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0002-9297/99/6502-0033$02.00

Am. J. Hum. Genet. 65:562–566, 1999

A 22q11.2 Deletion That Excludes *UFD1L* and *CDC45L* in a Patient with Conotruncal and Craniofacial Defects

To the Editor:

Microdeletions of chromosome 22q11.2 occur with a high frequency in the general population, with an estimated incidence of 1/3,000–1/4,000 (Burn and Goodship 1996). They have been shown to be associated with the malformation phenotypes of velocardiofacial syndrome (VCFS [MIM 192430]), DiGeorge syndrome (DGS [MIM 188400]), and conotruncal anomaly face syndrome (CAFS [MIM 217095]) (Emanuel et al. 1999*a*). Deletions of this region have also been demonstrated in some patients with the autosomal dominant form of Opitz G/BBB syndrome (MIM 145410) (McDonald-McGinn et al. 1995). Significant phenotypic overlap is found among these entities, including conotruncal cardiac defects, craniofacial anomalies, learning disabilities, and cleft palate. The spectrum of clinical findings shows considerable variability, even within families (McLean et al. 1993).

Although the overwhelming majority (>85%) of patients have deletions of the same ~3-Mb region (Emanuel et al. 1999*a*), several reports have described patients with atypical, shorter deleted segments nested within the large typically deleted region (TDR) (Levy et al. 1995; Kurahashi et al. 1996; O'Donnell et al. 1997; McQuade et al. 1999). Recently, a small, 20-kb deletion within the TDR was reported in a patient with a classic VCFS/DGS phenotype. This smaller deletion disrupts the *UFD1L* and *CDC45L* genes, the products of which (in particular, *UFD1L*) have been suggested to play important roles in craniofacial and cardiac development resulting in the phenotype (Shaikh et al. 1999; Yamagishi et al. 1999). However, several of the aforementioned patients (some of whom have cardiac and craniofacial defects) have deletions that do not include the region containing these genes. These observations suggest that additional sequences within the TDR affect early craniofacial and cardiac morphogenesis. Additionally, a patient with features of DGS and with a microdeletion that falls outside the TDR but that does not overlap with any of the known deletions was recently described (Rauch et al. 1999). This patient had craniofacial abnormalities and an interrupted aortic arch (type B) with truncus arteriosus, the same defect seen in the patient described by Yamagishi et al. (1999). The report by Rauch et al. further emphasizes the likelihood that the 22q11.2-related cardiac defects are unlikely to result from defects involving a single gene within the TDR.

We have identified a patient, CH98-18 (Emanuel et al. 1998), with a novel deletion of chromosome 22q11.2. His deletion is distal to the usual 3-Mb deletion found in most patients with VCFS and appears to overlap with a portion of the deleted region described by Rauch et al. (1999). The deletion does not overlap with any of the previously described "minimal critical regions" for VCFS/DGS. The patient was born to a 33-year-old mother at 35 wk gestation. The pregnancy was complicated by a weight gain of 70 lbs and premature rupture of membranes. The baby was delivered by cesarean section, because of breech presentation, with Apgar scores of 7 at 1 min and 8 at 5 min. Physical examination at birth was notable for an appropriate-for-gestational-age infant with hypertelorism, posteriorly rotated ears, micrognathia, a loud cardiac murmur, hypospadias, descended testes, single palmar creases, and 5th-finger clinodactyly bilaterally. Renal and cranial ultrasounds were normal. Echocardiography showed the presence of truncus arteriosus type II and a ventricular septal defect. Borderline hypocalcemia was also present. The patient had surgical repair of his truncus arteriosus at age 3 wk and a replacement graft at age 7 mo.

