The pit, the cleft and the web

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Orofacial clefts (cleft lip and/or palate) are among the most common birth defects in humans, affecting up to 1 in 500 infants at birth. The cause of the most common syndromic clefting condition, Van der Woude syndrome, has now been identified as haploinsufficiency of the gene encoding interferon regulatory factor 6 (*IRF6*). Furthermore, dominant-negative mutations of *IRF6* lead to webbing of the skin in popliteal pterygium syndrome, demonstrating beyond doubt that these syndromes are allelic.

As a first year resident in pediatrics in 1980, I remember vividly the birth of a child with a midline cleft of the lip. When asked by the parents about the causes of their son's lip anomaly, I felt ill prepared to answer them, as the genetic and environmental causes of non-syndromic cleft lip and/or palate (CL/P) were largely unknown. In the accompanying paper[1], Shinji Kondo and colleagues report the identification of disease-causing mutations in *IRF6* in two syndromes: one known for the characteristic lip pits and clefts of the lip and/or palate (Van der Woude syndrome, VWS), and an allelic condition with orofacial clefting, genital anomalies and webbing of the skin (popliteal pterygium syndrome, PPS). VWS is the most common autosomal dominant disorder in humans associated with CL/P (PPS is actually quite rare), and lessons learned from this analysis may have implications for the more common non-syndromic CL/P and for genetic studies of other complex traits.

The underlying etiologies of clefting syndromes are only beginning to be elucidated, although genetic factors as causes of CL/P were first defined in 1942 by Fogh-Anderson[2] and confirmed by various segregation analyses[3,4]. These early genetic and embryologic studies suggested that there are different causes for clefts of the lip with or without clefts of the hard palate and clefts of the soft palate only (CPO; Figs 1 and 2). Each category can be subdivided into syndromic and non-syndromic forms. The majority of CL/P are non-syndromic, as are about half of CPO. No disease-causing mutations have been identified in non-syndromic clefting. Among the syndromic cases, more than 350 mendelian disorders are known to have CL/P or CPO as part of their clinical presentation[5]. In addition, recurring chromosomal anomalies and teratogens contribute to causes of syndromic CL/P and CPO (ref. 5). Recently, the underlying causes of several clefting syndromes in humans have been identified.

Lessons from the gene hunt

The connection between pits of the lower lip and CL/P was first described by Van der Woude in 1954 (ref. 6). The search for the VWS gene has followed traditional positional cloning strategies, including linkage and cytogenetic analysis, and mapping of VWS to chromosome 1q32-q41. Fine mapping of a VWS critical region resulted in a 350-kb genomic interval encompassing more than 20 candidate genes[7].

The unique approach that allowed the identification of *IRF6* as the gene mutated in VWS was the use of a pair of monozygotic twins discordant with respect to VWS. A nonsense mutation in *IRF6* in the affected twin not present in the brother or parents was the first step to finding an additional 45 mutations in other VWS families. Monozygotic twinning is common and occurs in about 1 in 200 births. A somatic mutation resulting in an altered phenotype in one member of a pair of monozygotic twins is a fortuitous finding for rare mendelian disorders[8]. This approach may aid in the identification of genes that contribute to common complex traits, including hypertension and diabetes, as well as behavioral differences such as attention deficit hyperactivity disorder.

PPS and VWS have overlapping clinical features, including CL/P and lip pits, as well as a similar map position on chromosome 1q. Individuals with PPS were tested and found to have mutations in *IFR6*. The phenotypic differences between VWS and PPS can be striking—PPS includes genital anomalies and, in extreme forms, a skin web from the heel to the buttocks—but are consistent with the *IRF6* expression pattern in the developing palate before fusion, the external genitalia and the skin[1].

