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# Deletion of chromosome 21 and normal intelligence: molecular definition of the lesion

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**Summary.** Application of a method for the fine structure analysis of unbalanced chromosomal rearrangements using quantitative Southern blot analysis has established that an individual of normal intelligence and largely normal appearance has a significant interstitial deletion of chromosome 21. Using high resolution cytogenetic analysis and molecular analysis with five single copy DNA sequences unique to chromosome 21 and a probe for human *SOD1* (CuZn, superoxide dismutase), we find that the deletion extends from the border of bands 21q11.1–11.2 and extends to the border of bands 21q21.2–q21.3. The latter border is established molecularly by the presence of two copies of *SOD1*, previously mapped to band 21q22.1, and of four single copy sequences known to be located distal to this region. The presence of *SOD1* was confirmed by enzyme dosage analysis. These findings demonstrate that deletion of close to 20,000 kb of autosomal material is compatible with normal intelligence. Further, they suggest that chromosome 21 may include a large region of relative developmental neutrality whose molecular basis may now be investigated. Because of the limits of even high resolution cytogenetic analysis, fine structure molecular analyses of this type will be necessary to reliably detect and define similar small chromosomal deletions or insertions. The molecular definition of such aneuploidy provides the basis for increasing the resolution of the human physical genetic map.

## Introduction

Human chromosome 21 has been a major focus for scientific investigation for two reasons. It is the smallest human chromosome, containing only about 1.7% of the human genome or about 54,000 kb (Korenberg and Engels 1978). Further, trisomy 21 results in Down syndrome, the most common human autosomal aneuploidy and a major cause of mental retardation and congenital heart disease (reviewed in Epstein 1986). To date, more than 100 single-copy random probes and greater than 20 expressed genes have been mapped to chromosome 21. In spite of this, very little is known about the molecular bases of primary nondisjunction, aneuploidy, and the biology of the associated defects.

In this report we describe the application of the molecular tools that are now available to the problems of human aneuploidy and chromosome organization. We describe our observation that deletion of close to 20,000 kb in as complex a eukaryote as man is compatible with the development of normal intelligence. We also discuss the implications of our findings for human chromosome mapping and for out understanding of chromosome organization.

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## Materials and methods

### *Cell lines*

Lymphoblastoid and fibroblast cell lines were established from a patient, DEL21JC, with an interstitial deletion of chromosome 21 (Korenberg et al. 1986). Human diploid DNA derived from male lymphocytes was used as control. The cell lines were grown in Earle's minimal essential medium with 10% fetal calf serum and L-glutamine (2 m*M*). High molecular weight DNA was prepared by standard techniques (Maniatis et al. 1982).

### *DNA probes*

All six DNA probes used are single-copy DNA sequences, five unique to human chromosome 21 and one unique to chromosome

17. *D21S46* (probe SF85) and *D21S48* (probe SF105) are single-copy DNA sequences previously mapped to the region 21pter–21q21.2 (Korenberg et al. 1987); *D21S47* (probe SF103) maps in the region 21q21.2–qter (Korenberg et al. 1987) and more specifically, proximal to 21q22.2 (J. R. Korenberg and T. Falik-Borenstein, unpublished). The gene *SOD1* for Cu/Zn superoxide dismutase has been previously mapped to the region 21q22.1–q22.2 (Sinet et al. 1976); *D21S39* (probe SF13A) and *D21S42* (probe SF43) are located in the region 21q21.2–qter (Korenberg et al. 1987), specifically to mid 21q22.3 (Korenberg et al. 1986; J. R. Korenberg and T. Falik-Borenstein, unpublished). Probe HHH202 for *D17S33* (Nakamura et al. 1987), used as the reference DNA sequence, was a generous gift from Y. Nakamura. Probes were isolated by preparative gel electrophoresis and labeled with 32P-dCTP by oligonucleotide priming to a specific activity of $1–2 \times 10^9$ cpm/μg according to the manufacturer's specifications (Amersham Multiprime Kit).

