Am. J. Hum. Genet. 66:1705–1709, 2000

Report

The Novel Genetic Disorder Microhydranencephaly Maps to Chromosome 16p13.3-12.1

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We studied a large consanguineous Anatolian family with children who exhibited hydranencephaly associated with microcephaly. The children were severely affected. This novel genetic disorder is autosomal recessive. We used autozygosity mapping to identify a locus at chromosome 16p13.3-12.1; it has a LOD score of 4.11. The gene locus is within a maximal 11-cM interval between markers D16S497 and D16S672 and within a minimal critical region of 8 cM between markers D16S748 and D16S490.

Anencephaly (MIM 206500), the most common congenital malformation of the brain, is a neural-tube defect resulting in limited development of the cerebrum and, occasionally, of other brain regions. Both environmental factors (Yen and MacMahon 1968; Farag et al. 1989) and autosomal recessive inheritance of a major-gene defect (Farag et al. 1986; Shaffer et al. 1990) have been suggested as contributing to the etiology of anencephaly. Hydranencephaly (MIM 236600) occurs when spinocerebellar fluid (SCF) seems to have partially replaced some of these tissues. However, microcephaly, a condition in which the affected individual's head circumference is >3 SD below the mean for the age and sex of the child, is not commonly associated with hydranencephaly.

In this study, we mapped the gene responsible for severe hydranencephaly associated with microcephaly, a condition that we call "microhydranencephaly" (MHAC [Human Gene Nomenclature Committee database]), in a single large consanguineous Anatolian family. We mapped the gene at chromosome 16p13.3-12.1 by use of homozygosity mapping (Lander and Botstein 1987), also called "autozygosity mapping." The family has three surviving affected children, and data on the pedigree strongly suggested an autosomal recessive inheritance of the trait. Other striking phenotypic features were severe mental and motor retardation, as well as very small body size and very small occipital-frontal circumference (OFC). No other abnormality could be detected in the affected individuals.

The family that we analyzed was very large, with several first-cousin marriages (fig. 1). At the end of this study, the age of the oldest affected child (individual IV-5 in fig. 1) was 6.5 years; the ages of other two were 4.6 and 4.5 years old (individuals IV-7 and IV-6, respectively, in fig. 1). The youngest of these three children was diagnosed with microcephaly at birth, and the family stated that the condition had also been present at birth in the other two affected children, as well as in a child who had died at the age of 6 mo (individual VI-3 in fig. 1). Whether the two fetuses lost in miscarriages also had the condition could not be assessed.

All three affected children were extremely small, with no malformations other than microhydranencephaly. Height, weight, and OFC are given in table 1. The youngest patient, who was age 4.5 years at the time of the study, was the most striking case: his height corresponded to a sex mean of 7 mo, his weight to 1 mo, and his OFC to prenatal. Results of karyotyping, urine analysis, complete blood count, folic acid and vitamin B_{12} analysis, urine-blood amino acid chromatography, blood-gas analysis, and liver and renal function tests were normal for all three patients. One of the mothers and her affected daughter (individuals III-1 and IV-5, respectively, in fig. 1) had tested negative for *Toxoplasma gondii,* rubella virus, cytomegalovirus, and herpes simplex virus.

Received September 20, 1999; accepted for publication February 9, 2000; electronically published April 4, 2000.

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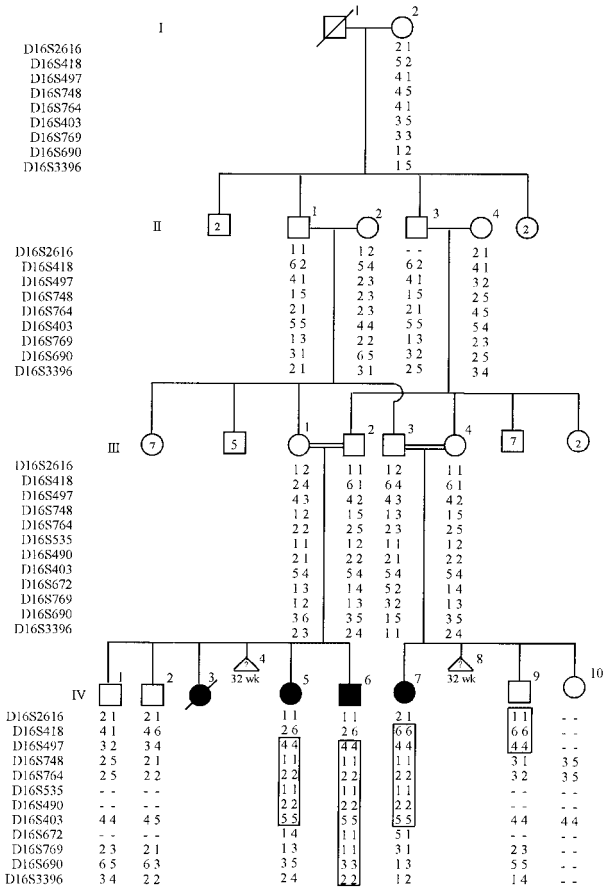

