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Molecular Characterization of a 130-kb Terminal Microdeletion at 22q in a Child with Mild Mental Retardation

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Summary

We have analyzed a recently described 22q13.3 microdeletion in a child with some overlapping features of the cytologically visible 22q13.3 deletion syndrome. Patient NT, who shows mild mental retardation and delay of expressive speech, was previously found to have a paternal microdeletion in the subtelomeric region of 22q. In order to characterize this abnormality further, we have constructed a cosmid/P1 contig covering the terminal 150 kb of 22q, which encompasses the 130-kb microdeletion. The microdeletion breakpoint is within the VNTR locus D22S163. The cloning of the breakpoint sequence revealed that the broken chromosome end was healed by the addition of telomeric repeats, indicating that the microdeletion is terminal. This is the first cloned terminal deletion breakpoint on a human chromosome other than 16p. The cosmid/P1 contig was mapped by pulsed-field gel electrophoresis analysis to within 120 kb of the arylsulfatase A gene, which places the contig in relation to genetic and physical maps of the chromosome. The acrosin gene maps within the microdeletion, ~70 kb from the telomere. With the distal end of chromosome 22q cloned, it is now possible to isolate genes that may be involved in the overlapping phenotype of this microdeletion and 22q13.3 deletion syndrome.

Introduction

Cytogenetically visible deletions of 22q13.3 are associated with generalized developmental delay, normal or accelerated growth, hypotonia, severe delays in expressive speech, and mildly dysmorphic facial features (Nesslinger et al. 1994). Molecular studies of seven cases revealed no correlation between the severity of the phenotype and the proximal extent of deletion. The smallest region of overlap between the 22q13.3 deletion patients (critical region) extends from below locus D22S97 proximally to below arylsulfatase A (ARSA). Since ARSA is the most distally mapped locus on chromosome 22q (Dumanski et al. 1991; Collins et al. 1995), it has not been possible to assess the extent of the deletions below this locus.

The critical region of the 22q13.3 deletion syndrome covers a genetic distance of 25.5 cM (Nesslinger et al. 1994), which represents ⩾5 Mb as measured by pulsed-field gel electrophoresis (PFGE) (H. E. McDermid, unpublished data). This large size therefore makes impractical a search for genes that may be involved in the production of the phenotype of this syndrome. However, microdeletions within a critical region have been used in many deletion syndromes to narrow the focus of gene searches (Greger et al. 1993; Huber et al. 1994; Levy et al. 1995). Such a microdeletion of 22q13.3 has recently been reported. In a study of 99 mentally retarded individuals screened for subtelomeric chromosomal rearrangements, Flint et al. (1995) described a 12-year-old boy (NT) with delayed expressive speech, mild mental retardation (IQ = 64), normal facial features, and a negative family history for mental retardation. Although his high-resolution karyotype was normal, the paternal allele of the minisatellite probe D22S163 (MS607) was found to be deleted. ARSA and other 22q13.3 probes were present in two copies, indicating that NT carried a microdeletion.

We now describe the molecular characterization of this microdeletion, which spans the terminal 130 kb of 22q. A cosmid/P1 contig covering the deleted region was constructed, allowing the localization of the deletion breakpoint and the mapping of the acrosin gene (ACR) to the region. Analysis of the deletion breakpoint revealed that it is actually within locus D22S163, which therefore is only partially deleted. The breakpoint was healed by the addition of telomeric sequences. With the distal end of chromosome 22q cloned, it becomes feasible to isolate the genes that may be involved in the

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overlapping phenotype of this microdeletion and 22q13.3 deletion syndrome.

Material and Methods

Cell Lines

The establishment of Epstein Barr virus (EBV)–transformed lymphoblastoid cell lines for NT and his parents has been described elsewhere (Flint et al. 1995). The normal control lymphoblastoid cell line (GM03657) was obtained from the Coriell Human Genetic Mutant Cell Repository. The fibroblasts from FB, who has the 22q13.3 deletion syndrome (Nesslinger et al. 1994), were used for comparison of the copy number of probes in NT's microdeletion region.

Probes

The following probes were used in this study: CP8, a partial cDNA of ARSA (Herzog et al. 1990); pHU4A, a cDNA of ACR (Baba et al. 1989); D22S163 (probe MS607), a minisatellite probe (Armour et al. 1990); and D21S15 and D21S110, two reference probes on chromosome 21 (Stewart et al. 1985; Spinner et al. 1989). Three probes were produced from cosmid N66C4: a 5.0-kb *Hin*dIII fragment (H5.0), a 1.2-kb *Xho*I fragment (Xh1.2), and a 7.0-kb *Hin*dIII/*Eco*RI fragment (HE7.0).