### *Southern blot hybridization*

For each determination, two separate agarose gels were run. Each gel consisted of six lanes each of patient and control DNAs arranged in alternating lanes. A 4-μg sample per lane of each DNA was digested with *Eco*RI according to the manufacturer's directions (Bethesda Research Laboratories), subjected to electrophoresis through 1.2% agarose gels in 1 × TAE (40 m*M* Tris-acetate, 20 m*M* Na Acetate, 1.6 m*M* EDTA, pH 7.5) buffer and, transferred to nylon membranes (Nytran, Amersham) by standard techniques (Southern 1975). Membranes were dried and crosslinked at a distance of 20 cm with short wavelength ultraviolet radiation (LUX box). After prehybridization, membranes were hybridized simultaneously with probes for four or five DNA sequences unique to chromosome 21 in addition to the chromosome 17 reference DNA sequence, *HHH202*. One membrane was hybridized to probes for *D21S16, D21S46, D21S39,* and *D21S42*. The second was hybridized to probes for *D21S13, D21S46, D21S48, D21S47,* and *SOD1*. Prehybridization and hybridization were carried out at 65°C in buffer containing 7% SDS, 1% bovine serum albumin, 1 m*M* EDTA, and 0.5 *M* sodium phosphate, pH 7.0. After overnight hybridization, membranes were washed (final conditions of 0.1 × SSC (sodium citrate for 30 min at 55°C). The membranes were exposed to Kodak XAR-5 X-ray film with one intensifying screen. Multiple exposures were obtained such that the densities of all bands fell into the most linear part of the X-ray film as judged by comparison to a standard scale. The standard scale was generated by densitometric analysis of XAR-5 film exposed with a National Bureau of Standards penetrometer (data not shown).

### *Data analysis*

Autoradiograms were scanned with a Helena EDC densitometer, and the area under the curves integrated by computerized linear interpolation. The gene copy number of a given DNA probe in the deletion cell line was calculated by normalization of its hybridization signal with that of the reference probe HHH202 and the control DNA as follows. The densitometric hybridization signal for each test DNA sequence was divided by that for the reference sequence (HHH202) in each lane of DNA from cell line DEL21JC and from the control. The resulting ratio from each lane of DEL21JC DNA was then divided by the ratio obtained from the neighboring control lane DNA to generate a standardized ratio. This resulted in six standardized ratios for each test DNA sequence, which were averaged to yield the ratio shown in Table 1. These were then analyzed by single tailed *t*-test for significant difference from 0.5, consistent with one copy of the test probe and from 1.0, consistent with two copies of the test probe in DNA from the deletion cell line. All data analyses utilized log transformations of the raw densitometric data to approximate better the assumption of normality inherent in the statistical analysis.

### *Superoxide dismutase-1 (SOD-1) assays*

The control fibroblast strains used have been previously described (Weil and Epstein 1979). They were obtained from autopsy specimens of fetuses and were stored at −160°C prior to use. Strains 255 (diploid) and 256 (trisomic) were derived from a pair of unrelated aborted fetuses matched for gestational age. Cells were grown in Dulbecco's modified Eagle medium (Gibco, Grand Island, N. Y.) containing 10% fetal calf serum, gentamycin (0.1 ml/100 ml) and glutamine (1 ml/100 ml) in 5% $CO_2$ at 37°C. The cells were harvested at 70%–100% confluence and were washed three times in saline solution.

SOD-1 was assayed for its ability to inhibit the superoxide-mediated reduction of nitroblue tetrazolium (NBLT) according to the method of Salin and McCord (1974) and Fried (1975). The protein concentration was assayed according to the method by Lowry et al. (1951).

### *Cytogenetic analysis*

Initial karyotypes and high resolution chromosome G-banding were produced using standard techniques of peripheral blood leukocytes. G-banding was performed on chromosomes from fibroblasts as previously described (Fraser et al. 1987).