Motor development was normal. The patient sat at age 6 mo and walked at age 14 mo. However, he had expressive-speech delay, speaking his first words at age 21 mo. At age 26 mo, he had speech appropriate for an 18-month-old. During a recent physical examination at age 26 mo (fig. 1), short stature, microcephaly, a prominent glabella, partially attached earlobes, a broad nasal bridge, a broad nasal tip with a crease, hypoplastic nasal alae, anteverted nares, a featureless philtrum, a downturned mouth, a bifid uvula, and normal hearing and vision were noted. Endocrine evaluation including thyroid-function and growth-hormone panels was unremarkable. Immunologic studies including surface markers for T-cell, B-cell, and NK lineages, myeloid markers, leukocyte adhesion, and Wiskott-Aldrich–associated

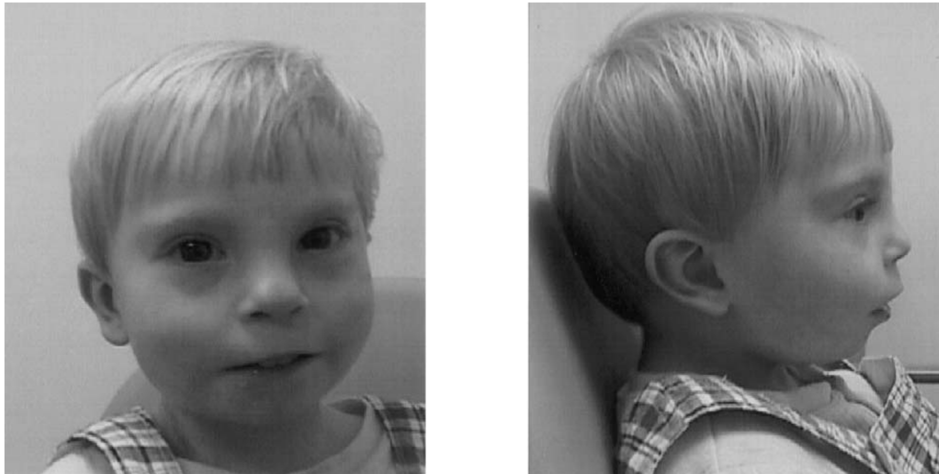

Figure 1 Photographs of patient CH98-18 at age 26 mo. Note hypertelorism, broad nose, and micrognathia.

proteins were all normal. Proliferative responses to mitogen-stimulation tests were also normal, as were functional-antibody responses.

GTG-banded chromosomes prepared directly from peripheral-blood lymphocytes showed a normal 46,XY karyotype, and FISH was negative for a deletion when the N25 (Oncor) probe was used (fig. 2*A*). In addition, CH98-18 did not have a deletion for a number of other cosmid-based FISH markers within the TDR, including D22S788, ZNF74, HCF2, and cHKAD26 (fig. 2*B*) (Emanuel et al. 1998). With the exception of cHKAD26, cosmids used for FISH were isolated by colony hybridization from the chromosome 22–specific cosmid library (LL22NCO3) generated at the Lawrence Livermore Laboratories. The cosmid that contains the cHKAD26 locus was provided by the Japanese Cancer Research Resources Bank. A cosmid, 83C5, containing the portions of the *UFD1L* and *CDC45L* genes deleted in the patient of Yamagishi et al. (Shaikh et al. 1999) was also used for FISH and was not deleted (data not shown). We determined that a cosmid located distally in the TDR (107D7) was not deleted (fig. 2*B*). Because the clinical findings in the patient, including truncus arteriosus, a bifid uvula, hypoplastic alar nasi, and a history of hypocalcemia, were consistent with those seen in VCFS, additional analysis was undertaken. Use of a series of cosmid and bacterial artificial-chromosome–derived probes for FISH demonstrated, initially with a cosmid for locus D22S801, that a deletion adjacent to the TDR was present (fig. 2*A*,*b* and *B*). Both of CH98-18's parents were analyzed by FISH with the cosmid for D22S801 and were found not to have a deletion, indicating a de novo origin of the deletion.

