SHORT REPORT

Identification of genetic heterogeneity in Refsum's disease

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Refsum's disease (MIM 266500) is a recessive disorder characterised by defective peroxisomal alpha-oxidation of phytanic acid. A Refsum's disease gene, phytanoyl-CoA hydroxylase (*PAHX*), has been localised to chromosome 10p13 between the markers D10S226–D10S223. This study investigated whether all cases of Refsum's disease were linked with chromosome 10p13. Eight genetically informative families comprising 92 individuals including 17 living patients with a Refsum's disease phenotype and initial plasma phytanic acid > 200 µmol/L were recruited. Linkage to the 10pter–10p11.2 region was investigated using a panel of eight dinucleotide repeat markers. Linkage analysis of this phenotypically identical cohort suggested that Refsum's disease was genetically heterogeneous ($Z_{\max} = 5.28$, $\alpha = 0.45$). Two subgroups were identified. One group of four families with eight affected individuals had a maximum multipoint lod score for linkage of 3.89 in the region D10S547 to D10S191, whilst in another three families with nine affected individuals linkage to this region was definitely excluded. Our results show that Refsum's disease is genetically heterogeneous, with up to 55% of cases not being linked to the *PAHX* gene locus at D10S547 to D10S223. This suggests that Refsum's disease, in common with other peroxisomal 'diseases', may be more accurately described as a heterogeneous syndrome. *European Journal of Human Genetics* (2000) 8, 649–651.

Keywords: Refsum's disease; phytanic acid; linkage; heterogeneity

Introduction

Refsum's disease (MIM 266500) is an autosomal recessive disorder characterised by defective peroxisomal alpha-oxidation of phytanic acid leading to presentation in adolescence with retinitis pigmentosa which progresses to eventual blindness. Other features with variable penetrance include anosmia, deafness, sensory neuropathy, ichthyosis, ataxia, peripheral skeletal or limb dysmorphism and cardiomyopathy.¹ A Refsum's disease gene, phytanoyl-CoA hydroxylase (*PAHX*), has been localised to chromosome 10p13 between the markers D10S226–D10S4223 near D10S191.^{2,3} Mutations

in *PAHX* have been identified in 13 cases of Refsum's disease.⁴ This study investigated whether all cases of Refsum's disease were linked with chromosome 10p13.

Materials and methods

Eight families comprising 92 individuals including 17 living patients with Refsum's disease were recruited, with ethical consent, from a specialist clinic. All the affected patients had the classic clinical features of Refsum's disease of retinitis pigmentosa, anosmia, sensory neuropathy and ataxia. All had visual deterioration after age 12 with initial night blindness and later developed other clinical signs. Deafness and short fifth metacarpals/metatarsals were present in 70% and 50% of cases, respectively, on presentation. All had an initial plasma phytanic acid concentrations > 200 µmol/L

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(reference limit $< 30 \mu\text{mol/L}$). All unaffected family members were reviewed and none had features of classical Refsum's disease. Their plasma phytanic acid concentrations were all $< 15 \mu\text{mol/L}$. Family 7 was consanguineous by reason of a first cousin marriage. EDTA blood samples were obtained from 50 living individuals in these families including all except two deceased affected individuals and two unaffected individuals. A panel of eight fluorescein-labelled dinucleotide repeat markers spanning 10pter-10p11.2 (Perkin Elmer, UK) was amplified from patient lymphocyte DNA by polymerase chain reaction and typed on a ABI310 analyser with Geneamp software (Perkin-Elmer Ltd, UK). Fluorescent scans were automatically scored and allele typings were confirmed by two independent observers blinded to the clinical data. Any markers showing ambiguous allele sizes were re-run. The final data was analysed by standard techniques using the GENEHUNTER linkage analysis program⁵ assuming complete penetrance for a recessive disorder and a mutation rate of 10^{-5} . The allele frequency for the Refsum's disease trait was assumed as 0.001 based on the prevalence of Refsum's disease of 10^{-6} in the UK. Primer sequences, allele frequencies and distances for chromosome 10 markers were taken from computerised database sources (www.genlink.wustl.edu). Heterogeneity in inheritance and admixture were assessed using the computer program HOMOG.⁶

Results

Statistical analysis of the two point linkage data for the families is shown in Table 1. Initial analysis revealed no linkage of Refsum's disease in the overall cohort to chromosome 10. Analysis for genetic heterogeneity allowing for admixture was conducted using the program HOMOG giving a result of $Z_{\text{max}} = 5.284$ at a distance (θ) of 0.25 from D10S249, and a proportion of linked families (α) of 0.45. Analysis of the components of χ^2 gave a value of $X^2 = 10.57$; $P < 0.001$ for heterogeneity confirming the presence of significant genetic heterogeneity in this cohort. Data on the conditional probability of linkage by pedigree is shown in

Table 1. In pedigrees 1–4 a maximum multipoint lod score for linkage of 3.89 to the region D10S547 to D10S191 could be obtained by *post hoc* analysis. Pedigrees and haplotypes of four families not linked to chromosome 10 are shown in Figure 1. In pedigrees 6–8 definite exclusion of linkage was found for the region D10S547 to D10S191 and D10S197 with multipoint lod scores < -5 .

Comparison of the clinical and biochemical features of Refsum's disease phenotype between the two groups of patients revealed no differences in clinical presentation manifest clinical symptoms, severity of disease and initial or post-treatment plasma phytanic acid concentration.

