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Reply
role of the dHAND–UFD1L pathway

Novelli *et al.* discuss the relevance of our recent findings regarding UFD1L in DiGeorge syndrome in the context of other work performed in the field[1]. We believe that the one or more genes involved in the 22q11.2 deletion phenotype should meet the following criteria: (1) be expressed during embryogenesis specifically in tissues affected in 22q11.2 deletion; (2) play an important role in development of cranial and cardiac neural crest cells during embryogenesis; (3) be deleted in individuals with 22q11.2 deletion; (4) one or more individuals with the DiGeorge phenotype but without a large deletion should have a smaller deletion or point mutation within *UFD1L*. Here we summarize our current perspective of the role of dHAND, UFD1L and CDC45L in the 22q11.2 deletion syndrome with the above criteria in mind.

Unlike traditional genetic approaches, our recent work utilized mouse and human studies to implicate UFD1L in the 22q11.2 deletion syndrome. The basic helix–loop–helix transcription factor dHAND (Ref. 2) provided an entry into understanding the molecular pathways regulating the region-specific development of the heart[3] and allowed the systematic identification of genes involved in developmental fields during cardiogenesis. Mouse knockout studies had suggested that a dHAND-dependent pathway might be involved in the 22q11.2 deletion phenotype. Specifically, *endothelin-1*-null mice had a phenotype similar to 22q11.2 deletion[4] and exhibited downregulation of dHAND (Ref. 5). In *dHAND*-null embryos, the neural crest defects were more severe, resulting in embryonic death[3,5]. Utilizing the *dHAND*-null model to identify dHAND-dependent genes by subtraction cloning, we found that the mouse homolog (*Ufd1*) of a yeast gene encoding ubiquitin fusion degradation gene protein 1 (Ref. 6) was regulated by dHAND (Ref. 7). The human homolog (*UFD1L*) mapped to the commonly deleted 1.5–2.0 Mb region known as the DiGeorge critical region (DGCR)[8] but was just outside a proposed 250-kb 'minimal critical region' (MDGCR)[9]. Sequencing and targeted mutations of genes in the MDGCR have not identified any strong candidate genes, suggesting that the critical gene(s) lie outside the 'MDGCR' (Ref. 10). FISH analysis of individuals with 22q11.2 deletion confirmed that haploinsufficiency of *UFD1L* was present in 182 out of 182 patients tested with DiGeorge 22q11.2 deletion syndrome[7], although there are a few rare atypical deletions that would not include UFD1L (Ref. 10). Finally, expression analysis of Ufd1 during mouse embryogenesis revealed that Ufd1 was not ubiquitous but, rather, was expressed specifically in tissues affected in 22q11.2 deletion[7].

Our screening for gene rearrangements and point mutations of *UFD1L* detected a DiGeorge-like patient with a 20-kb deletion encompassing exons 1–3 of *UFD1L* (Ref. 7). However, the *5′* region of *CDC45L* (exons 1–5)[11], which lies immediately telomeric to *UFD1L* (Ref. 12), was also deleted in this patient[7]. Thus, it remains possible that deletion of *CDC45L* also contributes to the DiGeorge phenotype in this patient. Screening for point mutations of *UFD1L* in other patients has failed to detect any mutations as yet (V. Garg, H. Yamagishi and D. Srivastava, unpublished). However, it is clear that deletions on other chromosomes can result in a similar phenotype[13]. In addition, mouse studies suggest that numerous genes are involved in neural crest development and, when mutated, cause DiGeorge-like defects[14]. Finally, vitamin A embryopathy and other environmental factors can also produce neural crest defects[15]. Given the numerous potential causes of cranial neural crest maldevelopment, negative data regarding *UFD1L* mutations in non-deleted patients is not particularly surprising, especially with small sample numbers.

