

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 HÜBNER¹

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*

Electronic-Database Information

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

Généthon, <ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/> (for genetic markers)

Marshfield Medical Research Foundation Center for Medical Genetics, <http://www.marshmed.org/genetics/> (for genetic markers)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for SMA1 [MIM 253300])

References

- Bertini E, Gadisseux JL, Palmieri G, Ricci E, Di Capua M, Ferriere G, Lyon G (1989) Distal infantile spinal muscular atrophy associated with paralysis of the diaphragm: a variant of infantile spinal muscular atrophy. *Am J Med Genet* 33:328–335
- Brunialti AL, Poirier C, Schmalbruch H, Guénet J-L (1995) The mouse mutation progressive motor neuropathy (*pmm*) maps to chromosome 13. *Genomics* 29:131–135
- Grohmann K, Hübner C, Saar K, Stoltenburg-Didinger G, Wienker T (1998) Diaphragmatic spinal muscular atrophy (SMAD) is not the homologue counterpart to murine progressive motoneuron disease (*pmm*). Paper presented at the 5th Workshop Neurogenetics in Germany, Freiburg, Germany, October 22–24
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363
- Lathrop GM, Lalouel JM (1984) Easy calculations of LOD scores and genetic risks on small computers. *Am J Hum Genet* 36:460–465
- Mellins RB, Hays AP, Gold AP, Berdon WE, Bowdler JD (1974) Respiratory distress as the initial manifestation of Werdnig-Hoffmann disease. *Pediatrics* 53:33–40
- Novelli G, Capon F, Tamisari L, Grandi E, Angelini C, Guerrini

P, Dallapiccola B (1995) Neonatal spinal muscular atrophy with diaphragmatic paralysis is unlinked to 5q11.2-q13. *J Med Genet* 32:216–219

Rudnik-Schöneborn S, Forkert R, Hahnen E, Wirth B, Zerres K (1996) Clinical spectrum and diagnostic criteria of infantile spinal muscular atrophy: further delineation on the basis of SMN gene deletion findings. *Neuropediatrics* 27:8–15

Saar K, Chrzanowska KH, Stumm M, Jung M, Nürnberg G, Wienker TF, Seemanová E, et al (1997) The gene for the ataxia-telangiectasia variant, Nijmegen breakage syndrome, maps to a 1-cM interval on chromosome 8q21. *Am J Hum Genet* 60:605–610

Schmalbruch H, Jensen H-J, Bjærg M, Kamieniecka Z, Kurland L (1991) A new mouse mutant with progressive motor neuropathy. *J Neuropathol Exp Neurol* 50:192–204

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

© 1999 by The American Society of Human Genetics. All rights reserved. 0002-9297/1999/6505-0031\$02.00

Am. J. Hum. Genet. 65:1462–1465, 1999

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.

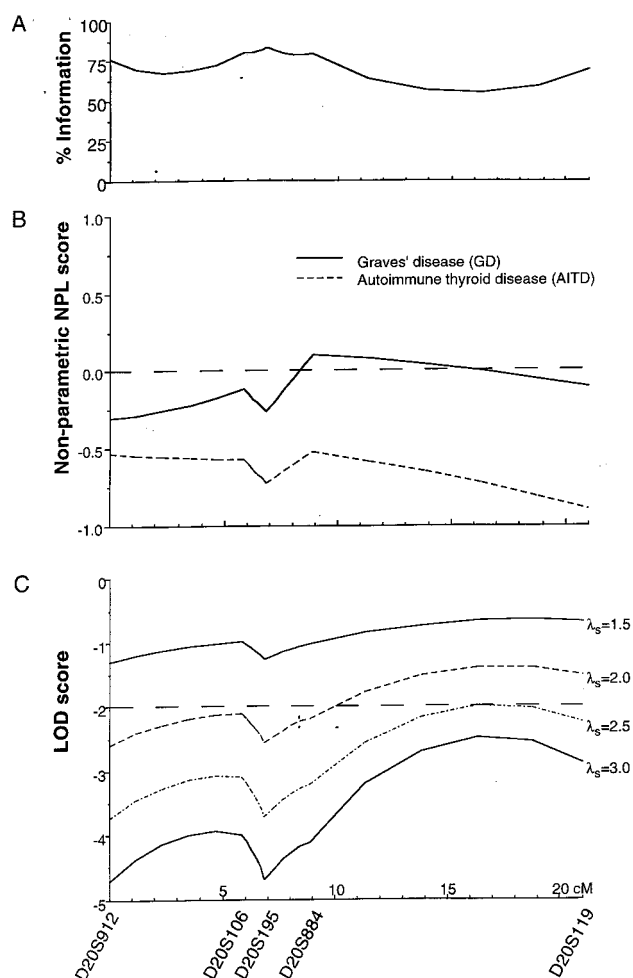


Figure 1 Linkage analysis in all 64 affected GD kindreds. *A*, Percentage information content shown at each of the map positions. *B*, Multipoint nonparametric linkage analysis of kindreds with GD for chromosome 20q13.11 markers. Genotyping was performed by PCR with fluorescently labeled Généthon markers (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. Non-parametric, 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

