

BRIEF REPORT

Genetic Compensation in a Human Genomic Disorder

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SUMMARY

Cytogenetic studies of the parents of a girl with the DiGeorge (or velocardiofacial) syndrome, who carried a deletion at 22q11.2, revealed an unexpected rearrangement of both 22q11.2 regions in the unaffected father. He carried a 22q11.2 deletion on one copy of chromosome 22 and a reciprocal 22q11.2 duplication on the other copy of chromosome 22. Genetic compensation, which is consistent with the normal phenotype of the father, was shown through quantitative-expression analyses of genes located within the genetic region associated with the DiGeorge syndrome. This finding has implications for genetic counseling and represents a case of genetic compensation in a human genomic disorder.

DELETIONS AND DUPLICATIONS AT 22Q11.2 REPRESENT CANONICAL EXAMPLES of human genomic disorders, as defined by Lupski in 1998.¹ Most patients presenting with the 22q11.2 deletion syndrome (22q11DS), also designated the DiGeorge syndrome (Online Mendelian Inheritance in Man [OMIM] number, 188400) and the velocardiofacial syndrome (OMIM number, 192430), carry a hemizygous recurrent deletion of a sequence 3 Mb in length at 22q11.2. This deletion is thought to result from nonallelic homologous recombination, occurring during meiosis and mediated by low-copy repeats on chromosome 22.^{2,3} The deletion is usually sporadic, but inherited deletions have been reported in 6 to 28% of patients with the syndrome.⁴⁻⁹ Duplications of the same 22q11.2 region, which have also been reported, result in a phenotype that has some features in common with 22q11DS.¹⁰⁻¹³ We studied the healthy parents of a girl presenting with 22q11DS to determine whether the deletion was sporadic or inherited, for purposes of genetic counseling.

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METHODS

CASE REPORT

The diagnosis of 22q11DS was suspected at birth in a female child of healthy parents because of seizures due to severe and persistent hypocalcemia, lack of the thymus, and facial dysmorphism. No cardiac malformation was apparent. We obtained written informed consent from all family members studied.

MOLECULAR CYTOGENETIC ANALYSIS

Fluorescence in situ hybridization (FISH) assays were performed on cultured peripheral-blood lymphocytes obtained from the proband, her parents, and her grandfather, with the use of the following probes: N25 from Cytocell covering *DGCR14* (the DiGeorge syndrome critical region gene 14) and partially covering *DGCR2* (the DiGeorge syndrome critical region gene 2) and *CLCTL1* (*Homo sapiens* clathrin, heavy chain–like 1); TUPLE1 from Vysis, a probe partially covering *HIRA* (the HIR histone cell-cycle regulation defective homolog A [*Saccharomyces cerevisiae*]) from Vysis, and T-box 1 gene (*TBX1*) from Kreatech. The probes covered the 22q11.2 region and were used according to the manufacturer's instructions. FISH analyses of 900 interphase nuclei from buccal mucosa of the proband's father only were performed with the use of the *TBX1* probe, as previously described.¹⁴

GENOMIC DNA ANALYSIS

Genomic DNA was extracted from peripheral-blood specimens with the use of the FlexiGene DNA kit (Qiagen). A quantitative multiplex polymerase-chain-reaction (PCR) assay of short fluorescent fragments (QMPSF), adapted from Jacquet et al.,¹⁵ was used to assess the numbers of copies of seven genes in total. Five are located within the 3-Mb genetic region associated with the DiGeorge syndrome, called the DiGeorge syndrome critical region — *GSC1* (the gooseoid homeobox 2 gene), *TBX1*, *ARVCF* (the armadillo repeat gene deletes in velocardiofacial syndrome), *ZNF74* (the zinc-finger protein 74 gene), and *SNAP29* (the synaptosomal-associated protein, 29-kD gene) — and two genes are located on either side of the 3-Mb region — *USP18* (the ubiquitin-specific peptidase 18 gene) and *UBE2L3* (the gene for ubiquitin-conjugating enzyme E2L3). The microsatellite markers *D22S420*, *D22S941*, *D22S264*, *D22S303*, *D22S257*, and *D22S533* were studied with the use of dye-labeled primers, capillary electrophoresis on a genetic analyzer (ABI3100, Applied Biosystems), and GeneScan software, version 3.7 (PE Applied Biosystems).

