Figure 3   Sequence of alleles present in family Z. Alleles 1 and 2 differ at position +3 of the intron. Allele 3 differs, at intronic position +17, from alleles 1 and 2. The mutation A1244G is present on the background of allele 1.

mutation. Finally, this case illustrates the danger, in linkage analysis, of overinterpreting slight phenotypic or pathological features, especially for carrier-status determination in X-linked diseases.

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Detection of an Atypical 22q11 Deletion That Has No Overlap with the DiGeorge Syndrome Critical Region

To the Editor:

Recent data indicate that the etiology of DiGeorge syndrome (DGS) and velo-cardio-facial syndrome (VCFS) is more complex than previously thought, with one recent report suggesting the existence of a second "critical region" within 22q11 (Kurahashi et al. 1996). Here we report another patient, with a mild phenotype, who has a deletion distinct from, and distal to, the established proximal critical region.

Interstitial deletions within human chromosome 22q11 have been described in patients with DGS (Scambler et al. 1991; Driscoll et al. 1992a), VCFS (Driscoll et al. 1992b; Scambler et al. 1992), and conotruncal anomaly face (CTAF) (Burn et al. 1993) and in a minority of patients with overlap between VCFS and Opitz GBBB (McDonald-McGinn et al. 1995). They also have been reported in many instances in which the full spectrum of these syndromes is not evident—for instance, in congenital heart disease (Wilson et al. 1992). However, in these cases there is usually facial dysmorphism typical of that seen in VCFS. Since both mild and severe malformations, as well as birth defects affecting distinct organ systems, can occur in the same family, these various abnormalities are generally thought to share the same genetic etiology—namely, haploinsufficiency for a gene(s) within 22q11.

Several investigators have compared 22q11 deletions, in order to establish a shortest region of deletion overlap (SRO). Comparison of the position and extent of deletions does not suggest any simple genotype/phenotype correlation. The interstitial deletions reported have almost all been large, involving the same region of ~2 Mb. Examination of terminal deletions enabled us to establish an SRO of ~500 kb (Halford et al. 1993). Others have narrowed this further, to ~250 kb (Gong et al. 1996). Although this region evidently contained more than one gene, the balance of evidence favored the hypothesis that the birth defects are secondary to haploinsufficiency of a single gene, since a balanced 2;22 translocation breakpoint, ADUBP, mapped within the SRO. Positional cloning efforts concentrated on identification of genes in the proximity of the ADUBP.

Three groups have reported cloning of sequences at or adjacent to the ADU breakpoint (Budarf et al. 1995; Demczuk et al. 1995; Wadey et al. 1995). However, no gene isolated from this region can be unequivocally implicated in DGS. Budarf and colleagues sequenced the disrupted sequences within ADU and identified an open reading frame disrupted by ADUBP, but no cDNA encoding this open reading frame could be isolated (Budarf et al. 1995). A gene, DGCR5, spanning the balanced translocation has been cloned, but it does not appear to code for a protein (Sutherland et al. 1996). The structural gene closest to the ADU breakpoint is DGCR2/IDD, encoding a transmembrane protein (Demczuk et al. 1995; Wadey et al. 1995). However, none of these sequences appears to have a point mutation in any patient without a 22q11 deletion.

Levy et al. (1995) and colleagues reported a child (patient G) with DGS and a 22q11 deletion that did not extend into the region disrupted in ADU. One possible interpretation (Levy et al. 1995) is that the ADU breakpoint exerts a position effect on a gene(s) located distally within the refined SRO. Most recently, Kurahashi et al. (1996) described a patient with CTAF and a deletion that did not overlap with the SRO located proximally (see fig. 1, top), and they suggested the existence of a second critical region located distally within the frequently deleted region. The location and number of genes involved in VCFS/DGS is therefore currently in doubt.

In this paper we report on the mapping of a 22q11 deletion found in one member of a family referred on the basis of familial congenital heart defect. The deletion in this patient is distal to the proximal SRO but overlaps with the deletion reported by Kurahashi.

