The proband, a boy, was born after uncomplicated pregnancy at term by spontaneous vaginal delivery to a 38-year-old G2P1 Thai mother and a 30-year-old unrelated Thai father. Birth weight was 2,500 g. He was noted to have oral clefts since birth. His development was appropriate for age. Physical examination at the age of 8 months revealed length of 64 cm (-2 SD) and weight 7 kg (-1.5 SD). He had bilateral complete cleft lip and palate, and two conical elevations with lip pits on his lower lip (Fig. 1A).

His mother had cleft lip on the right side, bilateral complete cleft palate and lip pits on her lower lip (Fig. 1B). Three other members in the family, i.e., the maternal grandfather, an uncle and his daughter (first cousin) had various manifestations of the clefts and lower lip pits (Fig. 2).

Mutation analysis. After informed consent was received, peripheral blood (3 ml) was obtained from the boy and his mother and DNA was extracted by standard methods. Exons 3 to 8 and a part of exon 9 of *IRF6*, which contain its entire coding region, were amplified by standard PCR (4). The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, Ohio), according to the company recommendations, and sent for direct sequencing at the National Science and Technology Development Agency, Bangkok, Thailand.

Results and Discussion

Direct sequencing analysis of the PCR products revealed that the boy and his mother were heterozygous for deletion of cytosine at nucleotide position 1,234 [1234del(C)] in exon 9 of *IRF6* (Fig. 3). The mutation is expected to result in subsequent changes of 24 amino acids and truncation at amino acid 435 because of a frame shift.

In our family, the proband and his mother had clinically typical VWS. Both had cleft lip, cleft palate and lip pits, similar to the boy's affected first cousin (Fig. 2). However, the proband's maternal grandfather had cleft lip and lip pits, while a proband's uncle had only lip pits. This variable expressivity has been known in this syndrome. Previous studies showed that lip pits were the most common manifestation, being present

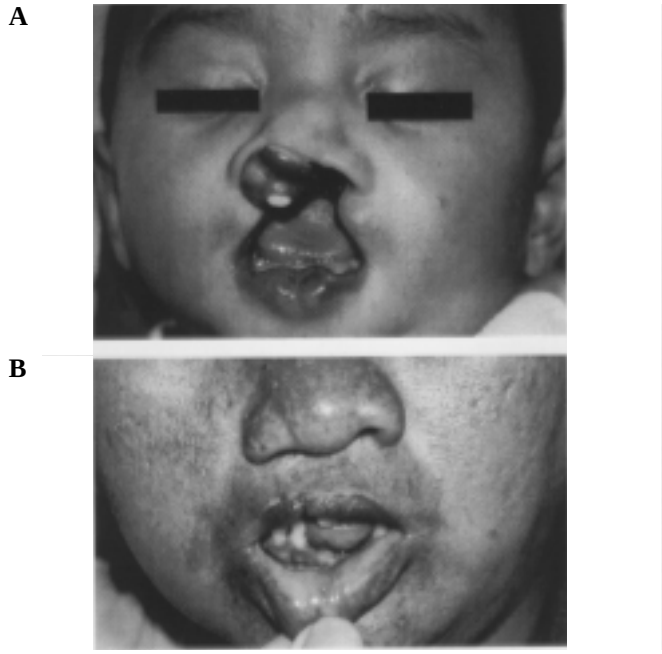

Figure 1. A, the proband has cleft lip, cleft palate and lip pits. B, the proband's mother has surgically repaired cleft lip, unrepaired cleft palate and a lip pit on her lower lip.

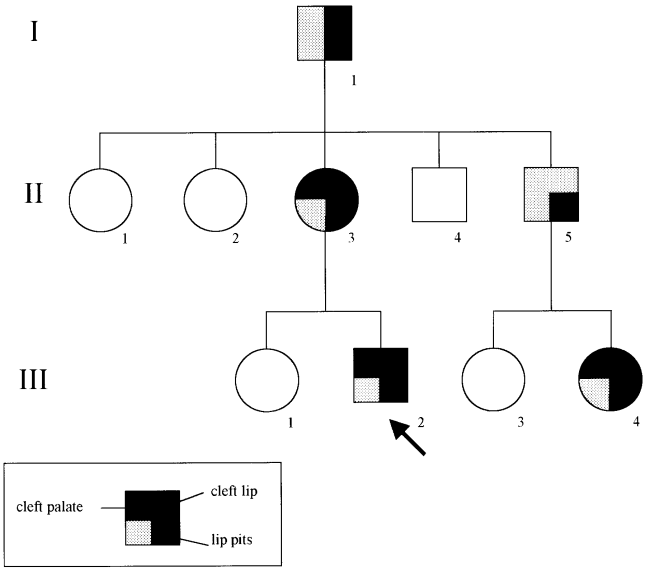

Figure 2. Pedigree of the family. Unaffected individuals (open symbol), proband (arrow), and individuals with VSW (gray) are indicated. Symbols representing specific manifestations are shown below the pedigree.