GENE-EXPRESSION ANALYSIS

RNA was extracted from peripheral-blood lymphocytes with the use of Trizol reagent (Invitrogen) and was subjected to reverse transcription, with the use of avian myeloblastosis virus reverse transcriptase (AMV-RT, Finzyme). Expression of *DGCR8* (the DiGeorge syndrome critical region gene 8) and *DGCR6L* (the DiGeorge syndrome critical re-

gion gene 6–like gene), as well as expression of *USP18*, located at the proximal boundary outside the DiGeorge syndrome critical region, was assessed by means of a real-time quantitative PCR assay. The procedure used the genes encoding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the TATA box binding protein (*TBP*) as reference genes, QuantiTect Primer Assays (Qiagen), and a LightCycler 480 System (Roche).¹⁶

RESULTS

FISH with the use of probes covering the 22q11.2 region confirmed the 22q11.2 microdeletion in the proband (Fig. 1A and 1D). We carried out the same analyses in the parents, to determine whether the deletion was sporadic or inherited. Normal staining of *TBX1* was observed on metaphasic and interphasic chromosomal spreads of peripheral-blood cells from the mother (Fig. 1B and 1E). Unexpectedly, *TBX1* staining in cells from the father revealed the absence of signal on one chromosome 22 and an enlarged signal on its homologue in all observed metaphases (Fig. 1C). In 86% of interphasic nuclei from the father's cells, two distinct *TBX1* probes were clearly observed, always close together (Fig. 1F), in contrast with their random location in the mother's interphasic nuclei (Fig. 1E). Similar results were obtained with the N25 and TUPLE1 probes (data not shown).

These results, suggestive of a homogeneous 22q11.2 deletion on one chromosome 22 and a 22q11.2 duplication on the other chromosome 22 in the proband's father, led us to perform similar analyses in peripheral-blood cells from the proband's paternal grandfather. (Peripheral-blood cells from the paternal grandmother were not available for study.) The cells from the paternal grandfather displayed normal staining in the metaphases and in interphasic nuclei (data not shown). Both paternal grandparents were healthy. FISH analyses, using the *TBX1* probe, of 900 interphasic nuclei from the buccal mucosa of the father failed to detect any nucleus with even a single *TBX1* spot suggestive of an isolated 22q11.2 deletion. The observation in each nucleus of two *TBX1* spots close to one another suggested the presence, within the father's buccal mucosa cells, of a homogeneous 22q11.2 deletion–duplication rearrangement.

We used a QMPSF assay to determine the numbers of copies of various genes in the region of interest (Fig. 2A). The assay confirmed, in the in-