Figure 1 Haplotypes for 11 markers at 16p, for affected branches of the family. Marker order and relative distances (minimal to maximal [in cM]) are D16S2616-(1–1.5)-D16S418-(1.5–7)-16S497-(0–1)-D16S748-(2–3)-D16S764-(5–6)-D16S535-(0)-D16S490-(2)-D16S672-(8)-D16S769-(13)-D16S690-(11)-D16S3396, as deduced from Genome Database. The marker D16S403 maps somewhere in the region of markers D16S535, D16S490, and D16S672.

Other clinical features of the patients were spastic quadriplegia, multiple joint contractures, bilateral pes equina varus, myoclonic seizures, and athetosis. Intracranial calcification, cataracts, and renal dysplasia were not detected. The motor achievements were limited and accomplished only by arms, head, and legs. None of the patients could sit, even with support, at the time of the last examination in the study. They could not chew, so they needed to be fed soft food. Common physical features were sloping forehead, exophthalmia, large auricles, and receding chin (fig. 2). Frequent respiratory and skin infections were reported. The patients could not develop eye contact. They could not say any meaningful words, uttering only short cries.

Computed tomography gave similar results for all three patients. The calvaria and basal bones of the skull were normal. The pons, cerebellar hemispheres, forth ventricle, posterior fossa, and basal ganglia were normal. Falx cerebri was present. Agenesis of corpus callosum and extensive cerebral dysplasia and atrophy were observed. The parenchyma at the anterior compartments of the cerebrum was thin and had been replaced by SCF. Severity of symptoms increased with the age of the patients. Cerebral hemispheres were not present, with the exception of basal ganglia, and SCF was present in their place. No other brain defect was observed.

In addition to these common features, other observations and results of analyses for individual patients were as follows. Patient 1 (IV-5), despite being the oldest and having been assessed, by computed tomography, as the most severely affected of the patients, had the least severe clinical phenotype. She could sit, with support, at age 6 mo and at the time of a previous clinical examination 3 years ago, but not at the time of the last examination in this study. Deep-tendon reflexes were hyperactive, and the upper extremities were hypertonic. In the last examination, she could perform a crawling rotatory motion with support of the elbows, when laid on her stomach. She recognized her parents and expressed joy. She was observed to self-mutilate: she constantly bit her hands. When she did so, her face expressed pain, but she could not free the hand. The parents noted one seizure when she was age ~7–8 mo.

Patient 2 (IV-7) is the cousin of patient 1. During the last visit that we conducted, she was completely inactive and rarely presented a social smile. Her parents remember only one episode of seizure.

Patient 3 (IV-6) is the brother of patient 1 and the most severely affected of the three probands. He never presented a social smile. His parents deny observing any seizures. Doctors attempted to diagnose the disorder prenatally by use of serial ultrasonographic investigation, and the fetus was reported to be normal at 8, 14, and 19 wk of pregnancy.

DNA was isolated from peripheral blood lymphocytes by standard methods (Miller et al. 1988). The genome-wide search was accomplished by use of the 156 mark-

Table 1

Sex, Age, Height, Weight, and OFC of Affected Individuals

		Observed (Mean) Value[a]		
Patient (Sex)	Age	Height (cm)	Weight (kg)	OFC (cm)
1 (F)	6 mo	64[b] (64.5)	5.7[c] (7.4)	32.5[d] (42.8)
	6.5 years	93[d] (114)	9.8[d] (21.3)	40.1[d] (50.5)
2 (F)	4.6 years	72[d] (104)	9.0[d] (17.2)	35.2[d] (49.7)
3 (M)	4.5 years	68[d] (104.4)	3.8[d] (17.6)	30.9[d] (50.7)

[a] Mean values are means for age and sex.
[b] 0 SD.
[c] −2 SD.
[d] ≪−3 SD.