DNA Studies

DNA extraction, digestion, electrophoresis, transfer, and hybridization were done as described by Nesslinger et al. (1994). The copy number of a probe was preferably determined by RFLP analysis of NT and his parents. For noninformative probes, quantitative analysis of Southern blots was performed, comparing NT to a normal individual and a 22q13.3 deletion patient (FB). For each probe, five replicates were analyzed as described by Nesslinger et al. 1994, by using control probes D21S15 or D21S110 for comparison.

PFGE

PFGE was performed using a contour-clamped heterogeneous electrical field–DRII apparatus (BioRad). Cells from three normal individuals were embedded in agarose and processed as described by McDermid et al. (1993). DNA was digested with *Not*I or *Nru*I and separated by PFGE at 150 V with a 120-s switch time for 23 h followed by 180-s switch time for 23 h. The resulting blot was probed with HE7.0 (fig. 1). The blot was then stripped of signal and reprobed with ARSA.

Construction of a Cosmid Contig Spanning the NT Microdeletion Region

Two cosmid contigs that together span the NT microdeletion region were constructed independently by screening two different cosmid libraries (fig. 1). The construction of the "B" contig from the chromosome 22–specific library ICRFc106 is described elsewhere (Ning et al. 1996). It started from a P1 clone containing the 22q telomere and extended proximally. The prefix ICRFc106 has been left off each cosmid name, for brevity. The "A" contig was begun from overlapping cosmid clones N85E7 and N66C4, which were isolated from the chromosome 22–specific library LL22NC03 (De Jong et al. 1989) by probing with D22S163. Cosmid end fragments were isolated by detecting *Sma*I-digested vector-insert junction fragments with a vector probe on Southern blots. These vector-insert junction fragments were then used to identify *Xho*I and *Xho*I/*Hin*dIII fragments flanking but not including vector sequences. End fragments were tested for copy number in NT by RFLP or densitometric analysis before being used to isolate overlapping cosmid clones within the microdeletion region. A *Bam*HI restriction map of both contigs was constructed by using *Bam*HI fragments as probes against *Hin*dIII and/or *Sma*I cosmid digests electrophoresed and Southern blotted. This allowed unambiguous assignment of contiguous *Bam*HI fragments.

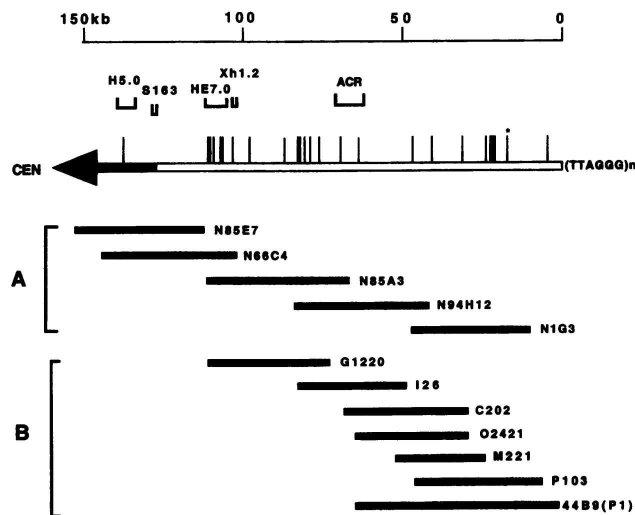

Figure 1 Cosmid/P1 contig, spanning the NT microdeletion. The upper bar (with the arrow) shows the chromosome, with the deleted region represented by an open bar. Vertical lines on the chromosome represent *Bam*HI sites. *A*, Cosmid contig, constructed by screening the chromosome 22–specific library LL22NC03. *B*, Cosmid contig, constructed by screening the chromosome 22–specific library ICRFc106 (modified from Ning et al. 1996). In contig *B*, the full name of each cosmid is "ICRFc106," followed by the designations shown in the figure. Locus D22S163 is abbreviated as "S163." The *Bam*HI site marked with an asterisk (*) was found only in 44B9.

FISH

Chromosome preparations were made from lymphoblast cultures by conventional methods. Probe labeling, suppression of repetitive sequences, denaturation, hybridization, and fluorescence detection were performed

as described by Kuwano et al. (1991). DNA from cosmid P103 was labeled with digoxigenin-11dUTP by nick-translation. A chromosome 22 alpha-satellite probe generated by using a mouse-human hybrid containing a single human chromosome 22 (Weier et al. 1991) was labeled with biotin-16-dUTP and used as a chromosome 22 control. Rhodamine antidigoxigenin and fluorescein isothiocyanate (FITC)–avidin were used to detect digoxigenin and biotin-labeled probes, respectively. Slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Analysis was performed using a Zeiss Axiophot microscope equipped with filters to detect rhodamine, FITC, and DAPI separately, as well as a triple band pass filter set to detect signals simultaneously. Images were collected and merged using a cooled CCD camera (KAF 1400, Photometrics) and IP Lab Spectrum software.