Interestingly, both VWS and PPS have been observed in the same family. Although there are several examples of allelic conditions (different craniosynostosis syndromes owing to *FGFR2* mutations (OMIM 101200, 123500, 101600), or craniosynostosis and short stature syndromes

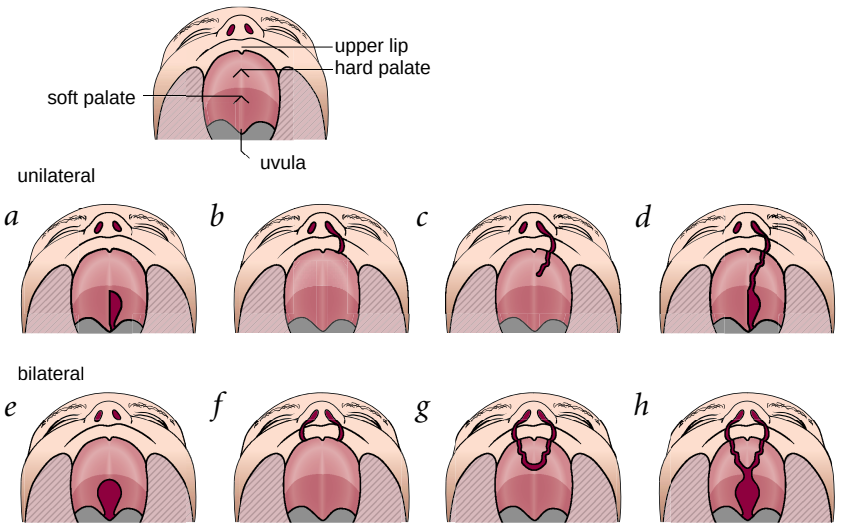

Fig. 1 Diagram of various types of orofacial clefting. Normal lip and palate in top panel. ***a*,*e***, Unilateral and bilateral cleft of the soft palate only. ***b*,*c***, Cleft lip and various degrees of cleft lip and cleft palate involving the hard (*c, g*) and soft (***d***, *g*) palate (CL/P). Note that unilateral and bilateral CL/P can be easily differentiated (*b, c, f, g*), whereas this is not the case for unilateral or bilateral clefts of the soft palate.