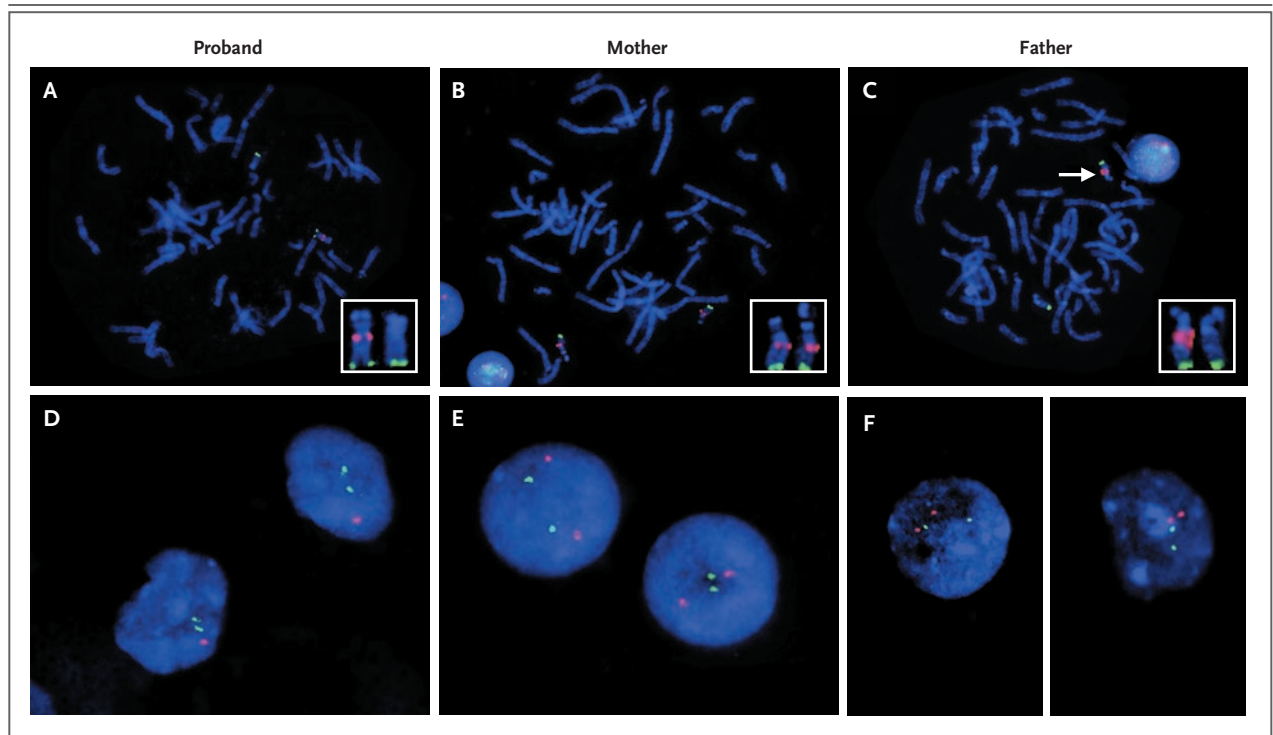


Figure 1. Results of Fluorescence In Situ Hybridization Analysis of Cultured Peripheral-Blood Lymphocytes from the Proband with the 22q11.2 Deletion, Her Mother, and Her Father.

The chromosomes shown for the proband, mother, and father are metaphasic in Panels A, B, and C, respectively, and interphasic in Panels D, E, and F, respectively. The red dots are T-box 1 gene (*TBX1*) probes, and the green dots are 22qter control probes. The insets in Panels A, B, and C provide close-up views of both copies of chromosome 22 in metaphase. The arrow in Panel C indicates the suspected *TBX1* duplicated signal in the father's lymphocyte. The percentages of interphasic nuclei containing one *TBX1* probe and two *TBX1* probes were 100% and 0% for the proband, respectively; 0% and 100% for the mother (with the normal genotype), respectively; and 14% and 86% for the father, carrying the 22q11.2 del-dup rearrangement. We had expected to find two *TBX1* probes in all cells from the father; however, since the two *TBX1* genes are close together as a result of the duplication, two *TBX1* probes could overlap one another and appear as a single spot.

dex case, the classic 3-Mb microdeletion between low-copy repeat A and low-copy repeat D on chromosome 22 and, in the father, the presence of two copies of each of the genes tested within the 22q11.2 region (Fig. 2B).