## Results

### *Clinical case*

The patient was an 8-year-old boy who initially presented with small testes. Figure 1 shows photographs of the patient and his normal parents. His father was 45 and mother 24 years old at the time of his birth. Pregnancy, labor and delivery were normal. Birth weight was 5 lb 3 oz. Early developmental milestones were normal except for delayed walking (18 months), a left inguinal hernia, and febrile seizures until the age of 4 years. The patient is in age-appropriate classes in school. Family history was negative for mental retardation. Both parents are normal, the father having held steady jobs as a laborer. The mother is described as quite intelligent, having worked to support her family while a teenager in Hong Kong and taken English classes after her move to the west, and as a good wife and mother. However, of the mother's three sisters, one has three healthy daughters, a 6-month miscarriage, and a stillborn, and a second sister had a son who died on day 1 of life. A third sister has not had children yet. The father has two normal sisters. The patient has a 3-year-old brother who is normal.

Physical examination revealed a normal-appearing boy who did not look like his parents or his normal younger brother. He had a slender habitus with a height of 124.5 cm (25%), a span of 126.5 cm (normal), a weight of 45 lb (10%), and a head circumference of 53.5 cm (just above 50%). His head was dolichocephalic, with a normal posterior hair whorl but with a rather high forehead with an upswept hairline. He had downslanting palpebral fissures and borderline telecanthus with an inner canthal distance of 6.2 cm (97%) and outer canthal distance of 11.9 cm. The palate was high and arched. The ears were of normal form and size (right 5.2 cm and left,

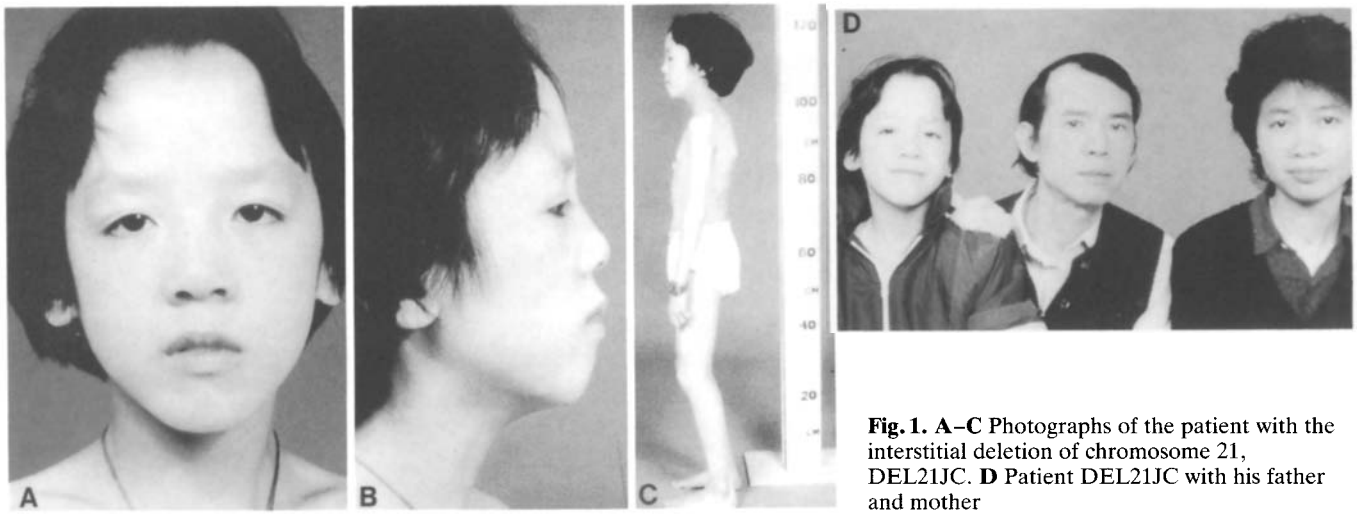


**Fig. 1. A–C** Photographs of the patient with the interstitial deletion of chromosome 21, DEL21JC. **D** Patient DEL21JC with his father and mother

5.3 cm, 10%). His right hand (13.0 cm long; palm 8.3 cm long, 6.2 cm in breadth) was somewhat smaller than his left (14.4 cm long; palm 9.3 cm long, 6.2 cm in breadth) and he had bilateral fifth finger clinodactyly. The feet were small (3%), measuring 19.1 cm right and 19.3 cm left.

