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Molecular Definition of a Region of Chromosome 21 That Causes Features of the Down Syndrome Phenotype

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Summary

Down syndrome (DS) is a major cause of mental retardation and heart disease. Although it is usually caused by the presence of an extra chromosome 21, a subset of the diagnostic features may be caused by the presence of only band 21q22. We now present evidence that significantly narrows the chromosomal region responsible for several of the phenotypic features of DS. We report a molecular and cytogenetic analysis of a three-generation family containing four individuals with clinical DS as manifested by the characteristic facial appearance, endocardial cushion defect, mental retardation, and probably dermatoglyphic changes. Autoradiograms of quantitative Southern blots of DNAs from two affected sisters, their carrier father, and a normal control were analyzed after hybridization with two to six unique DNA sequences regionally mapped on chromosome 21. These include cDNA probes for the genes for CuZn-superoxide dismutase (*SOD1*) mapping in 21q22.1 and for the amyloid precursor protein (*APP*) mapping in 21q11.2-21.05, in addition to six probes for single-copy sequences: *D21S46* in 21q11.2-21.05, *D21S47* and *SF57* in 21q22.1-22.3, and *D21S39, D21S42,* and *D21S43* in 21q22.3. All sequences located in 21q22.3 were present in three copies in the affected individuals, whereas those located proximal to this region were present in only two copies. In the carrier father, all DNA sequences were present in only two copies. Cytogenetic analysis of affected individuals employing R and G banding of prometaphase preparations combined with in situ hybridization revealed a translocation of the region from very distal 2lq22.l to 21qter to chromosome 4q. Except for a possible phenotypic contribution from the deletion of chromosome band 4q35, these data provide a molecular definition of the minimal region of chromosome 21 which, when duplicated, generates the facial features, heart defect, a component of the mental retardation, and probably several of the dermatoglyphic changes of DS. This region may include parts of bands 21q22.2 and 21q22.3, but it must exclude the genes *S0D1* and *APP* and most of band 21q22.1, specifically the region defined by *S0D1*, *SF57* and *D21S47*.

Introduction

Down syndrome (DS), a major cause of mental retardation and heart disease, is usually caused by the presence of an extra chromosome 21. In some cases, however, it is caused by the presence of only the distal half of chromosome 21, band q22 (Epstein 1986). This band has been called the "Down syndrome region," as defined by the presence of a subset of the major phenotypic features of the syndrome. These features include mental retardation, congenital heart disease, the characteristic facial appearance, and probably the hand anomalies and dermatoglyphic changes. Furthermore, there is some cytogenetic evidence that a duplication of only

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the subband 21q22.1 and perhaps 21q22.2 may generate most of these abnormalities (Poissonnier et al. 1976; reviewed in Epstein [1986]). The gene *SOD1,* for CuZn-superoxide dismutase, is the only cloned gene located in this region. Therefore, although its contribution to the DS phenotype is still unknown, it had assumed the role of being a "molecular marker" of DS.

Recently, we and others have described rare individuals with a "partial" DS phenotype who have an apparently normal karyotype but harbor a small amount of extra material from chromosome 21 (Huret et al. 1987; Korenberg et al. 1988*b*). Molecular analysis of these individuals has provided the opportunity to define the region of the chromosome responsible for the phenotypic features of Down syndrome. The conceptual basis for this approach has been extensively discussed (Epstein 1986, 1990). We now report a Japanese family with a chromosome 21 duplication (or "partial" trisomy 21) exhibiting many of the characteristic physical features of DS. These include the characteristic DS facies, congenital heart disease of the endocardial cushion type, and hand anomalies, in addition to other minor features. Mental retardation was also present. From our analysis of this family, we have significantly narrowed the region responsible for the listed physical components of the DS phenotype by first establishing the existence of partial trisomy 21, then by excluding the gene *APP* (amyloid precursor protein) from the trisomic region, and, finally, by excluding *SOD1* from the region of band 21q22.1 involved in the generation of the DS features (Korenberg et al. 1988*a,* 1988*b*). Moreover, by combining molecular characterization with in situ hybridization and high-resolution cytogenetic analysis, we have been able to narrow the responsible region of band 21q22.1 to such a degree that it is below the limit of cytogenetic detection. Finally, our findings exclude all known cloned genes and some random DNA sequences mapping in band 21q22.1 from the region of duplication defined by this family and establish the basis for specifying molecular markers for the region of chromosome 21 responsible for the phenotypic features of DS listed above.

Material and Methods

Molecular

The DNA sequence probes used in these investigations are all unique to human chromosome 21. FB68L is a cDNA probe corresponding to the 3′ end of the APP locus (Tanzi et al. 1987). The cDNA probe for *SOD1* was a gift from Y. Groner. Probes for *D21S39, D21S42, D21S43, D21S47,* and *SF57* are single-copy DNA sequences unique to chromosome 21 (Korenberg et al. 1987). Probes were isolated as DNA fragments by preparative gel electrophoresis and labeled by oligonucleotide priming to a specific activity of $2–5 \times 10^9$ cpm/microgram according to the manufacturer's specifications (Amersham, Arlington Heights, IL).

