Letters to the Editor

Am. J. Hum. Genet. 65:1457–1459, 1999

About the "Pathological" Role of the mtDNA T3308C Mutation…

To the Editor:

Numerous mtDNA mutations have been associated with the mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS) syndrome (MIM 540000). These include transitions at nucleotide positions (nt) 1642, 3243, 3252, 3256, 3271, 3291, 3308, and 9957, and a 4-bp deletion beginning at nt 14787. For some of these mutations (A3243G, C3256T, and T3271C), the causal relationship with the phenotype has been confirmed, whereas for others, the status is still provisional (MITOMAP). The T3308C mutation in the NADH dehydrogenase subunit 1 (ND1) is a member of the "provisional" group and was described in a Spanish subject affected by MELAS and bilateral striatal necrosis. This mutation changes the highly conserved methionine 1 to a threonine, was heteroplasmic in both the proband and her asymptomatic mother, and was absent in 130 normal and other-disease controls (Campos et al. 1997). More recently, a homoplasmic T3308C mutation has also been reported in a colorectal tumor, in which it was associated with two other somatic homoplasmic transitions, T710C and T1738C. It has been suggested that these mutations could have a functional effect in mitochondrial selection (Polyak et al. 1998). However, doubts about the pathological significance of the T3308C mutation have been raised by a study involving 37 Portuguese patients with a clinical phenotype of mitochondrial encephalomyopathies and 150 Portuguese control subjects. The T3308C mutation was observed in two patients and in four controls (Vilarinho et al. 1999). In all cases it was homoplasmic.

To better define the role of this putative pathological mutation, we did a detailed analysis of the mtDNA background on which the T3308C had been reported. By sequence analysis of several tRNA genes and their surrounding sequences, we determined that, in addition to the T3308C mutation, the mtDNA of both Portuguese patients harbored the combination of mutations T1738C, T5655C, G7521A, A10398C, and A14769G and a dinucleotide deletion at nt 514–515. We observed the same mutations in the two Spanish patients (in the meantime a second Spanish patient had been found) and in the four Portuguese controls who tested positive for the mutation. Thus, these results indicated that all these mtDNAs were members of the same mtDNA haplogroup and that most likely they shared the T3308C mutation by descent. Intriguingly, this haplogroup harbored the combination of mutations T3308C and T1738C, similar to the case reported by Polyak et al. (1998). The search in our samples for the third somatic mutation (T710C) found in the colorectal tumor was negative.

To identify the mtDNA haplogroup harboring the mutation T3308C, sequence analysis of the mtDNA control region between nt 16090 and 16375 was performed in the eight T3308C samples (table 1). This analysis revealed a consensus motif (16126–16187–16189–16223–16264–16270–16278–16293–16311) that is typical of the West African haplogroup L1b (Watson et al. 1997; Rando et al. 1998), thus allowing us to classify Portuguese and Spanish mtDNAs with the T3308C mutation within this haplogroup. It has been determined elsewhere, by high-resolution restriction analysis (Tor-

Table 1

mtDNA Control Region Variation in Iberian Patients and Controls

Sample ID	Origin	Control Region Variation[a]
1	Portuguese patient	126, 187, 189, 215T, 223, 264, 270, 278, 311
2	Portuguese patient	126, 187, 189, 223, 264, 270, 278, 293, 311
3	Spanish patient	126, 187, 189, 223, 264, 270, 278, 293, 311, 360
4	Spanish patient	126, 187, 189, 223, 264, 270, 278, 293, 311
5	Portuguese control	104, 187, 189, 223, 270, 278, 289, 293, 311
6	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311
7	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311
8	Portuguese control	126, 187, 189, 223, 264, 270, 278, 293, 311

[a] Nucleotide positions (−16000) between nt 16090 and 16375, different from the Cambridge Reference Sequence (Anderson et al. 1981). Mutations are transitions (T↔C, A↔G), unless the base change is specified explicitly.

roni et al. 1996, 1997), that haplogroup L1b is defined by the RFLP motif: +185 *Taq*I, +2349 *Mbo*I, −2758 *Rsa*I, +3592 *Hpa*I, −3693 *Mbo*I, −7055 *Alu*I, +10394 *Dde*I, +10806 *Hin*fI (Chen et al. 1995; Rando et al. 1998; A. Torroni, unpublished data). Therefore, we selected, among our African population samples, all those (a total of 48) who either by RFLP analysis or by control region sequencing had been classified as members of haplogroup L1b. Analysis of their status at nt 3308 revealed that all of them harbored the mutation. In contrast, control samples belonging to African haplogroups L1a, L1c, and L2 were found to lack the mutation. These results indicate that the T3308C mutation defines exclusively by descent haplogroup L1b mtDNAs, and it is very ancient since L1b probably originated in western Africa ~12,000–19,000 years ago (Watson et al. 1997; Rando et al. 1998). Thus, Spanish and Portuguese mtDNAs with the T3308C mutation are of African origin, and their presence probably reflects the arrival of North Africans during the Mesolithic Age (8000 B.C.) and/or during the Arabic rule that started at ~800 A.D. (Arnaiz-Villena et al. 1997). If we take into account that haplogroup L1b frequencies in populations of western Africa are in the range of 10%–20% (Watson et al. 1997; Rando et al. 1998), the observed frequency in the Portuguese population (~2%–3%) indicates a significant influence of North Africans in the Iberian gene pool.

In conclusion, the T3308C mutation is an ancient marker of a common West African haplogroup, and all Iberian subjects with this mutation who were affected by mitochondrial encephalomyopathies harbored haplogroup L1b mtDNAs. This finding is difficult to reconcile with a role of this mutation in disease expression and further indicates that haplogroup classification of patients' mtDNAs, followed by a search for the putative disease mutation in phylogenetically closely related control mtDNAs, is a crucial step in the identification of mtDNA disease mutations. Furthermore, the observation that the elimination of the methionine codon AUA at position 1 of the ND1 subunit is common in some human populations suggests that the maintenance of that codon is not so critical in our species. Possibly this is because the third codon (AUG) of the human ND1 subunit also encodes for a methionine, and the ND1 subunit of L1b mtDNAs, although it might be shortened by two amino acids, apparently still retains its functionality. However, it is intriguing that the same combination, T3308C–T1738C, that characterizes haplogroup L1b has also occurred in a colorectal tumor as new somatic mutations. This is especially noteworthy when it is taken into account that T1738C occurs in the 16S rRNA, a gene involved in the translation process, and that the T3308C mutation might indeed affect the translation process of ND1 on non-L1b mtDNA backgrounds. This observation raises again the possibility of polygenic models in which certain mtDNA mutations can be functional and maintained in the population only if they occur in combination with other specific mtDNA mutations.

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HUGO ROCHA,[1] CARLOS FLORES,[2] YOLANDA CAMPOS,[3] JOAQUÍN ARENAS,[3] LAURA VILARINHO,[1] FILIPPO M. SANTORELLI,[4] AND ANTONIO TORRONI[5,6]

[1]*Unidade de Biologia Clínica, Instituto de Genética Médica Jacinto de Magalhães, Porto, Portugal;* [2]*Universidad de La Laguna, Tenerife, Spain;* [3]*Centro de Investigación, Hospital Universitario "12 de Octubre," Madrid;* [4]*Molecular Medicine, Children's Hospital Bambino Gesù, and* [5]*Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza," Rome; and* [6]*Istituto di Chimica Biologica, Università di Urbino, Urbino, Italy*

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Address for correspondence and reprints: Dr. Antonio Torroni, Istituto di Chimica Biologica, Universitá di Urbino, 61029 Urbino, Italy. E-mail: torroni@axcasp.caspur.it

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Diaphragmatic Spinal Muscular Atrophy with Respiratory Distress Is Heterogeneous, and One Form Is Linked to Chromosome 11q13-q21

To the Editor:

Diaphragmatic spinal muscular atrophy (SMA) has been delineated as a variant of infantile SMA (SMA1 [MIM 253300]) (Mellins et al. 1974; Bertini et al. 1989). The most prominent symptoms are severe respiratory distress resulting from diaphragmatic paralysis with eventration shown on chest x-ray and predominant involvement of the upper limbs and distal muscles. In contrast to classic SMA1, in diaphragmatic SMA the upper spinal cord is more severely affected than the lower section. The *pmn* mouse presents with progressive motor neuronopathy and a disease that closely resembles diaphragmatic SMA (Schmalbruch et al. 1991). The *pmn* locus has been mapped to murine chromosome 13 (Brunialti et al. 1995).

