Am. J. Hum. Genet. 60:997–999, 1997

Possible Narrowed Assignment of the Loci of Monosomy 21–Associated Microcephaly and Intrauterine Growth Retardation to a 1.2-Mb Segment at 21q22.2

To the Editor:

In a recent issue of the *Journal,* Chettouh et al. (1995) reported a trial assignment of 23 monosomy 21–associated clinical manifestations to seven regions on chromosome 21. Since we encountered a patient with partial monosomy 21 and analyzed the phenotype-karyotype correlation in the patient, we report here the results of our study. The patient, a boy, was born at the 38th wk of gestation as the second child to unrelated parents. Apgar scores were 8 at 1 min and 9 at 5 min. Intrauterine growth retardation was evident—that is, his birth weight was 2,295 g (−1.8 SD), his length was 45 cm (−1.5 SD), and his head circumference was 30.5 cm (−1.5 SD). At his birth, both parents were 30 years old. Asthma developed at age 7 mo. When first seen by us at age 1 year 7 mo, his weight was 11.2 kg (+1.2 SD), his length was 78 cm (−1.4 SD), and his head circumference was 43.8 cm (−2.5 SD). In addition, the following abnormalities were observed: microcephaly, low-set ears, prominent nose, micrognathia, short neck, and mental retardation. Chromosome analysis revealed a de novo 46,XY,inv del(21)(p11.2→q22.1::p12→pss) karyotype (fig. 1*a*), indicating a monosomy for 21q22.1→qter. The abnormal chromosome has a double satellite (pss) derived from the patient's mother.

We analyzed the deletion extent of the rearranged chromosome 21, with FISH. Clones used in the FISH study included nine cosmids—Q98A3, Q36D3 (*D21S17*), Q34F2 (*D21S334*), Q46D6, Q41B8 (*LA230E8*), Q65D5 (the single-minded II gene [*SIM2*]), Q55C11, c103G03010 (*D21S333*), and c102G11119—and three bacteriophage P1–derived artificial chromosomes (PACs)—27A22 (*CBR*), 307O17 (*D21S394*), and 30K7 (*D21S407*). They were selected from 4-Mb-sized contigs that encompass a region containing genes responsible for the characteristic facies and mental retardation of Down syndrome (Osoegawa et al. 1996) (fig. 1*b*). Cosmid and PAC clones were grown in Luria-Bertani medium containing 25 μg kanamycin/ml, and their DNAs were extracted by an automatic plasmid isolation system (PI-100; Kurabo) and were treated with 2.5 μg RNaseA/ml. Metaphase chromosomes were obtained with the standard cytogenetic method from an Epstein-Barr virus–transformed lymphoblastoid cell line of the patient. Cosmid and PAC DNAs (1 μg) labeled with biotin-16-dUTP (Boehringer-Mannheim) by use of nick translation were hybridized to the patient's chromosomes, together with 10 μg human Cot-1 DNA (GIBCO-BRL) to suppress repetitive sequences and with 10 μg sheared salmon-sperm DNA as a carrier, as described elsewhere (Egashira et al. 1996). FISH signals were detected with fluorescein isothiocyanate–conjugated avidin (Vector Laboratories), and chromosomes were counterstained with propidium iodide. Photomicroscopy was performed under a fluorescence microscope by use of two filter combinations, G-2A and B-2A (Nikon). Of the 12 clones used, 5 (Q98A3, Q36D3, 27A22, c103G03010, and Q34F2) showed FISH signals on both the normal and rearranged chromosome 21, whereas the remaining 7 clones (Q46D6, Q41B8, Q65D5, Q55C11, 307O17, 30K7, and c102G11119) gave signals only on the normal chromosome 21 (fig. 2). The results indicated that the rearranged chromosome 21 lacked a segment distal to *D21S334*.