DNA was isolated (Korenberg et al. 1987) from lymphoblastoid cell lines derived from DS family members and from normal unrelated placenta used as a control. Each DNA was digested with the restriction enzyme *Eco*RI (Bethesda Research Laboratories) according to the manufacturer's directions, size separated by 1% agarose gel electrophoresis, and transferred to nylon membranes (Hybond; Amersham) by standard techniques (Maniatis et al. 1982). Multiple gels were run, each containing at least three lanes of DNA from a given family member and two to three lanes of control DNA. The nylon membranes were then treated as previously described (Korenberg et al. 1989) and hybridized simultaneously with two to five of the DNA sequences described above. The hybridization probe mix always included a probe for *D21S46* as an internal chromosome 21 reference probe. This sequence has been previously mapped to band 21q21 or proximal (Korenberg et al. 1987) and refined (Korenberg et al. 1986, 1989, 1990) to band 21q11.2-21.05. Both assignments were quite proximal to the cytogenetically defined duplicated region and, therefore, likely to be present in two copies in both DS patients and controls. This has been confirmed both by the present analysis and by subsequent experimental comparison to a chromosome 17 single-copy DNA sequence (data not shown).

Autoradiographs were generated by exposure of Kodak XAR film for the time required to bring the band signals to an approximately linear range of the film. This range was determined using a National Bureau of Standards penetrometer (data not shown). The results from each autoradiogram resulting from a single gel were analyzed separately to generate independent estimates of DNA sequence copy number, and the data from at least two autoradiograms were averaged and statistically analyzed.

Each lane of the autoradiogram was scanned by a Helena EDC densitometer, and the areas under each peak were integrated by computer. The copy number of each sequence is calculated as follows. First, the ratio of the density of the unknown band to that of band *D21S46* (the reference sequence) in the patient's DNA is calculated. Second, the same ratio is calculated in

the placental control DNA. Third, the standardized ratio, which represents the relative copy number of the unknown and reference sequences, is then calculated by dividing the ratio in the patient's DNA by the ratio in the control DNA. This is done separately for each lane of DNA, and the results are averaged for each patient across all lanes and statistically analyzed by the one-tailed *t*-test.

Cytogenetics and In Situ Hybridization

The probe for DNA sequence *D21S39* was radiolabeled by nick translation as described in Magenis et al. (1985) to a specific activity of 3.75×10^7 dpm/μg using [^{3}H]dTTP (65 Ci/mmol) and [^{3}H]dCTP (60 Ci/mmol; Amersham).

Lymphoblastoid cells from the father (III-1) and affected individuals IV-1 and IV-2 were synchronized with amethopterin to obtain an adequate number of cells in early metaphase (Yunis and Chandler 1977). Chromosome analyses were performed using trypsin G-banding and fluorescent R-banding with chromomycin A_3/distamycin A (Schweizer 1980).

In situ hybridization, silver-grain analysis, and photography were done as described elsewhere (Magenis et al. 1985). After development, the slides were R-banded using a modification (Magenis et al. 1985) of the technique of Schweizer (1980).

Results

Clinical

The pedigree of the three-generation Japanese family in which DS is segregating and photographs of the four affected individuals are shown in figures 1 and 2,

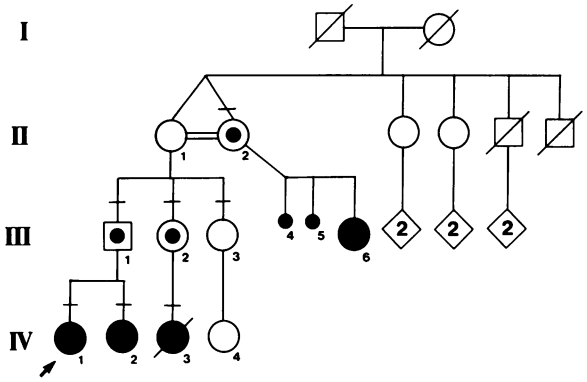


Figure 1 Pedigree of family with Down syndrome. ♂/□ indicates individuals studied; ⊙/⊡ indicates balanced translocation t(4:21) carriers; ●/■ indicates Down syndrome patients.

