

Fig. 2 *MSX1* nonsense mutation. **a**, Reverse sequence of a part of exon 1 of *MSX1* showing the substitution producing the Ser104 Stop. **b**, *MboII* digestion after PCR amplification with forward and reverse primers specific for exon 1 (ref. 12). The mutation causes loss of an *MboII* site, resulting in separation of a larger band following agarose electrophoresis.

gous in all 12 affected family members (clefting and/or missing teeth), but not in the three unaffected. Further, the Ser104stop mutation was not present in 102 control chromosomes.

The Arg239Pro *MSX1* protein has a perturbed structure, a reduced thermostability, was inactive *in vivo* and did not antagonize the activity of wild-type protein¹⁴. It was concluded that haploinsufficiency was the probable cause for the mutant phenotype and not a dominant-negative affect, as originally proposed¹². Our finding of a stop mutation in exon 1, proximal to the important Antp homeodomain in exon 2, is also likely to lead to haploinsufficiency.

The phenotype in the Dutch family presented here appears to be more severe than that described for the Arg239Pro missense mutation in *MSX1* (reported as a

Arg31Pro substitution in exon 2 of *MSX1*), and apparently gives rise solely to tooth agenesis¹² in all 12 affected family members¹². The pattern of tooth agenesis was almost identical in the two families. In the Dutch family, however, the presence of clefting variations in 4 of 12 affected individuals demonstrates the variability in penetrance and expressivity of this feature. Previous studies suggested aetiological distinction between isolated cleft palate and cleft lip with or without cleft palate¹⁵. In the Dutch *MSX1* family, combinations of cleft palate only and cleft lip and cleft palate are present. This is consistent with the linkage disequilibrium observed for both types of clefting with *MSX1* (ref. 8). It may be that the absence of clefting in the Arg239Pro family is a sampling affect and that availability of more affected individuals would have eventually revealed clefting defects in that family also. Again, differences in genetic background or environment might be responsible for clefting differences between the two families.

We conclude that the mutant phenotype of the family presented here has orofacial clefting similar to that of the *Msx1*-mutant mouse⁹, in addition to the dental agenesis¹². Although this confirms *MSX1* as a candidate gene for orofacial clefting, it is necessary to ascertain

further human families exhibiting association between *MSX1* mutations and orofacial clefting and dental agenesis.

Acknowledgements

We thank P.L. Pearson for his suggestions in preparing this manuscript.

Marie-José H. van den Boogaard, Marinus Dorland, Frits A. Beemer & Hans Kristian Ploos van Amstel

Department of Medical Genetics, University Medical Center Utrecht, The Netherlands.

Correspondence should be addressed to M.-J.H.v.d.B. (e-mail:

M.J.H.vandenBoogaard@dmg.azu.nl).

- Schutte, B.C. & Murray, J.C. *Hum. Mol. Genet.* **8**, 1853–1859 (1999).
- Vanderas, A.P. *Cleft Palate J.* **24**, 216–225 (1987).
- Murray, J.C. *et al.* *Cleft Palate Craniofac. J.* **34**, 7–10 (1997).
- Croen, L.A., Shaw, G.M., Wasserman, C.R. & Tolarová, M.M. *Am. J. Med. Genet.* **79**, 42–47 (1998).
- Tolarová, M.M. & Cervenka, J. *Am. J. Med. Genet.* **75**, 126–137 (1998).
- FitzPatrick, D. & Farral, I.M. *J. Craniofac. Genet. Dev. Biol.* **13**, 230–235 (1993).
- Christensen, K. & Mitchell, L.E. *Am. J. Hum. Genet.* **58**, 182–190 (1996).
- Lidral, A.C. *et al.* *Am. J. Hum. Genet.* **63**, 557–568 (1998).
- Satokata, I. & Maas, R. *Nature Genet.* **6**, 348–356 (1994).
- Satokata, I. *et al.* *Nature Genet.* **24**, 391–395 (2000).
- Wilkie, A.O.M. *et al.* *Nature Genet.* **24**, 387–390 (2000).
- Vastardis, H., Karimbux, N., Guthua, S.W., Seidman, J.G. & Seidman, C.E. *Nature Genet.* **13**, 417–421 (1996).
- Hewitt, J.E., Clark, L.N., Ivens, A. & Williamson, R. *Genomics* **11**, 670–678 (1991).
- Hu, G. *et al.* *Mol. Cell. Biol.* **18**, 6044–6051 (1998).
- Fraser, F.C. *Am. J. Hum. Genet.* **22**, 336–352 (1970).

Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2

Generalized epilepsy with febrile seizures plus type 2 (GEFS+2, MIM 604233) is an autosomal dominant disorder characterized by febrile seizures in children and afebrile seizures in adults. We describe here two mutations of the gene encoding

the neuronal voltage-gated sodium channel (*SCN1A*), Thr875Met and Arg1648His, that co-segregate with the disorder in two families with GEFS+ linked to chromosome 2q. These mutations identify a new disease gene for human inherited epilepsy.

Patients with GEFS+ express a variable phenotype combining febrile seizures, afebrile generalized seizures (tonic-clonic, absence, myoclonic or atonic) and partial seizures¹. GEFS+ type 1 (MIM 600235) was associated with a mutation in *SCN1B*, encoding the β 1-subunit of the voltage-gated sodium channel². GEFS+2 was recently mapped to a 20-cM region of chromosome 2q24–q33 in two French families^{3,4}. The sodium channel α -subunit gene cluster⁵ on chromosome 2q24 contains three neuronal genes (*SCN1A*, *SCN2A* and *SCN3A*), encoding proteins

a

```

MEQTVLVPPG PDSFNFTRE SLAAIERRIA EEKAKNPKPD KKDDDENGFK 50
PNSDLEAGNK LPFIYGDIIP EMVSEPLEDL DPYYINKKTF IVLNKGKAIF 100
RFSATSALYI LTPFNPLRKI AIKILVHSLEP SMLIMCTILT NCVFMTSMNP 150
PDWTKNVEYT FPGIYTFESL IKIIRAGPCL EDFTFLRDPW NMLDFTVITF 200
AVVTFEVDLG NVSALRTRFRV LRALKTTISVI EGLKTIIVGAL IQSVKKLSDV 250
MLTVFVCLSV FALIGLQLFM GNLRNKCIQW PPTNASLEEH SIEKNITVNY 300
NGTLINETVF EFDWKSYYIQD SRYHYFLEGF LDALLCGNSS DAGQCPEGYM 350
CVKAGRNPNY GYTSFDTFWV AFLSLFRMLT QDFWENLYQL TLRAAGKTYM 400
IFFVLVIFLG SFYLINLILA VVAMAYEBQN QATLEBAEQK EAEFQOMIEQ 450
LKKQQAQQ AATATASEHS REPSAAGRLS DSSSEASKLS SKSAKERRNR 500
RKKRKKQEQS GGEEKDEDEF QKSESEDSIR RKGFRSIFSG NRLTYEKRYS 550
SPHQSLLSIR GSLFSPRRNS RTSLSFSTRGR AKDVGSEPDF ADDEHSTFED 600
NESRRDLSLV PRRHGERRNS NLSQTSRSTR MLAVFPANGK MHSTVDCNGV 650
VSLVGGSPVP TSPVGLLPE VIIDKPAITD NGMTTETEMR KRSSSPHVS 700
MDFLEDSQR QRAMSTIASIL TNTVELEBS RQKCPCCWYK FSNIFLWDCC 750
SPYWLKVKHV VNLVMDPPV DLAITICIVL NTLFMAHEHY PMTDHFNVL 800
TVGNLVPTGI FTAEMFLKII AMPYYYQPE GWNIFDGFIV TSLVELGLA 850
NVEGLSVLRS FRLLRVFKLA KSWPTNMLI KIIGNSVGLR GNLTLVLVLI 900
VIFAVVGMG LKGSYKDCV KIAISDCQLP RHMMDPFHS FLIVRVLCC 950
EWIETWDCM EVAQAMCLT VFMMVMVIGN LVVLLNLFAL LLSFSADNL 1000
AATDDNEMN NLQIVADRMH KGVAVYKRKI YEFIQQSFR KOKILDEIKP 1050
LDDLNNKDS CMSNHTAEG KDLDYLKDVN GTTSGIGTGS SVEKYIDES 1100
DYMSFINNPS LTVTVPIAVG ESDFENLNTD DFESEDLBE SKEKLNESSE 1150
SSEGSTVDIG APVEEQPVVE PBETLEPEAC PTRGCVCQRFK CCQINVEEGR 1200
GRQWNLRRT CPRIVEHNFV ETFIVEMILL SSGALAFEDI YIDQRKTIKT 1250
MLEYADKVT YIFILEMLLK WVAQGYQYVF TNAWCWLDFL IVDVSLVSLT 1300
ANALGYSELG ATKSLRTRLA LRPLRALSRF EGMRVVNAL LGAIPSIMNY 1350
LLVCLIFWLI FSTMGVNLFA GKFYHCINTT TGRDFIEDV NNHTDCLKLI 1400
ERNETARWKN VKNVFNDFV GYLSLQVAT FKGWMDIMYA AVDSRVELQ 1450
PKYEESLYMY LYFVIFILG SFPTLNLFIG VIIDNFQKQ KFGGQDIFM 1500
TEEQKYVNA MKKLGSKKPO KPPIPRGNKF QGMVFDETVR QVFEDISTMIL 1550
ICLNMTVMV ETDDQSEYVT TILSRINLVF IVLFTGECVL KLISLRHYFF 1600
TIGWNIPDFV VVILSIVGMF LAELIBKVFV SPTLFRVRL ARIGRIHL 1650
KGAKGIRTL FALMMSLPA FNIGLLPLV MFYIAFGMS NFAYVKREVG 1700
IDDNFNFTF GNSMICLQFI TISAGWDGLL APILNSKPPD CDPKNVNEGS 1750
SVKGDGCGNS VGIFFFVSYI IISPLVVNM YIAVILENS VATEESAEP 1800
SEDDPEMFYE VWEKPPDAT QFMFEKLSQ FAAALEPPLN LPQPNKQLI 1850
AMDLPVSGD RHCLDILPA FTKRVLGESG EMDALRIQME ERFMASNPSK 1900
VSYQPITTL KRQEQEVS AV IIRAYRRLH LKRTVKQASF TYNKNKIKGG 1950
ANLLIKEDMI IDRINENSIT EKTDLTMTA ACPPSYDRVT KPIVEKHEQE 2000
GKDEKAKG* 2009
    
```