Because mutation analyses in patients might not be informative, functional analyses are required for further confirmation of a role for UFD1L in neural crest development. Ufd1p functions in a post-ubiquitination step in yeast[6], although its precise role is unknown. Identification of the proteins normally degraded by the UFD pathway will be essential to understanding its role during development. We propose a functional model (Fig. 1) in which downregulation of UFD1L activity results in accumulation of certain proteins and excessive apoptosis or maldevelopment of neural crest cells, as observed in *dHAND*-null branchial arches[5] and aortic arch arteries (H. Yamagishi and D. Srivastava, unpublished). A genetic model has been proposed that assumes a functional architecture of 22q11.2 that is disrupted even in disparate deletions of 22q11 (Refs 1, 10). This model might explain the handful of rare patients who have deletions of 22q11.2 that do not involve *UFD1L*. Even in this genetic model, one or more genes involved in neural-crest development must be functionally affected. In light of current developmental and

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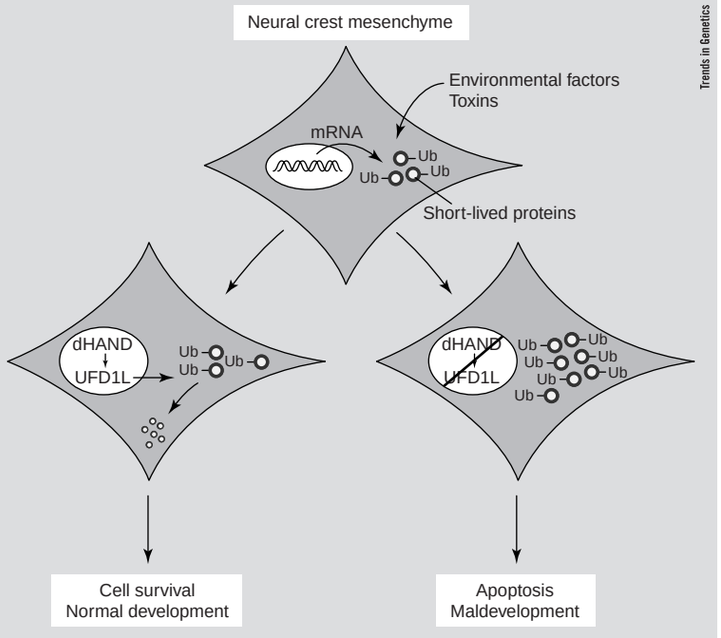
FIGURE 1. Model for dHAND–UFD1L-dependent neural-crest development



A combination of environmental factors and genetic pathways can result in the presence of proteins whose half-life is regulated tightly by ubiquitin-dependent proteolytic pathways. Environmental factors, such as vitamin A, can activate gene transcription directly or can accumulate in the cytosol. dHAND normally activates UFD1L in neural-crest-derived cells, resulting in the appropriate degradation of a subset of ubiquitinated proteins. In the absence of sufficient UFD1L activity, some proteins accumulate and can cause maldevelopment or apoptosis of neural-crest-derived cells. Abnormal development of neural-crest-derived cells would result in the neural-crest defects observed in 22q11.2 deletion syndrome. Abbreviation: Ub, ubiquitin.

genetic data, disruption of UFD1L function alone, or in combination with CDC45L and/or HIRA (Ref. 16), is the most likely etiology for most defects observed in DiGeorge syndrome. Further testing of the above models might solve the long mystery of DiGeorge 22q11.2 deletion syndrome and lead to better genetic counseling and new intervention for patients.

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Inferring the direction of evolutionary changes of genomic base composition

Identifying precisely the evolutionary mechanisms differentiating the substantial guanine and cytosine (GC) content between different organisms remains elusive. For instance, the overall GC content of *Mycobacterium tuberculosis*[1] and *Mycobacterium leprae* genomes are 65% and 57% (Ref. 2), respectively. When 146 orthologous genes are considered, the GC at the 3rd base codon position (GC3) for each species is 79% and 66%, respectively. This corresponds to approximately 20% [= (79%–66%)÷66%] difference of GC content, which appears drastic particularly when we take into account the fact that these species share about 80% amino acid identity for these genes. Thus, it is of extreme interest to see how such difference of GC content took place even for these relatively closely related species. In particular, we want to know if the difference in GC3 between them was caused by: (1) a sharp increase or decrease in GC3 content in either *M. tuberculosis* or *M. leprae* after divergence from their common ancestor, or (2) a moderate increase in one and a moderate decrease in the other. In other words, we want to know if it is possible to infer the GC content of the common ancestor for this pairwise species comparison and thus to identify