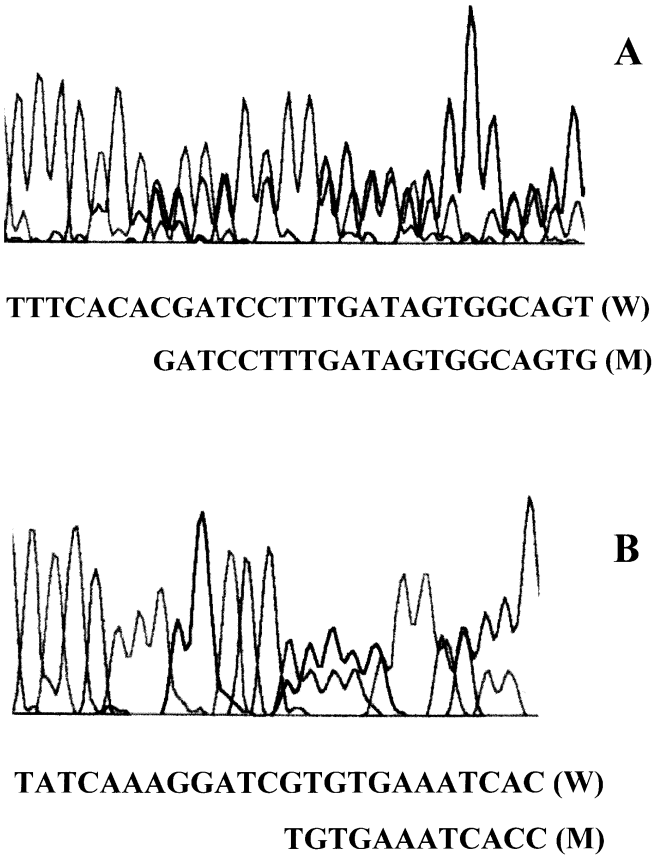

Figure 3. A, the sense and B, the antisense sequence electropherograms in the proband. Note the wild-type sequence (W) can be observed along with the mutant sequence (M).

in 88% of affected individuals and the only manifestation in 64% of them (5). Oral clefts occurred in 21% and penetrance was 96.7% (5). All five affected members in our family were in one clefting phenotype, i.e., the cleft lip with or without cleft palate (CL/P). The CL/P and isolated cleft palate (CP) are genetically distinct. The mixed phenotype with CL/P and CP in the same family is very rare in non-syndromic oral clefts and not found in the majority of patients with syndromic clefts (6). Although not seen in our family, VWS is one of such few syndromic forms of oral clefts that manifest either clefting phenotype.

The IRF6 protein is one of the nine members in a family of transcription factors. They share a highly winged-helix conserved DNA-binding domain (amino acids 13-113) and a less conserved protein-binding domain (amino acids 226-394) named the Smad-interferon regulatory factor-binding domain (SMIR). No obvious functional domain has been found in the C-terminus region. However, since the mutation identified in our family was a heterozygous 1234del(C) that results in a truncated protein without 56 amino acids at the C-terminus, certain functional domain(s) may exist in this region. In fact, Kondo *et al* (4) also reported a missense mutation in the C-terminus region.

IRF6 presumably forms dimers before it binds to other transcription factors and their DNA targets (7). Most IRF are regulators of host defense after viral infection (8). Distinctively, IRF6 functions in a pathway for normal development of the lip, palate, skin and external genitalia (4). IRF binding sites were found in the promoter of *MSX1*, mutations of which are associated with orofacial clefting (9). In addition, IRF6 may interact with Smads, a family of transcriptional factors known to transduce TGF-ßs, the gene product of which is required for palatal fusion (10). These observations suggest that IRF6, MSX1, and TGF-ßs may be involved in a common pathway.

Previous studies demonstrated that protein-truncation mutations, as observed in our family with VWS, and large

deletions encompassing the entire *IRF6* resulted in VWS (4,11,12). On the other hand, missense mutations in the DNA-binding domain that directly contact DNA were associated with another dysmorphic syndrome, popliteal pterygium syndrome (PPS; OMIM 119500). PPS is another autosomal dominant disorder characterized by orofacial manifestations similar to VWS as well as by skin and genital abnormalities (13). Therefore, it has been hypothesized that haplo-insufficiency of *IRF6* disrupts orofacial development, while dominant-negative mutations disturb development of the skin and genitalia. The 1234del(C) found in our VWS family, which is expected to result in a truncation protein, supports the hypothesis.

In summary, we describe a VWS Thai family with a novel protein truncation mutation presumably resulting in haplo-insufficiency of the *IRF6*. This observation further supports that *IRF6* is the gene responsible for VWS across different populations and that haploinsufficiency of the gene disturbs development of the lip and palate.

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