Here we report on nine patients from three families with diaphragmatic SMA following autosomal recessive inheritance. The diagnosis of diaphragmatic SMA was made on the basis of clinical criteria (Rudnik-Schöneborn et al. 1996). Family 1 is of Lebanese origin; family 2, German origin; and family 3, Italian origin. We obtained DNA samples from these families after receiving informed consent, in accordance with the Declaration of Helsinki.

In family 1 (fig. 1*A*), the parents are first cousins. The first affected son died, at the age of 10 wk, of suspected sudden infant death syndrome (SIDS). One daughter presented, at the age of 6 wk, with feeding difficulties and progressive respiratory distress. Chest x-ray showed eventration of the diaphragm. Mechanical ventilation was initiated at the age of 8 wk. She developed progressive muscular atrophy with complete paralysis of the upper and lower limbs and mild contractures of the knee and ankle joints. Three other children, nonidentical twin daughters and the youngest daughter, died of respiratory failure—the twins at the age of 8 and 9 wk and the youngest daughter at the age of 8 wk. Autopsy specimens were taken from gastrocnemius muscle in both twins and from the upper spinal cord in one twin. Skeletal-muscle histology revealed neurogenic atrophy without signs of reinnervation. Ultrastructurally, the motor end plates lacked nerve terminals and showed postsynaptic degenerative changes characterized by deep invaginations. The diameter of anterior spinal roots was reduced in the upper spinal cord. The remaining motor neurons showed chromatolysis. These findings offer two different pathophysiological concepts: (1) degeneration of the anterior horn cells of the spinal cord with neurogenic muscular atrophy suggests dying-forward atrophy, and (2) presynaptic and postsynaptic signs of motor end-plate degeneration suggest dying-back atrophy. In family 2 (fig. 1*B*), the first child had severe muscular hypotonia and died, at the age of 9 wk, of cardiorespiratory failure. The third child has been mechanically ventilated since the age of 3 mo. In family 3 (fig. 1*C*), which has been reported in detail elsewhere (Novelli et al. 1995), the gene locus for SMA1, on chromosome 5q, has been excluded. Both affected sibs presented with respiratory insufficiency right after birth and with the typical signs of diaphragmatic SMA.

First, we confirmed that, in families 1 and 2, there is no linkage of the trait to markers of the SMA locus on 5q11.2-q13.3, as there is in family 3. Second, the orthologous regions corresponding to the murine *pmn* gene region on human chromosomes 1q and 7p were excluded as gene loci responsible for the disease (Grohmann et al. 1998).

To locate the gene locus for diaphragmatic SMA, a whole-genome scan was undertaken in family 1. Microsatellite analysis was performed, by standard semiau-

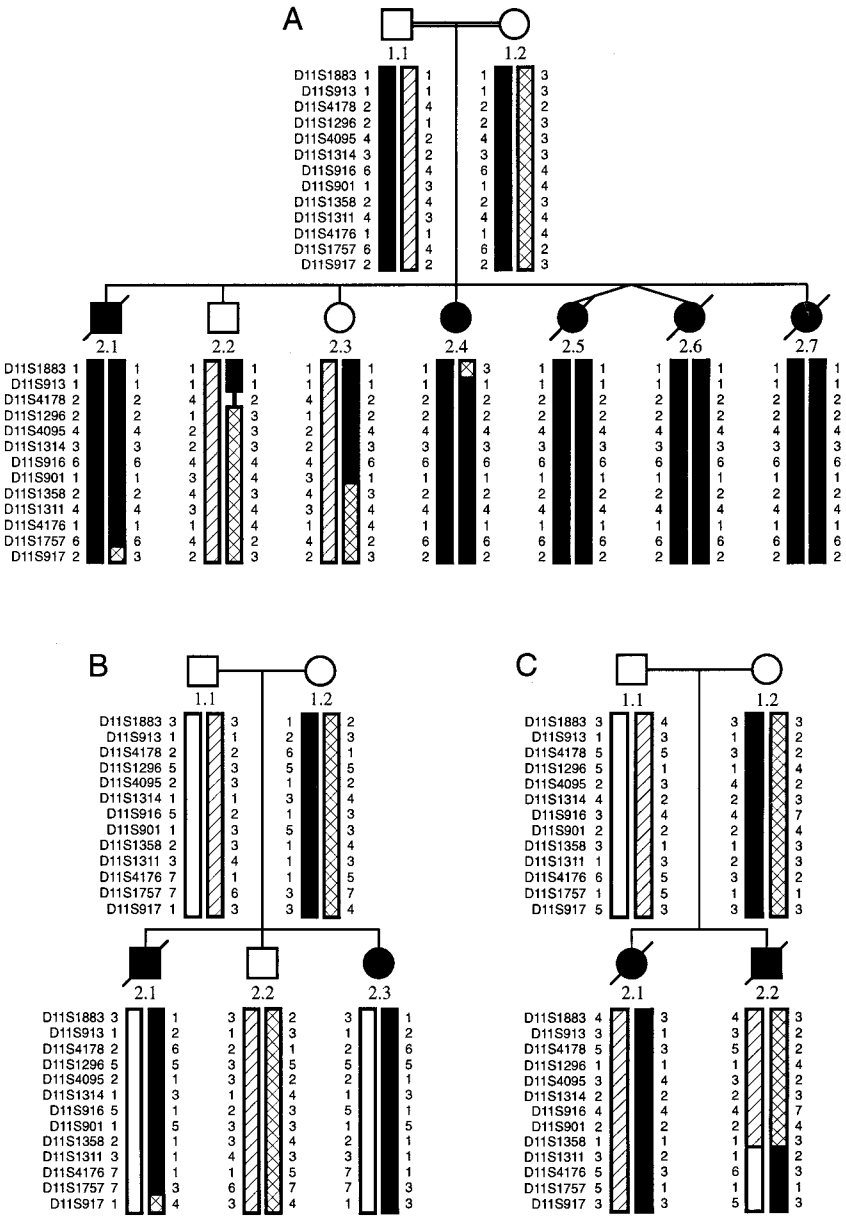

Figure 1 Haplotypes in families with diaphragmatic SMA subtypes. *A*, Family 1 (Lebanese origin): age at onset, 6–10 wk. *B*, Family 2 (German origin): age at onset, 9–12 wk. *C*, Family 3 (Italian origin): onset at birth. Haplotype analysis indicated that the cosegregating segment of the *SMARD* locus is flanked proximally by marker D11S1883 and distally by marker D11S917. Family 3 has no linkage to the *SMARD* locus. Blackened squares represent affected males; unblackened squares, unaffected males; blackened circles, affected females; unblackened circles, unaffected females; double line (in *A*), consanguinity.

tomated methods, by an ABI 377-Sequencer, and the results were processed by GENESCAN software, as described elsewhere (Saar et al. 1997). The whole-genome linkage scan was performed with the use of 340 polymorphic fluorescence–labeled markers spaced at ~10-cM intervals throughout the autosomal part of the genome. Subsequent fine mapping was performed with eight additional microsatellite markers. Markers were chosen from the Généthon final linkage map. Two-point parametric linkage analyses were performed with the LINKAGE package, version 5.2 (Lathrop and Lalouel 1984), under the following assumptions: a regular, fully penetrant autosomal recessive trait locus with a disease-allele frequency of .002 and no phenocopy rate, codominant marker loci with uniformly distributed allele frequencies, and standard recombination rates. Multipoint

Table 1

LOD-Score Values at Standard Recombination Rates for Markers on Chromosome 11q in Lebanese Family 1

			LOD Score at θ =						
Marker	Position[a]	Heterozygosity[b]	.00	.01	.05	.10	.15	.20	.30
D11S1883	68.5	.73	$-\infty$	−.87	−.24	−.03	.06	.09	.09
D11S913	70.9	.57	1.15	1.13	1.04	.93	.81	.69	.43
D11S1296	71.0[c]	.50	3.16	3.10	2.86	2.55	2.23	1.91	1.22
D11S4095	71.0	.64	3.16	3.10	2.86	2.55	2.23	1.90	1.22
D11S4178	71.5	.67	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S1314	77.5	.77	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S916	80.1	.72	2.96	2.90	2.67	2.38	2.07	1.76	1.09
D11S901	89.8	.82	3.16	3.10	2.86	2.55	2.23	1.90	1.22
D11S1358	96.3	.75	3.16	3.11	2.88	2.59	2.28	1.96	1.28
D11S1311	97.5	.75	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S4176	97.5	.82	1.75	1.72	1.58	1.40	1.22	1.04	.66
D11S1757	98.1	.65	3.16	3.11	2.88	2.59	2.28	1.96	1.28
D11S917	100.9	.80	$-\infty$	−.28	.30	.45	.47	.44	.30

[a] Sex-averaged genetic coordinates on chromosome 11 (cM), according to the Généthon map.
[b] Estimated value.
[c] Estimated from the genetic maps of the Marshfield Medical Research Foundation Center for Medical Genetics.

analysis was performed with the GENEHUNTER program, version 1.3 (Kruglyak et al. 1996).