Family A (pedigree in fig. 1, bottom) was screened with 22q11 markers, since three children had congenital heart defects. One child (II.3) had died as a consequence of the heart defects, and no sample was available for analysis. She had an inlet muscular ventricular septal
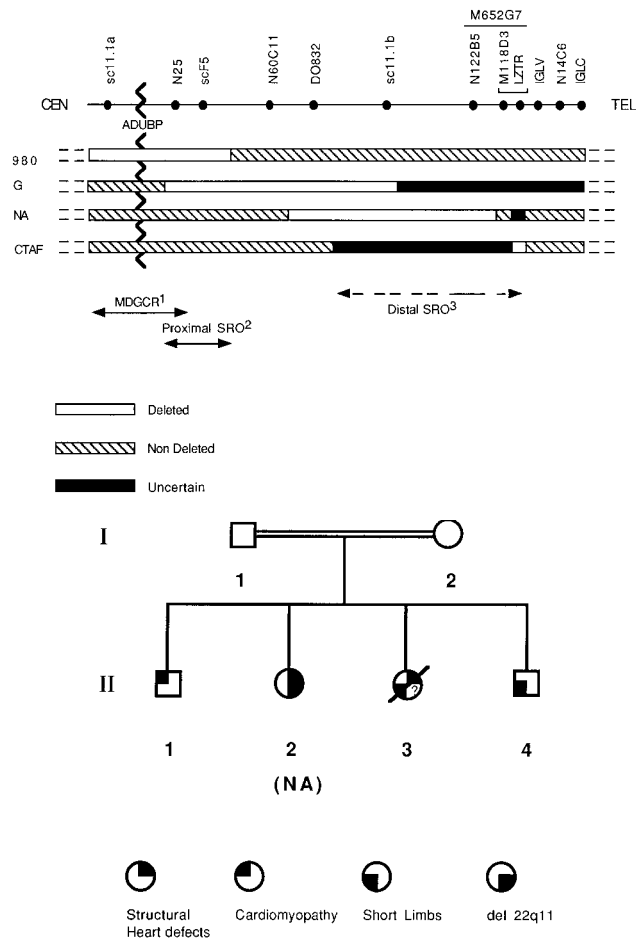
Figure 1 *Top,* Map of 22q11 deletion breakpoints. The relative position of 22q11 markers is shown at the top of the panel; and the positions of various deletion breakpoints are given at the bottom of the panel, with the ADUBP depicted by the jagged line. The superscript "1" and "2" indicate MDGCR, as defined by Gong et al. (1996), in relation to the SRO seen in patients whom we have analyzed—namely, GM00980 and G. The superscript "3" indicates the putative second SRO, as proposed by Kurahashi et al. (1996), distal to and distinct from the proximal SRO. The deletion analyzed here (NA) overlaps with the distal SRO and is similarly distinct from the proximal SRO. *Bottom,* Pedigree of family A.