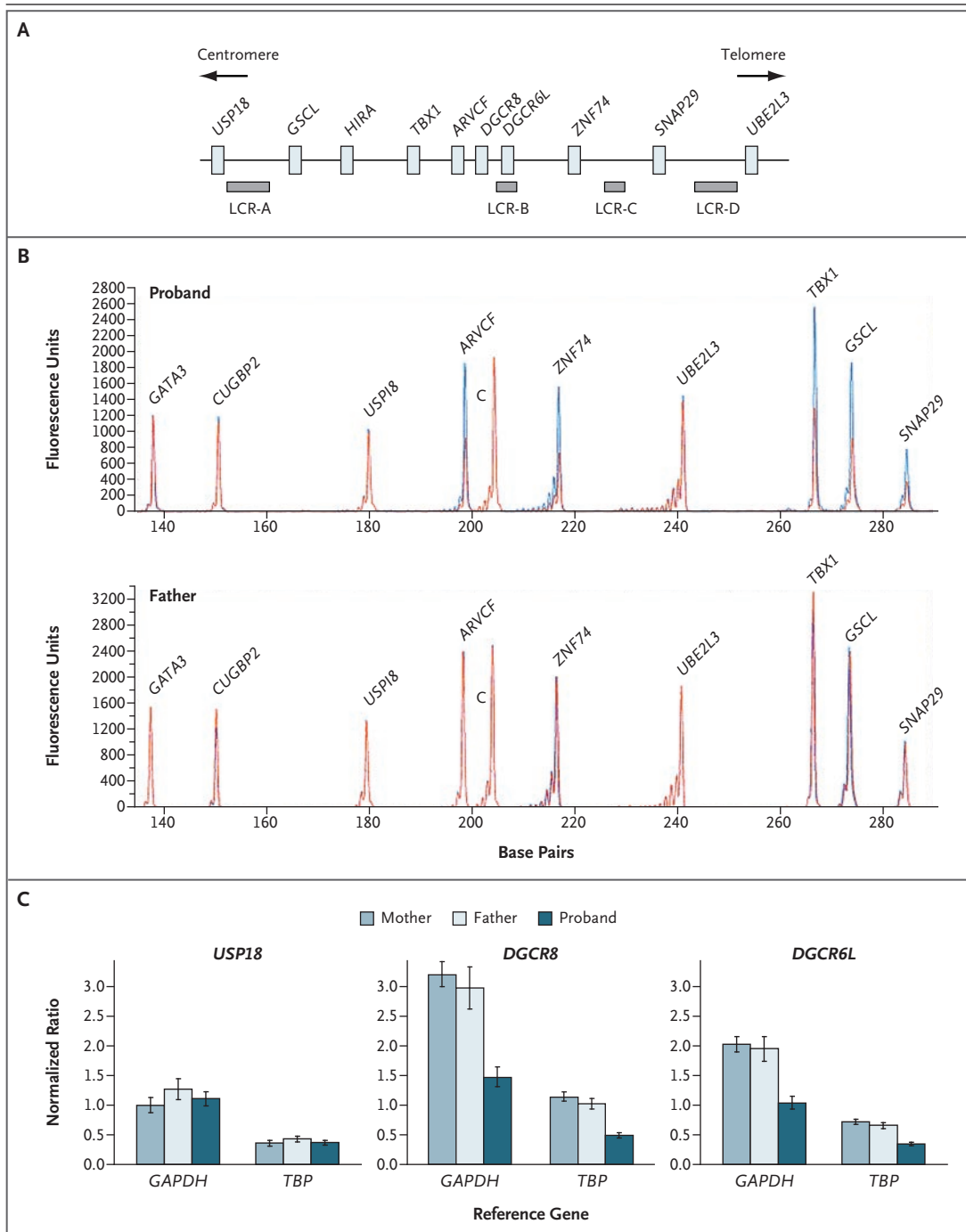
To determine whether the father's duplication compensated for the deletion, we measured the expression of genes localized within the genetic region associated with the DiGeorge syndrome, using a quantitative reverse-transcriptase PCR assay. Using the *GAPDH* and *TBP* reference genes, we found levels of expression of *DGCR8* and *DGCR6L* decreased in the affected girl, as compared with her mother, whereas her father had levels of expression of these genes similar to those in his wife (Fig. 2C).

To determine the origin of the rearrangements observed in the father, we analyzed the segregation of six microsatellite markers on chromo-

some 22 within the family (Fig. 3). The detection of alleles of different parental origin for the pericentromeric marker *D22S420* in the proband's father (Fig. 3) allowed us to rule out paternal uniparental disomy of chromosome 22. Maternal uniparental disomy of chromosome 22 in the proband's father can also be ruled out, on the basis of the microsatellite alleles observed for markers *D22S941* and *D22S264* in the proband's father, paternal aunt, and paternal grandfather.