We and others have implicated blocks of duplicated DNA sequence containing BCRL and GGTL elements in the mechanism etiologic for the 22q11.2 deletions (Emanuel et al. 1998, 1999*b*; Edelmann et al. 1999). We predicted, on the basis of the presence of additional BCRL and GGTL duplicated sequences distal to the TDR, that CH98-18's deletion might involve one of these elements. Thus, the extent of his deletion was investigated on the basis of the map of the region immediately distal to the TDR. This region contains the immunoglobulin-λ light-chain locus (IGLL), within which are located BCRL4 and a copy of GGTL (Kawasaki et al. 1995). The IGLL locus has been completely characterized in a cosmid contig (Kawasaki et al. 1995) and has been sequenced (Kawasaki et al. 1997). Using the cosmid and sequence reagents, we moved distally from D22S801 (LN80) into the IGLL to detect the deletion end point (DEP). To insure that the germline genomic configuration of this region was being investigated, all studies were performed on cultured peripheral-blood lymphocytes. We determined that, although cosmid 61E11 is deleted, 102D1 is the first cosmid not deleted in CH98-18 (fig. 2*A* and *B*). In the sequenced contig, these cosmids are separated by ~40 kb that contain BCRL4 and GGTL. Using DNA sequence information for 102D1, we designed PCR-based 2.0-kb FISH probes and determined that both ends of this cosmid, which is immediately distal to the BCRL4/GGTL duplicated sequence in the IGLL locus, are present (GenBank). These probes are present, then, on both homologues. Results for one of these probes are shown in figure 2*A*. Thus, our patient's telomeric DEP involves a duplicated-sequence block that contains BCRL4 and GGTL sequences. The presence of a DEP in the vicinity of the BCRL4/GGTL duplication suggests unequal crossing-over in the formation of the patient's deletion. Additional cosmids distal to 102D1 were examined by FISH, and all were present on both