The testes were small (1.5 cm × 1.9 cm at 7 years 8 months). The phallus was normal. Patchy eczema was noted involving the flexion surfaces of the elbow, wrist, and finger joints. The fingernails were ridged and pitted. Neurological evaluation was normal except for motor function. Tight hamstrings were noted resulting in abnormal posture with slight flexion at the hips and knees. Neuropsychological testing was performed at 7 years 11 months. Formal motor testing using the Bruininks-Oseretsky Test of Motor Proficiency revealed a minor deficit in fine motor and slightly more pronounced deficit in gross motor skills. Deficits included poor balance, running speed, and agility, and bilateral coordination and upper limb coordination were below average. Fine motor performance was notable for normal visual motor control (1 year above age performance on the Motor Free Visual Perception Test) but below average response speed and below average upper limb speed and dexterity. Visual acuity and hearing were normal.

Extensive neuropsychological testing revealed an intellectually capable child with standardized test results within age-appropriate limits on all tests given. The results follow. The Beery Developmental Test Visual Motor Integration was normal. Scaled scores on the Wechsler Intelligence Scale for Children (WISC-R) were within the average range (8–12) with highest scores in similarities (11), arithmetic (11), and comprehension (11), a single 7 in object assembly, and all other subtests 9–11. Verbal performance and full scale I.Q. were all in the average range. However, this was felt to be a minimum estimate of his intellectual potential because the test is normalized on children who are native speakers of English whereas the patient had spoken English for less than 3 years and continued to speak only Cantonese at home. The Peabody Picture Vocabulary Test of receptive English vocabulary and the Test of Language Development – Primary placed his performance in the low average range. However, his perceptual/organizational language ability was normal as tested by the Illinois Test of Psycholinguistic Abilities. Further, his verbal reasoning skills were 1.5 years above age using the Verbal Absurdities subtest of the Detroit Test of Learning Apitude. The Durrell analysis of reading (Spelling subtest) revealed function at grade 3, one grade ahead of his class. All testing revealed excellent cooperation and motivation with a meticulous style that, along with his slight motor deficits, contributed to slow handwriting and reticence to participate in sports.

Endocrinologic evaluation initially done for concerns about short stature at age 5.5 years was normal but revealed small testes. Further testing at age 8 years was also normal, including thyroid, blood electrolytes, complete blood count, and tests of the hypothalamic pituitary gonadal axis.

The EEG was minimally dysrhythmic, perhaps more so bifrontally, but had no epileptiform discharges.

In summary, the patient was an essentially normal-appearing boy of normal intelligence who had a history of a hernia, mild body asymmetry, small testes, and minor physical anomalies accompanied by mild motor system dysfunction.

*Cytogenetic analysis*

Initial cytogenetic analysis done as a part of the endocrine evaluation revealed a probable interstitial deletion of the long arm of chromosome 21. High resolution banding (Fig. 2) and complete analysis of 50 cells demonstrated that 21q11.2 and most of the prominent proximal G band, q21, was missing in the deleted chromosome. A small dark band that is seen close to the centromere most likely consists of the entirety of band 21q21.3. Therefore, the proximal margin of the deleted region is likely at the border of 21q11.1–q11.2. The distal margin of the deleted region is most likely at the border of 21q21.2–q21.3. The distal bands q22.1, q22.2, and q22.3 appear normal.