Genomewide linkage scanning of family 1 revealed linkage of diaphragmatic SMA only to markers on chromosome 11q13-q21. In the following, we name this subtype of diaphragmatic SMA "spinal muscular atrophy with respiratory distress" (SMARD). For the markers D11S1296, D11S4095, D11S901, D11S1358, and D11S1757, a maximum two-point LOD score of 3.16 at recombination fraction (θ) 0 was obtained. The two-point LOD scores for 13 markers on chromosome 11q are summarized in table 1. Haplotype analysis revealed a recombination event in individual 2.4 that placed the disease locus distal to marker D11S1883 (fig. 1*A*). The crossing-over in individual 2.1 placed the disease locus proximal to marker D11S917. Consistent with parental consanguinity, all affected siblings from family 1 were autozygous for all markers within the cosegregating segment. Multipoint linkage analysis with the use of 13 markers yielded a maximum LOD score of 3.86, which clearly places the disease locus between D11S1883 and D11S917 (Généthon map positions 68.5 cM and 100.9 cM).

In family 2, the two affected sibs shared two identical parental haplotypes in the SMARD cosegregating segment on 11q13-q21, a finding that supports the assignment of the *SMARD* locus to this region (fig. 1*B*). In family 3, haplotype analysis was inconsistent with linkage to the markers tested (fig. 1*C*). Thus, this locus was excluded as being responsible for the disease in this family. Our finding that diaphragmatic SMA with onset at age 6–12 wk is linked to chromosome 11q markers in two apparently unrelated families from different countries (families 1 and 2) but that diaphragmatic SMA with onset at birth does not show such linkage (family 3) suggests that diaphragmatic SMA is both clinically and genetically heterogeneous.

The prevalence of diaphragmatic SMA is unknown. However, in a series of >200 patients with early-onset SMA, ~1% presented with diaphragmatic SMA and did not have a deletion of the survival motor-neuron gene (*SMN*) on chromosome 5q (Rudnik-Schöneborn et al. 1996). Considering the case history of the affected son from family 1 who had suspected SIDS, we presume that some of those infants with SIDS may possibly have been misdiagnosed. We are currently looking for further patients with SMARD, to refine the large cosegregating region on chromosome 11q.

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KATJA GROHMANN,[1] THOMAS F. WIENKER,[5] KATHRIN SAAR,[7] SABINE RUDNIK-SCHÖNEBORN,[8] GISELA STOLTENBURG-DIDINGER,[3] RAINER ROSSI,[4] GIUSEPPE NOVELLI,[9] GUDRUN NÜRNBERG,[7] ARNE PFEUFER,[2] BRUNHILDE WIRTH,[6] ANDRÉ REIS,[7] KLAUS ZERRES,[8] AND CHRISTOPH HÜBNER[1]

[1]*Clinic of Neuropediatrics and* [2]*Institute of Laboratory Medicine, Charité, Humboldt University,* [3]*Department of Neuropathology, University Hospital Benjamin-Franklin, and* [4]*Children's Hospital Neukölln, Berlin; Institutes of* [5]*Medical Statistics and* [6]*Human Genetics, University of Bonn, Bonn;* [7]*Microsatellite Center, Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany;* [8]*Department of Human Genetics, Technical University, Aachen, Germany; and* [9]*Department of Biopathology and Diagnostic Imaging, Tor Vergata, University of Rome, Rome*

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Address for correspondence and reprints: Dr. C. Hübner, Clinic of Neuropediatrics, Charité, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: christoph.huebner@charite.de

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Further Evidence for a Susceptibility Locus on Chromosome 20q13.11 in Families with Dominant Transmission of Graves Disease

To the Editor:

The susceptibility loci for Graves disease (GD [MIM 275000]), which is a common complex trait (Brix et al. 1998), have been difficult to define (Roman et al. 1992; McLachlan 1993; Davies 1998; Farid 1998; Vaidya et al. 1999). Tomer et al. (1998) recently found evidence for linkage of GD to markers on the long arm of chromosome 20 (MIM 603388), with a peak multipoint LOD score of 3.5 at the marker D20S195. Their linkage analysis was performed by both parametric and nonparametric methods, and their cohort of 53 families with at least two first-degree relatives affected with autoimmune thyroid disease (AITD) was derived from the

Table 1

Phenotypes of Affected Sib Pairs with AITD

Sib-Pair Type	No. with Phenotype		
	GD-GD[a]	GD-AH[b]	All AITD
Full	66	6	72
Half	5	0	5
Total	71	6	77

[a] Sib pairs with GD only.

[b] Sib pairs with mixed GD and autoimmune hypothyroid. Families were selected on the basis of two affected sibs with GD. GD-AH sib pairs make up additional members of the same families.

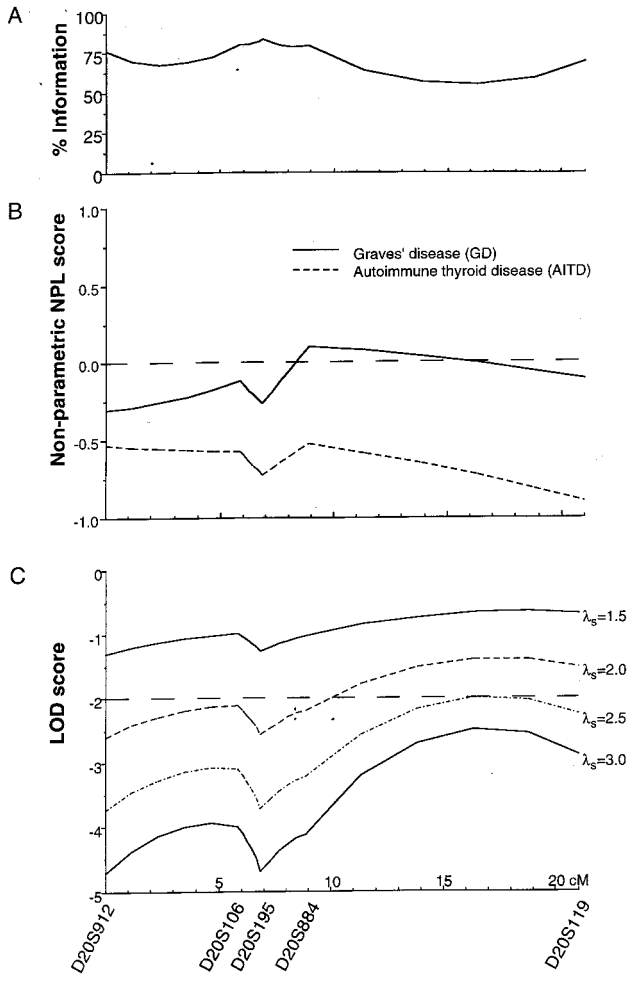

Figure 1 Linkage analysis in all 64 affected GD kindreds. *A,* Percentage information content shown at each of the map positions. *B,* Multipoint nonparametric linkage analysis of kindreds with GD for chromosome 20q13.11 markers. Genotyping was performed by PCR with fluorescently labeled Généthon markers (Dib et al. 1996) and was resolved by use of a laser detection system (ABI). Linkage analysis was performed by the "score all" function of GENEHUNTER (Kruglyak et al. 1996), with either GD or AITD as the affected phenotype. The marker order and genetic distances shown are derived from our own data and correspond closely to the sex-averaged Généthon and Marshfield Medical Research Foundation Center for Medical Genetics maps (Dib et al. 1996; Broman et al. 1998). There is no evidence for linkage of GD or AITD to any of the five markers studied. *C,* Exclusion mapping of chromosome 20q13.11 as a GD-susceptibility region. The "exclude" function of GENEHUNTER was used to plot the probability (LOD score) that 20q13.11 contained a hypothetical GD locus with λ_s values of 1.5, 2.0, 2.5, and 3.0 at each position of the marker map (Kruglyak and Lander 1995; Kruglyak et al. 1996). All affected sib pairs were used. There is no evidence to suggest linkage (LOD score > 0) for a locus of $\lambda_s = 1.5$, and a locus of $\lambda_s = 2.5$ can be formally excluded (LOD score < −2.0) from this region.

North American, Italian, Israeli, and British populations (Tomer et al. 1998).