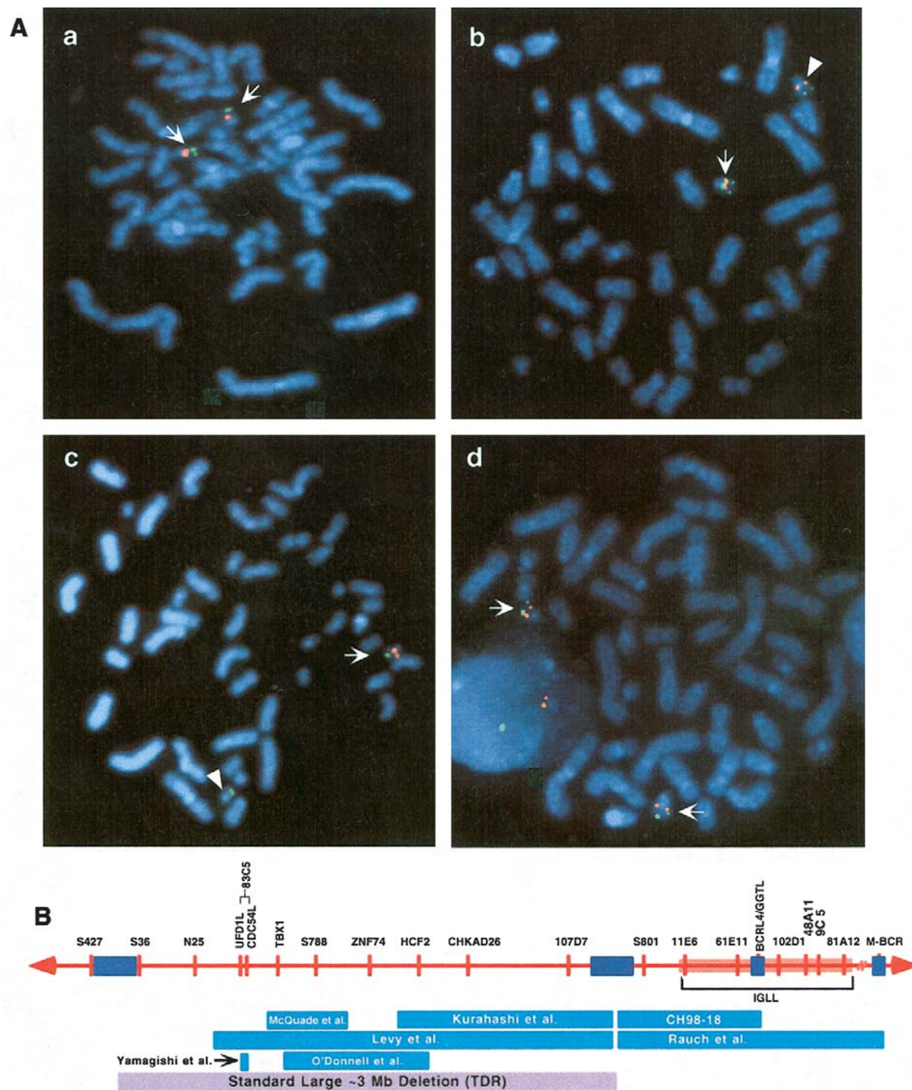

Figure 2 *A,* Dual-color FISH for DEP positioning in patient CH98-18. All metaphase spreads are hybridized with a control cosmid containing the distal marker D22S39. D22S39 was biotinylated and detected with avidin-FITC to identify chromosome 22 (seen at the telomeric end of chromosome 22, in all panels [*green*]). Most test cosmids were labeled and detected with digoxigenin-rhodamine–conjugated probes (*red*), except D22S801, which was biotinylated and then detected with avidin-FITC (*green*). In *a,* N25 (Oncor) is shown (*red*) and is present on both homologues (*arrows*). In *b,* cohybridization with two test cosmids, D22S36 (*red*) and D22S801 (*green*), shows them as overlapping signals on the normal chromosome 22 (*arrow*), with the control signal (D22S39) (*green*). Absence of the FITC test signal on the other chromosome 22 (*arrowhead*) indicates a deletion of D22S801. In *c,* cosmid 11E6 (*red*) is not detected on one of the chromosomes 22 (*arrowhead*) but is present on the normal homologue (*arrow*). In *d,* a PCR-derived probe from the distal end of cosmid 102D1 (*red*) is present on both chromosome 22 homologues (*arrows*). The 102D1 probe was generated by PCR using primers designed from sequence (for nucleotides 34424–36498) available in GenBank (accession number D86994). The PCR product was cloned by ligation into a TA cloning vector (Invitrogen), and the resultant plasmid was labeled by nick translation. *B,* Composite breakpoint map of 22q11.2, depicting the relative locations of several reported atypical deletions (*turquoise bars*). The typical deletion is also shown (*lavender bar*). Within the map of the TDR, genes and markers that identify the cosmids used for FISH experiments are depicted. Addresses of cosmids from the IGLL map (Kawasaki et al. 1995) that were used for FISH experiments are also shown. Individual duplicated-sequence blocks are also shown (*dark blue*). The IGLL locus is indicated by the bracket below the map. The cosmids from within the IGLL locus that are used for FISH are indicated on the top line over the region shaded in light red.

homologues (data not shown). These cosmids included 48A11, 9C5, 75C12, and 81A12 from the previously published IGLL contig (Kawasaki et al. 1995), as well as the commercially available M-BCR probe (Oncor). The region distal to 102D1, extending to the M-BCR probe, was deleted in the family described by Rauch et al. (1999).

