Letters to the Editor

Am. J. Hum. Genet. 65:247, 1999

Mutations of *UFD1L* Are Not Responsible for the Majority of Cases of DiGeorge Syndrome/Velocardiofacial Syndrome without Deletions within Chromosome 22q11

To the Editor:

Deletions of chromosome 22q11 are associated with a wide spectrum of congenital malformation, encompassed by the acronym "CATCH22" (cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcemia on chromosome 22), including velocardiofacial syndrome (VCFS; MIM 192430), DiGeorge syndrome (DGS; MIM 188400), and conotruncal-anomaly face (Emanuel et al. 1998). The major anomalies include outflow-tract congenital heart defects, hypoplasia of the parathyroids and thymus, craniofacial dysmorphism, and learning/behavioral problems (Ryan et al. 1997). Many of these are thought to be due to a defective neural-crest contribution during development. The DiGeorge chromosomal region (DGCR) is entirely cloned (Carlson et al. 1997) and sequenced, and several genes have been reported mapping to the region. Mutation screens of genes mapping to the proximal end of this region, termed the "minimal DiGeorge chromosomal region" (MDGCR; Gong et al. 1996), have been negative (Wadey et al. 1995; Gong et al. 1997; Gottlieb et al. 1997; Lindsay et al. 1998). Attention therefore has turned to the regions adjacent and distal to the MDGCR. Recently, the gene *UFD1L* was proposed as the major gene haploinsufficient in this group of syndromes (Yamagishi et al. 1999). *UFD1L* is downstream of *dHAND*, a gene known to be involved in control of the development of structures affected in DGS, and *Ufd1l* is expressed in the branchial arches, frontonasal mass, and outflow tract. In addition, a single patient has been reported with a de novo deletion affecting *UFD1L* and the neighboring gene, *CDC45L2* (Yamagishi et al. 1999). *CDC45* is required for initiation of DNA replication in yeast, and *CDC45* mutants are nonviable. However, *CDC45L2* expression is not altered in *d-HAND* −/− embryos. On the basis of these findings, Yamagishi and colleagues concluded that *UFD1L* haploinsufficiency (perhaps with some contribution from *CDC45L2*) causes DGS.

We conducted mutation screens, in both *UFD1L* and *CDC45L2*, as a three-center collaboration. *UFD1L* was screened by direct sequencing of 12 patients in London, by direct sequencing of all exons and 900 bp of the 5′ UTR in 20 patients in Rome, and by DGGE of 7 patients' DNA in Rotterdam. Local ethical review and consenting procedures were followed. The majority of patients were chosen on the basis of the presence of two or more features of the 22q11 deletion syndromes, but with no detectable deletion of 22q11 or of the DGSII region of 10p13 (Daw et al. 1996). The Rome series contained six patients with an isolated (i.e., nonsyndromic) interrupted aortic arch, a congenital heart defect commonly associated with the deletion. These patients were included because point mutations may be associated with a narrower spectrum of malformation than deletion and—since *UFD1L* was specifically identified as a *d-HAND* target—because congenital heart defects might be especially significant. The previously described patient with a balanced 2;22 translocation in association with DGS (patient ADU; Augusseau et al. 1986) was also screened. *UFD1L* primers and conditions are available from the collaborating centers, and the genomic organization of *UFD1L* and the resources for exon PCR amplification have been described elsewhere by Novelli et al. (1998). In London, 24 patients were similarly screened for *CDC45L2* mutations; primers and PCR conditions are available on request, and genomic organization has been published previously (McKie et al. 1998). No mutations of either gene were detected. We did, however, detect a number of sequence variants. Within the 5′UTR of *UFD1L* we found a single polymorphic sequence, initially detected by SSCP and subsequently shown to involve an A→G transition, located at the −277 position (with respect to the first base of the initiation codon). Screening of 25 unrelated controls generated a heterozygosity value of .40. Within *CDC45L2* we detected an A→G transition 22 bp upstream of exon 17 (at intron 16, with heterozygosity of .3) and a G→T transversion 24 bp into intron 18 (heterozygosity of .5). In addition, Southern analysis of 42 patients was conducted, with four different restriction-enzyme digests (*Hin*dIII, *Eco*RI, *Kpn*I, and *Bam*HI), in

an attempt to ascertain rearrangements similar to the *UFD1L/CDC45L2* deletion reported elsewhere. This analysis included all of the London patients screened for point mutations, as well as an additional 18 patients. No rearrangements or deletions were detected, although four RFLPs were observed. Finally, mice with hemizygous targeted mutations of *Ufd1l* were normal (A. Baldini, personal communication).