BAL31 Analysis

Agarose-embedded DNA from NT was used to avoid DNA shearing. Plugs of 50 μl were added to the BAL31 digest reaction mix and adjusted to a final volume of 1 ml. DNA was digested with 20 U BAL31 (New England Biolab) at 30°C for 0, 0.5, 1, and 1.5 h. DNA plugs were then equilibrated in ice-cold 100 mM EGTA (ethylene glycol-bis[β-Aminoethyl ether] N, N, N′, N′-tetraacetic acid) to inactivate BAL31, followed by an ice-cold TE (10 mM Tris pH8.0, 1 mM EDTA) wash before digestion with *Eco*RV. DNA plugs were then loaded in a 0.6% agarose gel and separated by standard electrophoresis, Southern blotted, and probed with D22S163.

Cloning and Sequencing of PCR Products

The sequence of D22S163 locus (Armour and Jeffreys 1991) was used to design a primer to amplify the region adjacent to the deletion breakpoint. A 25-nt forward primer (5′ GTGACTTGACTTCTCTGAACCTTGG 3′) was used in conjunction with a reverse primer containing a telomeric sequence (5′ TATGGATCCCTA-ACCCTAACCCTAACCC 3′). Amplification conditions were 30 cycles of 94°C (1 min), 60°C (1 min), and 72°C (3 min) in 100-μl reaction with *Taq* polymerase purchased from Boehringer Mannheim and the manufacturer's buffer. PCR products were cloned into M13 and sequenced with forward and reverse primers on an ABI 373 sequencing machine. Six independent clones were sequenced in both orientations. Normal sequence was obtained by sonicating the cosmid N66C4, size separating the fragments, and subcloning a 2-kb fraction into M13. The M13 library was plated out, and plaques were transferred to Hybond N (Amersham International) nylon membrane. Filters were then hybridized, with the forward primer used for PCR and positive plaques sequenced on the ABI sequencer.

Results

PFGE Localizes the NT Microdeletion Region to within 120 kb of ARSA

Flint et al. (1995) detected a paternal deletion of locus D22S163 (MS607) in NT but not of ARSA or other 22q13.3 probes. D22S163 had previously been mapped to 22q by somatic cell hybrid analysis and linked to CRI-L1272 (D22S18) by genetic analysis (Armour et al. 1990). However, the exact location of D22S163 was unknown. In order to determine the location of D22S163 relative to ARSA, the most distal probe in chromosome 22 genetic and physical maps (Dumanski et al. 1991; Collins et al. 1995), we used PFGE of DNA from normal individuals. The resulting blots were probed with HE7.0, a fragment from a cosmid (N66C4) containing D22S163 (fig. 2*A*). The resulting *Not*I and *Nru*I banding patterns were identical to the same blot stripped and probed with ARSA (fig. 2*B*). The smallest *Not*I band (120 kb, arrow) gives a maximal distance between the two probes. Densitometric analysis (Flint et al. 1995) and FISH (Ning et al. 1996) results both indicate that ARSA is not deleted in NT. Therefore, the NT microdeletion is within 120 kb of ARSA, although these results did not indicate whether it lays proximally or distally.

Production of a Cosmid Contig from D22S163/P1 to the 22q Telomere

To characterize the NT microdeletion fully, we constructed contigs that span the deleted region. One cosmid walk started from a P1 clone containing the 22q telomere and walked proximally (fig. 1, contig *B*) (Ning et al. 1996). A second cosmid walk (fig. 1, contig *A*) started from the cosmid clones N85E7 and N66C4,

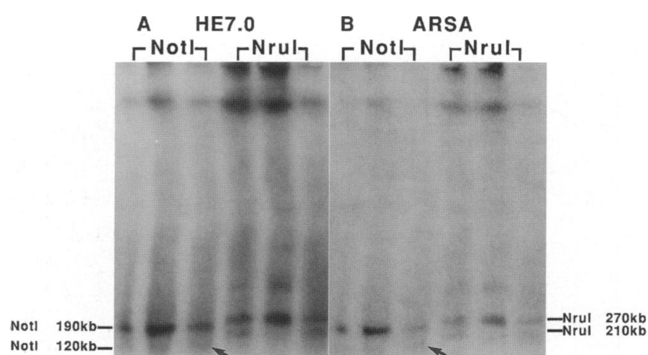

Figure 2 Localization of the NT microdeletion to within 120 kb of ARSA. DNA from three normal individuals was digested with *Not*I or *Nru*I and was then separated by PFGE under conditions stated in Material and Methods. The resulting blot was probed with the HE7.0 fragment (fig. 2) from N66C4 (*A*). The blot was then stripped of signal and reprobed with ARSA (*B*). The arrows indicate the smallest *Not*I band (120 kb) containing both probes. Bands were sized by comparison to lambda concatamers.