Thus, our patient has a novel deletion of chromosome 22q11.2 and features of VCFS, including hypoplastic alar nasi, a bifid uvula, and truncus arteriosus, a cardiac lesion characteristic of the deletion. In a previous study, ~35% of patients with persistent truncus arteriosus were demonstrated to have standard deletions of 22q11.2 (Goldmuntz et al. 1998). To determine whether CH98-18's distal deletion might be related to his particular heart defect, we subsequently analyzed 15 other patients with truncus arteriosus who did not have a deletion when the N25 probe was used. None of these patients had a deletion of D22S801 (B. S. Emanuel, unpublished data). Furthermore, patient CH98-18 has hypertelorism, anteverted nares, a grooved nasal tip, and hypospadias, which, although reported in VCFS, are more commonly described in Opitz G/BBB syndrome (Robin et al. 1996). Opitz G/BBB is a heterogeneous disorder first described in 1969 (Opitz et al. 1969*a*, 1969*b*) and linked to the X chromosome (MIM 300000) and 22q11 (Robin et al. 1995). The X-linked form has subsequently been associated with mutations of the *MID1* gene located on Xp22 (Quaderi et al. 1997; Gaudenz et al. 1998). Patients with features of Opitz G/BBB and an autosomal dominant mode of inheritance have also been reported (Opitz 1987; Robin et al. 1995). Because of phenotypic overlap with the 22q11.2 deletion, several patients were assayed for and were found to have deletions of 22q11.2 (McDonald-McGinn et al. 1995). These patients all had the typical 3-Mb deletion commonly seen in VCFS/DGS. Analysis of several additional Opitz G/BBB cases without TDR deletions has failed to demonstrate a distal deletion similar to the one described in the present report (B. S. Emanuel, unpublished data).

Therefore, the deletion reported here is atypical of that seen with a VCFS/DGS phenotype, and it is also atypical of those deletions that have been seen with autosomal dominant Opitz syndrome. It is notable that CH98-18's deletion does not encompass the region containing the *UFD1L* and *CDC45L* genes. He does, however, like the patient described by Rauch et al., have a cardiac defect similar to that seen in the patient described by Yamagishi et al. These cases with deletions distal to the TDR argue against the hypothesis that *UFD1L* and *CDC45L* alone are sufficient to cause the cardiac defect and craniofacial features typically seen in VCFS/DGS. It is more likely that our patient's phenotype is the result of haploinsufficiency of other genes located in this distal deleted region, which may, perhaps, function with *UFD1L* and *CDC45L* in a common developmental pathway. Alternatively, our patient's deletion raises the possibility of either a "position effect" on genes within the TDR or a more complex mechanism etiologic for features of the disorder, because his deletion is distal to the TDR and appears not to include any of the genes described within the TDR.

Our patient's deletion is contained within the deletion described by Rauch et al. (1999); however, the phenotypes are not concordant. Both patient CH98-18 and the proband from the study by Rauch et al. have persistent truncus arteriosus. Nonetheless, there are no signs of Optiz G/BBB in any members of the family (including the proband) described by Rauch et al. However, since all of the subjects described in their report were female, only the laryngoesophageal anomalies and hypertelorism would be pertinent findings.

Thus, it becomes apparent, when one is studying such "atypical" patients, that delineating a minimal critical region as causal for the VCFS/DGS phenotype spectrum may be of limited applicability. Findings from this patient and the others discussed here imply that disruption of more than one gene most likely contributes to the phenotype. Further definition of the mechanisms that lead to these deletions and of the involvement of the duplicated-sequence blocks found in the region (Emanuel et al. 1998; Edelmann et al. 1999) would enable a greater understanding of (1) why these deletions and their subsequent phenotypes are so frequently encountered and (2) the role that sequences on 22q11.2 have in their etiology.

Acknowledgments

This work was supported by National Institutes of Health grants DC02027 and HL51533 (to B.S.E, H.M., and E.H.Z.) and HD 26979 (to B.S.E.) from the Mental Retardation and Developmental Disabilities Research Center. S.C.S. is supported by Medical Genetics Research Training grant MH1-T32-GM-08638.

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Electronic-Database Information

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

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for cosmid 102D1 sequence)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for DGS [MIM 188400], VCFS [192430], CAFS [217095], and Opitz syndrome [145410]).

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0002-9297/99/6502-0034$02.00