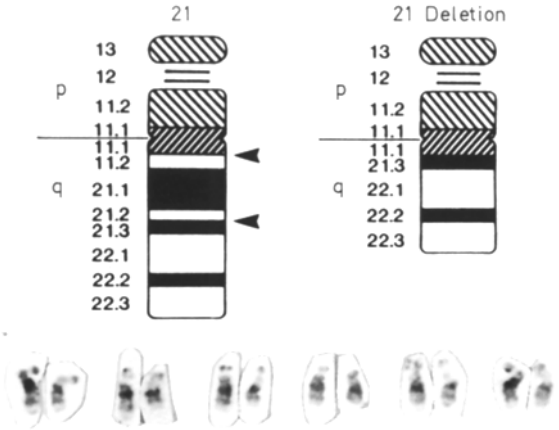


**Fig. 2.** Ideogram and photographs of the normal *(left)* and deleted *(right)* chromosomes 21 in patient DEL21JC. The *arrow heads* shown in the ideogram of the normal chromosome mark the positions of the deletion breakpoints. Each of the six pairs of chromosomes shows normal *(left)* and deleted *(right)* chromosomes 21 from patient DEL21JC. The remainder of the karyotype was normal

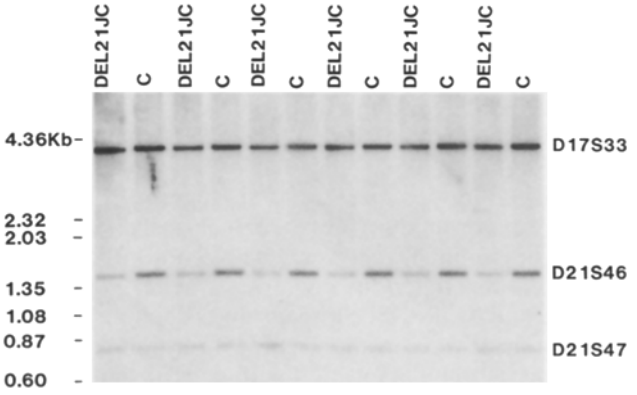


**Fig. 3.** Autoradiogram of representative Southern blot used to map chromosome 21 DNA sequences. Replicate lanes of DNAs from the patient *(DEL21JC)* and control *(C)* are indicated. *D17S33* is the chromosome 17 reference sequence; all others are unique to chromosome 21

**Table 1.** Chromosome 21 DNA sequence copy number in DEL21JC

| DNA sequence | Copy no. | Ratio[a] | 95% Confidence limits | Test for significance | |
|---|---|---|---|---|---|
| | | | | 0.5, $P=$ | 1.0, $P=$ |
| *D21S46* | 1 | 0.42 | (0.34, 0.54) | 0.15 | 0.00 |
| *D21S48* | 1 | 0.37 | (0.18, 0.75) | 0.32 | 0.02 |
| *D21S47* | 2 | 1.29 | (0.75, 2.22) | 0.01 | 0.28 |
| *SOD1*[b] | 2 | 1.00 | (0.56, 1.79) | 0.04 | 0.99 |
| *D21S39* | 2 | 1.17 | (0.91, 1.50) | 0.00 | 0.18 |
| *D21S43* | 2 | 1.14 | (0.97, 1.35) | 0.00 | 0.09 |

[a] The ratio is calculated with reference to chromosome 17 DNA sequence, *HHH202*. Each ratio is based on six paired measurements of densitometric signals of the reference and test DNA sequences determined in DNAs from the cell line *DEL21JC* and from control

[b] Reference DNA sequence was *D21S39*, three lanes each of DEL21JC and control

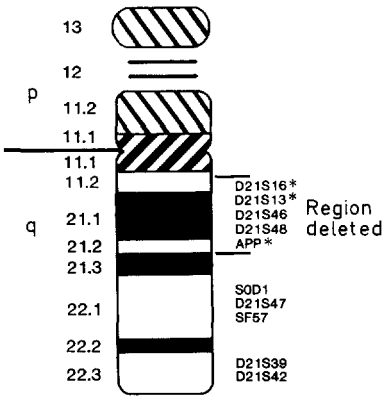


**Fig. 4.** Chromosome 21 physical map of DNA sequences defining the deleted region in DEL21JC. Data for *starred* sequences are presented elsewhere [Korenberg et al. 1989; Pulst et al. (in press)]

Chromosomal analysis of the patient's fibroblasts revealed the same interstitial deletion as in lymphocytes with no evidence of mosaicism. The chromosomes of the parents and brother were normal.