PENETRANCE	LOD SCORE			
	Without Heterogeneity		With Heterogeneity	
	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

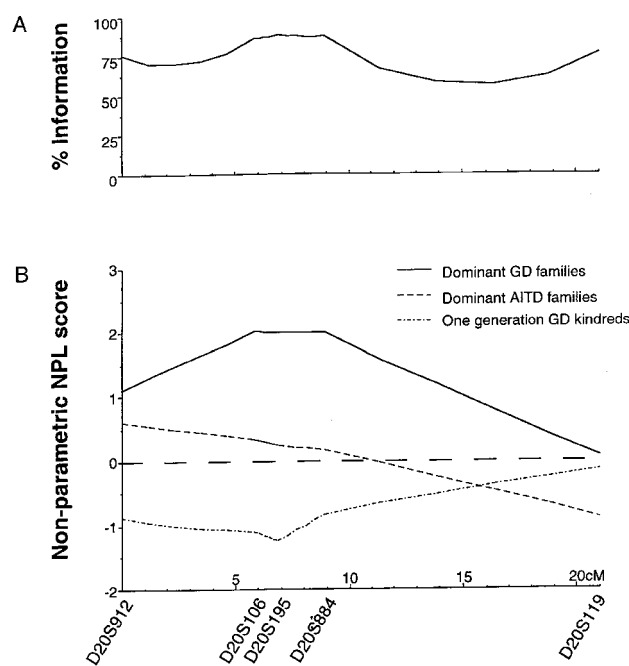


Figure 2 Linkage analysis of the subset of 12 GD kindreds with dominant transmission of GD, and other groups. A, Percentage information content for the 12 families with dominant transmission of GD is shown at each of the map positions. B, Multipoint nonparametric linkage analysis of the subsets of GD 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).

Acknowledgments

We are grateful to Dr. Yaron Tomer for sharing his results with us prior to publication and to Drs. D. Carr, D. M. Large, and D. Trump, The British Thyroid Foundation, and Thyroid Eye Disease for help with recruitment of patients. We also thank an anonymous reviewer for helpful comments. This work was supported by The Wellcome Trust.

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-TAYLOR¹

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

Electronic-Database Information

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

Généthon, <http://www.genethon.fr/>
Marshfield Medical Research Foundation Center for Medical Genetics, <http://www.marshmed.org/genetics/>
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])

References

- Brix TH, Kyvik KO, Hegedus L (1998) What is the evidence of genetic factors in the etiology of Graves' disease? a brief review. *Thyroid* 8:727–734
- Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am J Hum Genet* 63:861–869
- Davies TF (1998) Autoimmune thyroid disease genes come in many styles and colors. *J Clin Endocrinol Metab* 83:3391–3393
- Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154
- Farid NR, Balazs C (1998) The genetics of thyroid associated ophthalmopathy. *Thyroid* 8:407–409

- Gaffney PM, Kearns GM, Shark KB, Ortmann WA, Selby SA, Malmgren ML, Rohlf KE, et al (1998) A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci USA* 95:14875–14879
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363
- Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57:439–454
- McCarthy MI, Kruglyak L, Lander ES (1998) Sib-pair collection strategies for complex diseases. *Genet Epidemiol* 15:317–340
- McLachlan SM (1993) The genetic basis of autoimmune thyroid disease: time to focus on chromosomal loci other than the major histocompatibility complex (HLA in man). *J Clin Endocrinol Metab* 77:605A–605C
- Moser KL, Neas BR, Salmon JE, Yu H, Gray-McGuire C, Asundi N, Bruner GR, et al (1998) Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc Natl Acad Sci USA* 95:14869–14874
- Roman SH, Greenberg D, Rubinstein P, Wallenstein S, Davies TF (1992) Genetics of autoimmune thyroid disease: lack of evidence for linkage to HLA within families. *J Clin Endocrinol Metab* 74:496–503
- Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TF (1998) A new Graves disease-susceptibility locus maps to chromosome 20q11.2. *Am J Hum Genet* 63:1749–1756
- Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, Large DM, et al (1999) The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum Mol Genet* 8:1195–1199
- Werner SC (1977) Modification of the classification of the eye changes of Graves' disease: recommendations of the ad hoc committee of the American Thyroid Association. *J Clin Endocrinol Metab* 44:203–204

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

© 1999 by The American Society of Human Genetics. All rights reserved.
0002-9297/1999/6505-0032\$02.00

Am. J. Hum. Genet. 65:1465–1469, 1999

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-