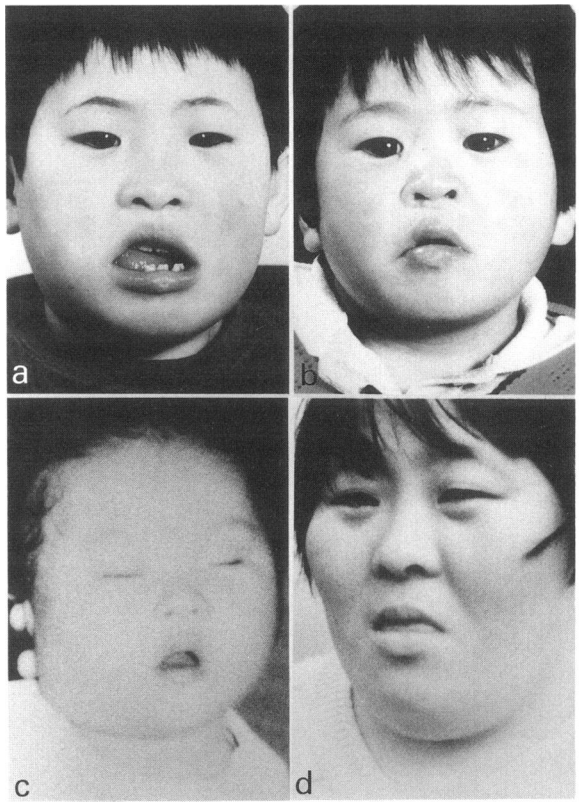


Figure 2 Photographs of the four affected individuals. *a*, Patient IV-1 at 6 1/2 years; *b*, patient IV-2 at age 2 years; *c*, patient IV-3 at 1 mo; *d*, patient III-6 at 30 years.

respectively, and the clinical findings are summarized in table 1. Taken together, the four patients exhibit the characteristic facial features of DS and are retarded; two have congenital heart disease, and two have most of the characteristic dermatoglyphic findings. One of the four had a history of hypotonia.

Cytogenetics and Molecular Analyses

Initial high-resolution G-banding analysis of the chromosomes of IV-1 and IV-2 revealed an apparently normal karyotype, with two normal chromosomes 21. However, high-resolution cytogenetic analysis of the father (III-1) of patients IV-1 and IV-2, and of the mothers of patients IV-3 and III-6 (fig. 1), revealed an abnormality of chromosome 21 in the region 21q22.1-22.2 in a karyotype that otherwise appeared normal.

To define the genetic content of the potentially duplicated region of chromosome 21 in these patients and its familial transmission, we performed a molecular analysis of the DNA of IV-1 and IV-2 and of their clini-

Table 1

Features of Affected Individuals

	IV-1	IV-2	IV-3	III-6
Age at evaluation (years)	6.5	2	Death at .5	34
Microcephaly	−0.9 SD	−1.8 SD		
Flat occiput	−			
Hypotonia	−	−	+	
Lax ligaments	+	+		
Poor suck at birth	+			
Delayed milestones	Motor speech	Speech		
Short stature	−1.8 SD	−1.4 SD		
Failure to thrive	+	−		
IQ	42 (at 6 years)		Retarded	
Dementia	−	−		−
Flat facies	+	+	+	+
Upslanted palpebral fissures	+	+	+	+
Epicanthic folds	+	+	+	+
Telecanthus	+	+		
Flat nasal bridge	+	+	+	+
Dentition abnormal	+			
Macroglossia	+	+		
High palate	−			
Open mouth	+			
Ears cupped or low set	−	−		
Short neck	−			
Heart disease	ASD		ECD	
Broad hands	+			
Brachydactyly	+	+		
Fifth-finger clinodactyly	+			
Dermatoglyphics:				
Finger pads	10 UL	3 UL, 7 W		
Third interdigital loops	+	+		
Hypothenar patterns	Bilateral	Bilateral		
Distal axial triradius (t")	+(left)	Bilateral		
Single transverse palmar crease	Bridged left crease			
Hallucal fibular loop	Bilateral	Bilateral		
First interdigital loop	Bilateral	Bilateral		
Sole open field patterns	Bilateral	Bilateral		

NOTE.—Presence (+) or absence (−) of each feature is noted as indicated. Features for which no information is available are indicated by blank spaces. ASD = atrial septal defect; ECD = endocardial cushion defect; UL = ulnar loop; W = whorl.

cally normal father. For this purpose we used a fine structure molecular map of chromosome 21 constructed by isolating 13 single-copy DNA sequences unique to chromosome 21 and establishing their regional location using a somatic cell hybrid and then a panel of DNAs from cells aneuploid for only parts of chromosome 21 (Korenberg et al. 1986, 1987, 1989; J. R. Korenberg and T. Falik-Borenstein, unpublished data).