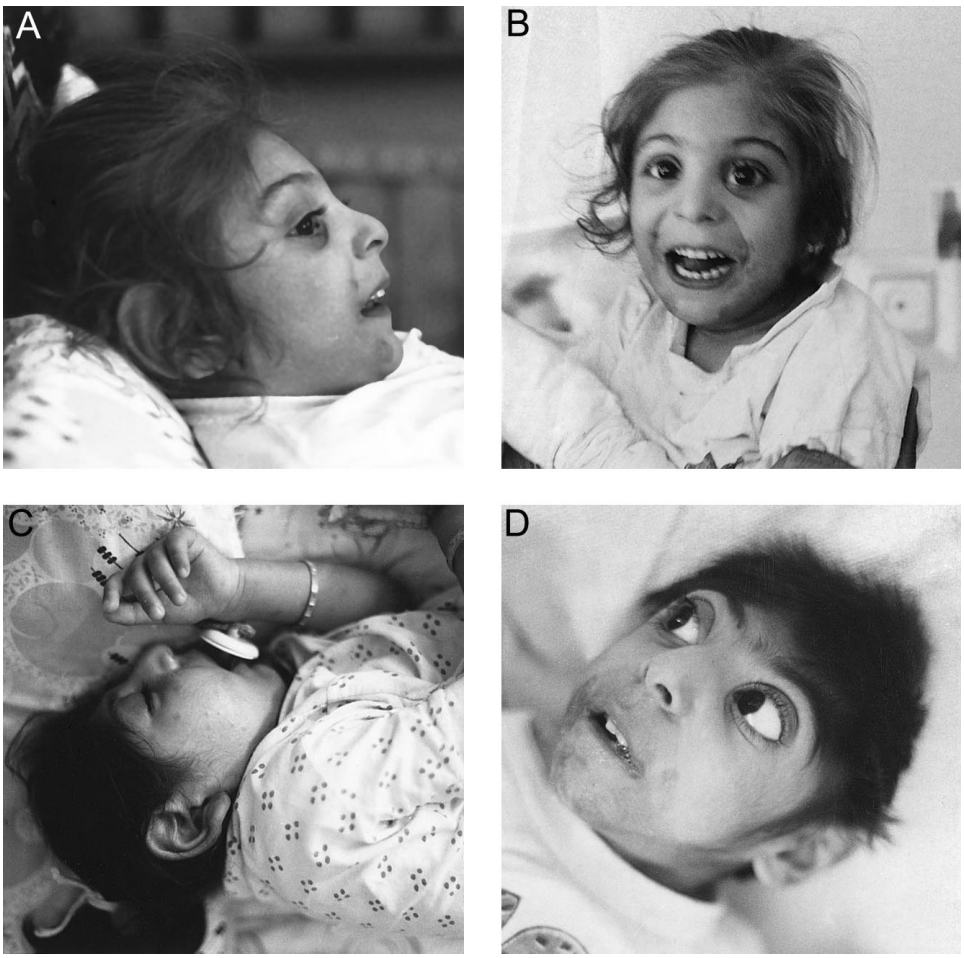

Figure 2 Photographs of patient 1 (*A* and *B*), patient 2 (*C*), and patient 3 (*D*)

ers, at a density of 25 cM, with Human Screening Set, version 8a, from the Cooperative Human Linkage Center. All markers used in the study were purchased from Research Genetics. Alleles were resolved by electrophoresis on a denaturing polyacrylamide gel at 40°C–45°C and were visualized by silver staining as follows: the gel was incubated in 0.1% silver nitrate for 8 min, washed with water, and incubated in freshly prepared 1.5% NaOH, 0.01% $NaBH_4$, and 0.4% formaldehyde until the bands became visible. The gel was then soaked in 2.5% Na_2CO_3 for 10 min, to stop the reaction, followed by soaking in 5% glycerol, to gain elasticity.

