Orofacial Clefting: Update on the Role of Genetics


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Abstract. Orofacial Clefting: Update on the Role of Genetics. Introduction: Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects in the world. Prevalence varies between populations, with an average of 1/700. CL/P has a major clinical impact, requiring surgical, dental, orthodontic, speech, hearing and psychological management throughout childhood. The aetiology of CL/P is mostly unknown, and it is thought that both genetic and environmental factors play a role. Several causative genes for inherited syndromic forms of CL/P have been identified, and some recent studies have shown that these genes also contribute to the occurrence of isolated forms. Van der Woude syndrome (VWS) is one of the best models for non-syndromic CLP. It is an autosomal dominant disorder characterised by the presence of pits on the lower lip in addition to CL/P. Pits are the only feature distinguishing VWS from isolated clefts. Interestingly, in numerous VWS patients, the lip pits are very small and not readily diagnosed, thus mimicking isolated CL/P.

Mutations in the IRF6 gene were shown to be the major genetic cause of VWS. Results: We performed direct sequence analysis of IRF6 on samples from a large European cohort and identified mutations in 27 (80%) families. This shows that IRF6 is the major causative gene of VWS in Europe also. Moreover, it is the gene to study when a seemingly isolated CL/P patient has minor signs, such as lip pits, since the identification of a mutation in IRF6 is associated with an increase in the risk of having a child with CL/P from 4-6%, the risk of transmission of an isolated cleft, to 50%, the risk of transmission of a dominant Mendelian disorder like VWS. Moreover, we studied the association of isolated CL/P with the IRF6 locus using two variants in a set of 195 patients from Belgium. As in an American study, a clear association was observed. This suggests that IRF6 also contributes to the occurrence of sporadic, isolated CL/P, even if no mutation in the gene can be identified in such patients.

Conclusion: In conclusion, genes that are mutated in familial syndromic forms of CL/P may be predisposing genetic factors to sporadic isolated CL/P. Due to technological advances and the availability of the human genome sequence, we have now the opportunity to try and unravel the genetic factors behind the various forms of CL/P.

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Introduction

Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects in the world. Its aetiology is complex since several genetic factors interact with environmental triggers. Prevalence varies widely according to geographic origin and ethnic background, with an average of 1/700 live births. The treatment of cleft lip and palate is burdensome and may extend over several years. It requires a team approach including plastic surgeons, orthodontics, speech therapists, and others. Complications may occur with the cleft, such as recurrent ear infections, displaced teeth, poor speech, and lip and nasal deformities. Embryological and epidemiological studies suggest that clefts of the lip and the primary palate have a different mechanism from the clefts of the secondary palate. The genetic factors involved in cleft lip with or without cleft palate (CL/P) are therefore thought to be different from those of the cleft palate only (CPO). In addition, clefts can be divided into non-syndromic, meaning that the cleft is the only sign in the patient, and syndromic when it is present together with other anomalies. Seventy per cent of CL/P and 50% of CPO cases are non-syndromic.

Several genetic factors involved in clefts have been identified on the basis of expression analysis, animal models and role in known human clefting syndromes. A recent study showed that point mutations in six candidate genes, FOXE1, GLI2, MSX2, SKI, SATB2 and SPRY2, may contribute to 6% of isolated cleft lip and palate cases, especially in bilateral cleft lip and palate, and if there is a positive familial history.

Van der Woude syndrome (VWS, OMIM# 119300), a dominantly inherited disorder, mimics isolated clefts. Pits on the lower lip in 80% of cases are the only signs distinguishing it from non-syndromic CL/P. However, hypodontia can be present in 25% of cases. It is the most common Mendelian form of cleft lip and palate, and accounts for 2% of all cleft cases. Mutations in the IRF6 gene (Interferon Regulatory factor 6), localised in 1q32.2, have been shown to be responsible for VWS. IRF6 belongs to a family of 9 transcription factors that share a highly-conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain called SMIR. The popliteal pterygium syndrome (PPS, OMIM# 119500) is a rarer syndrome, allelic to VWS. It combines VWS signs with cutaneous and genital anomalies. The spectrum of phenotypic variability in the two syndromes is wide, even within families. The same mutation can cause different levels of severity, varying from the presence of small, barely visible lip pits, to serious skin anomalies combined with the presence of a cleft. Interestingly, some patients have only cleft lip and/or palate, and can therefore be considered to be phenocopies of isolated clefts. This raises the question of whether IRF6 plays a role in the occurrence of non-syndromic isolated cleft lip and palate.

We investigated the prevalence of mutations in IRF6 in a European VWS/PPS cohort, as well as the association of IRF6 with European non-syndromic isolated CL/P cases.

Van der Woude and PPS syndrome

We recruited a total of 31 families with a clinical diagnosis of VWS. In 10 of them, at least one child was affected with PPS syndrome. A total of 76 affected and 30 non-affected individuals were included. Screening for IRF6 consisted of DHPLC coupled to sequencing. A mutation was identified in each of the 10 PPS families and in 21 of the 31 VWS families (De Lima R and Desmyter L: personal communications) (Figure 1). Interestingly, the Arg84 amino acid was mutated in 6 out of the 10 PPS families. This residue is similar to Arg82 of IRF1, which is important for binding to the GAAA DNA core sequence of target genes.
position are therefore expected to disrupt DNA binding functions of IRF6.