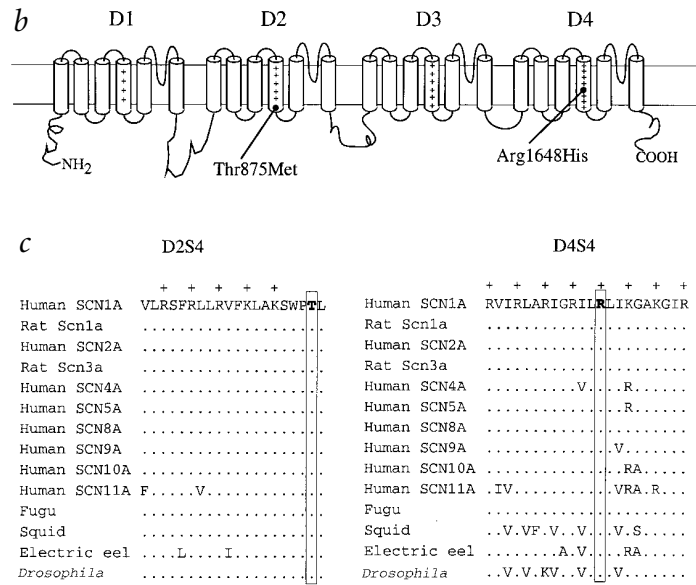


Fig. 1 Sequencing, exon organization and evolutionary conservation of *SCN1A*. **a**, Amino acid sequence and intron/exon organization. The transmembrane segments (S; underlined), the intron locations (triangles) and the mutant residues Thr875 and Arg1648 (asterisks) are indicated. **b**, The mutations Thr875Met and Arg1648His are located in D2S4 and D4S4 (arrows). **c**, Evolutionary conservation of the residues Thr875 and Arg1648 (boxed). GenBank accession numbers from the top are as follows: M22253, M94055, Y00766, M81758, M77235, AB027567.1, X82835, AF117907.1, AF188679, D37977, L19979, M22252, M32078.

with 85% amino acid sequence identity, that are candidates for GEFS+2.