*Molecular analysis*

The presence of such a large chromosome deletion in a relatively normal individual caused concern that the apparently deleted material was in fact located elsewhere in the genome, but below the limit of cytogenetic detection. Investigation of this possibility utilized chromosome-21-specific molecular probes located within and outside the deleted region. To date, there are only two cloned human expressed genes mapping in this approximate region, that for SOD1, and for APP (the amyloid precursor protein). Therefore, additional single-copy DNA sequences were utilized. Previously, 14 chromosome-21-specific random single-copy probes were isolated and used in conjunction with partially aneuploid human cell lines to construct a detailed physical map of chromosome 21. With 15 aneuploid cell strains (Korenberg et al. 1986, 1987, 1989; Pulst et al. 1989; T. Falik-Borenstein and J. R. Korenberg, unpublished) and one somatic cell hybrid, we may unambiguously assign any chromosome-21-specific DNA sequence to one of 16 regions of chromosome 21. Two (for *D21S46* and *D21S48*) may be located in the region of the proximal breakpoint (q11–q21) and one, for *D21S47*, in addition to the gene for *SOD-1*, is located in the region of the distal breakpoint, bands q21.2–q22.1. Further, the two probes for *D21S39* and *D21S42*, located in band q22.3, define a region likely present in DEL21JC.

We first defined the distal breakpoint using SOD1, *D21S47, D21S39*, and *D21S42*. A representative autoradiogram is shown in Fig. 3. A darker autoradiographic exposure was used for the analysis of *D21S47*.

Table 1 shows the results for *SOD1* and similar analyses with five other probes. Figure 4 illustrates the results from this study combined with other results showing that DEL21JC was deleted for the gene *APP* (Korenberg et al. 1989) and DNA sequences for *D21S16* and *D21S13* (Pulst et al., in press). For *SOD1*, the normalized ratio

**Table 2.** SOD-1 activity in fibroblasts. The mean SOD-1 activity (units/mg protein) in fibroblasts from the patient DEL21JC compared to diploid and trisomy 21 fibroblasts, and the relative SOD-1 activity of the deletion 21 and the trisomy 21 fibroblasts versus the diploid fibroblasts. Each mean was the result of six replicate enzyme assays. The mean activity in diploid vs in DEL21JC fibroblasts was not different by *t*-test ($P = 0.001$)

| | SOD-1 units/mg protein (SE) | Relative activity to diploid |
|---|---|---|
| Diploid | 9.30 (0.80) | 1.0 |
| DEL21JC | 8.55 (0.45) | 0.92 |
| Trisomy | 15.61 (1.62) | 1.68 |

was consistent with a copy number of two indicating that *SOD1* was retained in the deleted 21. This finding was confirmed by analysis of enzyme activity (Table 2). These results place the distal breakpoint within or proximal to band q22.1. Further analysis established that the DNA sequence recognized by the probe for *D21S47* was also retained in two copies. As expected from the cytogenetic analysis, DNA sequences recognized by the probes for *D21S39* and *D21S42*, were also found to be present in two copies.

The proximal breakpoint was next defined. DNA sequences recognized by the probes for *D21S48* and *D21S46*, previously mapped in the region overlapping the proximal breakpoint were missing in the deleted chromosome. These probes, then, in addition to those shown in Fig. 4, served to establish that chromosomal material was indeed missing from the genome in the region indicated cytogenetically.

## Discussion

From our molecular analysis, we conclude that the patient has a deletion of part of chromosome 21. We have further defined the position of the proximal break as at the border of 21q11.1–q11.2, and the position of the distal break as the border of 21q21.2–q21.3. This leads to a deletion of bands 21q11.2–q21.2. This placement of the deletion breakpoints is now slightly proximal to the cytogenetic analysis of DEL21JC previously reported (Korenberg et al. 1989). This change in breakpoint assignment results from the preparation and analysis of longer chromosomes with consequently higher cytogenetic resolution than was achieved in the previous analysis. Moreover, further molecular analyses of DEL21JC have established DNA sequences for *D21S16* and *D21S13*, thought to map close to the centromere, as the most proximal deleted regions in DEL21JC (Pulst et al., in press; Gardiner et al. 1990). These findings suggest that the proximal breakpoint may be located even more centromeric than indicated by current cytogenetic analysis.