which were isolated using a probe from D22S163. End fragments of these cosmids were tested by densitometric or RFLP analysis, to assess copy number in NT. Densitometric analysis showed that the proximal end of N85E7 was not deleted in NT (data not shown). However, the distal end fragment of N66C4 (Xh1.2, fig. 1) showed a paternal deletion by RFLP analysis (fig. 3). The cosmid walk was therefore extended from the distal end of N66C4 for three rounds of end-fragment walking. The two contigs overlap by about three cosmid lengths (~100 kb) and span ~150 kb between the proximal end of N85E7 and the 22q telomere. Since ARSA was not present by hybridization within the combined contig, D22S163 maps distal to ARSA. This also places ARSA at a maximum distance of 250 kb (120 kb + 130 kb) from the 22q telomere.

FISH Indicates That the NT Microdeletion Is Terminal

When the cosmid walk reached the N94H12/N1G3 region, densitometric analysis could no longer be used to determine the copy number in NT; presumably this is because of the presence of the subtelomeric repeats shared with other chromosomes (Ning et al. 1996). As a result, FISH was used to test for deletion in NT. The most distal cosmid of the contig (P103) was labeled and hybridized to NT metaphase spreads, in combination with the chromosome 22 alpha-satellite probe to mark the centromere of both chromosome 22s. P103 hybridized to many chromosome ends, as well as 2q13, the fusion site of two ancestral chromosomes (Ijdo et al. 1991). Only one chromosome 22 hybridized to P103 (fig. 4). This result indicates that the entire cosmid contig is deleted in NT distal to D22S163.

Localization of the Breakpoint of the NT Microdeletion

Since the proximal end of N85E7 was not deleted, while the distal end of N66C4 showed a paternal deletion by RFLP analysis, these results indicated that the breakpoint of the NT microdeletion is within the N66C4 and N85E7 region. An additional probe (H5.0), which is 6.5 kb proximal to D22S163 (fig. 1), showed no deletion by densitometric analysis (data not shown), further narrowing down the breakpoint. In order to look for DNA rearrangement fragments indicative of the breakpoint, genomic DNAs from a normal control and the NT family were digested with *Hin*dIII, *Sma*I, *Eco*RV, and *Ngo*MI, electrophoresed, blotted, and hybridized to the D22S163 probe. Novel smeared bands in the 9–11-kb range were present in *Hin*dIII and *Sma*I digestions of NT DNA (fig. 5). This smear is characteristic of breakpoint-junction fragments fused with telomeric sequences that are heterogeneous in length (Wilkie et al. 1990). D22S163 also detected the presence of a large rearrangement band in NT with *Eco*RV and *Ngo*MI digests (fig. 5). The presence of these wide rearrangement bands suggested that NT has terminal deletion and that the breakpoint is within D22S163. One discrepancy between this study and that of Flint et al. (1995) is that we detected *Hin*dIII-, *Sma*I-, *Eco*RV-, and *Ngo*MI-rearrangement fragments with the D22S163 probe, while Flint et al. (1995) detected a paternal deletion with the same probe, using *Sau*3AI. We hypothesized that under normal electrophoretic conditions, the smaller *Sau*3AI-smeared rearrangement band could be diffused

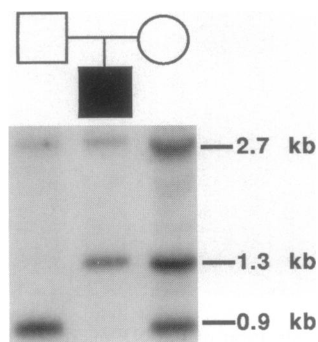

Figure 3 RFLP analysis, showing that Xh1.2 is deleted in NT. The autoradiograph shows the NT family genomic DNA digested with *Taq*I and probed with Xh1.2. Each individual shows a constant 2.7-kb band. The father is homozygous for the 0.9-kb allele, and the mother has both the 1.3-kb and 0.9-kb allele. NT inherited the 1.3-kb allele from the mother but no allele from the father.