We initially analysed *SCN2A* because mutation of the mouse orthologue results in a seizure disorder (J. Kearney *et al.*, pers. comm.). Complete sequencing of the 26 exons of *SCN2A* did not reveal any mutations in the two families^{3,4} with GEFS+2 (unpublished data). We determined the coding sequence of human *SCN1A* by aligning the rat cDNA sequence^{6,7} with genomic sequence (from the Human Genome Center at Washington University; GenBank AC010127). The deduced amino acid sequence of the 2,009-residue human *SCN1A* protein and the positions of introns are shown (Fig. 1a). Human *SCN1A* is highly conserved, with 98% amino acid sequence identity to the rat coding sequences. The intron/exon organization of *SCN1A* is

identical to that of *SCN8A* on chromosome 12q13 and probably corresponds to that of the ancestral gene⁵. We confirmed the location of *SCN1A* within the candidate region for GEFS+2 by typing the GB4 radiation-hybrid panel with primers for intron 21, further localizing *SCN1A* to the 4-cM interval between D2S156 and D2S399. The 26 exons of *SCN1A* were amplified from genomic DNA of 1 affected and 1 unaffected individual in each of the 2 families. We detected an unusual conformer of exon 15 in the affected individual III-9 in family 1 using conformation-sensitive gel electrophoresis⁸. Sequencing demonstrated heterozygosity for a C→T transition, C2624T, resulting in the amino acid substitution Thr875Met (Fig. 2a). We assayed C2624T in the rest of the pedigree by the loss of an *AccI* site in the mutant allele. We found 11 affected individuals and the obligate carrier to be heterozygous for the mutation, whereas the 4 unaffected relatives carry two normal alleles (Fig. 2c). Subject III-2 does not carry the mutation, confirming the double recombination event previously reported³.

Analysis of *SCN1A* exons in individual IV-6 from family 2 identified a nucleotide substitution, G4943A, in exon 26 that

results in the amino acid substitution Arg1648His (Fig. 2b). We assayed for Arg1648His by loss of a *MaeII* site and found it to co-segregate with GEFS+ in family 2 (Fig. 2d). Mutation analysis for at-risk members confirmed that individual IV-7 was a phenocopy, as suggested by haplotype reconstruction⁴. We interpret the presence of the mutation in the asymptomatic individual III-10 as an example of the incomplete penetrance of GEFS+. We did not detect C2624T and G4943A in more than 100 controls.

The phenotype of each individual in these two families has been described^{3,4}. Generalized seizures were observed in 5 of 11 individuals with the Thr875Met mutation in family 1 compared with 11 of 12 individuals carrying Arg1648His in family 2. The latter mutation thus appears to have a more severe clinical effect.

The mutant residues Thr875 and Arg1648 are located in S4 transmembrane segments of the sodium channel α -subunit, which is comprised of four homologous domains (D1–D4), each containing six transmembrane segments (S1–S6; Fig. 1b). The functional importance of Thr875 and Arg1648 is supported by their evolutionary conservation in other mammalian gene family members and in lower vertebrates and invertebrates (Fig. 1c). The S4 segments of voltage-gated channels contain multiple positively charged amino acids that are known to have a role in channel gating.