Monosomy for a segment distal to 21q22.3 results in few significant phenotypic effects (McGinnis et al. 1992; Pangalos et al. 1992). In contrast, a more proximally

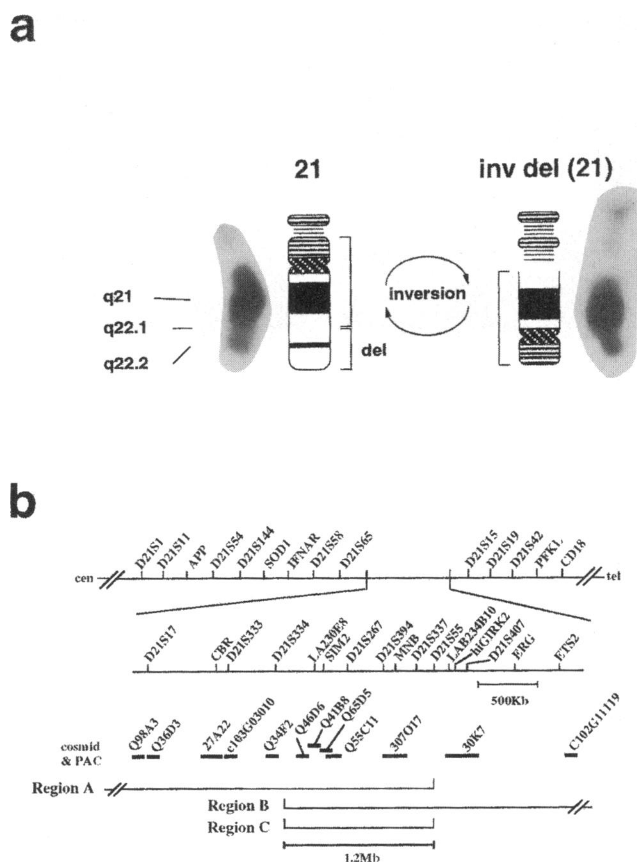

Figure 1 Rearranged chromosome, inv del(21), of the patient (*a*) and regional map around 21q22.2 (*b*). Region A is a segment where the putative loci for intrauterine and postnatal growth retardation, microcephaly, low-set ears, and cleft palate were mapped (Chettouh et al. 1995). Region B is the region of deletion in our patient, and Region C is a segment to which the putative loci for intrauterine growth retardation, microcephaly, and low-set ears are assigned.

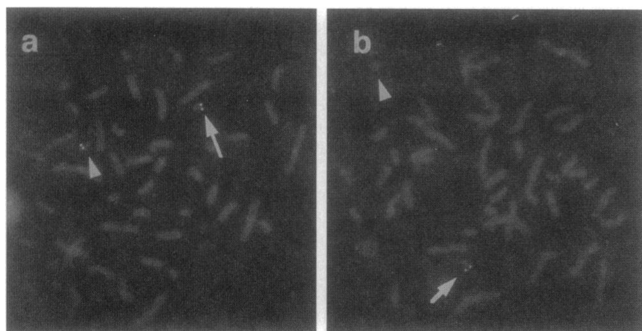

Figure 2 FISH analysis of the patient's chromosomes by use of cosmid Q34F2 (*D21S334*) (*a*) and cosmid Q41B8 (LA230E8) (*b*) as probes. Arrows indicate the normal chromosome 21, and arrowheads indicate inv del(21).

extended deletion involving a 21q22.1-qter segment seems to lead to growth retardation and microcephaly (Yamamoto et al. 1979). These findings may provide support for localizing putative loci for growth retardation and for microcephaly to 21q22. Chettouh et al. (1995) tentatively mapped the putative loci for intrauterine and postnatal growth retardation, microcephaly, low-set ears, and cleft palate to a region at 21q22.2, between the markers *D21S11* and *D21S55:* we refer to this region as "Region A" (fig. 1*b*). Our patient, who lacked a segment (Region B) that is distal to *D21S334,* presented intrauterine growth retardation, microcephaly, low-set ears, micrognathia, short neck, and mental retardation. When the phenotype map by Chettouh et al. (1995), the data by Yamamoto et al. (1979), and a physical map by Osoegawa et al. (1996) are all combined, microcephaly, intrauterine growth retardation, and low-set-ear loci may be confined to Region C—that is, between *D21S334* and *D21S55*—and may span an ~1.2-Mb region (fig. 1).