Figure 3 shows four segments of the map, which are represented by eight DNA sequences. To analyze the extent of the suspected chromosome 21 duplication in the family being studied, we investigated the copy number of each of these sequences. *D21S46* and *APP* are located in 21q11.2-q21.05, outside the previously defined classical DS phenotype region, and *APP* is located below *D21S46* (Korenberg et al. 1987, 1989; Patterson

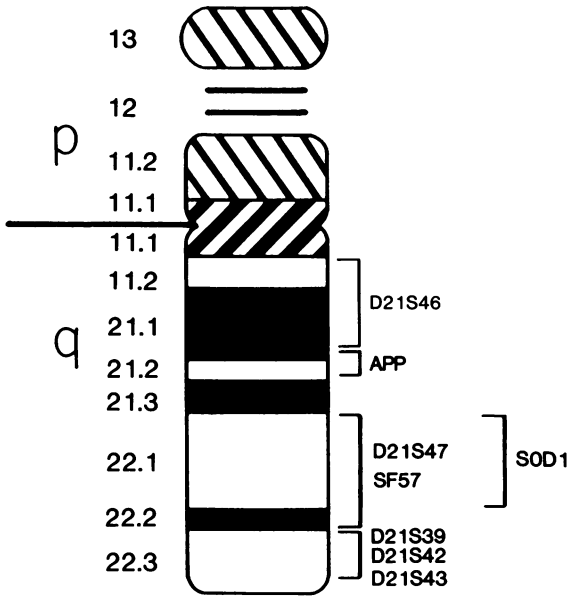


Figure 3 Physical map of chromosome 21 showing location of the chromosome 21–specific DNA sequences analyzed in the present study.

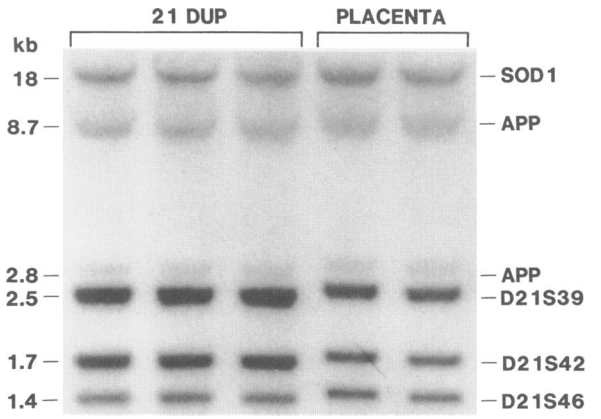


Figure 4 Autoradiogram of DNAs from patient IV-1 and from a control hybridized to chromosome 21 DNA sequences. The three lanes on the left, labeled 21 DUP, each contain DNA from this patient, and the two on the right contain control DNA. Probe names are indicated at the right and *Eco*RI fragment sizes are indicated on the left of the autoradiogram.

et al. 1988); *SOD1* maps to the region including 21q22.1 (Sinet et al. 1976). Two single-copy random DNA sequences, *SF57* and *D21S47,* also map to the region including band 21q22.1 through proximal q22.3 (Korenberg et al. 1987; J. R. Korenberg and T. Falik-Borenstein, unpublished data). Finally, *D21S39, D21S42,* and *D21S43* are located in mid–band 21q22.3 (J. R. Korenberg and T. Falik-Borenstein, unpublished data).

We determined the relative copy number of specific DNA sequences using quantitative Southern blot dosage analyses as described above. Figure 4 presents an illustrative autoradiogram used for the analysis of DNA sequences *D21S46* (the reference sequence), *D21S47, SF57, D21S42,* and *D21S39.* Table 2 shows the results of the statistical analysis of the results obtained with these sequences, as well as *D21S43* and the genes *SOD1* and *APP.* The copy number of each DNA sequence is presented along with the standardized ratio obtained for DS patients IV-1 and IV-2 and their normal father

Table 2

Chromosome 21 DNA Sequence Copy Number

Map Position and DNA Sequence	Mean Ratio (±SE)			Copy Number		
	IV-1	IV-2	Carrier Father	IV-1	IV-2	Carrier Father
Q11.2–21.05:						
FB68L (*APP*)	.91 (.044)	1.0	.8	2	2	2
Q22.1:						
SOD1	1.01 (.034)	1.1	.9	2	2	2
D21S47.....	.93 (.04)	NT	NT	2	...	...
SF57	.94 (.07)	NT	NT	2	...	...
Q22.3:						
D21S39.....	1.53 (.035)*	1.8**	1.1	3	3	2
D21S42.....	1.6 (.04)*	1.74**	1.1	3	3	2
D21S43.....	1.83 (.16)*	NT	NT	3	...	...

Note.—NT = not tested.
*Different from 1.0 by *t*-test; $P < .0001$.
**Different from 1.0 by *t*-test; $P < .05$.