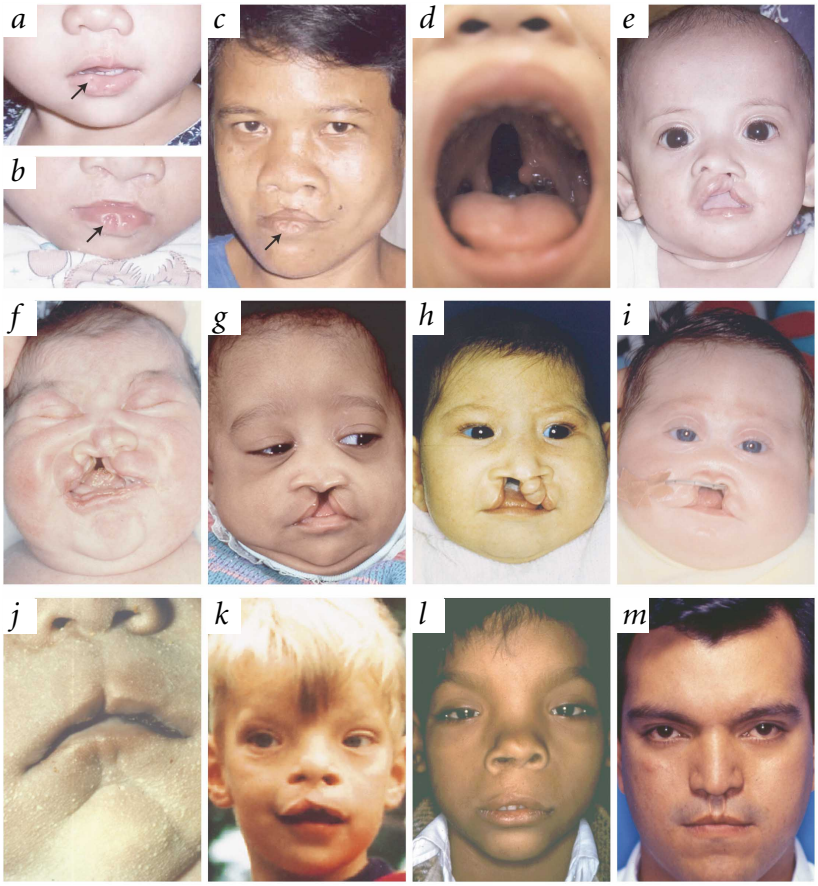

Fig. 2 Individuals with various types of orofacial clefting with known and unknown etiologies. ***a–c***, Van der Woude syndrome. Note pits on lower lip (arrows) in individuals with repaired unilateral (*a*) and bilateral (*b*, *c*) clefts of the upper lip. ***d***, Non-syndromic cleft palate only (CPO). ***e***, Non-syndromic unilateral cleft of the lip and palate. ***f–m***, Syndromic clefting: unilateral CL/P in Smith-Lemli-Opitz syndrome owing to mutations in *DHCR7*; holoprosencephaly owing to mutations in *PTCH1* (***g***, midline CL), *TGIF* (***h***, bilateral CL/P), and *SHH* (***i***, bilateral CL/P); oral-facial-digital syndrome type I owing to mutations in *CXORF5* (***j***, note midline notch of upper lip and facial milia); Opitz GBBB syndrome owing to loss of function mutation of *MID1* (***k***, repaired unilateral CL); craniofrontonasal syndrome due to changes in a putative gene in Xp22 (***l***, repaired unilateral CL in; ***m***, bilateral pseudo CL). (Figures 2*a–e* were provided by Sandy Daack Hirsch and Katie Krahn, University of Iowa.)

owing to different mutations in *FGFR3* (OMIM 602849, 100800, 146000)), rarely do different syndromes segregate in the same family. Even more striking is the fact that 'mixed' clefting (either CL/P or CPO), rarely seen together in non-syndromic clefting in the same family, has been observed in VWS families with the same mutations, pointing to a different mechanism in the etiology of syndromic (VWS) and non-syndromic clefting. As lip pits are variable findings in VWS and may not be present at all in some VWS kindreds, families with several affected individuals with CL/P in the absence of pits may have mutations in *IRF6*, especially if they have mixed cleft types.

Non-syndromic clefting

The mutations in *IRF6* are for the most part specific, predicting loss of function as the most common mechanism for the orofacial anomalies in VWS. Dominant-negative mutations that alter the DNA-binding properties may underlie the skin and genital anomalies in PPS. Perhaps the most intriguing sequence variant in *IRF6* is the substitution of an isoleucine for an evolutionarily conserved valine residue at codon 274 in a protein-binding domain (Smad-Interferon Regulatory Factor binding domain, SMIR). This variant was found in people with VWS and also in 3% of Caucasians and 22% of Asians. Although usually considered polymorphisms that are not associated with an abnormal phenotype, occasionally these sequence variants lead to functional alterations, as seen both in people with laterality defects and in control individuals who have the Arg78Trp substitution in *CFC1* (ref. 9). Thus, the Val274Ile substitution in IRF6 may contribute to disease susceptibility either as an allelic modifier or in other, still unknown, ways. Specifically, this sequence variant in IRF6 may well have larger implications as a modifying allele in the etiology of non-syndromic CL/P, a hypothesis that is supported by transmission/disequilibrium test analysis of families with non-syndromic orofacial clefts and linked markers from the VWS region[10]. Progress in the next few years should help to identify the causes of non-syndromic CL/P or CPO. And if the young man with cleft lip who I saw as a newborn 22 years ago comes for genetic counseling, better answers should be at hand. □

1. Kondo, S. *et al. Nat. Genet.* **32** (2002); advance online publication, 3 September 2002 (doi:10.1038/ng985).
2. Fogh-Anderson, P. *Inheritance of Harelip and Cleft Palate* (Munksgaard, Copenhagen, 1942).
3. Murray, J.C. *Clin. Genet.* **61**, 248–256 (2002).
4. Spritz, R.A. *Curr. Opin. Pediatr.* **13**, 556–560 (2001).
5. Gorlin, R.J. *et al. Syndromes of the Head and Neck* (Oxford University Press, New York, 2001).
6. Van der Woude, A. *Am. J. Hum. Genet.* **6**, 244–256 (1954).
7. Schutte, B.C. *et al. Genome Res.* **110**, 81–94 (2000).
8. Machin, G.A. *Am. J. Med. Genet.* **61**, 216–228 (1996).
9. Bamford, R. *et al. Nature Genet.* **26**, 365–369 (2000).
10. Houdayer, C. *et al. Am. J. Med. Genet.* **104**, 86–92 (2001).