We have examined this chromosomal region in a homogeneous cohort of 71 affected GD sib pairs derived from 64 multiplex British GD kindreds (146 subjects with GD, 20 with autoimmune hypothyroidism [MIM 140300], and 72 unaffected). In six families, an additional sibling had autoimmune hypothyroidism, resulting in a total of 77 affected sib pairs with AITD (i.e., either GD or autoimmune hypothyroidism) (table 1). Parents (n = 49) and unaffected sibs (n = 36) were studied wherever available. All subjects were white, and >95% of the grandparents were from the mainland United Kingdom or were of Irish origin. The clinical definitions of GD and autoimmune hypothyroidism were identical to those described elsewhere (Tomer et al. 1998). Fifty-four (37%) of the patients with GD had significant thyroid-associated orbitopathy (class 3 or worse) (Werner 1977). Background allele frequencies were derived from typing of DNA obtained from local subjects without evidence of autoimmune disease. Nonparametric, parametric, and exclusion-mapping analyses were performed with the use of the GENEHUNTER package, version 2.0 (Kruglyak et al. 1996). For parametric analyses, a population frequency of 1% for GD was assumed, with a nonsusceptibility-genotype penetrance of .005, and allele frequencies were varied, according to Hardy-Weinberg equilibrium, for each susceptibility-genotype penetrance studied.

Multipoint nonparametric analysis with the use of five microsatellite markers spanning a 21-cM area of 20q13.11 showed no evidence to support linkage in the 71 GD sib pairs, with a peak NPL (nonparametric linkage) score of 0.1 occurring at the marker D20S884 (fig. 1). We were able to formally exclude (LOD score< −2.0) a hypothetical GD locus with a $\lambda_s > 2.5$ from this entire region (fig. 1). Parametric analysis was performed both with and without the assumption of heterogeneity, with both recessive and dominant models. There was no evidence for linkage of GD to this region at disease penetrances of 30%, 60%, or 90%, with either model of inheritance, in the 71 sib pairs (table 2).

The ascertainment strategy (at least two affected sibs with GD) used to recruit families for our study was different from that (at least two affected first-degree relatives with AITD) used by Tomer et al., such that their cohort of families was likely to contain many more affected parent-offspring kindreds (Tomer et al. 1998). We speculated that such affected parent-offspring kindreds might have enriched their cohort for families segregating dominant loci and that this difference in ascertainment might explain the apparent discrepancy between our findings, if the susceptibility locus segregated as a dominant (McCarthy et al. 1998). Therefore, we investigated linkage both in a subgroup of 12 families (38 subjects

Table 2

Peak Multipoint Parametric LOD Scores for the Chromosome 20q13.11 Markers in the 64 Families with GD and in the Subset of 12 Kindreds with Dominant Transmission of GD

	LOD Score			
	Without Heterogeneity		With Heterogeneity	
Penetrance	Dominant	Recessive	Dominant (α)	Recessive (α)
All 64 kindreds:				
30%	−6.44	−9.28	.01 (.05)	.00 (.00)
60%	−8.08	−13.60	.01 (.05)	.00 (.00)
90%	−9.03	−16.49	.01 (.05)	.00 (.00)
12 Dominant kindreds:				
30%	.72	.12	1.05 (.73)	.37 (.58)
60%	.40	−.20	1.06 (.68)	.34 (.50)
90%	.22	−.43	1.06 (.66)	.33 (.49)

with GD) who had apparent dominant transmission of GD from parent to offspring and in a subgroup of 28 families with dominant transmission of AITD from parent to offspring (75 subjects with GD and 17 with autoimmune hypothyroid). Multipoint nonparametric analysis in the 12 families with dominant transmission of GD showed a 4-cM plateau suggestive of linkage, with a peak NPL score of 2.02 (P = .023) occurring at the marker D20S106 (fig. 2). This was not observed in the larger subgroup of 28 families with parent-to-offspring transmission of AITD (fig. 2). Parametric analysis in the subgroup with dominant transmission of GD, with the assumption of heterogeneity, showed a peak LOD score of 1.06 occurring at the marker D20S884 with a dominant model (table 2).

Our study provides some evidence to support the presence of a GD-susceptibility locus in this region of 20q13.11 (Tomer et al. 1998), and we show that this locus appears to be important only in families with dominant inheritance of GD. The small number of such kindreds that we have studied precludes a reliable estimate of the strength of effect of this locus, but our ability to detect the effect using only 12 families with this structure, coupled with the 1:0 allele-sharing ratio of 69% between the sib pairs with GD, suggests that it may have a strong effect. In contrast, our families with affected subjects with GD in only one generation and our families with dominant transmission of AITD do not show evidence of linkage to this locus (figs. 1 and 2). Analysis of a larger cohort of kindreds with dominant transmission of GD is necessary to confirm the presence of this susceptibility locus for GD. However, the recent mapping of a susceptibility locus for systemic lupus erythematosus (MIM 152700) to this region of chromosome 20 in two different mixed American cohorts (Gaffney et al. 1998; Moser et al. 1998) suggests that this region may harbor a polymorphism(s) that is important in other autoimmune disorders. In addition, our study illustrates

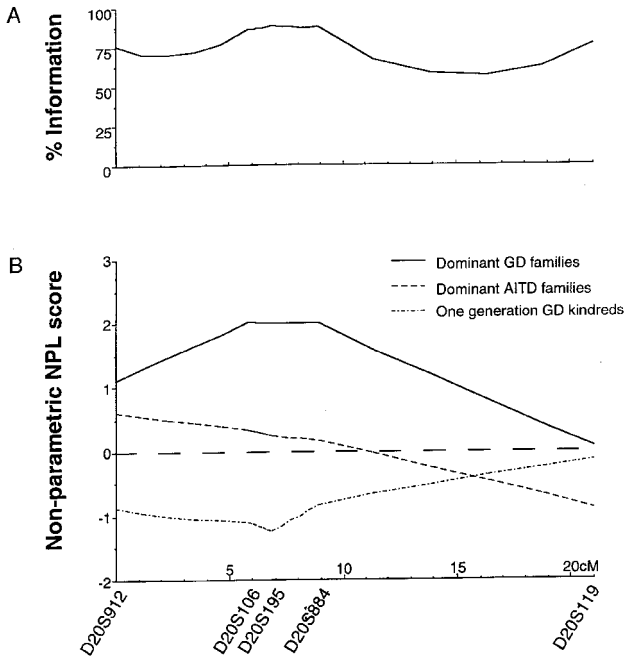

Figure 2 Linkage analysis of the subset of 12 GD kindreds with dominant transmission of GD, and other groups. *A*, Percentage information content for the 12 families with dominant transmission of GD is shown at each of the map positions. *B*, Multipoint nonparametric linkage analysis of the subsets of GD kindreds for chromosome 20q13.11 markers. Genotyping and linkage analysis were performed as described in figure 1. There is an ~4-cM region of excess allele sharing between markers D20S106 and D20S884, encompassing D20S195, in the families with dominant transmission of GD (*unbroken line*). The maximum evidence for linkage, an NPL score of 2.02 (P = .023), occurs at marker D20S106. In contrast, there is no evidence to support linkage either in subsets of families with dominant transmission of AITD (i.e., including kindreds with transmission from a parent with autoimmune hypothyroid to offspring with GD, or vice versa) or in families with only one generation affected by GD (*dashed lines*).

that the ascertainment strategies employed in the collection of cohorts of kindreds with complex disorders may have a marked effect on the ability to detect a given susceptibility locus (McCarthy et al. 1998).

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SIMON H. S. PEARCE,[1] BIJAYESWAR VAIDYA,[1] HELEN IMRIE,[1] PETROS PERROS,[1] WILLIAM F. KELLY,[2] ANTHONY D. TOFT,[3] MARK I. MCCARTHY,[4] ERIC T. YOUNG,[1] AND PAT KENDALL-TAYLOR[1]

[1]*Department of Endocrinology, School of Clinical Medical Sciences, University of Newcastle upon Tyne, United Kingdom;* [2]*Diabetes Care Centre, Middlesbrough General Hospital, Middlesbrough, United Kingdom;* [3]*Endocrine Unit, Royal Infirmary of Edinburgh, Edinburgh; and* [4]*Section of Endocrinology, Division of Medicine, Imperial College School of Medicine at St. Mary's, London*

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Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GD [MIM 275000], GD susceptibility locus 2 [MIM 603388], Hashimoto disease [MIM 140300], and systemic lupus erythematosus [MIM 152700])

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Address for correspondence and reprints: Dr. Simon Pearce, Department of Medicine, 4th Floor, Leech Building, The Medical School, Newcastle upon Tyne, NE2 4HH, United Kingdom. E-mail: spearce@hgmp.mrc.ac.uk

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Primary Autosomal Recessive Microcephaly: Homozygosity Mapping of MCPH4 to Chromosome 15

To the Editor:

Microcephaly is a condition in which the head circumference is smaller than <3 SD below the mean for age. Syndromic microcephaly is found in a number of environmental, chromosomal, or single-gene disorders. Non-