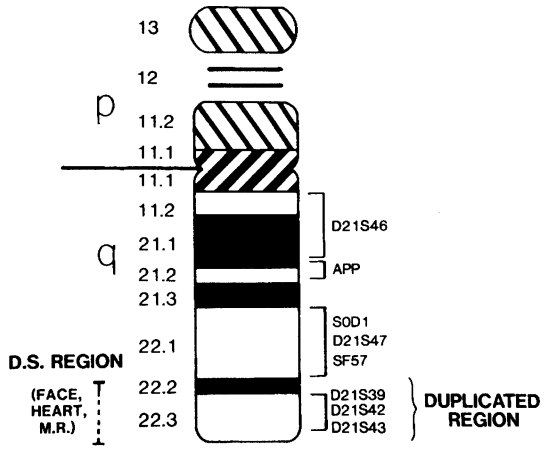
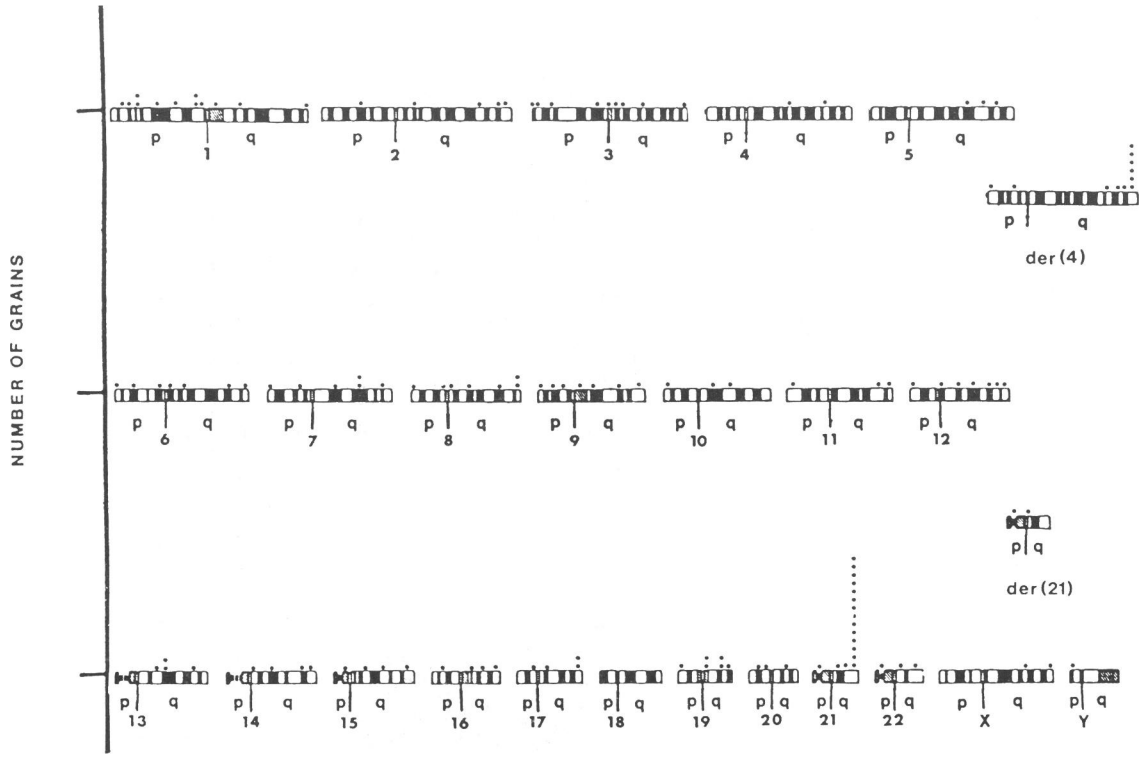


Figure 5 Physical map of chromosome 21 showing location of DS phenotypic region defined by the present study.

III-1. In the two affected individuals, *D21S39, D21S42,* and *D21S43* are present in three copies, whereas *SF57, D21S47, SOD1,* and *APP* are present in only two. Therefore, as summarized in figure 5, in this family there is a duplication which includes bands 21q22.2 and 21q22.3 but excludes the region of band 21q22.1 defined by our probes. *SOD1, APP,* and the sequence *D21S46* have been excluded from the region of the duplication. CuZn-SOD enzyme activities in red blood cells were found to be normal in IV-1 and IV-2 and in their parents (data not shown).

To detect the presumed translocated segment, *in situ* hybridization was performed on cells from DS patients IV-1 and IV-2 and from their normal father III-1, using the probe for *D21S39* which we had shown to be in the duplicated region. The results shown in figure 5 confirmed the presence of a balanced translocation between chromosomes 4 and 21 in the normal father (III-1). A low background and the presence of all grains only over chromosomes permitted accurate assignment of map position. Of the total grains observed in a single individual, no chromosomal band had more than a single overlying grain except the following. In 100 cells examined from the father (fig. 6) there were 136 grains, of which a significant proportion of grains were seen on the normal chromosome 21 (14% of the cells; 10% of the grains) and at the telomeric region of the derivative chromosome 4 (6% of the cells; 4.3% of the grains), but not on the derivative chromosome 21 or the normal 4, suggesting the presence of 4;21 translo-