Further molecular analyses are necessary to reveal whether the deleted chromosome 21 arose in the father, as might be suggested by his advanced age at conception. If so, molecular definition of the breakpoints may elucidate the potential mutational mechanisms in older males. The deleted region appears to include most of band q21. However, the true size of the deleted region is best estimated from a knowledge of the pulsed-field map of chromosome 21 coupled with the molecular analysis of a hamster-human hybrid cell line constructed from DEL21JC (Gardiner et al. 1990). Recent pulsed-field gel analysis of this hybrid indicates that the deletion region may include almost 20,000 kb (Gardiner et al. 1990). This represents the summation of all *Not*I fragments mapping within the deleted region extending from *D21S16* through *D21S54*.

Our findings contradict the strongly supported assumption (Schinzel 1984) that deletions of nonpolymorphic chromosomal material are associated with grave phenotypic effects. The partial deletion of chromosome 21 observed in DEL21JC includes the region from band q11 to the border of bands q22.2–22.3, a region of close to 20,000 kb. This case joins a case of partial deletion of 5p14, a region of about 4,000–6,000 kb (Overhauser et al. 1986) as molecularly defining the only regions of the human genome in which hemizygosity is known to be compatible with normality, as in the 5p14 deletion, or a close-to-normal phenotype, as seen in DEL21JC. Deletion of a third genomic region, 13q21, may also be compatible with normal phenotype (Couturier et al. 1985) and awaits molecular confirmation. Finally, it is of interest that the deletion in our patient closely overlaps a region associated with a normal phenotype when trisomic (Daniel 1979).

What distinguishes these genomic regions from those that have severe phenotypic abnormalities when hemizygous? They could have fewer genes, genes with broader limits of normal function, or perhaps genes whose expression could be dosage compensated. Phenomenologically, they share the chromosomal features long noted by cytogeneticists to be associated with genetic "inactivity" (reviewed in Korenberg and Rykowski 1988; Korenberg et al. 1978; Holmquist 1989). They are all G bands that replicate their DNA very late in the DNA synthetic period. At present, only the gene map of chromosome 21 is dense enough to evaluate the regional genetic content of these bands. From this analysis, we find that the small number of genes in the region may explain the mild phenotype. That is, although the deleted region of DEL21JC includes DNA fragments equaling almost one-half of the pulsed-field map (Gardiner et al. 1990), it includes only 1 of the 14 cloned genes of known function now mapped on chromosome 21, the gene for the amyloid precursor protein, APP. In contrast, deletion of band 21q22, a region of similar size, includes 13 of the 14 cloned genes on chromosome 21, and is accompanied by devastating phenotypic consequences (see for example, Yamamoto et al. 1979). Further, individuals carrying deletions extending from 21q21 and including the *SOD1* region of q22.1 are also seriously affected. Finally, there are two deletions closely overlapping that of DEL21JC cytogenetically (Reynolds et al. 1985; Roland et al. 1990). The first case (Reynolds et al. 1985) is associated with multiple congenital abnormalities similar to those seen in the monosomy 21 cases. However, molecular analysis of a somatic cell hybrid that contains the rearranged 21 from

