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Genome-wide linkage screen of a consanguineous multiple sclerosis kinship

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Multiple sclerosis (MS), like Alzheimer's disease (AD) and Parkinson's disease (PD), is a common neurological disorder thought to be caused by the interaction of several genes with unknown environmental factors. In both AD and PD the identification of disease forms inherited in a classic Mendelian fashion has helped investigators elucidate pathogenetic mechanisms. In this study a whole-genome screen, with an average of 608 successful genotypes per person, was performed on nine members of a consanguineous family: the index case, three of her siblings and her daughter, all of whom have been diagnosed with definite MS; as well as the parents of the index case (first cousins), one of her five healthy siblings and her husband (who is also her first cousin). Nonparametric linkage analysis was performed on genotyping data. Based on the presence of consanguinity, the a priori hypothesis was that the disease is transmitted in an autosomal recessive fashion in the pedigree. Linkage analysis revealed a suggestive logarithm of odds (LOD) score of 2.29 on the long arm of chromosome 9. Four of five affected family members were identically homozygous for a haplotype under this peak, spanning approximately 43 cM, while the fifth affected subject and all unaffected family members were heterozygous for the haplotype.
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Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). The aetiology of MS is unknown but has been postulated to involve a T helper 1 (Th1)-mediated autoimmune event leading to the destruction of myelin-producing oligodendrocytes.[1] Epidemiological studies have demonstrated the importance of genetic factors in MS, but so far there has been no convincing evidence for any single locus affecting either susceptibility or outcome, except for the human leucocyte antigen (HLA) class II region on the short arm of chromosome 6 – a region found to be linked to or associated with the risk of MS in several different studies.[2]

The hopes of investigators only a few years back of identifying susceptibility genes in MS by way of linkage analysis of a large number of families or sibling pairs, or by large case-control studies, have been somewhat shattered by disappointing results. One hope now lies in finding a kinship in which MS is inherited in a classic Mendelian fashion and in tracking down the gene responsible for the phenotype in that kinship.[3] Although susceptibility to MS in the majority of cases is thought to be conferred in a polygenic manner, the identification of such a monogenic exception to the rule could prove to be of immense value for understanding disease pathogenesis, as has been the case in both Parkinson's disease (PD) and Alzheimer's disease (AD).[4,5] Understanding the pathogenesis of MS could in turn provide the basis for much-needed novel therapeutic approaches.

In this study, a genome-wide linkage analysis was performed on a kinship in which MS seems to display a monogenic inheritance pattern. The family is of Middle Eastern origin with many family members having emigrated to Sweden. Some of them now live a few miles south of Stockholm, within the catchment area for Huddinge University Hospital (HUH).

Materials and methods

Study protocol

All patients and relatives in this study gave informed consent. This study was approved by the ethics committee of Huddinge University Hospital.

Huddinge kinship

Figure 1 shows the family pedigree of members of the Huddinge kinship. There are two instances of consanguinity in the pedigree: the index case's parents are first cousins, and of their nine children, four have been diagnosed with definite MS; in addition, the index case

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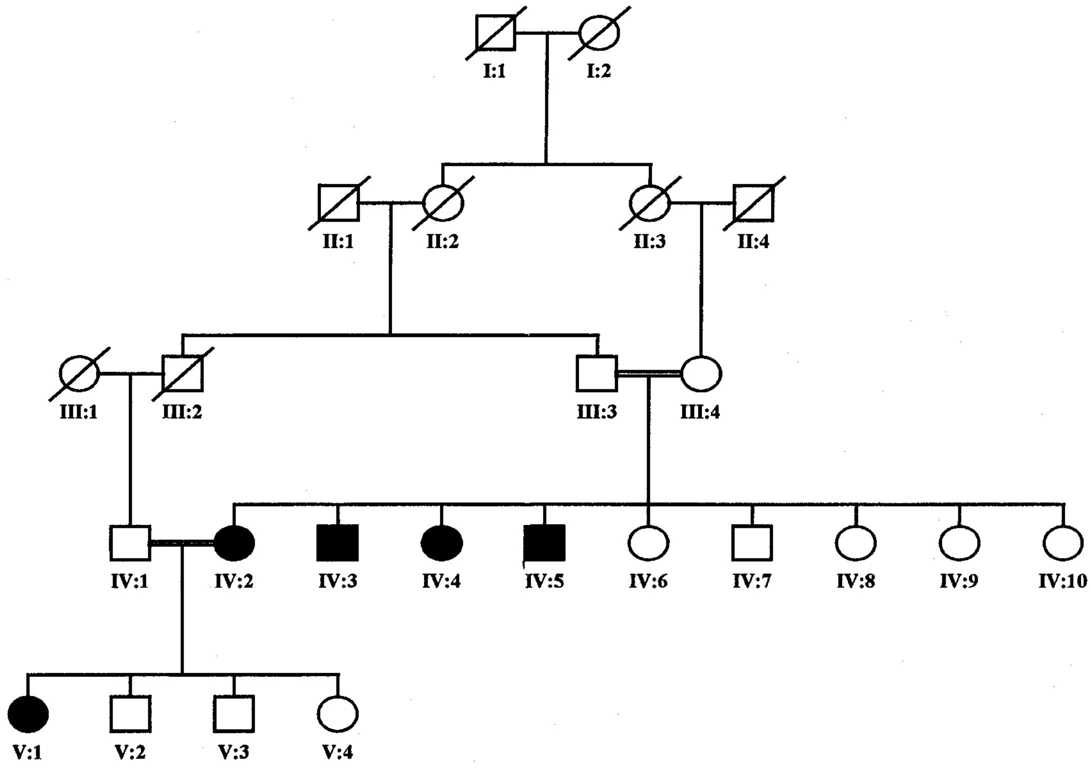

Figure 1 *Family tree of Huddinge kinship. Arrow denotes index case. Filled symbols denote family members afflicted with MS and double lines consanguineous marriage (in both cases between first cousins).*

is married to her first cousin and their oldest child has been diagnosed with definite MS.