The three affected individuals were analyzed and were found to be homozygous for shared alleles for nine markers: D1S1665, D2S1384, D3S2460, D5S1725, D5S1505, D8S1477, D12S375, D16S764, and D17S1301. The parents of the affected children were then genotyped for these markers, to investigate whether the loci possessed true shared homozygosity. Only marker D16S764 seemed to be a true candidate for the gene locus. To test this hypothesis, marker D16S748, which is 7 cM from D16S764, was employed. All four parents were found to be informative. On analysis with 11 other nearby markers (2 of which were not informative), a true shared region of homozygosity was confirmed. The order of the markers and the relative distances between them are given in figure 1. All three affected individuals were homozygous for markers D16S497, D16S748, D16S764, D16S403, D16S535, and D16S490, as well as for the same haplotype, consistent with linkage to chromosome 16p13.3-12.1. Since healthy individual IV-9 carried the same haplotype, in the homozygous state, for markers D16S418–D16S497, marker D16S497 was excluded from the gene locus. As a result, the gene region was localized to a maximal 11-cM region between markers D16S497 and D16S672 and to a minimal critical region of 8 cM between markers D16S748 and D16S490.

A total of 46 individuals were typed. The haplotypes were constructed manually, under the assumption of minimum recombination. The odds ratios from inde-

pendent cases (a total of three affected and four unaffected children in the two core families) were multiplied, to obtain a final odds ratio of 12,945. This means that the probability that the haplotype has been inherited, by chance (i.e., not in association with the genetic disorder), in the homozygous state, by the affected individuals but not the unaffected individuals is 1:12,945. This gives a LOD score ($\log_{10}$ of the odds ratio) of 4.11 with 0 recombination, a value much higher than the acceptable minimum of 3.00. Our use of a single consanguineous family for gene localization eliminates the need for computer-based statistical analysis.

The haplotype was traced through the four grandparents and a common great-grandmother (individual I-2 in fig. 1). Since the common great-grandmother did not carry the haplotype, we conclude that her deceased husband must have been the carrier.

In total, the patients have 33 cousins, not including themselves and their siblings. At the time of the study, three of the cousins had various neurological disorders. A 15-year-old cousin had advanced contractures in all extremities and scoliosis that developed in the last few years, and she was a carrier of the MHAC haplotype. The other two were not carriers. One had dorsal meningomyelocele, and his sister had encephalomeningomyelocele. These conditions are types of spina bifida, a developmental abnormality that is known to be associated with anencephaly.

Several known and unknown genes have been localized between markers D16S497 and D16S672, which flank the MHAC locus (see the Web sites of The Genome Database and The Institute for Genome Research). The best candidate gene is GSPT1, a GTP-binding protein that facilitates G1-to-S phase transition (Hoshino et al. 1989). Another gene (PDE1B) codes for a phosphodiesterase, with homology to the dunce learning and memory gene of *Drosophila melanogaster* (Bolger et al. 1993). The CDR2 gene codes for a cerebellar degeneration–related autoantigen, which is a leucine zipper DNA-binding protein associated with Yo syndrome (Fathallah-Shaykh et al. 1991). This autoimmune disorder causes paraneoplastic cerebellar degeneration. Two genes (ABCC1 and ABCC6) are associated with multiple drug resistance and are members of an adenosine triphosphate–binding cassette superfamily of transport systems (Cole et al. 1992; Longhurst et al. 1996). CRYM codes for μ-crystallin, which, in humans, is not a lens-structural protein and is expressed in neural tissue, retina, muscle, and kidney (Kim et al. 1992).

Since no family with a similar phenotype has been reported elsewhere, we consider MHAC to be a novel genetic disorder. However, it is also possible that the disease may have resulted from a very mild mutation in a gene responsible for hydranencephaly, thus allowing postnatal survival. If so, microcephaly observed in the family would be secondary to severe hydranencephaly. Our hope is that this report will initiate the identification of more families afflicted with the disorder and will aid clinical diagnosis of affected individuals and allow management of the condition in afflicted families. Characterization of the gene involved in this disorder should contribute significantly to an understanding of the molecular basis of the neurodevelopment and evolution of the cerebrum, the brain region responsible for higher intellectual functions.

Acknowledgments

We are grateful to the individuals in the family, for their participation. This work was supported by grants from the State Planning Organization, Academy of Sciences of Turkey, and the Boğaziçi University Research Fund. G.N.K. was a fellow of the Scientific and Technical Research Council of Turkey.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://lpg.nci.nih.gov/CHLC (for Human Screening Set, version 8a)

Genome Database, The, http://gdbwww.gdb.org (for genes and location of markers at 16p13.3-12.1)

Human Gene Nomenclature Committee, http://www.gene.ucl.ac.uk/nomenclature (for MHAC)

Institute for Genome Research, The (TIGR), http://www.tigr.com (for unknown genes at 16p13.3-12.1)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for anencephaly [MIM 206500] and hydranencephaly [MIM 236600])

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