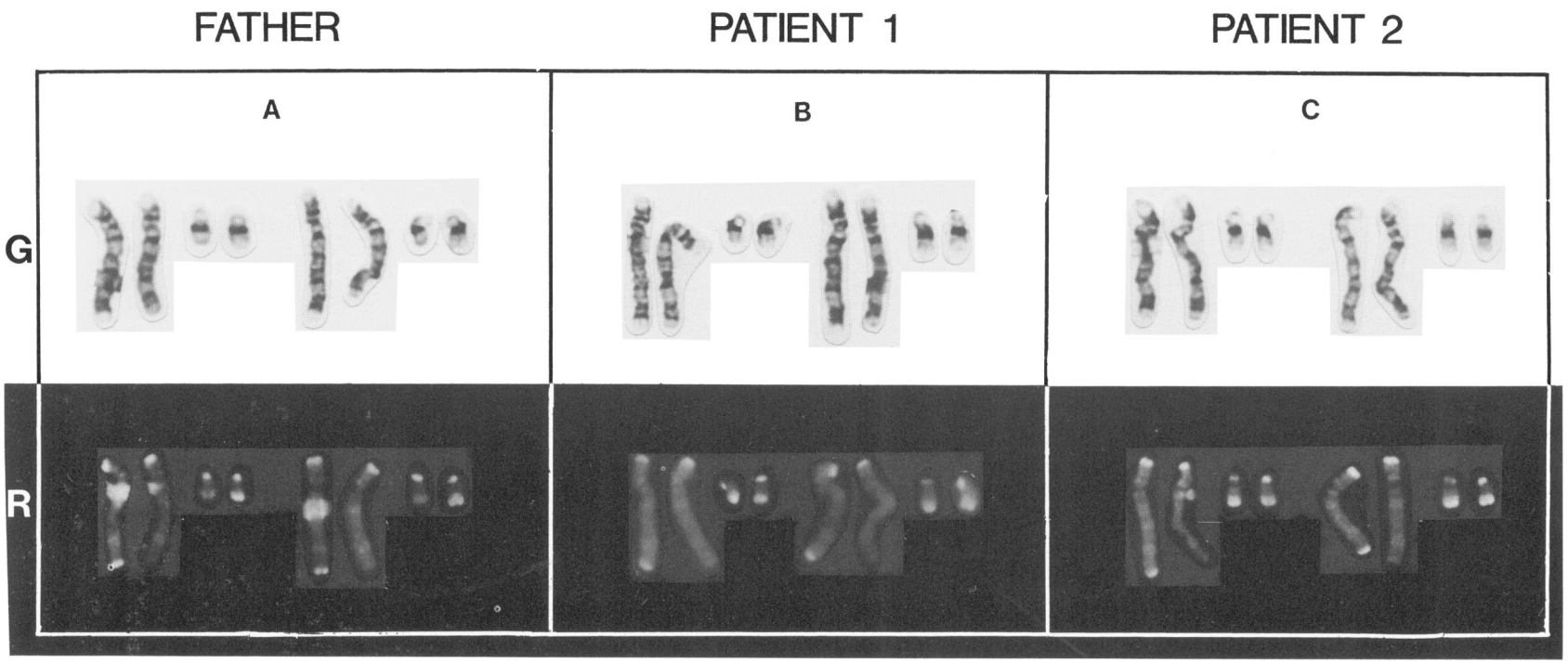


Figure 7 Homologous chromosome pairs 4 (*left*) and 21 (*right*) from patients IV-1 (*B*) and IV-2 (*C*) and from their normal carrier father, III-1 (*A*), shown with G-banding (*upper panels*) and R-banding (*lower panels*) at the ~550 band stage. The father's R-banded chromosomes are shown on the left lower panel. It is clear that the highly fluorescent tip of the long arm is present only on one, not both, of the chromosomes 21, and that the normally dull tip of 4q is now highly fluorescent. In contrast, the two patients' R-banded chromosomes shown in the middle and right panels reveal brightly fluorescent tips on both 21s in addition to one of the 4s. Although difficult to appreciate, all of band 21q22.2 is absent from the abnormal chromosome 21, presumably translocated to chromosome 4, and the tip is replaced by a dull band. Further, the tip of chromosome 4q is abnormal with the telomeric G band shown on the right of each pair replaced by a longer R band, originating from chromosome 21. Further, the proximal G band, 4q34, appears larger but single, presumably the result of the apposition of the chromosome 21 G band 21q22.2. The patients each carry two normal chromosomes 21 and one normal chromosome 4 plus the derivative 4 in contrast to their father, who carries only one normal chromosome 21, one normal chromosome 4, and both the derivative 21 and derivative 4 in the balanced state.