Table 1

Phenotypic Data of the Microcephaly Kindred

Characteristics	Proband	Affected Sister	Affected Brother	Affected Brother
Age (years)	22	21	19	16
Height (cm)	157	152	156	157
Weight (kg)	35	44	39	36
Head circumference (cm)	44.5	47	48	45
Head circumference relative to mean for age (SD)	<−6	<−5	<−5	<−6

syndromic, isolated microcephaly is also etiologically heterogeneous, and micrencephaly—that is, a generalized reduction of the brain mass causing a small skull without craniosynostosis—appears as a distinct subtype within this group. When micrencephaly is the only or the leading pathological alteration, it is referred to as "primary microcephaly," or "microcephalia vera" (Ross and Frias 1977; Baraitser 1997). Mental retardation ranges from moderate to severe in primary microcephaly, although motor development may be normal during the first years of life. Linear and ponderal growth is often impaired, and, although the cause for this finding is not clear, a deficiency of growth hormone (GH) has been implicated in some cases (Dacou-Voutetakis et al. 1974). When familial, primary microcephaly often appears to be transmitted as an autosomal recessive disorder (MIM 251200) with a significant proportion of cases associated with parental consanguinity and with an incidence of 1/30,000–1/250,000 (Van den Bosch 1959, and references therein). Microcephaly may not be present until late in the third trimester of pregnancy, so prenatal diagnosis is problematic (Tolmie et al. 1987). Genetic heterogeneity has long been suspected, on the basis of subtle phenotypic differences among families (Cowie 1960). Recently, a locus for primary microcephaly, MCPH1, has been identified at 8p22-pter by homozygosity mapping (see below), and evidence for locus heterogeneity has been shown (Jackson et al. 1998). MCPH2 is ascribed to 19q13 in the Human Gene Nomenclature Database, in which the as-yet-unpublished locus MCPH3 has also been registered.

Homozygosity mapping in consanguineous families is based on the assumption that a rare mutation is inherited from a common ancestor via both parents, so that affected siblings are homozygous by descent, for polymorphic markers close to the disease locus. Comparison of genotypic data, both between and within subjects, makes it a powerful strategy that needs only a few affected individuals in order to map a recessive disorder (Lander and Botstein 1987). We now report homozygosity mapping of a new locus, MCPH4, to chromosome 15, in a newly ascertained family.

The propositus is a male 22 years of age who presented, at age 4 mo, with microcephaly and left cryptorchidism. He was a first child born after an uneventful pregnancy and delivery to healthy young Moroccan parents, who are first cousins once removed and who both have normal head circumferences and an otherwise unremarkable family history. No craniosynostosis was present in the patient, and the initial psychomotor devel-

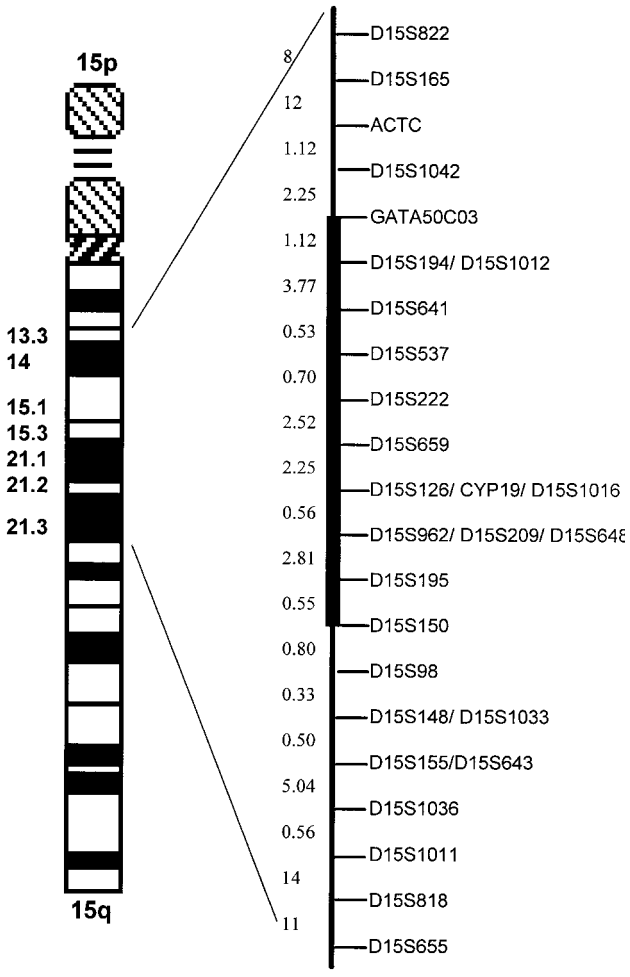

Figure 1 Genetic map of chromosome 15 markers. Distances are shown in centimorgans. The distance between markers ACTC and D15S98 is 19 cM. The blackened bar indicates the MCPH4 candidate region.

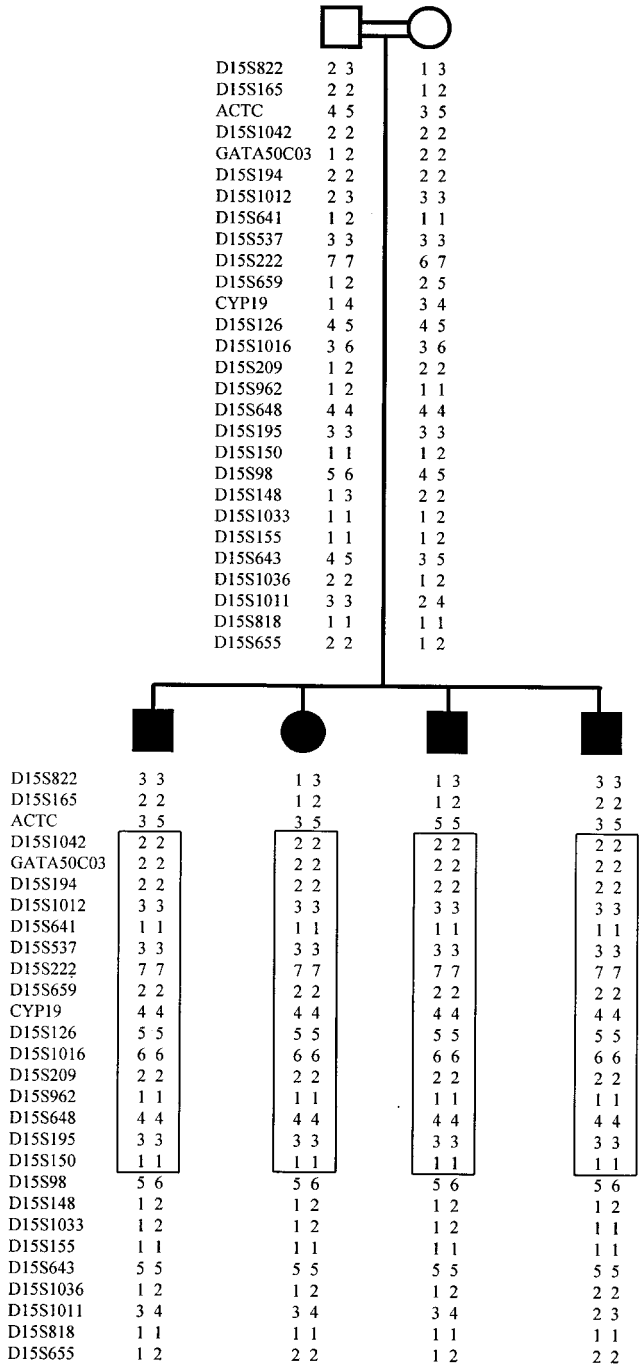

Figure 2 Haplotypes in the microcephaly kindred. Blackened symbols represent affected individuals. The region of homozygosity is boxed.

opment was normal. In early childhood, growth was at the 3d centile for height and weight, and microcephaly was <6 SD below the mean for age (41.5 cm at age 4.0 years). Mental retardation was noted. In late childhood, IQ measures were consistently ~≤50. A brain computed-tomography scan showed large cerebral ventricles and no cerebral malformation or neuronal ectopia. A partial deficiency of GH secretion was demonstrated by dynamic testing with insulin, glucagon, and GH-releasing factor. Therapeutic GH supplementation at age 11–13 years produced no change in the growth curves. The pubertal development was normal. The patient now has a kind, collaborative, cheerful personality. A sister and two younger brothers presented with an identical picture of microcephaly and with height and weight growth at approximately the 3d centile (table 1). Minor malformations were noted in the youngest boy (epicanthal folds, single palmar creases, a left preauricular tag, and myopia) but not in the sister and other brother. GH treatment in the youngest brother, at age 4–7 years, yielded no appreciable effect on growth. Results of karyotypes of blood lymphocytes were normal. Extensive metabolic workups gave normal results. The levels of maternal blood glucose and phenylalaninemia were strictly normal.