defect, septum primum atrial septal defect, patent ductus arteriosus and dextrarotation, and hypothyroidism. She also had slightly short limbs, but a skeletal survey revealed no specific dysplasia. The parents were normal on examination and echocardiography but were first cousins. Patient NA (II.2) had normal limbs and, at birth, a ventricular septal defect that did not require surgical intervention and closed spontaneously. No immune studies have been conducted, since there is a history of typical childhood infections only. There is no documented instance of hypocalcemia, but parathyroid-function studies were not performed. The palate and facies are normal, and there is no nasal intonation to the speech. Learning and behavior are normal. Child
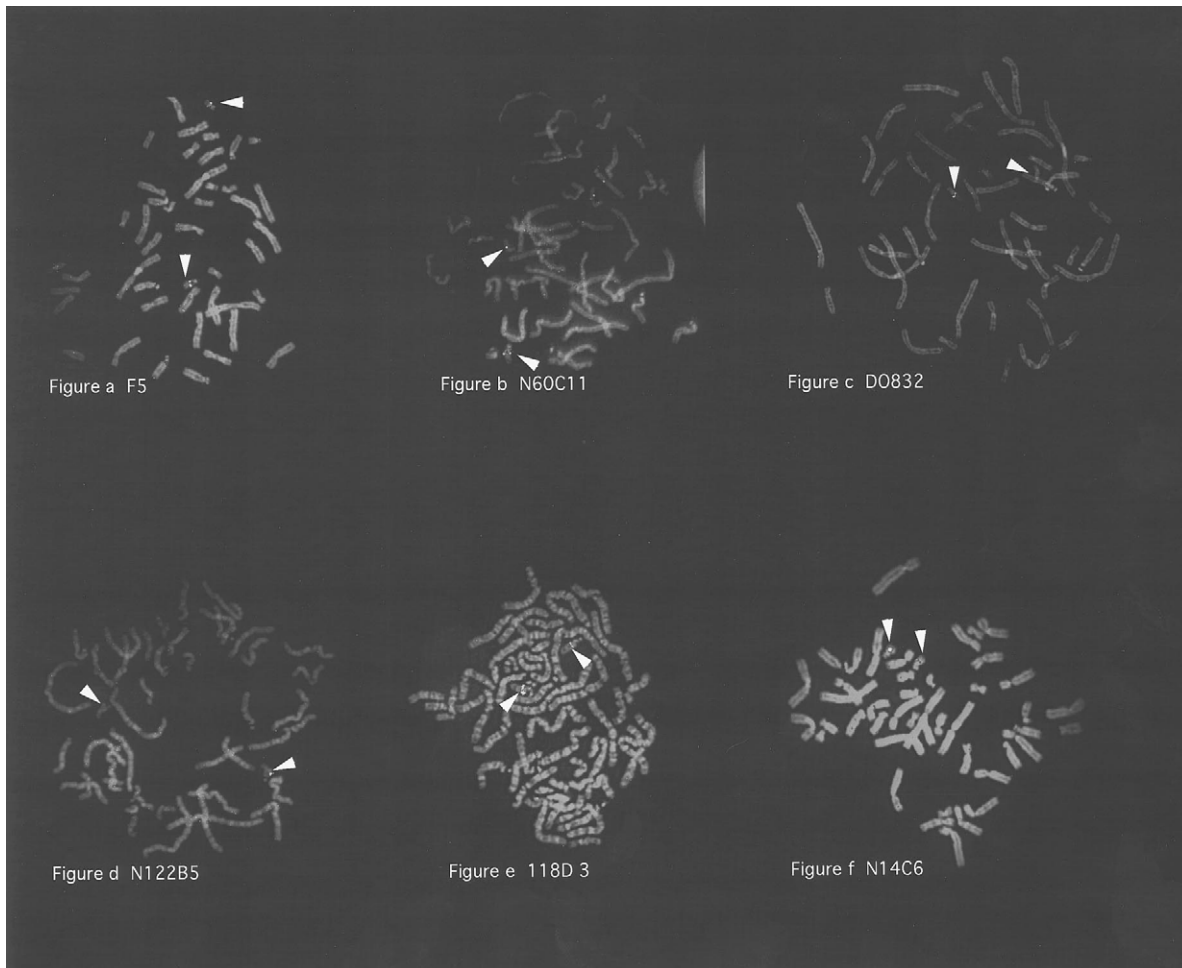
Figure 2   FISH analysis of patient NA's metaphase chromosomes, with 22q11 markers. Arrowheads are used to indicate chromosome 22. Panels *a* and *b* show, respectively, markers scF5 and N60C11, both of which detect signal on both chromosomes 22; these markers map proximal to the deletion. Panels *c* and *d* show, respectively, markers DO832 and N122B5, which detect signal on only one chromosome 22; these markers are within the deletion. Panels *e* and *f* show, respectively, markers 118D3 and N14C6, which detect signal on both chromosomes 22; these markers are distal to the 22q11 deletion. The chromosome 22 centromeric marker D22Z1 (which also detects chromosome 14) has been used to identify both chromosomes 22 in some hybridizations, including those in which there is test probe signal only on the nondeleted chromosome (*c* and *d*).