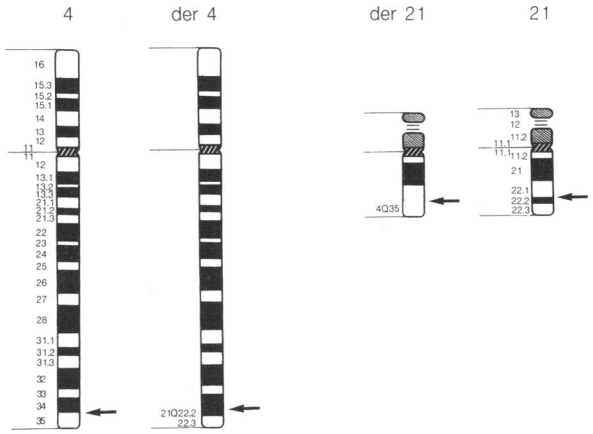


Figure 8 GBG-banded ideogram of the 4;21 translocation shown in fig. 7. Arrows indicate the breakpoints on distal chromosome 21q22.1 at the 21q22.2 border and on chromosome 4q35 proximal.

cation. In 100 cells examined from IV-1, there were 119 grains found with 8 grains (7% of grains) over chromosomes 21 and 6 grains (5%) over the derivative 4. Similarly, in 80 cells examined from IV-2, there were 111 grains found. Fifteen of these were over the distal 21 (18.8% of cells; 13.5% of grains), and 12 (15.0% of cells; 10.8% of grains) were on the distal tip of the derivative 4. These findings indicated the presence of a 4;21 translocation chromosome in addition to two normal chromosomes 21. The presence of the 4;21 translocation was confirmed with R-banding, and the breakpoints were defined with high-resolution G-banding as being in very proximal 4q35 and in distal 21q22.1 at the 21q22.2 border (Harnden and Klinger 1985) (figs. 7 and 8). At the level of resolution employed (about 550 bands/haploid genome), the unitary nature of the distal G-band, 4q34, suggests that the apposed break in chromosome 21 was below the limit of cytogenetic detection in distal 21q22.1 or in 21q22.2 as schematized in figure 8. Therefore, affected individuals, in addition to having a duplication of distal chromosome 21q, have a deletion of 4q35.

Discussion

We have described a three-generation family segregating four individuals with the phenotypic features of DS, three of them shown to be associated with a chromosome 21 rearrangement and the fourth untested. Using quantitative Southern blotting we first established that the duplicated region excludes DNA sequences mapping in 21q21 (*APP, D21S46*) and in 21q22.1 (*SOD1* and probably *D21S47, SF57*), but includes DNA sequences likely mapping in band 21q22.3 (*D21S39, D21S42, D21S43*). Using *D21S39* as a probe for in situ hybridization, we next established the existence of a 4;21 translocation, carried in the balanced state in intervening relatives in the pedigree, but in the unbalanced state in two of the individuals with phenotypic DS, resulting in partial trisomy 21. High-resolution cytogenetic analysis then excluded most of band 21q22.1 from the duplicated region, greatly narrowing the chromosome 21 region responsible for the facial features, heart defects, and probably the dermatoglyphic changes characteristic of DS seen in this family.

The Down Syndrome Region

The complete panoply of phenotypic features seen in Down syndrome (Epstein 1986) includes a large set of physical and biochemical features, but a subset of these is of obvious diagnostic, clinical, and social significance, namely, the facial appearance, congenital heart disease, and mental retardation, respectively. Previous work has shown that this subset of features may be produced by duplication including only band 21q22, as initially suggested by Niebuhr (1974), and perhaps only band 21q22.1-21q22.2 for the facial appearance and mental retardation (Poissonnier et al. 1976; Summitt 1981; Epstein 1986; Park et al. 1987). The importance of the 21q22 region for the production of the classical facial features of DS is emphasized by the lack of these features in individuals partially trisomic for other regions of chromosome 21. For example, duplication of 21pter-proximal q21 may be associated with a normal phenotype (Daniel 1979). Similarly, duplication of 21pter-q21 (likely of a larger region than the former case) does not appear to produce the characteristic DS subset of features but, rather, a different subset that shares only mental retardation in common, in addition to marked microcephaly, short stature, and hypoplastic nails (Park et al. 1987). Although these features may occasionally be a part of the DS phenotype, they are not constant or specific (Jackson et al. 1976) and therefore have not contributed significantly to the diagnosis of DS.

The precise assignment of a phenotypic feature to a particular region of chromosome 21 is limited by the resolution of cytogenetic analysis. Moreover, phenotypic mapping is made more difficult by the variability of phenotype of individuals with full trisomy 21. For example, only 40% of such cases have congenital heart disease. Indeed, in the published literature there is only

one case of partial trisomy 21 with congenital heart disease (Miyazaki et al. 1987). This variable expressivity is particularly important in that it limits phenotypic mapping power, because a phenotypic feature may be mapped by its presence but not excluded by its absence. In addition, cases of partial trisomy are rare, frequently associated with unbalanced translocations, and, therefore, usually accompanied by other aneuploidy which complicates the assignment of a particular feature to a region of chromosome 21. Consequently, although there is general agreement on the necessary involvement of band 21q22 in generating the characteristic facial appearance, mental retardation, and heart disease, there is little information on further subdivision of this region.

