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Genetic linkage of Meleda disease to chromosome 8qter

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Meleda disease (mal de Meleda) MIM *248300 is an autosomal recessive disorder, clinically characterised by transgressive palmoplantar keratoderma, hyperhidrosis and perioral erythema. It was first described on the Adriatic island of Meleda, where it was relatively common. The prevalence in the general population is estimated to be 1 in 100 000. Linkage analysis of two large consanguineous families from Algeria, including 10 affected individuals, showed strong evidence for localisation of Meleda disease to chromosome 8qter with a maximum two-point lod score for D8S1751 of 8.21 at $\Theta = 0$. Analysis of homozygosity regions and recombination events places the gene in a region of at least 3 cM, telomeric to D8S1727. A common haplotype was observed in the two families, suggesting a founder effect.

Keywords: mal de Meleda; Meleda disease; keratosis palmoplantaris transgrediens; chromosome 8qter; homozygosity mapping

Introduction

Mal de Meleda (MdM) MIM *248300¹ was first described by Neumann in 1898 on the island of Meleda.² This autosomal recessive disorder was relatively common in inhabitants of this island in Dalmatia, Yugoslavia, which served as an isolation island for infectious diseases such as leprosy until 1808.³ In order to localise the gene, we applied homozygosity mapping⁴ to two large consanguineous families.

MdM is clinically characterised by palmoplantar keratoderma and transgressive pachyderma, which involves the palms and soles and also the back of the

hands and feet. Brachydactyly with short cone-shaped fingers and nail abnormalities such as koilonychia or pachyonychia are frequently associated features. Hyperhidrosis, perioral erythema and lichenoid plaques are also noted.^{5–7} The evolution of this disorder is often progressive. Histological features are acanthosis, pseudospongiosis and small papillar bodies. A greatly thickened corneal layer, increased stratum lucidum, mild apparent dysplasia of the basal layer and an expanded granular layer are also typical of the disorder. The sweat glands are often twice the normal size.

Materials and Methods

Blood samples for DNA extraction were collected from 27 members of the two families. We used standard procedures for DNA extraction from whole blood.

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Fluorescent Genome Mapping and Non-fluorescent Genotyping

Fluorescent PCR-primers⁸ spaced at an average distance of 15 cM were used for whole-genome screening. Genotyping for other microsatellite markers in the region of interest was carried out using unlabelled primers. The rest of the procedure was the same as that recently described by Fischer *et al.*⁹

Linkage Analysis

Linkage analysis was performed using the LINKAGE 5.1 program assuming autosomal recessive inheritance, a full penetrance and a disease frequency of 1 per 100 000. Two-point lod scores were calculated with the MLINK program.¹⁰ Consanguineous loops were incorporated into the pedigree files. The allele frequencies used were based on the 27 individuals from the two Algerian families.

Radiation Hybrid Mapping

Radiation hybrid (RH) mapping was carried out according to the procedure described by Gyapay *et al* using the GB4 panel.¹¹ Multipoint calculations on this data were performed using the RHMAP package.¹²

Results

Clinical Data

We studied two large consanguineous families from Algeria (Figure 1A and 1B). All 27 family members were examined by a dermatologist (BB) and clinical data documented. Ten individuals were affected and 17 non-affected. In family A (sibships A1 and A2) and family B, the parents are first cousins, whereas in sibship A3 of family A, no consanguineous relationship between the parents was known. The clinical features of the two families were relatively homogeneous. The early age of onset of the disease was marked by first symptoms of keratoderma palmoplantaris between birth and 20 days after birth. The transgressive character appeared later in the evolution of the disorder, between 1 and 3 years of age. Four individuals showed early progressive evolution between the age of 1 and 8 years, whereas three individuals showed this feature later between 17 and 20 years of age. All the patients had nail abnormalities and four of them had polydactyly.

Linkage and Haplotype Analysis

We first undertook a genome-wide scan of the large consanguineous family A. The marker AFMa082wh9 (D8S1836) showed strong evidence for linkage to chromosome 8qter with a maximal lod score of 4.70 for $\Theta = 0$. The application of additional markers in a 15 cM interval suggested the existence of a larger homozygous region. The localisation was confirmed using the second

consanguineous family B. The maximum pairwise lod score for the two families for marker D8S1751 was 8.21 for $\Theta = 0$ (Table 1). The pedigrees and haplotypes of markers from the linked region are shown in Figures 1A and 1B. The haplotypes were constructed assuming the most parsimonious linkage phase. We established the smallest co-segregating region in the two informative families using haplotype analysis (Figure 2). The centromeric limit is defined by D8S1727. No recombination event enabled us to define the telomeric limit of the homozygous region, in which the last marker is D8S1836.

The analysis of parental phases in haplotypes of children showed several recombination events, which made it necessary to change the order of genetic markers relative to the Généthon human genetic linkage map.⁸ Haplotype analysis in our families suggested a more centromeric position for markers D8S1704 and D8S1729. The order was established by verification of the genotypes of the CEPH families and by radiation hybrid mapping using the GB4 panel¹¹ and the MaxLike program.¹² We propose D8S1837–D8S1741–D8S1717–D8S1704–D8S1729–D8S1713–D8S1744–D8S1727–D8S1751–D8S1836 as the new order.