II.1 had a cardiomyopathy but no stigmata of VCFS or DGS and had normal limbs; cardiomyopathy is not a feature of 22q11 deletions. A fourth child, born after the chromosome analysis, had the short-limbed phenotype.

A routine diagnostic test revealed that NA was dizygous for the marker N25 (D22S75) but hemizygous for cosmid D0832 (see map in fig. 1, *top*). In order to confirm and extend these unexpected findings, a series of markers from across the commonly deleted region (Collins et al. 1995; Morrow et al. 1995) were examined to map the proximal and distal extents of deletion. FISH analysis demonstrated dizygosity for markers sc11.1, scF5 (fig. 2*a*), and N60C11 (fig. 2*b*). Markers D0832 (fig. 2*c*) and N122B5 (fig. 2*d*) detected hemizygosity in patient NA, whereas sequences detected by 118D3 and N14C6 were dizygous (fig. 2*e* and *f*).

NA is hemizygous for cosmid N122B5 and dizygous for the mega-YAC 118D3. Interrogation of the Sanger center database revealed that both these markers are contained within the mega-YAC M652G7; therefore, the distal deletion breakpoint must be within mega-YAC M652G7. No analysis with HKAD was conducted. However, the HKAD cosmid and the mega-YAC M652G7 both contain the LZTR gene, so the distal extents of these two atypical deletions are similar.

FISH analysis demonstrates that patient NA has an atypically small 22q11 deletion. This is the third 22q11 deletion that does not extend into the region disrupted by the ADU balanced-translocation breakpoint, but, more strikingly, the deletion does not overlap with the previously described SRO for DGS/VCFS. The distal extent of our SRO is defined by the deletion seen in cell

line GM00980 (Halford et al. 1993). Cosmid N60C11 is dizygous in GM00980 and therefore maps distal to the SRO (Halford et al. 1993). N60C11 is also dizygous in patient NA, mapping proximal to the deletion. Both parents had normal chromosomes and were dizygous for N25 and D0832. These observations were corroborated by microsatellite analysis (B. Morrow, unpublished data).

In the absence of a complete transcription map of 22q11, it is not possible to state unequivocally that distinct genes are affected in ADU, in patients G and NA. However, since the distance from the ADU breakpoint to the proximal boundary of the NA deletion is ⩾500 kb, this does seem unlikely. Since the deletion in NA is likely to overlap with the deletion observed in the CTAF patient of Kurahashi et al. (1996), it seems most probable that the phenotype in these two patients is secondary to haploinsufficiency of the same gene(s).

The mild phenotype observed in NA is worthy of comment, since, apart from the previous history of having had a VSD, she could be described as unaffected. Her sib had a more complex congenital heart defect, but no material was available for analysis. Evidently there is the possibility of a germinal mosaicism in this family, and there may be an independently segregating recessive mutation resulting in a mild shortening of the limbs. Interestingly, the CTAF patient with a small distal deletion also had a heart defect (pulmonary atresia and tetralogy of Fallot), and no hypoparathyroidism or cell-mediated immune deficiency was reported. It is possible that deletions within the distal "critical" region are more likely to result in a restricted phenotype, for which karyotype analysis might not be requested. In any case, examination with the commercially available probes would not detect their deletions.

It might be argued that the mild heart defect is due to factors other than a 22q11 deletion. In this case, it must be concluded that the deletion has no effect even though it covers approximately the same region as the CTAF distal deletion (Kurahashi et al. 1996). The hypothesis that distal deletions predispose to less severe defects than proximal deletions is robust against this objection. The number and location of genes involved in DGS/VCFS remains in doubt, with the identification of these genes made more difficult by the existence of nonoverlapping deletions and the likelihood of position effects within the region (Sutherland et al. 1996).

