DiGeorge syndrome: an enigma in mice and men

One out of 4000 children are born with cardiac and facial abnormalities and are missing part of chromosome 22 (22q11.2). This condition, often called DiGeorge syndrome (DGS), is the most common human deletion syndrome and the second most common genetic cause of congenital heart defects[1]. Thymic and parathyroid anomalies are often present, together with characteristic heart and facial defects. Because of the diverse clinical features, it is possible that more than one gene on 22q11.2 contributes to the observed phenotype. However, many of the embryonic regions affected in 22q11 deletion are derived from a common migratory population of cells that arise from the neural folds (neural crest cells), suggesting that disruption of a molecular pathway regulating neural crest development might account for many aspects of 22q11 deletion syndrome.

Substantial resources have been devoted to identifying the genes involved in this syndrome. Genetic analyses of hundreds of patients suggest that the size of 22q11 deletion, typically 3 million base pairs, does not correlate with the type of defects observed. As a result, the human genetic focus has shifted to studying patients with characteristic phenotypic features but without a detectable 22q11 deletion. Surprisingly, extensive mutation analysis of such 'non-deleted' patients has failed to reveal even a single individual, worldwide, who harbors a point mutation in any of the genes (which number close to 30) in the commonly deleted region. This finding might suggest that the vast majority of non-deleted patients have genetic or epigenetic causes of neural crest disruption that are unrelated to 22q11. Adding further complexity to the human genetic approach are the ten reported patients that harbor large, atypical deletions of 22q11 that do not overlap with one another[2].

Two recent reports have begun to take alternative approaches to identifying the genes involved in this enigmatic syndrome. Our group had been studying the function of a transcription factor, dHAND, required for survival of the types of neural crest-derived cells affected in 22q11 deletion syndrome[3–5]. In a screen for genes downregulated in *dHAND* mutant mice, we identified *UFD1L*, which had previously been mapped to 22q11 by Novelli's group. We subsequently demonstrated specific *UFD1L* mRNA expression in the neural-crest-derived structures most affected in 22q11 deletion syndrome, including the pharyngeal arches, cardiac outflow tract and aortic arch arteries (specifically, the fourth aortic arch artery). A small 20 kb deletion in a 'non-deleted' DiGeorge patient (JF) encompassing portions of *UFD1L* and a neighboring gene, *CDC45L*, provided further evidence that *UFD1L* and/or *CDC45L* might be involved in the etiology of 22q11 deletion syndrome[6]. As Novelli points out, this is not evidence that loss of one copy of *UFD1L* alone is sufficient to cause the phenotype; however, the results would suggest that a molecular pathway involving *UFD1L* contributes to the 22q11 phenotype.

The recently published contribution by Baldini's group[7] has further clarified the potential etiologies of 22q11 deletion syndrome. By generating a mouse mutant lacking 14 of the genes in the presumed 'DiGeorge critical region' (DGCR), they were able to recapitulate defects of the cardiac outflow tract and fourth aortic arch artery observed in the syndrome. Unfortunately, no other phenotypic features were found, and even the arch defects were incompletely penetrant (25%). It remains to be determined whether genes outside this region are important for other features of the syndrome, or if species-specific factors influence the discordant phenotypes observed in mice and men. Their deletion (*Df1*) encompassed *Ufd1l* and *Cdc45l* but also removed 12 other genes. Interestingly, heterozygosity of *Ufd1l* alone was not sufficient to cause aortic arch defects in mice, although loss of both alleles of *Ufd1l* resulted in embryonic lethality. As Baldini suggests, further studies will be required to clarify the role of *UFD1L* in the 22q11.2 deletion phenotype. Whether heterozygosity of *Ufd1l* and *Cdc45*, as suggested by patient JF, is sufficient to cause aortic arch defects in mice is currently being tested in our laboratory.

Ultimately, the most important information will come from understanding the function of the various genes in the commonly deleted region. *HIRA*, a gene located 50 kb away from *UFD1L*, encodes a protein that interacts with the homeodomain protein Pax3, and might play a functional role in neural crest development in chick embryos[8,9]. The yeast homolog of HIRA (Tup1) plays a role in cell cycle-dependent histone regulation. Although neither Baldini's nor our studies have addressed HIRA's role in 22q11 deletion, it remains conceivable that functional disruption of HIRA contributes to the phenotype. The precise role of Ufd1l and Cdc45l in vertebrates remains unknown as well; however, in yeast they are involved in a ubiquitin-dependent degradation pathway and cell cycle regulation, respectively. Numerous studies suggest convergence of degradation and cell cycle pathways, raising the possibility that Ufd1l, Cdc45l and perhaps HIRA function in a common pathway during development. Whether heterozygosity of the three genes in combination disrupts neural crest development will soon be testable in genetically engineered mice.