DISCUSSION

The data for the father of a child with 22q11DS are consistent with a 22q11.2 deletion on one chromosome 22 and a 22q11.2 duplication on the other. As expected, the expression of genes located within the genetic region associated with the DiGeorge syndrome was reduced by half in the af-



ected proband. Conversely, expression levels of these genes in the father's lymphocytes were similar to those observed in the mother's lymphocytes, suggesting that the phenotypic effects of the 22q11.2 deletion in the father were balanced by the 22q11.2 duplication. This compensatory effect has already been shown in a mouse model of the human 22q11.2-deletion region: congenital heart

defects found in mice that are heterozygous for a deletion encompassing the murine region homologous to the human 22q11.2 DiGeorge syndrome critical region are corrected by cross-breeding with mice that are heterozygous for a *Tbx1* duplication.¹⁸ Beckmann et al.¹⁹ recently hypothesized that copy-number variation might explain reduced penetrance of some disease-causing mutations.

Figure 2 (facing page). Genes in the 22q11.2 Region — Locations, Quantification of Copy Number, and Expression.

Panel A shows the positions of the genes on 22q11.2 that were studied, as well as the low-copy repeat (LCR) segmental duplications A through D.¹⁷ The distances are not to scale. Panel B shows the 22q11.2 rearrangements in the proband and her father, as detected by means of a quantitative multiplex polymerase-chain-reaction (PCR) assay of short fluorescent fragments (QMPSF) specific to the 22q11.2–10p14 regions. In each plot, the electropherogram of the proband (top) or her father (bottom) (shown in red) is superimposed on that of a normal control (shown in blue) by adjusting the peak heights obtained for the control amplicon (“C”) for the proband or father and the control to the same level. The location of the 22q11.2 deletion — between LCR-A and LCR-D — was confirmed in the proband (top), as was the existence of two copies of each gene within the 22q11.2 region in the proband’s father (bottom). In Panel B, in addition to the targeted genes on 22q11.2 (defined in the text), the copy numbers of genes for GATA binding protein 3 (*GATA3*) and CUG triplet repeat, RNA binding protein 2 (*CUGBP2*), both located on 10p14, are shown. Panel C shows the results of gene-expression analysis of two genes localized in the DiGeorge syndrome critical region (*DGCR8* and *DGCR6L*) and of one gene at the proximal boundary (*USP18*). *GAPDH* and *TBP* were used as reference genes. The data are expressed as the ratio of the complementary DNA (cDNA) level for the target gene to the cDNA level for the reference gene, averaged for a quantitative reverse-transcriptase (RT) PCR assay run in duplicate. (The results of a second RT-PCR assay were similar.) Expression of *DGCR8* and *DGCR6L* genes is clearly decreased in the proband, whereas the father’s and mother’s expression patterns are similar. The I bars represent 1 SD.

These authors suggested that the phenotype of a dominant loss-of-function mutation could be rescued by the gain of a copy-number variation, resulting in increased gene expression. Our study validates this prediction and suggests that, considering the density of copy-number variations in the human genome, the presence of compensatory copy-number variations may explain why some loss-of-function mutations have low penetrance.

The simultaneous duplication and deletion in the proband’s father can be explained by nonallelic homologous recombination occurring either at a prezygotic or postzygotic stage. One hypothesized mechanism is nonallelic homologous recombination between low-copy repeat A and low-copy repeat D on chromosome 22, occurring during the first meiosis in one grandparental germ line, followed by meiotic nondisjunction and uni-