The parents and patients gave informed consent to the genomic study, and DNA was extracted from peripheral-blood leukocytes. In a first analysis, we studied markers from the 8p region, where MCPH1 maps (Jackson et al. 1998), and found no evidence for linkage. A genome-wide screen was then launched by use of a set of microsatellite markers from the Cooperative Human Linkage Center human screening set, Weber version 9 (Research Genetics). From this set of 386 markers, 239 (mainly tetranucleotides) were selected to span the entire genome in intervals of ≤30 cM, since this spacing is sufficient for detection of homozygosity by descent in a family with this coefficient of inbreeding (Terwilliger et al. 1997). A pooling approach was employed for the initial screen. The DNAs of the parents were pooled in one sample, and the DNAs of the affected children were pooled in another (Arbour et al. 1997). Subsequently, these pools were typed by PCR amplification using 6 ng of each individual's DNA in a 15-μl final volume, followed by PAGE and silver staining (Budowle et al. 1988). Seven loci in the affected siblings—on 2q, 8p, 12p, 12q, 13q, and 15q—were identified as homozygous by state. These loci were then analyzed in the individual subjects, with additional, closely spaced markers (<2 cM apart). Marker order was obtained from the Center for Medical Genetics map, the Cooperative Human Linkage Center map, GeneMap '98, and the Genetic Location Database. When minor discrepancies between the various maps were observed, radiation-hybrid mapping was performed to determine the most probable order, by use of the GeneBridge 4 panel (Research Genetics) and the RH mapping program of the Whitehead Institute for Biomedical Research/MIT Center for Genome Research.

Six of the seven initial regions (listed above) were not consistently homozygous at each polymorphic locus, indicating, for the initial marker locus, identity by state

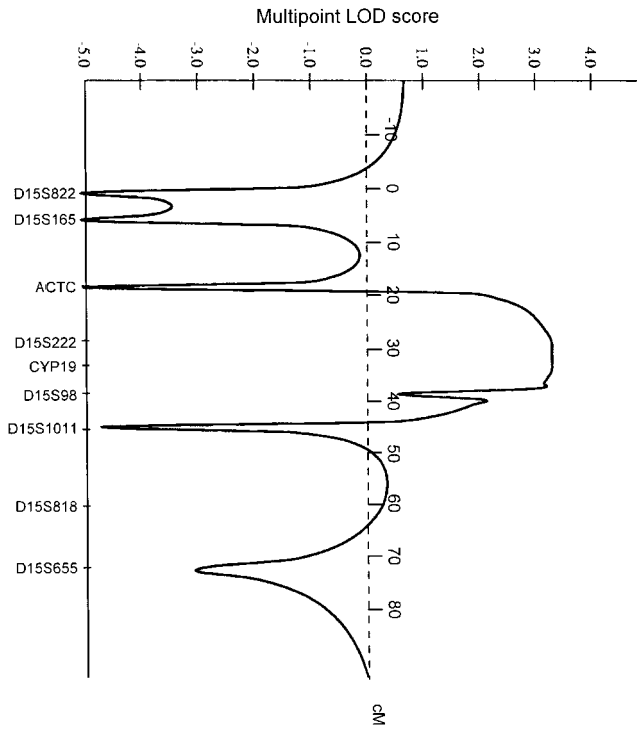

Figure 3 Results of MAPMAKER/HOMOZ multipoint linkage analysis. A maximum multipoint LOD score of 3.29 is observed at CYP19.

rather than identity by descent. Conversely, one of the seven markers initially identified on chromosome 15q was found to be part of a genomic segment (fig. 1) where all informative markers were homozygous in the affected siblings but were heterozygous in the parents. This was consistent with identity by descent and homozygosity for a disease haplotype (fig. 2).

Multipoint linkage analysis was performed by use of the MAPMAKER/HOMOZ algorithm software (Kruglyak et al. 1995), under the assumption of a fully penetrant disease with an allele frequency of .002. Allele frequencies for each polymorphic marker of the candidate region were evaluated by genotyping 30 unrelated individuals from the same ethnic population. This analysis provided a maximum multipoint LOD score of 3.29 (fig. 3). Heterozygosity was found in one of the affected siblings for marker ACTC and in all four affected siblings for marker D15S98 (fig. 2), indicating that recombination events had occurred at both loci. Thus, a minimal critical region—that is, the smallest region found to be identical by descent, in all affected siblings—of 19 cM was observed between markers ACTC and D15S98. Because of uninformativeness of parental markers for its boundaries, however, the critical region might be as small as 5.3 cM, encompassing D15S222 and D15S962.

Although linkage to this candidate region should be confirmed in additional families, our results present strong evidence for the presence of a new gene, MCPH4, at15q15-q21, a mutation of which presumably affects an aspect of neuronal proliferation. Although, during the past few years, knowledge of neuronal migration and brain-patterning defects such as holoprosencephaly or schizencephaly has increased, the molecular defects of neuronal proliferation are still poorly known. Considering the complexity of this process, locus heterogeneity is not unexpected (Walsh 1999). Identifying the genes implicated in primary microcephaly may prove particularly useful, since proper animal models for the developmental defects affecting the growth of the hemispheres are lacking, in part because of its human-specific nature.

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C. Ruth Jamieson,[1] Cédric Govaerts,[2] and Marc J. Abramowicz[3]
[1]*Laboratoire de Génétique Médicale,* [2]*IRIBHN, and* [3]*Service de Génétique Médicale, Université Libre de Bruxelles, Brussels*

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Cooperative Human Linkage Center, http://lpg.nci.nih.gov/CHLC (for microsatellite markers used for the genome screen)
GeneMap'98, http://www.ncbi.nlm.nih.gov/genemap98/ (for order and distances of markers on chromosome 15)
Genetic Location Database, http://cedar.genetics.soton.ac.uk/ (for order and distances of markers on chromosome 15)
Human Gene Nomenclature Committee, http://www.gene.ucl.ac.uk/nomenclature/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for microcephaly [MIM 251200])
Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www-genome.wi.mit.edu/ (for RH mapping of markers on chromosome 15)

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Address for correspondence and reprints: Dr. C. Ruth Jamieson, Laboratory for Medical Genetics, Campus Erasme–ULB, Building C, 808, Lennik, B-1070 Brussels, Belgium. E-mail: rjamieso@ulb.ac.be

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Association of *RET* Protooncogene Codon 45 Polymorphism with Hirschsprung Disease

To the Editor:

The *RET* protooncogene (MIM 164761) is expressed in human tissues of neural crest origin and has been recognized as a susceptibility gene for several autosomal inherited diseases, such as Hirschsprung disease (HSCR [MIM 142623]) and multiple endocrine neoplasia type 2 syndromes (MEN 2 [MIM 171400]) comprising medullary thyroid carcinoma (MTC [MIM 155240]) as an obligatory feature (Eng et al. 1997). Of the patients with HSCR, 10%–40% have been reported to harbor germline mutations of the *RET* protooncogene, which are primarily point mutations scattered throughout the extracellular domain and within the intracellular tyrosine kinase domain of *RET* (Edery et al. 1994*b;* Romeo et al. 1994; Angrist et al. 1995; Seri et al. 1997). MEN 2 syndrome germline mutations of the *RET* protooncogene have been found to affect exons 10, 11, and 13–16 (Donis-Keller et al. 1993; Mulligan et al. 1993; Carlson et al. 1994; Eng et al. 1994; Hofstra et al. 1994; Bolino et al. 1995). Functional studies have demonstrated that *RET* mutations that characterize the autosomal dominant–inherited MEN 2 cause activation of the RET-signaling pathway, often in a constitutive manner or by altering the substrate specificity (Borrello et al. 1995; Santoro et al. 1995; Ceccherini et al. 1997; Pasini et al. 1997; Chappuis-Flament et al. 1998). In contrast, *RET* mutations found in HSCR presumably result in either RET-protein truncation or functional inactivation of the molecule. Although loss of one allele in some patients with HSCR suggests haploinsufficiency (Martucciello et al. 1992), the retention of one wild-type allele in patients with HSCR who have an inactivating *RET* mutation seems to explain the presumed autosomal dominant inheritance and implicates a dominant-negative action of the mutated *RET* allele (Badner et al. 1990; Cosma et al. 1998).