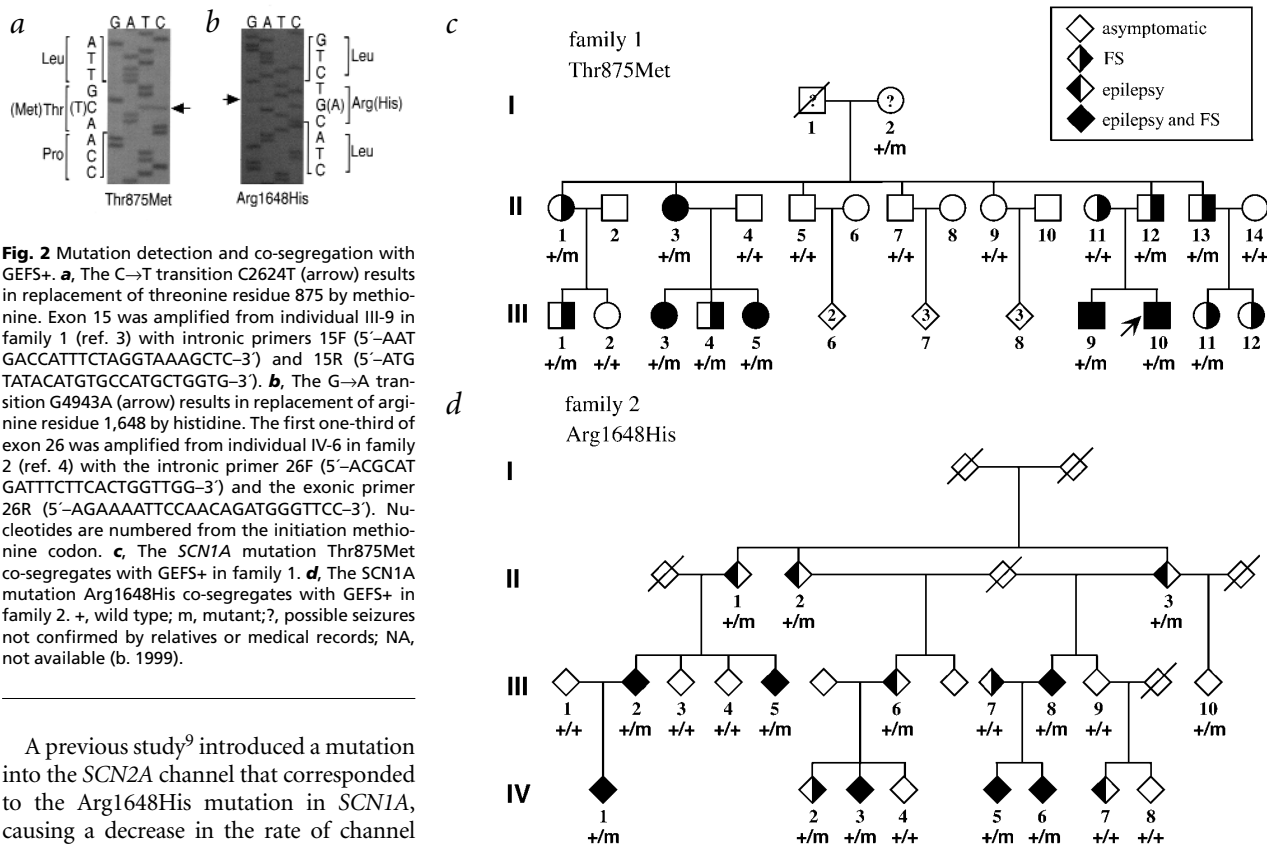


Fig. 2 Mutation detection and co-segregation with GEFS+. **a**, The C→T transition C2624T (arrow) results in replacement of threonine residue 875 by methionine. Exon 15 was amplified from individual III-9 in family 1 (ref. 3) with intronic primers 15F (5'-AAT GACCATTTCTAGGTAAGCTC-3') and 15R (5'-ATG TATACATGTGCCATGCTGGT-3'). **b**, The G→A transition G4943A (arrow) results in replacement of arginine residue 1,648 by histidine. The first one-third of exon 26 was amplified from individual IV-6 in family 2 (ref. 4) with the intronic primer 26F (5'-ACGCAT GATTCTTCACTGGTTGG-3') and the exonic primer 26R (5'-AGAAAATTCCAACAGATGGGTTC-3'). Nucleotides are numbered from the initiation methionine codon. **c**, The *SCN1A* mutation Thr875Met co-segregates with GEFS+ in family 1. **d**, The *SCN1A* mutation Arg1648His co-segregates with GEFS+ in family 2. +, wild type; m, mutant; ?, possible seizures not confirmed by relatives or medical records; NA, not available (b. 1999).

A previous study⁹ introduced a mutation into the *SCN2A* channel that corresponded to the Arg1648His mutation in *SCN1A*, causing a decrease in the rate of channel inactivation. This suggests that Arg1648His may reduce the rate of inactivation of *SCN1A*, resulting in increased Na⁺ influx, increased excitability of neurons in the central nervous system and increased seizure susceptibility. The loss-of-function mutation of the β 1-subunit in GEFS+ type 1 also indirectly decreases the rate of inactivation of sodium channel α -subunits².