**Table 3.** Phenotypic features of DEL21JC and other chromosome 21 deletion syndromes

| | Cases of partial monosomy 21[a] | | Present case DEL21JC[b] | Deletion 21q22–qter[c] |
|---|---|---|---|---|
| | 1 | 2 | | |
| Severe mental retardation | 5/5 | 5/5 | – | + |
| Hypertonia | 5/5 | | + | + |
| Microcephaly | 5/5 | | – | + |
| *Eyes* | | | | |
| Antimongoloid slant | 6/6 | 4/5 | + | |
| Microphthalmia | | | – | + |
| *Face* | | | | |
| Prominent nasal bridge | 6/6 | | – | + |
| Broad base of nose | 5/5 | | + | |
| Large nose | 4/5 | | – | |
| Micrognathia | 6/6 | 5/5 | – | + |
| High arched palate | 3/4 | 2/2 | + | |
| Cleft lip/palate | 2/3 | | – | |
| Ears large | 5/5 | 5/5 | + | |
| Ears malformed (large lobes) | | | + | + |
| "Fish-shaped" mouth | 5/5 | | + | |
| *Extremities* | | | | |
| Nail anomalies | 2/5 | | Pits | |
| Skeletal malformation | 5/5 | 3/4 | | |
| Flexion deformity Malposition of fingers & toes | | 5/5 | + | + |
| *Others* | | | | |
| Growth retardation | 5/5 | 5/5 | – | + |
| Hypospadias | 3/4 | | – | |
| Inguinal hernia | 2/5 | | + | |
| Small testes | – | | + | |
| Cryptorchidism | 2/4 | | – | |
| Pyloric stenosis | 3/5 | | – | |
| Congenital heart diesease | | | | |
| Eczema | | | + | + |
| Thrombocytopenia | 3/4 | | – | |
| Eosinophilia | 2/4 | | – | |
| Hypogammaglobulinemia | 2/3 | | | |

[a] Number of cases affected versus number of cases described with particular features as summarized in [1] Warren and Rimoin (1970) and [2] Abeliovich et al. (1979)
[b] Presence (+) or absence (–) of feature is noted in single case reports
[c] Yamamoto et al. (1979)

this case, revealed that the chromosome 21 regions deleted include parts of band 21q22.3 in addition to the region defined cytogenetically (Gardiner et al. 1990). Further molecular analysis of the patient's DNA is necessary to determine whether additional chromosome 21 regions were deleted from the patient's abnormal chromosome during the formation of the hybrid. The second case of deletion similar to that of DEL21JC (Roland et al. 1990) is associated with some cognitive impairment. Genes in the nonoverlap region may thus be important in determining the degree of mental impairment, although other genetic and epigenetic factors may also contribute.

We suggest that the G band, 21q21, with few human genes, late replication, and few serious effects when deleted, may represent the extreme case of the more general genetic organization of human chromosomes. The genesis and evolutionary stability of this organization is unknown, but it appears to be molecularly characterized by few expressed genes, DNA of relatively high adenine and thymine content, a predominance of long, interspersed, repeated DNA sequences (LINES) and a relative paucity of short interspersed repeated sequences (SINES; Singer 1982; Goldman et al. 1984; Bernardi et al. 1985; Korenberg and Rykowski 1988; Gardiner et al. 1990). Molecular study of the region defined by this deletion is in progress and may provide further clues to the significance of this overall genomic organization and its effects on human development.

It is of interest to determine which phenotypic features may be due to genes located in the deleted region. Therefore, it is notable that this patient is missing the gene for APP (Korenberg et al. 1989). Deletion of *APP* is therefore compatible with normal intelligence. The DNA sequences most closely linked to the chromosome 21 locus for familial Alzheimer disease (FAD), *D21S16*, and *D21S13*, are also deleted (Pulst et al., in press). This suggests that DEL21JC may be missing the FAD gene itself. Table 3 summarizes the phenotypic effects seen in cases of partial and possibly complete monosomy 21. Our patient shows some of the facial (downslanting palpebral fissures, downslanting mouth, large ears), skeletal, and other features, including the inguinal hernia. In addition, he has small testes and some fine and gross motor dyscoordination. Therefore, genes affecting the shared features may be located in the deleted region. It is also of interest that the deletion in our patient does not overlap the molecular region responsible for the classical phenotypic features of Down syndrome bands q22.1–qter (Korenberg et al. 1990; McCormick et al. 1989; Rahmani et al. 1989). Therefore, the downslanting palpebral fissures of the monosomy 21 seen in our patient cannot be due to the same genes causing the upslanting palpebral fissures of Down syndrome. This is molecular evidence against the type-contretype theory of aneuploid phenotype (reviewed in Epstein 1986).

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