The positions of two candidate genes in the region, plectin (PCN), MIM *601282,¹ and glutamate-pyruvate transaminase (GPT), MIM *138200,¹ were tested using the radiation hybrid map method, but the resolution of this method was not sufficient to map them precisely. However PCN and GPT are clearly linked to two markers (D8S1751 and D8S1836) in the homozygous region and the most likely position of both PCN and GPT is telomeric from D8S1836.

Discussion

The analysis of two families affected by MdM which had the same geographical and ethnic background showed linkage to locus 8qter. We concluded that in this population a single gene is responsible for the disorder. The smallest co-segregating region between markers D8S1727 and D8S1836 was defined by recombination events in children. The non-affected individual IX in family B presents the same homozygous disease haplotype as the affected patients for centromeric markers, but as a result of a recombination event centromeric to D8S1751, he had a heterozygous haplotype for the two telomeric markers. Assuming full penetrance we therefore consider that the disease gene is localised in the

Table 1 Combined pairwise lod score table for mal de Meleda in two families (A, B) for markers in the common linked interval

D-number	AFM-number	lod scores at recombination fraction Θ								Z_{max}	Θ_{max}
		0.0	0.01	0.05	0.1	0.2	0.3	0.4	0.5		
D8S1837	a083wg1	-6.23	-4.14	-1.78	-0.81	-0.19	-0.07	-0.03	0.00	0.00	0.5
D8S1741	b007zd5	-2.05	0.91	2.18	2.38	1.96	1.21	0.47	2.38	2.38	0.1
D8S1717	a191xf9	-5.14	-1.05	0.72	1.21	1.09	0.54	0.06	1.26	1.26	0.14
D8S1704	a119zh1	-3.38	-1.25	0.13	0.55	0.55	0.27	0.03	0.62	0.62	0.14
D8S1729	a288zf1	-7.21	-1.58	0.26	0.82	0.89	0.58	0.20	0.95	0.95	0.15
D8S1713	a184yg5	2.01	2.39	2.59	2.41	1.79	1.08	0.44	2.60	2.60	0.04
D8S1744	b014xe1	5.59	5.90	5.79	5.22	3.78	2.26	0.90	5.95	5.95	0.02
D8S1727	a240yd9	3.24	3.60	3.70	3.40	2.50	1.51	0.62	3.74	3.74	0.03
D8S1751	b046zc1	8.21	8.02	7.27	6.31	4.39	2.53	0.94	8.21	8.21	0.0
D8S1836	a082wh9	7.06	6.90	6.25	5.44	3.80	2.23	0.87	7.06	7.06	0.0

Figure 2 Length of the smallest homozygous region in the two families and the limits of the common linked interval (shaded). The centromeric limit (qCEN) is defined by the marker D8S1727 in family B; the telomeric limit (qTEL) cannot be defined and is represented by the dotted end of the arrow. The distances between the markers are indicated in cM on the left border.

telomeric part of the linked region. However, we cannot exclude a larger homozygous region extending further into the telomeric part of the chromosome, because no other telomeric markers are available to frame the disease interval. A common haplotype was observed in the two families, suggesting a founder effect. Moreover, they had the same geographical origin and an ancestral relationship cannot be excluded.

MdM belongs to the heterogeneous group of skin disorders characterised by palmoplantar keratoderma (PPK). The classification is principally based on histological and clinical findings and differentiates diffuse, focal and punctate PPK's from palmoplantar ectodermal dysplasias.¹³ Moreover the isolation of genes implicated in these disorders has helped in comprehension of the pathogenic mechanisms involved. Three of these PPKs with an autosomal dominant mode of inheritance are linked to keratin or involucrin clusters

on different chromosomes. Mutations in *keratin* genes 6a, 9, 16 or 17 are responsible for four of these disorders.¹⁴⁻¹⁷ In the homozygous linked region, however, no keratin genes are present. Thirty ESTs which were mapped using RH methodology,¹¹ were present in the region of homozygosity; one of them is expressed in keratinocytes (HUMNK041). There are three good candidate genes in the neighborhood such as the gene for plectin, the gene for focal adhesion kinase 2, MIM *601212,¹ and the gene for glutamate-pyruvate transaminase. Plectin is a 500-kDa intermediate filament binding protein involved in cytoplasmic cross-linking functions.¹⁸ Mutations in the plectin gene have already been shown to be responsible for autosomal recessive epidermolysis bullosa simplex associated with muscular dystrophy.^{19,20} The deficiency in plectin leads to defective anchorage of the cytoskeleton to the plasma membrane and results in cell fragility. Focal adhesion kinase 2 is a cytoplasmic-protein tyrosine kinase which is activated by ligand binding to different receptors such as integrin and growth factors. FAK2 is expressed in all organs, but most abundantly in brain. GPT, also known as alanine aminotransferase, catalyses the reversible reaction of L-alanine and alpha-ketoglutarate to L-glutamate and pyruvate. A close linkage between GPT and the autosomal dominant Ogna type of epidermolysis bullosa simplex MIM *131950¹ was described by Olaisen *et al.*²¹ There is also an aminotransferase implicated in the pathologic mechanism of a focal PPK with autosomal recessive inheritance. The Richner-Hanhart syndrome or occulo-cutaneous tyrosinosis is due to a deficiency of hepatic tyrosine-aminotransferase and results in an accumulation of tyrosine in the peripheral blood.

A PPK clinically very similar to MdM, Naxos disease, MIM *601214,¹ was recently described on the Greek island of Naxos.²² This PPK is, however, associated with

arrhythmogenic cardiomyopathy with right ventricular dysplasia.

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