In order to understand the genotype-phenotype correlation better, we pooled our data with those of collaborators in different countries (310 VWS and 37 PPS). The data were allocated on the basis of origin to two cohorts of unrelated families affected with VWS/PPS. The first cohort was obtained from Sao Paulo, Brazil (113 VWS and 1 PPS) and the other was of mixed geographic origin (197 VWS and 36 PPS) (De Lima R: personal communication). The mixed cohort was primarily Northern European in origin, as it includes 152 families from the United States, 31 families from Belgium, 7 families from Germany, 3 from the United Kingdom, 2 from Thailand, one from the Philippines and one from Brazil. A meta-analysis was conducted separately on the two cohorts. Overall, an IRF6 mutation was identified in 69% of families with VWS, and 97% of families with PPS. The distribution of mutations causing VWS was not random, with exons 3, 4, 7, and 9 accounting for 80%. In total, 87 protein-truncating mutations scattered throughout the gene and 127 missense mutations concentrated in the DNA-binding and protein-binding domains were identified (De Lima R: personal communication). None of these mutations have been observed in the CEPH diversity panel. In addition, PolyPhen and SIFT analyses that predict the likelihood of altered function on the basis of sequence conservation between species and similar proteins suggest that the identified amino acid changes are more likely to be damaging to gene function than any substitution at random (p < .001) (Table 1).

As deletions are missed in an exon-screen-based mutation search, this could explain an important fraction of the non-IRF6 mutated VWS/PPS. Moreover, other elements in the region may be important for IRF6 function and could possibly be mutated in some patients. They should therefore be sequenced. The detection of these mutations will be clinically important for the families in which no intra-exonic IRF6 mutation has been identified. The identification of VWS mutations in other parts of the IRF6 region may also provide clues to the regulation of IRF6 expression and function.

**Isolated non-syndromic cleft lip and palate (CL/P)**

Since the phenotype of Van der Woude syndrome overlaps with that of isolated CL/P, it is hypothesized that IRF6 may play a role in non-syndromic isolated clefts. A study showed that variations at the IRF6 gene were responsible for 12 per cent of the genetic contribution to cleft lip and palate, and tripled the risk of recurrence of CLP in families that already had one child with a cleft.

### Table 1

<table>
<thead>
<tr>
<th>Mutation effect</th>
<th>VWS</th>
<th>Random</th>
<th>VWS</th>
<th>Random</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign / Tolerated</td>
<td>17</td>
<td>1232</td>
<td>18</td>
<td>1575</td>
</tr>
<tr>
<td>Possibly damaging</td>
<td>21</td>
<td>729</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probably damaging / not tolerated</td>
<td>58</td>
<td>805</td>
<td>78</td>
<td>1192</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>96</td>
<td>2766</td>
<td>96</td>
<td>2767</td>
</tr>
</tbody>
</table>

Chisquare: 46.18
p-value: <0.001

**Table 1**

<table>
<thead>
<tr>
<th>VWS IRF6 Mutations analysed by PolyPhen</th>
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<tbody>
<tr>
<td><strong>PolyPhen</strong></td>
</tr>
<tr>
<td>Benign / Tolerated</td>
</tr>
<tr>
<td>Possibly damaging</td>
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<tr>
<td>Probably damaging / not tolerated</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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</tbody>
</table>

| **SIFT**                               |
| Benign / Tolerated                     | 18  | 1575 |
| Possibly damaging                      | 729 |
| Probably damaging / not tolerated      | 1192|
| **Total**                              | 2767|

Chisquare: 54.77
p-value: <0.001
We collected data for 195 families from Belgium. Cleft occurred as a sporadic feature in 190 children and in five families the affected child had one affected parent or sibling. Altogether, we included 498 persons. Two single nucleotide polymorphisms (SNPs) were genotyped, one in the IRF6 gene, rs 2013162, and one 100Kbp 3’ of the gene, rs2235543. A transmission Disequilibrium Test (TDT) was conducted to see whether there was any distortion in the transmission of alleles to the affected children. The SNP located in IRF6 showed a significant association with clefting (p = 0.01), whereas the marker situated outside the gene was fairly uninformative, and demonstrated only a trend towards association (p = 0.3). We also performed haplotype-based transmission disequilibrium analysis to increase the power of our study. The haplotype containing the two frequent alleles of the two SNPs had the most significant distortion (p = 0.004). Linkage disequilibrium between the two markers showed that their association was not random (r² = 0.014 D’ = 0.427).

This study shows that IRF6 plays a role in non-syndromic CL/P. The two associated SNPs were also most strongly associated with CL/P in two different populations. Whether these SNPs are causative or whether they are in linkage disequilibrium with the variants causing disease is not known and is an issue requiring elucidation. As these results have been replicated in several studies, they give great power to the IRF6 association, contrary to inconsistent results that have accumulated from genetic studies of complex traits over the past decade.

**Conclusion**

Recent studies show that IRF6 is the major causative gene for VWS in Europe and most probably in the rest of the world as well. Moreover, IRF6 is the gene to study when a seemingly isolated CL/P patient has minor signs, such as lip pits. Physicians must look for the presence of these lip pits very carefully since they are sometimes not easy to detect. The identification of a mutation in IRF6 is associated with an increase in the risk of having a child with CL/P from 4-6%, the risk of transmission of an isolated cleft, to 50%, the risk of transmission of a dominant Mendelian disorder like VWS. This is important for correct genetic counselling.

The implication of IRF6 in the complex aetiology of isolated CL/P holds out hope for unravelling the complex aetiology of sporadic non-syndromic CL/P. A better understanding of the function of IRF6, its targets and the functional pathways is essential for a better understanding of cleft formation, as well as for unravelling other associated factors. All the genes mutated in other syndromic forms of CL/P are also important as they may be additional predisposing genetic factors for sporadic isolated CL/P. Due to the technological advances and the availability of the human genome sequence, the future seems very positive for unravelling the genetic factors behind isolated CL/P.

**References**


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