The Thr875Met mutation is located at the intracellular interface of segment D2S4 (Fig. 1b) within a conserved leucine heptad repeat that is also found in potassium and calcium channels. Threonine to methionine substitutions within the heptad repeat in D2S5 of *SCN4A* and D4S4 of *SCN5A* have been associated with hyperkalaemic periodic paralysis^{10,11} and long QT syndrome¹², respectively. The invariant sequence of this portion of D2S4 (Fig. 1c) demonstrates evolutionary selection against substitution of Thr875.

A role for sodium channels in the aetiology of epilepsy is indicated by the efficacy of sodium channel blockers as therapeutic agents for this disease¹³. The GEFS+2 mutations in *SCN1A* are the first in a human neuronal sodium channel α -subunit to be associated with disease. The incomplete penetrance and large intrafamilial variability of the phenotype suggest the segregation of modifying genes that may interact with environmental factors such as episodes of fever. In families with

GEFS+ type 1 or 2, the detection of mutations in at-risk newborns should permit prevention of febrile convulsions by the precocious treatment of all infections. Evaluation of the role of *SCN1A* in other monogenic and multifactorial epilepsy, including febrile seizures type 3 (FEB3) and another locus recently linked to GEFS+2 (refs 14,15), is a high priority. Development of molecular markers from the BAC clone containing *SCN1A* will provide increased sensitivity in evaluation of this channel as a susceptibility factor in polygenic pedigrees.

Acknowledgements

We thank L. Isom and L.K. Sprunger for helpful discussions, and F. Picard and J.-F. Prudhomme for participation in identification of family 2. This research was supported by NIH Grant NS34509 (Group 1), the Association pour le Développement de la Recherche sur les Maladies Génétiques Neurologiques et Psychiatriques (ADRMGNP) (Group 2) and the Swiss National Science Foundation (Group 3).

Group 1: Andrew Escayg^{1*}, Bryan T. MacDonald¹ & Miriam H. Meisler¹

Group 2: Stéphanie Baulac^{2*}, Gilles Huberfeld², Isabelle An-Gourfinkel^{3,4}, Alexis Brice^{2,5} & Eric LeGuern²

Group 3: Bruno Moulard^{6*}, Denys Chaigne⁷, Catherine Buresi⁶ & Alain Malafosse⁶

*These authors contributed equally to this work.

¹Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA.

²INSERM U289, Hôpital de la Salpêtrière, Paris, France. ³Centre d'Epilepsie, Hôpital de la Salpêtrière, Paris, France. ⁴Généthon, Evry, France. ⁵Consultation de Génétique Médicale, Hôpital de la Salpêtrière, Paris, France.

⁶Division de Neuropsychiatrie, Hôpitaux Universitaires de Genève, Chêne-Bourg, Switzerland. ⁷Clinique Sainte-Odile, Strasbourg, France. Correspondence should be addressed to M.H.M. (e-mail: meislerm@umich.edu).

- Scheffer, I.E. & Berkovic, S.F. *Brain* **120**, 479–490 (1997).
- Wallace, R.H. et al. *Nature Genet.* **19**, 366–370 (1998).
- Moulard, B. et al. *Am. J. Hum. Genet.* **65**, 1396–1400 (1999).
- Baulac, S. et al. *Am. J. Hum. Genet.* **65**, 1078–1085 (1999).
- Plummer, N. & Meisler, M.H. *Genomics* **57**, 323–331 (1999).
- Noda, M. & Numa, S. *J. Recept. Res.* **7**, 467–497 (1987).
- Smith, R.D. & Goldin, A.L. *J. Neurosci.* **18**, 811–820 (1998).
- Escayg, A. et al. *Am. J. Hum. Genet.* (in press).
- Kühn, F.J.P. & Greeff, N.G. *J. Gen. Physiol.* **114**, 167–183 (1999).
- Bulman, D.E. *Hum. Mol. Genet.* **6**, 1679–1685 (1997).
- Lehmann-Horn, F. & Jurkat-Rott, K. *Physiol. Rev.* **79**, 1317–1372 (1999).
- Wattanasirichaigoon, D. et al. *Am. J. Med. Genet.* **86**, 470–476 (1999).
- Catterall, W.A. *Adv. Neurol.* **79**, 441–456 (1999).
- Peiffer, A. et al. *Ann. Neurol.* **46**, 671–678 (1999).
- Lopes-Cendes, I.E. et al. *Am. J. Hum. Genet.* **66**, 698–701 (2000).