Microdeletion of the entire *IRF6* gene in a Sub-Saharan African's family with Van der Woude syndrome.

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Abstract

Introduction

Microdeletion of the entire Interferon regulatory factory 6 (*IRF 6*) gene is a rare cause of Van der Woude syndrome (VDW) with only few cases reported in medical literature. Its occurrence in multiple affected members of a family is exceptional.

Case presentation

Here we reported phenotype features of members of a Central African family with Van der woude syndrome consisting of labio-alveolar cleft, depressions of the lower lip with labial fistulae (lip pits), submucosal clefts and cleft palate.

Mutation analysis by means of MLPA and chromosomal microarray revealed a 374.070 kb,deletion encompassing the entire *IRF6* gene in 4 affected family members.

Conclusion

Microdeletion of the entire *IRF6* gene causes the cassical Van der woude syndrome phenotype.

Key words : Microdeletion, IRF6 gene, orofacial clefts, Van der Woude syndrome

Introduction

Cleft lip and/or cleft palate (CL/P) can be syndromic or non-syndromic. A syndromic cleft is associated with one or more major malformations and/or dysmorphism. Syndromic CL/P account for 5-7% of all orofacial clefts, while non-syndromic clefts are found in 93-95% of all orofacial clefts (Mossey et al., 2009, Mbuyi-Musanzayi et al., 2018). The most common syndromic form is Van der Woude syndrome (VDW, MIM 119300), occurring in 2% of all orofacial clefts, with a prevalence ranging from 1/35000 to 1/100 000 in the white population (Grosen et al., 2010). It is an autosomal dominant condition with high penetrance, but with marked inter- and intrafamilial variation of phenotype (Cheney et al., 1986). Clinically, VDW patients can present cleft lip, cleft lip with or without cleft palate (CL±P), cleft palate only (CP), submucous cleft palate, bifid uvula, congenital lower lip pits, with or without other features including syngnathia and ankyloblepharon (Gorlin, RJ and al., 2001). These facial anomalies are also seen in individuals with popliteal pterygium syndrome (PPS, OMIM 119500) which is characterized by pterygium in the popliteal region, ankyloblepharon filiforme, syngnathia, pyramidal skinfold overlying the nail of the hallux, syndactyly, oligodactyly, and skinfold over toenail (Mubungu et al., 2014, Schutte et al., 1993). Mutations in two genes have been described in patients with Van der Woude syndrome, most commonly in the *IRF6* gene (Sander et al., 1994, Schutte et al., 1999), more rarely in the *GRHL3* gene (Peyrard-Janvid et al., 2014). Most *IRF6* pathogenic mutations are sequence variants causing loss-of-function. Deletion of the entire *IRF6* gene is a very rare condition, occurring in less than 2% of all patients with VDW syndrome (Schutte et al., 1993). We here report a unique Central African family with typical VDW phenotype carrying an *IRF6* gene deletion.

Case report

The index patient of this family was 4 years old at the last consultation. She was born in a maternity in the health district of Lubumbashi as the 7th in a family of 8 children and 3 previous abortions. At birth, her father was 38 years-old, her mother was 31 years-old. The mother was illiterate. Mother presented an episode of malaria and typhoid fever during the first trimester of pregnancy, treated with quinine and ciprofloxacin at an unknown dose. The first prenatal consultation was at 25 weeks of gestation. She reported consumption of clay and alcohol during the first trimester of pregnancy. The patient was born at 40 weeks of gestation with a weight of 3150 grams (-0.18 SD), head circumference 34 cm (+0.10 SD), and length 51 cm (+0.99 SD). At 4 years of age, she had a BMI of 16.1 Kg/m², a weight of 16.5 kg (+0.15

SD), a head circumference of 51 cm (+0.47 SD) and a height of 1.10 m (+1.69 SD). She presented a right labio-alveolar cleft, depressions of the lower lip with two labial fistulae (lip pits) (Figure 1a and b). Her mother also had depressions in the lower lip (Figure 1c). Her brother II7 showed a non-operated cleft palate (Figure 1d), with a depression of the lower lip. Her brothers II9 and II11 (Figure 2) showed submucosal clefts with depressions of the lower lip (Figure 1e, f and g).

Figure 1. Van der woude syndrome a. Index with operated cleft lip, b. Salivary fistulas on the lower lip, c. The mother (I2) of the index with depressions (“pits”) in the lower lip, d. II7 with a cleft palate, e. Depressions of the lower lips in II9, f. submucosal cleft in II9, G. Depressions of the lower lips in II11.

Figure 2. Pedigree of the family with Van der Woude syndrome.

Genetic analyses.