Furthermore, several polymorphisms in the coding region of the *RET* protooncogene have been described. A panel of the most frequent polymorphisms has been reported by Mulligan et al. (1993), Ceccherini et al. (1994), and Sáez et al. (1998), comprising those in codons 45, 125, 432, 691, 769, 836, and 904. In the study by Ceccherini et al., the allele frequencies of these polymorphisms were evaluated in a normal control group. These data were confirmed by a study by Gimm et al. (1999), and similar allele frequencies of the codon 45 polymorphism have been described by Edery et al. (1994*a*), who focused on a control population only. All of the investigated polymorphisms are silent mutations, except for the codon 691 polymorphism, which results in a change in the amino acid residue, from glycine to serine. Bugalho et al. (1994) investigated the frequency of the codon 691 polymorphism in a small population of clinically defined sporadic medullary thyroid carcinomas (MTC) and found no significant differences from a normal control population. Elsewhere, Gimm et al. (1999) have investigated all seven *RET* polymorphisms in a population with sporadic MTC and have found an

Table 1

Allele Frequencies of Polymorphic Variants of *RET* in 62 Patients with Sporadic HSCR and in 156 Control Individuals

Exon	Nucleotide Change (Codon)[a]	Restriction Site Changed	Allele Frequency in[b] (%)		Statistic	
			Controls	Patients with HSCR	χ^2	P
2	GCG→GCA (A45A)	*Eag*I	76.3	26.6	93.064	<.001
3	GTC→GTA (V125V)	*Mbo*II	98.1	97.6	.108	.742
7	GCG→GCA (A432A)	*Bsm*I	72.4	74.2	.139	.709
11	GGT→AGT (G691S)	*Ban*I	79.8	89.5	5.811	.016
13	CTT→CTG (L769L)	*Taq*I	76.3	57.3	15.556	<.001
14	AGC→AGT (S836S)	*Alu*I	96.4[c]	100	4.575	.032
15	TCC→TCG (S904S)	*Rsa*I	80.1	88.7	4.540	.033

[a] The wild-type allele is underlined.
[b] Of the wild-type allele.
[c] Only 153 control individuals were tested.

overrepresentation of the rare codon 836 polymorphism, compared with the frequency in normal controls. Interestingly, in this study the rare germline codon 836–sequence variant seems to be associated with the presence of a common somatic M918T mutation in the corresponding tumor DNA of patients with sporadic MTC.

To reveal the potential impact that *RET* polymorphisms for etiology have for HSCR in particular, we investigated the genotype distribution of polymorphisms of codons 45, 125, 432, 691, 769, 836, and 904 of the coding region of the *RET* protooncogene in patients with HSCR but without a family history of the disease. The population that we studied comprised 62 individuals with sporadic HSCR who were from two different areas of Germany, around the cities of Dresden (n = 37) and Erlangen (n = 25). The male:female ratio of these individuals was 3.8:1. For inclusion in the study, histopathological criteria of HSCR were (*a*) increased acetylcholinesterase histochemical staining in nerve fibers, in suction biopsies of the rectal submucosa, and (*b*) absence of neuronal ganglia, in operative histochemical and histological evaluation of the aganglionic tract. Patients with additional features or associated diseases were excluded from the study. Anonymous healthy blood donors from each region served as controls (n = 117 for Dresden; n = 39 for Erlangen). Controls were not matched for age or race, although all individuals were white. There was, therefore, a slight potential for population stratification in the patients with HSCR, relative to that in the controls. Genomic DNA was obtained from leukocytes from peripheral venous blood samples isolated by standard protocols. The seven investigated exons were amplified from genomic DNA by use of primers and reaction conditions described by Ceccherini et al. (1994), for exons 2 (codon 45), 3 (codon 125), 11 (codon 691), and 14 (codon 836), and by Mulligan et al. (1994), for exons 7 (codon 432) and 13 (codon 769). To amplify exon 15 (codon 904), we generated a new primer pair (sense, 5′CCCCCGGCCCAGGTCTCAC-3′; antisense, 5′GCTCCACTAATCTTCGGTATCTTT-3′). All analyzed polymorphisms generate or destroy a restriction site of an endonuclease—namely, *Eag*I, *Mbo*II, *Bsm*I, *Ban*I, *Taq*I, *Alu*I, or *Rsa*I (Ceccherini et al. 1994). Genotypes were determined by digestion of the PCR product and electrophoresis on a polyacrylamide gel. In addition, these results from the patient population were confirmed by DNA-sequencing analysis by use of the Thermo Sequenase™ Fluorescent Cycle Sequencing kit (Amersham Pharmacia Biotech), according to the manufacturer's protocol. The sequencing primers were the same as the PCR primers, with an additional Cy5TM labeling, allowing sequence analysis on A.L.F. express devices (Amersham Pharmacia Biotech). Statistical analysis was performed with the Pearson χ^2 test. Written informed consent was obtained from all patients.

Our data revealed that allele frequencies of all polymorphisms in the control population were similar to those reported by Ceccherini et al. (1994), Gimm et al. (1999), and Edery et al. (1994*a*), suggesting that the allele frequency is similar in the German, European, and American populations tested, but the study does not include data of an ethnically diverse, nonwhite population. The genotype distribution for each of the seven polymorphic loci did not deviate significantly from Hardy-Weinberg equilibrium. Although the wild-type allele of the codon 45 polymorphism was detected in 76.3% of 312 control chromosomes, the same allele was found in 26.6% of 124 HSCR chromosomes, an almost inverted relationship (table 1) (for allele frequencies in patients with HSCR vs. those in controls, $\chi^2 = 93.06$, $P < .001$). This highly significant difference between these allele frequencies resulted from a strong overrepresentation of the homozygous codon 45–polymorphism variant in the population with HSCR (34 of 62 patients with HSCR,

vs. 9 of 156 controls). Ceccherini et al. (1994) found the wild-type allele of the codon 45 polymorphism in 71% of 104 chromosomes, the same frequency as later was reported, by Gimm et al. (1999), in an analysis of 96 chromosomes. Furthermore, we found this highly significant association of the codon 45 polymorphism also in the two independent populations with HSCR and in controls from the regions around Erlangen and Dresden (for the allele frequency in Dresden patients with HSCR vs. that in Dresden controls, $\chi^2 = 60.65$, $P < .001$; for the allele frequency in Erlangen patients with HSCR vs. that in Erlangen controls, $\chi^2 = 31.65$, $P < .001$).

Within the population with HSCR, a tendency toward overrepresentation of the codon 769 polymorphism, similar to that of the codon 45 polymorphism, was found, compared with the frequency in the controls (table 1). In addition, we found the codon 769 polymorphism to be associated with HSCR in both populations, compared with what was found in the controls (for the allele frequency of Dresden patients with HSCR vs. that in Dresden controls, $\chi^2 = 9.26$, $P = .002$; for the frequency in Erlangen patients with HSCR vs. that in Erlangen controls, $\chi^2 = 5.72$, $P < .017$).

Although in codons 45 and 769 the polymorphic allele was overrepresented in the population with HSCR, in codons 691, 836, and 904 we found the wild-type allele to be more frequent in the population with HSCR population than in the control group, although the difference was not statistically significant (table 1).

In this study we have demonstrated that the codon 45–polymorphism allele frequency is overrepresented in patients with sporadic HSCR compared with the normal population, a finding that is highly significant statistically. In agreement with our findings, Puffenberger et al. (1994) described a significant excess of this polymorphism (for allele frequencies, $\chi^2 = 12.08$, $P < .001$) on the HSCR haplotype that is transmitted to affected members of Mennonite families with HSCR. However, the predominant mutation identified in this kindred is a founder homozygous W276C *EDNRB* (MIM 131244) gene mutation, which is an interesting association in itself and supports the polygenic, complex inheritance of HSCR. In addition, one patient has been described with both an *EDNRB* mutation and a *RET* mutation that apparently result in aberrant *RET* RNA splicing (Auricchio et al. 1999).

The mechanism by which the silent codon 45 polymorphism may act in HSCR genesis is unknown, but speculations have been made regarding the possible mechanisms. It has, for instance, been proposed that the silent sequence variant could lead to aberrantly spliced products, resulting in a protein with a 21-amino-acid deletion in the extracellular domain, altering a part of the extracellular signal-peptide sequence (Borrego et al. 1998).

In addition, it has been suggested that a seemingly nonfunctional polymorphism may create an unstable downstream sequence, which results in a functional somatic mutation (Gimm et al. 1999). Such a mechanism has been observed in the *APC* (MIM 175100) gene in Ashkenazim with familial colorectal cancer, in which additional somatic mutations were more often found on the allele carrying a conservative amino acid change (I1307K) (Laken et al. 1997).