Where does this leave the molecular genetics of the 22q11 deletion syndromes? Interpretation of current data must consider that, although ⩾10% of deletions are inherited (Ryan et al. 1997), there is no good evidence for inheritance of DGS/VCFS in nondeletion cases. Furthermore, there are a large number of potential phenocopies of the condition (Emanuel et al. 1998). It is therefore possible that only a fraction of nondeleted cases have an etiology related to chromosome 22q11. Therefore, *UFD1L* must still be regarded as a good candidate for contributing to this complex multiple-malformation syndrome. However, it should be kept in mind that a number of genes might be acting to produce a combined haploinsufficiency, especially since other genes within the DGCR are also expressed in affected tissues. In the case of HIRA, for instance, the protein is known to interact with *PAX3,* a gene required for conotruncal septation in the mouse (Magnaghi et al. 1998), and antisense attenuation of HIRA expression in chicks yields an increased incidence of persistent truncus arteriosus (Farrell et al. 1999). However, as with *UFD1L,* mutations within *HIRA* have not been detected. Another consideration is the presence of distinct (i.e., nonoverlapping) rearrangements of 22q11, associated with very similar DGS-like phenotypes (Dallapiccola et al. 1996; Kurahashi et al. 1996; Sutherland et al. 1996; Rauch et al. 1999). Perhaps haploinsufficiency of more than one gene can cause the syndrome, or long-range effects induced by the rearrangements can down-regulate the expression of the relevant gene(s). The role of combinations of genes during development is being tested by chromosome engineering in the mouse (Lindsay and Baldini 1998), although it is conceivable that long-range effects will confuse analysis in the murine system. In agreement with other commentators (Baldini 1999; Hagmann 1999), we think it is too early to call "Closing Time" (Heller 1996) on "CATCH22" (Heller 1955).

Acknowledgments

We would like to thank the families and clinicians who made the study possible. Support was from the Birth Defects Foundation and the British Heart Foundation (to P.J.S.), Telethon Foundation grant E. 723 (to B.D. and G.N.), and the Dutch Heart Foundation and the Sophia Foundation for Medical Research (to C.M.). We would like to thank Drs. Antonio Baldini and Elizabeth Lindsay for patient referrals, helpful discussion, and providing critical data prior to publication. Access to PCR conditions can be obtained at the e-mail addresses that follow: meijers@ch1.fgg.eur.nl (for C.M.), rwadey@hgmp.mrc.ac.uk (for R.W.), and novelli@utovrm.it (for G.N.).

Roy Wadey,[1] Judith McKie,[1]
Charalambos Papapetrou,[1] Helen Sutherland,[1]
Frans Lohman,[2] Jan Osinga,[4] Ingrid Frohn,[3]
Robert Hofstra,[4] Carel Meijers,[2]
Francesca Amati,[5] Emanuela Conti,[5]
Antonio Pizzuti,[5] Bruno Dallapiccola,[5]
Giuseppe Novelli,[5] and Peter Scambler[1]

[1]*Molecular Medicine Unit, Institute of Child Health, London;* [2]*Department of Cell Biology and Genetics/Pediatric Surgery, Erasmus University, and* [3]*Department of Pediatrics, Division of Cardiology, Sophia Children's Hospital, Rotterdam, and* [4]*Department of Medical Genetics, State University of Groningen, Groningen, the Netherlands; and* [5]*Department of Biopathology and Diagnostic Imaging, Tor Vergata University and CSS-Mendel Institute, Rome*

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for VCFS [MIM 192430] and DGS [MIM 188400])

References

Augusseau S, Jouk S, Jalbert P, Priur M (1986) DiGeorge syndrome and 22q11 rearrangements. Hum Genet 74:206–206

Baldini A (1999) DiGeorge syndrome: is the solution in HAND? Nat Genet 21:246–247

Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R, Patanjali S, et al (1997) Molecular definition of 22q11 deletions in 151 VCFS patients. Am J Hum Genet 61:620–629

Dallapiccola B, Pizzuti A, Novelli G (1996) How many breaks do we need to CATCH on 22q11? Am J Hum Genet 59: 7–11