As the molecular basis for the 22q11 deletion syndrome becomes clearer, how will our findings impact individuals and families who have endured the hardships associated with chronic and often life-threatening disease? Unfortunately, fetal gene therapy approaches remain in the distant future. However, the wide variance of clinical features in individuals with 22q11 deletion might indicate that secondary genetic or environmental factors influence the final phenotype. Thus, regulation of secondary factors during fetal development in individuals genetically predisposed to abnormal neural crest development might allow attenuation of the disease. It is our hope that continued efforts to understand molecular pathways in mouse and chick neural crest development will ultimately lead to novel approaches for prevention of cardiac and craniofacial birth defects. Until then, scientists, clinicians and patients will continue to experience the roller coaster of hope and despair that have become a hallmark of the 22q11 deletion syndrome.

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UFD1L is not the monogenic basis for heart defects associated with the CATCH phenotype*

It is clearly possible that some aspects of the important and highly variable CATCH phenotype* are attributable to loss of a single allele. However, we and others have devoted much effort to searching for mutations in candidate genes in individuals with features suggestive of the CATCH phenotype, without success until the exciting report by Yamagishi *et al.*[1] of a 20 kb deletion involving the first three exons of *UFD1L*. The fact that this gene shows appropriate expression and was absent in a large series of cases was important supportive evidence but not proof of causality. Novelli *et al.*[2] clearly demonstrate the difficulty of the single gene hypothesis with their careful review of the reported deletions that do not leave any segment of the commonly deleted region capable of accounting for all cases. Our own interpretation was, therefore, that while *UFD1L* was likely to be a major player, the case for a contiguous gene defect remained strong. The recent report by Lindsay *et al.*[3] has further reinforced the rejection of a defect in *UFD1L* as 'the cause'; using the Cre–Lox system, they have generated mice that are deleted for a 1.2 Mb section of the murine region homologous to the human deleted region, from *Es2* to *Ufd1l*. Mice heterozygous for the Lox site inserts, in which the only genetic defect is disruption of *Es2* and *Ufd1l*, do not develop heart defects, but hemizygous cases manifest the classic heart malformations associated with DiGeorge syndrome, in particular, hypoplasia or apparent absence of the fourth arch artery. Mice that have the deleted segment on one chromosome and the reciprocal duplication on the other are rescued, despite the fact that *Ufd1l* is not restored to normal. This would appear to clinch the case.

Why then did the child reported by Yamagishi *et al.*[1] (JF) with only 20 kb missing have the classic CATCH phenotype, including interrupted aortic arch, truncus arteriosus, cleft palate, small mouth, low-set ears, broad nasal bridge, neonatal hypocalcaemia and T cell deficit? The deletion involved more than one gene; the human homologue of the yeast cell cycle protein CDC45 was also disrupted, but this ubiquitous gene product is unlikely to play a specific role in neural crest migration. It is frustrating that the only evidence presented for the deletion in this child was a *Hin*D-III digest of genomic DNA, which displayed apparent hemizygosity for a 12.3 kb fragment, the largest fragment on the gel. Restriction patterns with other restriction endonucleases were not presented and no novel fragment was identified. Given that genomic sequence is available for the entire region, it should now be relatively straightforward to generate a fragment spanning the deleted region in order to clarify the breakpoints. We are not aware of these results being published. The one certainty is that this story is not yet complete.

*We have recently revised the acronym CATCH22 to the CATCH phenotype to reflect unease at the 'no win' implication of the former label[4,5]. Retaining an all-encompassing acronym that stands for **C**ardiac **A**bnormality, **T**-cell deficits, **C**lefting and **H**ypocalcaemia can be defended because of the wide range of eponymous syndromes described in affected individuals. Some reflect the differing professional emphasis of those who provided the original reports; DiGeorge syndrome concentrates on the thymic and parathyroid hypoplasia while the Velocardiofacial syndrome reported by Shprintzen *et al.* took the craniofacial perspective. Takao *et al.* saw the phenotype from the cardiological angle with their Contruncal Anomaly Face syndrome while the lesser-known Strong syndrome was the first to emphasize the variable dominant disorder producing outflow tract, facial and psychiatric disturbance in different family members. Also included in the acronym are the less common and distinct phenotypes of Opitz G/BBB and Cayler asymmetric crying face syndrome, neither of which is exclusive to 22q11 deletion.

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