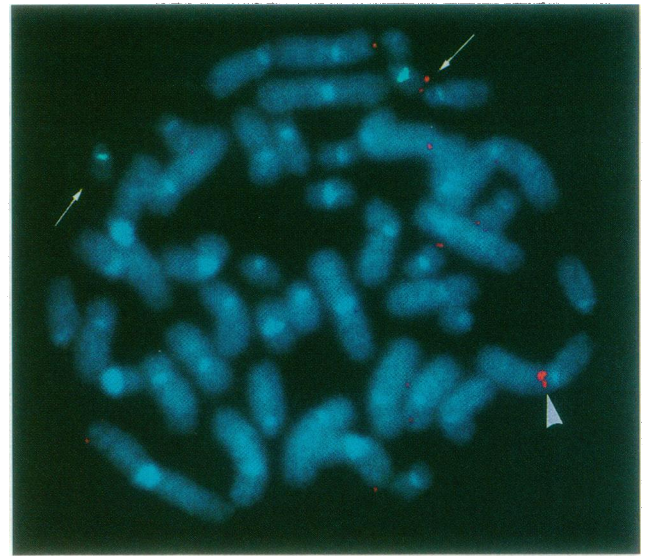

Figure 4 FISH, showing that the most distal cosmid in the contig is deleted in NT. A chromosome 22–specific alpha-satellite probe, appearing as green (FITC) centromeric signals, was used to identify the two chromosome 22 homologues. Cosmid P103, appearing as red signal (rhodamine), was observed on one homologue and deleted on the other homologue (arrows). Since cosmid P103 contains subtelomeric repetitive sequences, the red signals are also present on some telomeres of other chromosomes as well as chromosome 2q13 (arrow head).

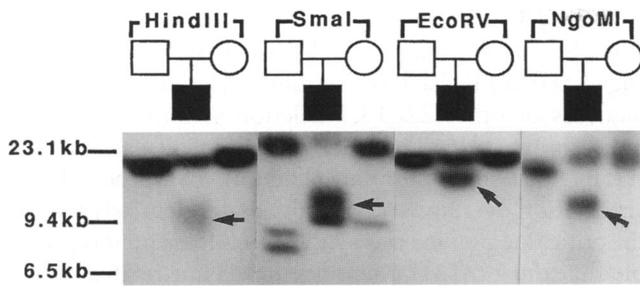

Figure 5 Rearrangement bands, detected by the D22S163 probe. The autoradiograph shows the NT family genomic DNA digested with *Hin*dIII, *Sma*I, *Eco*RV, and *Ngo*MI, followed by hybridization to the D22S163 probe. Novel smeared bands in the 9–11-kb range were present in *Hin*dIII and *Sma*I digest of NT DNA (arrows). *Sma*I also identifies a polymorphic locus with a 9.4-kb allele in NT and his mother and 8.0/9.0-kb alleles in the father, which confirms the paternal deletion of NT. D22S163 also detected the presence of large rearrangement bands in NT with *Eco*RV and *Ngo*MI (arrows). Slight differences in the apparent size of the large bands common to all individuals are due to electrophoresis artifact.

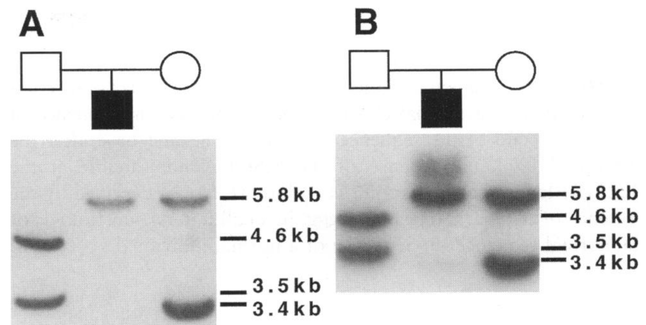

Figure 6 *Sau*3AI-digested NT family genomic DNA, hybridized to the D22S163 probe under two different electrophoretic conditions. In *A*, using conditions reported in Flint et al. (1995), NT appears to inherit one 5.8-kb allele from his mother but no allele from his father, suggesting a paternal deletion of D22S163 in NT. In *B*, electrophoresing the DNA over a much shorter distance and exposing the autoradiograph for a longer time, D22S163 detected a smeared rearrangement band in NT, indicating the microdeletion breakpoint is close to, or within, D22S163.

to the point of being not visible with a standard exposure (fig. 6*A*). We therefore repeated the *Sau*3AI digestions of the NT family DNAs and electrophoresed the fragments over a short distance to maximize the chance of seeing a smeared rearrangement band. Under these conditions, a novel smeared band was seen in NT (fig. 6*B*), indicating that D22S163 contains the breakpoint rather than being deleted. This also confirms the explanation given by Flint et al. (1995) as to why cosmids containing D22S163 did not show a deletion for NT when FISH was used, since only part of the cosmid is deleted.

BAL31 Analysis

If NT is a terminal deletion, then the rearrangement fragment should be sensitive to BAL31 exonuclease digestion. When agarose-embedded NT DNA was subjected to BAL31 digest from 0 to 1.5 h, followed by *Eco*RV digestion, the D22S163 probe detected a reduction in the size of the rearrangement band (fig. 7). The larger, normal band detected by D22S163 served as a control to show that the genomic DNA was intact. Since D22S163 is normally located 130 kb from the telomere, it is not sensitive to BAL31 digestion on a normal chromosome.