Daw SCM, Taylor C, Kraman M, Call K, Mao J, Meitinger T, Lipson A, et al (1996) A common region of 10p deleted in DiGeorge and velo-cardio-facial syndrome. Nat Genet 13: 458–460

Emanuel BS, Budarf BS, Scambler PJ (1998) The genetic basis of conotruncal heart defects: the chromosome 22q11.2 deletion. In: Rosenthal N, Harvey R (eds) Heart development, pp. 463–478. Academic Press, San Diego

Farrell M, Stadt H, Wallis K, Scambler PJ, Hixon RL, Wolfe R, Leatherbury L, et al (1999) Persistent truncus arteriosus is associated with decreased expression of HIRA by cardiac neural crest cells in chick embryos. Circ Res 84:127–135

Gong W, Emanuel BS, Collins J, Kim DH, Wang Z, Chen F,

Zhang G, et al (1996) A transcription map of the DiGeorge and velo-cardio-facial syndrome critical region on 22q11. Hum Mol Genet 5:789–800

Gong W, Emanuel BS, Galili N, Kim DH, Roe B, Driscoll DA, Budarf ML (1997) Structural and mutational analysis of a conserved gene (DGSI) from the minimal DiGeorge syndrome critical region. Hum Mol Genet 6:267–276

Gottlieb S, Emanuel BS, Driscoll DA, Sellinger B, Wang Z, Roe B, Budarf ML (1997) The DiGeorge syndrome minimal critical region contains a *Goosecoid*-like (*GSCL*) homeobox gene, which is expressed early in human development. Am J Hum Genet 60:1194–1201

Hagmann M (1999) A gene that scrambles your heart. Science 283:1091–1093

Heller JL (1955) Catch 22. Simon and Schuster, New York

——— (1996) Closing time. Simon and Schuster, New York

Kurahashi H, Nakayama T, Osugi Y, Tsuda E, Masuno M, Imaizumi K, Kamiya T, et al (1996) Deletion mapping of 22q11 in catch22 syndrome: identification of a second critical region. Am J Hum Genet 58:1377–1881

Lindsay EA, Baldini A (1998) Congenital heart defects and 22q11 deletions: which genes count? Mol Med Today 4: 350–357

Lindsay EA, Harvey EL, Scambler PJ, Baldini A (1998) *ES2,* a gene deleted in DiGeorge syndrome, encodes a nuclear protein and is expressed during early mouse development, where it shares an expression domain with a *Goosecoid*-like gene. Hum Mol Genet 7:629–635

Magnaghi P, Roberts C, Lorain S, Lipinski M, Scambler PJ (1998) HIRA, a mammalian homologue of *Saccharomyces cerevisiae* transcriptional co-repressors, interacts with pax3. Nat Genet 20:74–77

McKie JM, Wadey R, Sutherland H, Taylor K, Scambler PJ (1998) Direct selection of conserved cDNAs from the DiGeorge critical region: isolation of a novel CDC45-like gene. Genome Res 8:834–841

Novelli G, Mari A, Amati F, Colosimo A, Sangiuolo F, Bengala M, Conti E, et al (1998) Structure and expression of the human ubiquitin fusion-degradation gene (UFD1L). Biochem Biophys Acta 1396:158–162

Rauch A, Pfieffer RA, Leipold G, Singer H, Tigges M, Hofbeck M (1999) A novel 22q11.2 microdeletion in DiGeorge syndrome. Am J Hum Genet 64:658–666

Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Siedel H, Schuffenhauer S, et al (1997) Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet 34:798–804

Sutherland HF, Wadey R, McKie JM, Taylor C, Atif U, Johnstone KA, Halford S, et al (1996) Identification of a novel transcript disrupted by a balanced translocation associated with DiGeorge syndrome. Am J Hum Genet 59:23–31

Wadey R, Daw S, Taylor C, Atif U, Kamath S, Halford S, O'Donnell H, et al (1995) Isolation of a gene encoding an integral membrane protein from the vicinity of a balanced translocation breakpoint associated with the DiGeorge syndrome. Hum Mol Genet 4:1027–1034

Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D (1999) A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. Science 283: 1158–1161

Address for correspondence and reprints: Prof. Peter Scambler, Room 211, Molecular Medicine Unit, Institute of Child Health, London, WC1N 1EH, United Kingdom. E-mail: pscamble@hgmp.mrc.ac.uk

© 1999 by The American Society of Human Genetics. All rights reserved.

0002-9297/99/6501-0031$02.00