Discussion

Refsum's disease is a peroxisomal disorder associated solely with a deficiency of phytanic acid metabolism and can be caused by mutations in the *PAHX* gene which has been localised to the region D10S226 to D10S223.^{2–4} All but one case of Refsum's disease described to date have been found to be due to mutations in *PAHX* linked to these chromosome 10 loci, or not to complement cell lines from patients with Refsum's disease. The atypical case presented with classical features of Refsum's disease, but his fibroblasts complemented fibroblasts from patients with Refsum's disease and not those from patients with rhizomelic chondrodysplasia (RCDP).⁷

In our series of 17 cases in the UK, four pedigrees showed evidence of linkage to the region D10S547 to D10S191 where *PAHX* is located. This is consistent with previous reports and suggests a closer localisation of the *PAHX* gene between the markers D10S547 and D10S223. However, genetic heterogeneity was present, as 55% of the cohort did not show linkage to this region when evaluated at the best location for the group.

All these patients presented with a classical phenotype and could not be differentiated from the *PAHX*-linked group by clinical or biochemical criteria. However, the phenotype of Refsum's disease may be misleading as one consanguineous

Table 1 Two-point lod scores and heterogeneity analysis between chromosomes 10p loci and *PAHX* locus for 8 families with Refsum's disease

Marker	Family							
	1	2	3	4	5	6	7	8
D10S249	–	–	–	0.000	–	–	0.727	–
D10S591	–0.500	0.977	–	0.000	–	–	0.426	0.000
D10S189	0.500	–	–	–0.109	–	0.852	0.301	–
D10S547	–	0.250	0.426	0.000	–0.353	–	0.227	–
D10S191	0.500	1.579	1.931	0.125	–0.329	–	–	–
D10S1653	0.250	–	–	0.000	–	–	–	–
D10S548	0.375	–0.102	1.329	0.000	–0.144	–	–	–
D10S197	0.625	–	–	0.125	–	–	–	–
Likelihood of linkage	1.0	3.04	197.0	1.00	3.03	1.00	1.50	1.00
Conditional probability of linked type	0.721	0.969	0.986	0.522	0.277	< 0.001	< 0.001	< 0.001

Figure 1 Pedigrees and genotype data for markers spanning the *PAHX* candidate gene locus between D10S457 and D10S191 for four families with a Refsum's disease phenotype not linked to chromosome 10. Genetic distances between the markers are indicated. Deduced genotypes are shown in italics and brackets. Sites of likely meiotic recombination are marked with x. Dead or missing sibs are excluded from pedigrees with haplotypes for clarity: in family 5 one affected dead sib (II.3) has been excluded; in family 6 two dead unaffected (II.1 and II.4) and one dead affected (II.5) sibs have been excluded and one sib (II.2) could not be located. Parents in family 7 are consanguineous through a first cousin marriage.

family has been described with an atypical Refsum's disease phenotype but linked genetically to the *PAHX* region.^{8,9} Atypical clinical and biochemical features included leukodystrophy and pipecolic acidemia which are features of generalised peroxisomal assembly disorders presenting as the continuum between Zellweger's syndrome and infantile Refsum's disease (IRD) but not of the complementation group which includes Refsum's disease.¹⁰

Our results show that Refsum's disease is genetically heterogeneous with 55% of cases likely to be caused by mutations in genes other than *PAHX*. Taking our results and those of the other atypical cases, the clinical phenotypic and genetic data suggests that the constellation of features termed Refsum's disease are not homogeneous and, in common with other peroxisomal diseases,¹⁰ are more likely to represent a syndrome with multiple causes rather than a monogenic disorder.

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References

- Steinberg D: Refsum Disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn. McGraw Hill: New York, 1995, pp 2351–2369.
- Jansen GA, Ofman R, Ferdinandusse S *et al*: Refsum disease is caused by mutations in the phytanoyl-CoA hydroxylase gene. *Nat Genet* 1997; **17**: 190–193.
- Mihalik SJ, Morrell JC, Kim D, Sacksteder KA, Watkins PA, Gould SJ: Identification of *PAHX*, a Refsum disease gene. *Nat Genet* 1997; **17**: 185–189.
- Jansen GA, Ferdinandusse S, Skjeldal OH *et al*: Molecular basis of Refsum's disease: identification of new mutations in the phytanoyl-CoA hydroxylase cDNA. *J Inher Metab Dis* 1998; **21**: 288–291.
- Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and non-parametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996; **59**: 1347–1363.
- Terwilliger JD, Ott J: *Handbook of Human Genetic Linkage*. Johns Hopkins University Press: Baltimore, 1994.
- Moser AB, Rasmussen M, Naidu S *et al*: Phenotype of patients with peroxisomal disorders sub-divided into sixteen complementation groups. *J Pediatr* 1995; **127**: 13–22.
- Tranchant C, Aubourg P, Rocchiccioli F, Warter JM: A new peroxisomal disease with impaired phytanic acid and pipecolic acid oxidation. *Neurology* 1993; **43**: 2044–2048.
- Nadal N, Rolland M-O, Tranchant C *et al*: Localisation of Refsum disease with increased pipecolic acidemia to chromosome 10p by homozygosity mapping and carrier testing in a single nuclear family. *Hum Mol Genet* 1995; **4**: 1963–1966.
- Powers JM, Moser HW: Peroxisomal disorders: genotype, phenotype, major neuropathologic lesions and pathogenesis. *Brain Pathol* 1998; **8**: 101–120.