To detect chromosomal imbalances, microarray comparative genomic hybridization (CGH) was carried out in the cytogenetic laboratory of the Center for Human Genetics, UZ Leuven, using the 180k CytoSure ISCA v2 microarray (Oxford Gene Technology, OGT, Oxford, UK), according the manufacturer's instructions or using the 8x 60K CytoSure ISCA v2 microarray (AMADID 020040, Oxford Gene Technology, OGT, Oxford, UK). Visualization of results and data analysis were performed using the CytoSure Interpret Software (Oxford Gene Technology) and the circular binary segmentation algorithm.

Mutation analysis of the *IRF6* gene was initially performed in the genetics laboratory of the Center for Medical Genetics, UC Louvain. Mutations were searched in the entire coding region of the *IRF6* gene (NM_006147.3) by sequencing (Big DyeV3.1 / ABI 3500XL DX) followed by interpretation by the Mutation Surveyor v4.0 software. Deletions and duplications of all exons of the *IRF6* gene were analyzed by MLPA (MRC-MLPA Kit P-304-A1) with gel migration (sequencer ABI3500XL DX) and interpretation by the Genemarker software v2.6.3.

Mutation analysis of the *IRF6* gene by means of MLPA revealed a deletion encompassing the entire gene in 4 family members with Van der Woude phenotype. One unaffected family member showed no deletion of the gene. Chromosomal microarray analysis revealed a deletion of 374.070 kb, at position chr1:209.872.038-210.246.107 (Figure 3). This deletion encompassed 5 additional genes besides the *IRF6* gene: *HSD11B1*, *TRAF3IP3*, *C1ORF74*, *DIEXF* and *SYT14*.

Figure 3. Result of array-CGH showing the size and the location of the deletion in the family with Van der Woude syndrome.

Discussion

The *IRF6* gene plays an important role, both in syndromic and non-syndromic CL/P. Mutations in *IRF6* cause Van der Woude syndrome, an autosomal dominant syndromic condition with CL±P or CP, associated with lower lip pits. However, the manifestations can be highly variable, and sometimes, lower lip pits may be absent. Indeed, about 15% of patients with Van der Woude syndrome do not have lower lip depressions, and may thus be considered as non-syndromic CL/P when occurring sporadically (Burdick et al., 1985). Therefore, familial non-syndromic CL/P without lip pits may in fact be due to mutations in the *IRF6* gene. Mutations of the *IRF6* gene in familial non-syndromic CL/P are reported in 0.3% of non-syndromic orofacial clefts (Leslie et al., 2016). However, most mutations were found in families where there are both cases of CL±P and CP, or in families with multiple affected individuals, with clear autosomal dominant inheritance (Leslie et al., 2016).

In a family with typical autosomal dominant Van der Woude syndrome, a microdeletion involving the *IRF6* gene was observed. The majority of *IRF6* mutations described are point mutations, whereas microdeletions in 1q32-q41 are very rare conditions, with only 1-2% of Van der Woude syndrome cases (Bocian and Walker, 1987, Sander et al., 1994, Schutte and Murray, 1999, Kayano et al., 2003, Osoegawa et al., 2008, Salahshourifar et al., 2011, Kondo et al., 2002). All microdeletions in 1q32-41 including the *IRF6* gene are associated with Van der Woude syndrome, never with non-syndromic orofacial clefts (Bocian and Walker, 1987, Schutte and Murray, 1999, Houdayer et al., 2000, Osoegawa et al., 2008). Deletions including other genes than the *IRF6* gene may be associated with other anomalies (Bocian and Walker, 1987, Kayano et al., 2003, Osoegawa et al., 2008). However, in the present family, no additional features were observed besides the Van der Woude phenotype.

More than 300 mutations of the *IRF6* gene in different populations worldwide have already been reported as cause of Van der woude syndrome (Leslie et al., 2013a, Leslie et al., 2013b, Malik et al., 2014, Tan et al., 2013). Very few studies have reported mutations in the *IRF6* gene in sub-Saharan Africans (Butali et al., 2014) or in the descendants of the African people (Rutledge et al., 2010, Salahshourifar et al., 2012, do Rego Borges et al., 2015). Here, we report a microdeletion including the entire *IRF6* gene in a family with Van der Woude syndrome, a mutation which has not been observed before in Central Africa.

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Authors' contribution

Sébastien Mbuyi-Musanzayi: clinical examination, treatment of the patient, and redaction of article. Nicole Revencu: MLPA testing and manuscript corrections. Erick Kasamba Ilunga: DNA extraction. Prosper Lukusa Tshilobo: manuscript correction. Prosper Kalenga Muenze: manuscript correction. François Tshilombo Katombe: clinical examination. Hervé Reyckler: manuscript corrections. Koenraad Devriendt: clinical examination, diagnosis, and manuscript corrections.

Conflict of Interest

None

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