PCR Amplification of the Breakpoint Junction Sequence

To determine the sequence at the breakpoint of the NT microdeletion, DNA was amplified by PCR by using a D22S163 locus–specific primer and a primer that contains three telomeric repeats. PCR products were then cloned, and the breakpoint was sequenced (fig. 8*A*) and compared with a corresponding normal chromosome 22 sequence (fig. 8*B*). The breakpoint was located within the D22S163 locus and can be identified by the presence of telomeric repeats (ttaggg, in fig. 8*A*) substituted for the normal sequence. This result agrees with the localization of the breakpoint by Southern blot and BAL31 analyses. D22S163 consists of two distinct minisatellite arrays, namely, MS607A and MS607B (Armour and Jeffreys 1991). The flanking sequence surrounding the MS607A minisatellite array has been determined for 500 bp proximally (European Molecular Biology Library [EMBL] accession number X58043) and 866 bp distally (EMBL accession number X58044). The location of the breakpoint is 26 bp distal to the 3′ end of the 866-bp flanking sequence. Therefore, the breakpoint is 892 bp (866 bp + 26 bp) distal to the MS607A. The distance between minisatellite MS607A and MS607B is

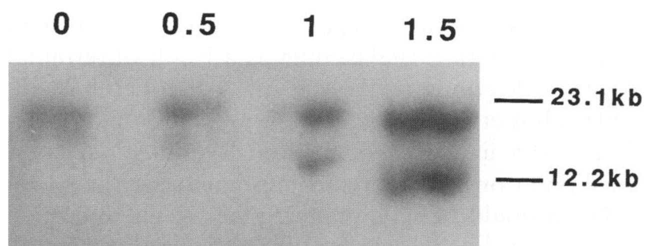

Figure 7 BAL31 sensitivity of the rearrangement band in NT. Genomic DNA from NT was digested with BAL31 before digestion with *Eco*RV. The number at the top of each lane indicates the incubation time of BAL31 (in hours). DNA was then separated by standard electrophoresis in a 0.6% agarose gel. The resulting blot probed with D22S163 shows a decrease in size of the rearrangement band after BAL31 digestion. The positions of DNA size markers from lambda phage digested with *Hin*dIII and the 1-kb ladder (BRL) are shown on the right.

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A5'AGGGGGTGGAGAGGGGGGTGGTGGAGagggttagggttagggtta3'
B5'AGGGGGTGGAGAGGGGGGTGGTGGAGGGGGGTGGTGGCACAGGGG3'
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Figure 8 Sequence at the NT microdeletion breakpoint, compared to that of a normal chromosome. *A*, Breakpoint sequence of NT. The 5′ end of the sequence starts from the first base after the 866-bp 3′ flanking sequence of the MS607A minisatellite at the D22S163 locus. The breakpoint is within D22S163, identified by the presence of telomeric repeats (ttaggg, in small letters) substituted for the normal sequence. *B*, Sequence of a normal individual.

not known, but an upper size is estimated to be ~1 kb (J. A. L. Armour, personal communication). If the NT deletion breakpoint is 891 bp distal to MS607A, it is either at the 5′ end or within the MS607B minisatellite.

Localization of ACR to the NT Microdeletion

PFGE studies of the region indicated that ARSA, D22S163, and the ACR locus all mapped to a 190-kb *Not*I fragment (data not shown). When the ACR cDNA probe was hybridized to a Southern blot containing *Bam*HI digests of all the cosmid DNAs, fragments of 4.6, 6.9, and 17 kb in cosmids N94H12, I26, and C202 were positive. The *Bam*HI fragment pattern matched the ACR restriction map reported by Vazquez-Levin et al. (1992). Therefore, the ACR locus maps to the NT microdeletion, ~60 kb distal to D22S163 and ~70 kb from the telomere (fig. 1). Densitometric analysis confirmed that ACR is deleted in NT (fig. 9), as well as the in 22q13.3 deletion patient FB.

Discussion

We have shown that NT carries a terminal microdeletion of the last 130 kb of chromosome 22q. Cloning of the breakpoint sequence and BAL31 analysis of rearrangement fragments suggest that the deletion breakpoint was healed by the addition of telomeric repeats. This explains the characteristic smeared rearrangement bands seen with *Hin*dIII, *Sma*I, and *Sau*3AI. The healing of broken human chromosomes by the addition of telomeric repeats has been described in individuals with α-thalassemia as a result of terminal 16p13.3 deletions (Wilkie et al. 1990; Lamb et al. 1993; Flint et al. 1994). However, the NT microdeletion is the first case of a cloned terminal deletion breakpoint on a human chromosome other than 16p.

The cosmid/P1 contig spanning the NT microdeletion serves to anchor the physical map of chromosome 22. PFGE analysis indicates that this contig lies ≤120 kb from ARSA, previously the most distally mapped locus on chromosome 22 (Dumanski et al. 1991; Collins et al. 1995). The order of known loci at the end of 22q is therefore ARSA-D22S163-ACR-telomere. This confirms the subtelomeric location of the VNTR at D22S163 used for detecting cryptic 22q terminal rearrangements (Flint et al. 1995).