If no pathogenic effect can be associated with the codon 45 *RET* polymorphism, then the possibility has to be considered that the base substitution is in linkage disequilibrium with an unknown functional variant upstream or downstream. For example, an *Msp*I RFLP of the *3′* end of the human *CYP1A1* (MIM 108330) gene has been shown to be in linkage disequilibrium with an adenine-to-guanine mutation at residue 462 in exon 7. The latter mutation causes an amino acid substitution, which results in increased enzymatic activity of CYP1A1 (Hayashi et al. 1991). Similarly, the silent codon 45 polymorphism may be either closely linked with a functional genetic variant or be functional itself.

Nevertheless, the observed difference in the homozygous genotype of the silent polymorphism—5.8% in the normal population of 156 individuals versus 54.8% in 62 analyzed patients with HSCR—suggests a strong association with the HSCR phenotype.

GUIDO FITZE,[1] MATTHIAS SCHREIBER,[4] EBERHARD KUHLISCH,[3] HANS K. SCHACKERT,[2] AND DIETMAR ROESNER[1]

Departments of [1]Pediatric Surgery and [2]Surgical Research and [3]Institute of Medical Informatics and Biometry, University of Technology Dresden, Dresden; and [4]Department of Pediatric Surgery, University of Erlangen, Erlangen, Germany

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Address for correspondence and reprints: Dr. Guido Fitze, Department of Pediatric Surgery, University of Technology Dresden, Fetscherstrasse 74, D-01307 Dresden, Germany. E-mail: Guido.Fitze@mailbox.tu-dresden.de

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The Sex Ratio in Familial Persistent Stuttering

To the Editor:

Stuttering is a speech disorder characterized by involuntary syllable repetitions, syllable prolongations, or interruptions, known as blocks, in the smooth flow of speech (World Health Organization 1992; Bloodstein 1995). Stuttering typically arises in young children, where it affects ⩾15% of children in the age range of 4–6 years (Bloodstein 1995). Stuttering often resolves spontaneously before adolescence, leading to a population prevalence of 1%–2% among adults. Stuttering beyond childhood is characterized by a significant bias toward males, with males outnumbering females by a ratio of 3:1–5:1 (Yairi et al. 1996).

Many studies support the view that inherited factors contribute to stuttering (Howie 1981; Yairi et al. 1996; Felsenfeld and Plomin 1997). As part of a linkage study to identify predisposing loci for this disorder, we assembled >100 small-to-medium–sized unrelated families with multiple cases of persistent stuttering, chosen to represent the typical presentation of familial stuttering in the adult population. In these families, we have observed a male-to-female ratio among the affected individuals that is strikingly different from the generally accepted ratio in the overall adult stuttering population.

Family ascertainment was designed to obtain the most diverse sample possible from the North American population. The NIH families were ascertained under NIH IRB-approved protocol 97-DC-0087, through a broad variety of appeals directed at stuttering interest groups, stuttering support groups, professional speech and language organizations, alumni of stuttering therapy programs—including intensive residential programs and part-time, outpatient programs—and the general public. The enrolled families included whites, African Americans, Hispanics, and Asians, with no evidence for over- or under-representation of any group compared to the general population. Among the identifiable probands in these families, 56% were male and 44% were female. We exhaustively ascertained and evaluated family members aged >8 years according to well-established diagnostic criteria for stuttering (Webster 1978; World Health Organization 1992), using videotaped speech samples and counting the number of stuttering-like dysfluencies, in both conversation and reading. In some cases, audio tape recordings were substituted. The standardized reading passage was 500 words in length and contained balanced numbers of each of the different classes of speech sounds. This tool has been used for >10 years and has well-established performance norms (R. Webster, personal communication; copy available, on request, from corresponding author). For individuals to be classified as affected, a score of ⩾4% dysfluent words (representing the 25th percentile among individuals who present themselves for stuttering therapy) was required in the individual's speech in both conversation and reading. In some cases, videotaped speech samples were not obtainable, and audio recordings of speech were substituted. By these criteria, 224 individuals were classified as affected in our families. Affection status, as determined by professional speech evaluation, was generally in agreement with self-reported affection status. The few discrepancies showed no evidence of bias between males and females. The affected individuals had an age range of 10–86 years, with a mean age of 39.9 years. Among these affected individuals, 137 are male and 87 are female, yielding a male-to-female ratio of 1.57.

To compare this ratio to the male-to-female ratio in the general stuttering population, we examined four different populations of unrelated, persistent stutterers. We chose four different groups of persistent stutterers, because each group was subject to individual ascertainment biases. For example, therapy programs are generally believed to ascertain males preferentially, while support groups are believed to attract more females, frequently affected mothers of affected children. We sought the largest available sources of such populations of stutterers and derived data from the clinical records of two large therapy programs, the Hollins Communications Research Institute (HCRI) and the American Institute for Stuttering (AIS), plus data on two groups, ascertained

Table 1

Numbers of Males and Females in Populations of Unrelated Persistent Stutterers, and χ^2 Analysis of the Differences in Gender Ratios between Groups

	No. of Patients in Population				
Sex	HCRI Alumni	NSP Members	SFA Records	AIS Alumni	Total
Males	810	285	131	826	2052
Females	156	112	52	212	532

Note.—Overall, familial cases versus general stuttering population $\chi^2 = 43$; df 1; $P < .00001$. Familial cases versus cases ascertained via therapy programs $\chi^2 = 63$. $P < .000001$. Familial cases versus cases ascertained without respect to treatment $\chi^2 = 13$. $P < .002$.

Table 2

Comparison of Sex Ratios in Familial Cases Versus Unrelated Cases Who Report No Family History

Group	No Family History	Males	Females	Male-to-Female Ratio
Familial, NIH	0	137	87	1.57
Unrelated, HCRI	468	410	58	7.07
Unrelated, AIS	435	360	75	4.8

without respect to treatment history, obtained from the records of the National Stuttering Project (NSP), and the Stuttering Foundation of America (SFA). The clinical affection status of individuals in the latter two groups was based on self-report, and all individuals were of age >14 years. The number of males and females in these sample populations is shown in table 1. As expected, these four groups displayed male-to-female ratios that were significantly different from each other. However, the combined overall male-to-female ratio is 3.8, which is in good agreement with numerous published estimates for persistent stuttering (Kidd et al. 1981; Bloodstein 1995; Janssen et al. 1996).

To obtain an estimate of the true sex-ratio difference between familial stuttering and sporadic stuttering, we made a closer examination of the data from HCRI and AIS and excluded individuals who reported any family history of stuttering (see table 2). Although using only alumni of therapy programs as a source of cases is susceptible to ascertainment and other potential biases, these data suggest that the differences in sex ratio between familial and sporadic persistent stuttering may be even greater than the values shown in table 1.

A potential source of bias in this study would result if, within the NIH families, females were significantly more likely to volunteer and be evaluated than were males. Information gathered from multiple members of each family gave no evidence that such bias occurred. Our results could also arise if, within our families in general, females significantly outnumbered males. In fact, counting all family members, there are slightly more males than females in our families (266 males and 216 females), providing additional support for the conclusion that the sex ratio in familial cases differs from the sex ratio in cases overall.

The observation in the NIH cohort of small families is similar to that made in at least one unusual family with persistent stuttering (Mellon 1991). In this large extended family, stuttering appears to segregate under the control of a single major gene, and affected members of this family exist in a male-to-female ratio of 1.6. While the great majority of cases of familial stuttering do not show such apparently simple genetic transmission, this similarity to the results in the NIH families suggests that genetic persistent stuttering may be unified by a consistent effect, in which males and females are more equally affected than is seen in the general stuttering population.

Regarding familial versus sporadic stuttering, our data agree with previous findings (Yairi et al. 1996) and suggest that approximately half of all cases of persistent stuttering are accompanied with a report of family history (468/966 from HCRI, 598/1,033 from AIS), while the remaining half appear to be sporadic. One possibility suggested by these data is that roughly half of all cases of stuttering is due to inherited causes, while the other half are due to poorly understood but nongenetic factors. This hypothesis is consistent with the view that persistent stuttering of nongenetic origin is largely a male disorder and may be related to a greater ability of females to overcome childhood stuttering (Ambrose et al. 1997). Genetic stuttering, in contrast, affects males and females more equally, which has important implications for genetic studies of this disorder that exclude young children. In particular, genetic-linkage studies will be much less obscured by the distortion in sex ratio, since this distortion is largely a phenomenon of sporadic stuttering.

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DENNIS DRAYNA,[1] JAMES KILSHAW,[1] AND JENNIFER KELLY[2]

[1]*National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, MD; and* [2]*Hollins Communications Research Institute, Roanoke, VA*

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Address for correspondence and reprints: Dr. Dennis Drayna, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 5 Research Court, Rockville, MD 20850. E-mail: drayna@nidcd.nih.gov

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