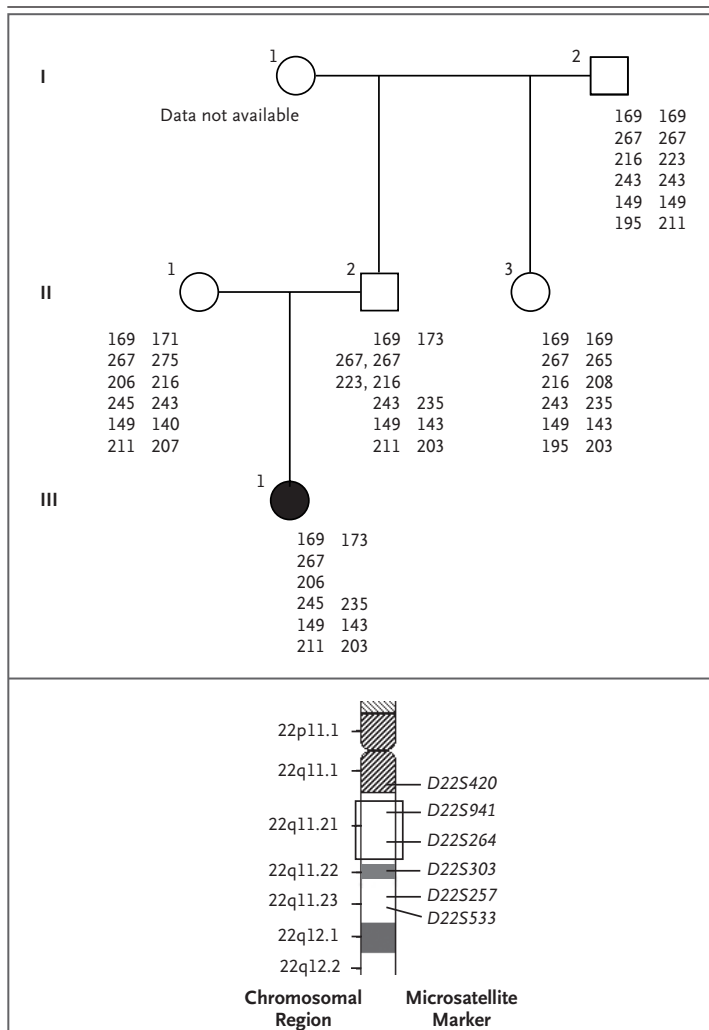


Figure 3. Pedigree of the Proband with the 22q11.2 Deletion and Selected Family Members, Based on Microsatellite Data.

Circles represent female family members, squares male family members, open symbols unaffected family members, and the solid circle the proband. The two columns of numbers next to each symbol indicate the alleles (expressed in base pairs) of each of the six microsatellite markers on chromosome 22, in the order in which the markers are diagrammed below the pedigree. The box delineated on chromosome 22 corresponds to the DiGeorge syndrome critical region. The heterozygosity of the pericentromeric *D22S420* marker in the proband’s father (Subject II-2) rules out uniparental disomy of chromosome 22 of this person.

parental heterodisomy after trisomy rescue in chromosome 22 (Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). However, this hypothesis is not supported by the results of microsatellite analyses within the family. Fertilization between a grandparental gamete carrying a de novo deletion and another carrying a de novo duplication is possible but very unlikely, given that the estimated rate

of de novo, locus-specific mutation is approximately 1.25×10^{-4} for the 22q11.2 deletion.²⁰ The father's genotype could also have resulted from a postzygotic, nonallelic homologous recombination (Fig. 2 in the Supplementary Appendix) occurring soon after conception and potentially resulting in a perfectly balanced 22q11.2 deletion–duplication genotype in the embryo and in chromosomal placental mosaicism (Fig. 3 in the Supplementary Appendix). This hypothesis is compelling, since it requires only one event.

Our study emphasizes the importance of FISH analyses, especially of interphasic nuclei, which quickly provided the correct cytogenetic diagnosis of the father's chromosomal rearrangement. Comparative genomic hybridization by means of

microarray analysis would have failed to detect this balanced anomaly.

Genetic compensation in the context of genomic disorders has tremendous clinical consequences for genetic counseling, given the 100% risk of unbalanced outcomes. Our case report highlights the importance of performing genetic investigations in both parents of any child presenting with a genomic disorder, even if the parents have normal phenotypes.

No potential conflict of interest relevant to this article was reported.

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