A patient with the 22q13.3 deletion syndrome (FB) was also shown to have a deletion of ACR (fig. 9). This suggests that FB also has a terminal deletion. Subsequent analyses of other 22q13.3 deletion syndrome patients have shown deletions of D22S163 in all cases tested (H. E. McDermid, unpublished data). Thus, the NT microdeletion overlaps and most likely falls entirely within the critical region for this syndrome. Although it is possible that the 22q13.3 microdeletion is not related to the phenotype of NT, this region may contain genes critical to the phenotypic features of NT that overlap with the 22q13.3 deletion syndrome: specifically, delayed speech and mental retardation.

The only gene mapped to the microdeletion to date is ACR, a serine protease present in the acrosome of the sperm head (Klemn et al. 1991). Protease inhibition studies suggest that ACR plays a key role in the acrosome-reacted binding of sperm to the zona pellucida and the penetration of sperm into the oocyte (Liu and Baker 1993; Takano et al. 1993). It is highly unlikely that the deletion of ACR is related to any present visible phenotype of NT. However, the reduction of ACR activity in sperm has been associated with male infertility (Mohsenian et al. 1982; Koukoulis et al. 1989), suggesting that the deletion of ACR may affect the fertility of NT and male 22q13.3 deletion patients.

We are currently searching for other genes deleted in NT. Subtelomeric regions of chromosomes are believed to be gene-rich (Saccone et al. 1992) and may have an average of one gene every 23.4 kb (Fields et al. 1993). We have cloned 130 kb from the 22q telomere; however, not all of this region will likely contain genes. FISH results indicate that cosmid P103 (fig. 1) cross-hybridizes with 2q13 and many chromosome ends and therefore contains subtelomeric repeats (fig. 4; Ning et al.

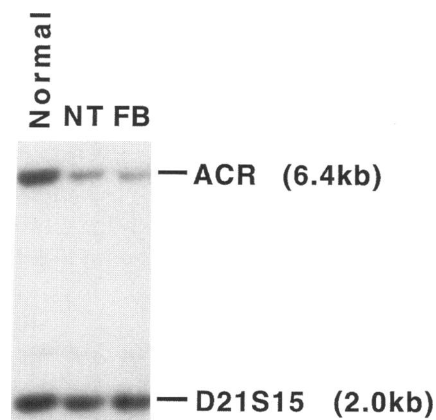

Figure 9 Densitometric analysis of ACR. The copy number of ACR was compared by densitometric analysis of *Taq*I-digested DNA from a normal individual, NT, and FB. The ACR gene probe was compared to a reference probe from chromosome 21 (D21S15). Both NT and FB indicate the presence of only one copy of ACR.

1996). Dosage analysis using the end fragment of N94H12 as a probe was unreliable, presumably because of the presence of subtelomeric repeats shared with other chromosomes. Although the NT microdeletion encompasses the terminal 130 kb of 22q, we estimate that at least the distal 60 kb (from the telomere to the end of N94H12) is probably rich in subtelomeric repeats. Therefore, only the proximal 70 kb may contain genes. Since the ACR locus has already been mapped to this region, we expect there are at least one to two genes in the microdeletion region yet to be mapped.

Acknowledgments

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References

Armour JAL, Jefferys AC (1991) STS for minisatellite MS607 (D22S163) Nucleic Acids Res 19:3158

Armour JAL, Povey S, Jeremiah S, Jeffreys AJ (1990) Systematic cloning of human minisatellites from ordered array charomid libraries. Genomics 8:501–512

Baba T, Watanabe K, Kashiwabara S-I, Arai Y (1989) Primary structure of human proacrosin deduced from its cDNA sequence. FEBS Lett 244:296–300

Collins JE, Cole CG, Smink LJ, Garrett CL, Leversha MA, Soderlund CA, Maslen GL, et al (1995) A high-density YAC contig map of human chromosome 22. Nature Suppl 377: 267–379

De Jong PJ, Yokobata K, Chen C, Lohman F, Pederson L, McNinch J, van Dilla M (1989) Human chromosome-specific partial digest libraries in κ and cosmid vectors (A2333). Cytogenet Cell Genet 51:985

Dumanski JP, Carlborn E, Collins VP, Nordenskjöld M, Emanuel BS, Budarf ML, McDermid HE, et al (1991) A map of 22 loci on human chromosome 22. Genomics 11: 709–719

Fields C, Adams MD, White O, Venter JC (1994) How many genes in human genome? Nat Genet 7:345–346