Region C contains both *SIM2* (Chen et al. 1995; Dahmane et al. 1995; Osoegawa et al. 1996) and the human minibrain gene (*MNB*) (Guimera et al. 1996). The sim protein in *Drosophila* is a master developmental regulator of the CNS midline lineage (Crew et al. 1988; Nambu et al. 1991), and the *sim* gene is required for early events in the midline cell development, including synchronized cell divisions, proper formation of nerve-cell precursors, and positive autoregulation of its midline expression. In situ hybridization of human *SIM2* or murine *Sim2* with the human and/or murine fetuses showed that the genes are expressed during an early fetal life, in the CNS and in other tissues, including the facial, skull, and palate primordia (Dahmane et al. 1995; Fan et al. 1996). It is speculated that *SIM2,* the human homologue of *Drosophila sim,* contributes to certain dysmorphic features, particularly the facial and skull characteristics, brain development, and/or mental retardation, in monosomy 21. In *Drosophila,* the *mnb* gene appears to play an essential role, during postembryonic neurogenesis, in regulating the number of distinct neuronal cell types (Tejedor et al. 1995). The human homologue, *MNB,* also may have a role in the process of generating neuronal cells in the brain, and its loss of function may be involved in microcephaly, as observed in partial monosomy 21.

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Acknowledgments

N.N. was supported, in part, by Grants-in-Aid for Scientific Research (08307019), for Priority Areas (08264228) and for the Human Genome Program (07NP0201), from the Ministry of Education, Science and Culture, and by a Grant-in-Aid from the Ministry of Health and Welfare, Japan.

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0002-9297/97/6004-0030$02.00

Am. J. Hum. Genet. 60:999–1001, 1997

Methylenetetrahydrofolate Reductase Thermolabile Variant and Human Longevity

To the Editor:

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Severe hyperhomocysteinemia due to MTHFR deficiency causes neurological abnormalities, mental retardation, arteriosclerosis, and thrombosis (Rosenblatt 1989). Mild hyperhomocysteinemia is considered to be an independent risk factor for occlusive arterial disease, including myocardial infarction (Kang et al. 1991; Stamfer et al. 1992). Hyperhomocysteinemia can be induced by disturbances in homocysteine remethylation and has been associated with a thermolabile variant of MTHFR (Kang et al. 1991; Engbersen et al. 1995). A C677T mutation in the MTHFR gene, which converts an alanine residue to a valine residue, results in an enzyme with decreased activity and increased thermolability (Frosst et al. 1995). Recent results show that this MTHFR C677T mutation is associated with hyperhomocysteinemia in cardiovascular patients (Frosst et al. 1995; Kluijtmans et al. 1996) and in subjects with risk factors for coronary artery disease (Jacques et al. 1996).

MTHFR thus has appeared to be a gene candidate for cardiovascular disease, one of the major causes of death. We therefore decided to investigate the relationship between longevity and MTHFR genetic variants in a centenarian population.

A population of 458 French centenarians (89% women and 11% men; mean age 100.71 ± 0.13 years), living at home or in institutions, was recruited through media advertising and organizations. They were compared to a control group of 374 French adults (49% women and 51% men; mean age 53.1 years; age range 20–70 years). All controls and centenarians were Caucasian and were living in France. Centenarians and controls from the CEPH pedigree (Schachter et al. 1994) were recruited throughout France. The regional distribution of centenarians and controls, respectively, was as follows: 34% and 18.7% from Paris and its suburbs; 17% and 14.3% from central France; 5% and 18.7% from northern France; 19% and 14.2% from eastern France; 20% and 21.9% from western France; and 5% and 12.2% from southern France. A population of 106 nonagenarians, recruited on the basis that they had at least one nonagenarian sibling still alive, also was studied. In each family, one of the siblings was selected at random so that all 106 nonagenarians were unrelated individuals.

The subjects were screened for the MTHFR C677T mutation by use of a PCR-based assay, as reported elsewhere (Frosst et al. 1995). The mutant allele (T677), denoted by a plus sign (+), gives *Hinf*1 restriction fragments of 175 bp and 23 bp, whereas the normal allele (C677), denoted by a minus sign (−), gives a single fragment of 198 bp.

Nonfasting blood samples were collected in EDTA-containing tubes and were centrifuged promptly. Plasma was stored at −70°C. Plasma folate levels were determined by use of radio-assay kits from Ciba-Corning. Total plasma homocysteine levels were determined by ion-exchange chromatography on a Beckman 6300 amino acid analyzer (Brattstrom et al. 1988).

The distribution of the MTHFR genotypes among the population is in Hardy-Weinberg equilibrium. The frequency of the +/+ genotype was lower among the centenarians (13.3%) than among the controls (18.5%), although the difference was not statistically significant ($\chi^2 = 4.31$; $P = .12$) (table 1). We tested a population of nonagenarians with a family history of longevity and also found that the frequency of the +/+ genotype was lower among the nonagenarians (11.4%) than among the controls ($\chi^2 = 3.33$; $P = .17$). When we pooled the