Our study has circumvented many of the above problems by the combined clinical, cytogenetic, and molecular analysis of a family with four cases of DS, all presumed to have the identical duplicated region. Although the affected members exhibit the phenotypic variability characteristic of trisomy 21, taken together they exhibit a broad subset of clinical DS features. We have, therefore, physically mapped the features seen in this family to the duplicated region that clearly involves 21q22.2-q22.3 and may include very distal 21q22.1, below the limit of cytogenetic detection. The features so mapped include the facial appearance (flat facial profile, upslanting palpebral fissures, epicanthal folds, flat nasal bridge, open mouth, protruding tongue), congenital heart defect of the endocardial cushion type, clinodactyly of the fifth finger, and probably the short fingers and dermatoglyphic changes (table 1). Neonatal hypotonia was noted only in patient IV-3, and this may have been due in part to her severe cardiac failure. This feature was, therefore, not mapped to the region under consideration.

These results are in agreement with those of McCormick et al. (1989) and may include regions in common with but below the limit of cytogenetic resolution of the patient of Poissonier et al. (1976), as analyzed in Rahmani et al. (1989). Both cases have some features of DS. However, study of the present family has also afforded an opportunity to define the chromosomal region responsible for the congenital heart disease in addition to the other phenotypic features of DS.

The necessary deletion (partial monosomy) of 4q35-4qter may also contribute to the phenotype seen in this family, particularly to the mental retardation. However, this deleted region is far smaller than any 4q deletion previously discussed. All of the three cases reported included a larger region, 4q32-qter (Fryns et al. 1981) or 4q33-qter (Mitchell et al. 1981; Stamberg et al. 1982), than that deleted in our family. These individuals had minor physical abnormalities (short nose, small mouth, cleft palate, small lower jaw, and limb abnormalities) in addition to *mild* developmental delays. Further, none of these had cardiac defects. Moreover, of the two reported patients in whom formal testing were done, both were of relatively high mental function. One had an IQ of 83–88 (within the low normal range), and the other, the one with the large 4q32-qter deletion, had an IQ of 68. Finally, although cardiac defects are seen with much larger deletions of 4q, none are of the endocardial cushion type seen in our family and characteristic of DS (reviewed in Mitchell et al. [1981]). These observations suggest that, although the 4q35 deletion in our patients may contribute to their physical features, both the endocardial cushion defect and some, though not necessarily all, of the mental impairment seen in our patients is produced by the duplication of chromosome 21. Finally, it is unlikely that genetic position effects of genes at the chromosomal breakpoints contribute significantly to the DS phenotype in this family, since the balanced translocation carriers are phenotypically normal.

For cytogenetic diagnostic purposes, it is important to note that the presence of a chromosome 21 translocation was strongly suspected from the pedigree and from the abnormal 21 observed in the normal father. However, it was still possible that the abnormal chromosome 21 carried an internal rearrangement predisposing to secondary meiotic duplication events that resulted in offspring with the appearance of DS. Indeed, no other karyotypic abnormality was detected by high-resolution G-banding until chromosome 4 was found to be abnormal by in situ hybridization and R-banding. This difficulty arises when translocations involve approximately equal pieces with similar banding patterns. In such cases, fluorescent R-banding may allow a differential degree of fluorescence among R-bands that may permit easy definition of a rearrangement. This caveat is well illustrated by our cases and has been emphasized in previous cases of 4;21 translocations (Dutrillaux et al. 1973; Mattei et al. 1981).

From our study we can conclude that the overexpression of both *APP* and *SOD1* is not necessary for the development of the phenotypic features seen in this family, including some elements of mental retardation. However, it is quite possible that the overexpression of *APP* contributes both to the neuropathology found in older individuals with trisomy 21 and to the dementia that sometimes results. Similarly, it is clear from ex-

periments with the *SOD1* transgenic mouse (Epstein et al. 1987) that the overexpression of *SOD1* may significantly alter both the expression of neurotransmitters (Elroy-Stein and Groner 1988) and neural histology (Avraham et al. 1988). Therefore, the potential importance of genes outside the molecular region defined in this report in the genesis of the overall phenotype of DS resulting from complete trisomy 21 cannot be underestimated (Epstein 1990). Nothing reported here is intended to imply that the region of duplication defined in the family being reported constitutes the entire segment of chromosome 21 responsible for the complete phenotype of DS. However, clinical and molecular investigation of such individuals should provide clues to defining the genetic basis of their phenotypes.

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