Flint J, Craddock CF, Villegas A, Bentley DP, Williams HJ, Galanello R, Cao A, et al (1994) Healing of broken human chromosomes by the addition of telomeric repeats. Am J Hum Genet 55:505–512

Flint J, Wilkie AOM, Buckle VJ, Winter RM, Holland AJ, McDermid HE (1995) The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet 9:132–140

Greger V, Woolf E, Lalande M (1993) Cloning of the breakpoints of a submicroscopic deletion in an Angleman syndrome patient. Hum Mol Genet 2:921–924

Herzog R, Holzmann K, Blin N (1990) A *Taq*I RFLP for the human arylsulfatase A gene. Nucleic Acids Res 18:6746

Huber I, Bitner-Glindzicz M, de Kok YJM, van der Maarel SM, Ishikawa-Brush Y, Monaco AP, Robinson D, et al (1994) X-linked mixed deafness (DFN3): cloning and characterization of the critical region allows the identification of novel microdeletions. Hum Mol Genet 3:1151–1154

Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA (1991) Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci USA 88:9051–9055

Klemn U, Müller-Esterl W, Engel W (1991) Acrosin, the peculiar sperm-specific serine protease. Hum Genet 87:635–641

Koukoulis GN, Vantman D, Dennison L, Banks SM, Sherins RJ (1989) Low acrosin activity in a subgroup of men with idiopathic infertility does not correlate with sperm density, percent motility, curvilinear velocity, or linearity. Fertil Steril 52:120–127

Kuwano A, Ledbetter SA, Dobyns WB, Emanuel BS, Ledbetter DH (1991) Detection of deletions and cryptic translocations in Miller-Dieker syndrome by in situ hybridization. Am J Hum Genet 49:707–714

Lamb J, Harris PC, Wilkie AOM, Wood WG, Dauwerse JHG, Higgs DR (1993) De novo truncation of chromosome 16p and healing with $(TTAGGG)_n$ in the α-thalassemia/mental retardation syndrome (ATR-16). Am J Hum Genet 52:668–676

Levy A, Demczuk S, Aurias A, Depètris D, Mattei M-G, Philip N (1995) Interstitial 22q11 microdeletion excluding the ADU breakpoint in a patient with DiGeorge syndrome. Hum Mol Genet 4:2417–2419

Liu DY, Baker HWG (1993) Inhibition of acrosin activity with a trypsin inhibitor blocks human sperm penetration of the zona pellucida. Biol Reprod 48:340–348

McDermid HE, Budarf ML, Emanuel BS (1993) Long-range restriction map of human chromosome 22q11-22q12 between the lambda immunoglobulin locus and the Ewing sarcoma breakpoint. Genomics 18:308–318

Mohsenian M, Syner FN, Moghissi KS (1982) A study of sperm acrosin in patients with unexplained infertility. Fertil Steril 37:223–229

Nesslinger NJ, Gorski JL, Kurczynski TW, Shapira SK, Siegel-Bartelt J, Dumanski JP, Cullen Jr RF, et al (1994) Clinical, cytogenetic, and molecular characterization of seven patients with deletions of chromosome 22q13.3. Am J Hum Genet 54:464–472

Ning Y, Rosenberg M, Biesecker LG, Ledbetter DH (1996) Isolation of the human chromosome 22q telomere and its application to detection of cryptic chromosomal abnormalities. Hum Genet 97:765–769

Saccone S, De Sario A, Della Valle G, Bernardi G (1992) The highest gene concentrations in the human genome are in telomeric bands of metaphase chromosomes. Proc Natl Acad Sci USA 89:4913–4917

Spinner NB, Eunpu DL, Schmickel RD, Zackai EH, McEldrew D, Bunin GR, McDermid HE, et al (1989) The role of cytological NOR variants in the etiology of trisomy 21. Am J Hum Genet 44:631–638

Stewart GD, Harris P, Galt J, Ferguson-Smith MA (1985)

Cloned DNA probes regionally mapped to human chromosome 21 and their use in determining the origin of nondisjunction. Nucleic Acids Res 13:4125–4132
Takano H, Yanagimachi R, Urch UA (1993) Evidence that acrosin activity is important for the development of fusibility of mammalian spermatozoa with the oolemma: inhibitor studies using the golden hamster. Zygote 1:79–91
Vazquez-Levin MH, Reventos J, Gordon JW (1992) Molecular cloning, sequencing and restriction mapping of genomic sequence encoding human proacrosin. Eur J Biochem 207: 23–26
Weier H-UG, Kleine H-D, Gray JW (1991) Labeling of the centromeric region on human chromosome 8 by in situ hybridization. Hum Genet 87:489–494
Wilkie AOM, Lamb J, Harris PC, Finney RD, Higgs DR (1990) A truncated human chromosome 16 associated with α-thalassemia is stabilized by addition of telomeric repeat $(TTAGGG)_n$. Nature 346:868–872