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Linkage Disequilibrium between the Spinocerebellar Ataxia 3/Machado-Joseph Disease Mutation and Two Intragenic Polymorphisms, One of Which, X359Y, Affects the Stop Codon

To the Editor:

Machado-Joseph disease (Takiyama et al. 1993) or spinocerebellar ataxia 3 (Stevanin et al. 1994*a*) (SCA3/MJD), is the most frequent form of autosomal dominant cerebellar ataxia type I, a heterogeneous group of neurodegenerative disorders of unknown etiology (Harding 1993; Sequeiros and Coutinho 1993; Dürr and Brice 1996; Dürr et al. 1996). The responsible mutation has been characterized as an unstable CAG-repeat expansion in the coding region of the MJD1 gene (Kawaguchi et al. 1994; Cancel et al. 1995). The molecular mechanism leading to repeat expansion remains unknown. However, Igarashi et al. (1996) recently suggested, on the basis of an intragenic polymorphism (987G→C) in the MJD1 gene, that an allelic interaction between the normal and expanded chromosomes may be implicated in the instability of the expanded CAG repeat. Until recently, the SCA3/MJD mutation was thought to originate in the Azores Islands. We now know that the disease is not restricted to patients of Portuguese descent and that several different mutations probably are involved, even in Portuguese pedigrees (Stevanin et al. 1995*a*; Takiyama et al. 1995; Gaspar et al. 1996; Iughetti et al. 1996). We report here a new intragenic poly-

morphism at the stop codon (1118A→C) of the MJD1 cDNA, resulting in the addition of 16 amino acid residues to the C-terminal domain of the gene product, that does not affect the phenotype. Analysis of the haplotypes defined by the 987G→C and 1118A→C polymorphisms revealed the existence of four different haplotypes associated with the SCA3/MJD mutation that have resulted from at least four ancestral mutations.

We have cloned cDNAs corresponding to the normal and expanded MJD1 alleles from a SCA3/MJD patient, by retro-transcription of brain mRNA followed by PCR. Direct sequencing of both cDNAs revealed at codon 359 a polymorphism that differs from the published MJD1 sequence (Kawaguchi et al. 1994) and that corresponds to a 1118A→C substitution (Ter→Y) predicting an SCA3/MJD protein containing 16 additional amino acid residues (YELHVIFALHYSSFPL). This result is in agreement with preliminary results obtained on cDNA from lymphoblastoid mRNA (Trottier et al. 1995) and from a brain library (Goto et al. 1996). The polymorphisms can be detected rapidly by differential PCR with the MJD52 primer (Kawaguchi et al. 1994) and either MJD-TAA (GCAAAAATCACATGGAGCTC<u>T</u>) or MJD-TAC (GCAAAAATCACATGGAGCTC<u>G</u>) for the 1118A→C polymorphism and either MJD-GGG (CTCTGTCCTGATAGGTCCC<u>C</u>) or MJD-CGG (CTCTGTCCTGATAGGTCCC<u>G</u>) for the 987G→C polymorphism. PCR amplifications were performed as described elsewhere (Cancel et al. 1995), except for annealing at 64°C and 63°C for the 1118A→C and 987G→C polymorphisms, respectively. PCR products were separated on 1% agarose gels and were visualized by UV exposure in the presence of 0.5 μg ethidium bromide/ml (fig. 1). PCR products from control subjects who were homozygous for a polymorphism on both normal chromosomes were run on 6% acrylamide gels for better resolution of the alleles and were visualized by autoradiography, after being blotted onto nylon membranes and hybridized with a γ^{32}P-labeled (CAG)$_7$ oligonucleotide.
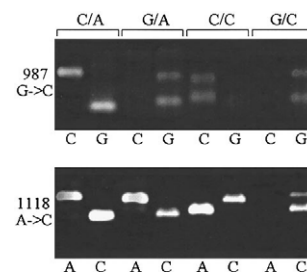

Figure 1 Detection of the four 987G→C/1118A→C haplotypes on expanded SCA3/MJD alleles. The four PCR amplifications (see text) for each subject were run on a 1% agarose gel containing 0.5 μg of ethidium bromide/ml, to separate normal and expanded alleles.