Clinical profile of the Huddinge kinship

Table 1 summarizes the clinical features of members of the Huddinge kinship afflicted with MS.

Patient IV:2 (index case)

This woman was born in the Middle East and emigrated to Sweden at the age of 25. Her parents are first cousins and she is the oldest of their nine children. Her previous medical history included chronic myalgia and seasonal allergic rhinoconjunctivitis. At the age of 36 she presented to the emergency ward of HUH with one week of slurred speech and numbness and weakness in her right arm and right leg. She reported that she had experienced similar symptoms on one occasion three years earlier. Upon physical examination she was found to have a slight right-sided limp and reduced sensitivity to pinprick and light touch in her right arm and right leg. No immunoglobulin (Ig) G or IgM antibodies against the flagellar antigen of *Borrelia burgdorferi* (the agent that causes Lyme neuroborreliosis) were found in either serum or cerebrospinal fluid (CSF) by enzyme-linked immunosorbent assay (ELISA). Isoelectric focusing and IgG-specific immunofixation (IEF) of the patient's CSF revealed oligoclonal IgG bands, and T2-weighted magnetic resonance imaging (MRI) brain scans uncovered multiple high-signal foci in the periventricular white substance bilaterally and a single lesion in the right thalamus. A second MRI exam three years later revealed a new gadolinium-enhancing lesion in the left parietal lobe. The patient thus fulfilled criteria recommended by Poser *et al.*[6] for laboratory-supported definite MS.

Patient IV:3

This brother of the index case was born in the Middle East and emigrated to Sweden at the age of 14. When he was 32, he was admitted to the neurology department of HUH complaining of tingling on the left side of his chest and abdomen and pain in his lower back radiating to his left leg. He also reported weakness in his left leg and, upon physical examination, displayed decreased sensitivity to vibration in his left foot. Six years earlier he had experienced a similar, transitory episode of left-sided hemidysesthesia, accompanied on that occasion by signs of left-sided peripheral facial palsy. Serological tests for *B. burgdorferi* were negative. MRI of the brain and spinal cord visualized two lesions, one adjacent to the posterior horn of the left lateral ventricle and one at the level of the third cervical vertebra on the right side. CSF analysis revealed mononuclear pleocytosis (10.3×10^6 cells per litre) and oligoclonal bands. The patient at age 35 developed instability in his right leg, in which tendon reflexes were increased upon examination. He was at this point judged to fulfil the Poser criteria for clinically definite MS.

Patient IV:4

This sister of the index case was born in the Middle East and emigrated to Sweden at the age of 13. She had sought

Table 1 Clinical features of members of Huddinge kinship affected with MS

Pedigree position	*Sex*	*Age at emigration*	*Age at onset*	*Previous illnesses*	*Signs and symptoms at onset*	*CSF analysis*	*MRI*	*Diagnosis (Poser et al.)*
IV:2	F	25	33	Allergic rhinoconjunctivitis, myalgia	Hemidysesthesia, dysarthria	OCB	Periventricular lesions, lesion in thalamus	LSDMS
IV:3	M	14	26	Cervical trauma	Hemidysesthesia, facial palsy	Pleocytosis, OCB	Periventricular lesion, lesion in spinal cord	CDMS
IV:4	F	13	34	Suspected viral meningitis	Acute vision loss, hemihyperesthesia	Pleocytosis, OCB	Lesions in brainstem, corpus callosum	CDMS
IV:5	M	6	27	Viral meningitis	Acute visual impairment, headache	Pleocytosis, OCB	Periventricular, subcortical lesions	CDMS
V:1	F	2	14	Minor hearing difficulties	Acute hemiparesis, facial palsy	Pleocytosis, OCB	Lesions in pons, corpus callosum	LSDMS

CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; OCB, oligoclonal IgG bands; LSD, laboratory supported definite; CD, clinically definite

medical attention at age 29 on account of headache, neck pain and nausea, but a suspicion of viral meningitis was never confirmed. She presented to the emergency ward of HUH with four days of unsteady gait and dizziness, as well as numbness in the entire left side of her body since the previous evening. A month and a half earlier she had experienced rapid loss of vision and retrobulbar pain in the right eye and was diagnosed with optic neuritis. Serological tests for *B. burgdorferi* were negative. CSF analysis revealed mononuclear pleocytosis (25.0×10^6 cells per litre) and oligoclonal bands, and MRI visualized four brainstem lesions and two lesions in the corpus callosum. She was diagnosed with clinically definite MS.

Patient IV:5

This patient, the brother and youngest sibling of the index case, was born in the Middle East and emigrated to Sweden at the age of six. He had been treated for viral meningitis at the age of 20 and presented to the emergency room seven years later with three days of acute-onset blurred vision in the right eye and headache. Serological tests for *B. burgdorferi* were negative. Two months later he experienced sensory disturbances in his right arm and was diagnosed with clinically definite MS. A MRI brain scan at that time revealed seven periventricular lesions and two subcortical lesions, and mononuclear pleocytosis (7.6×10^6 cells per litre) and oligoclonal bands were found in the CSF.

Patient V:1

This patient is the daughter and oldest child of the index case; her father is her mother's first cousin. She was born in the Middle East and moved with her family to Sweden at the age of two. She had a previous history of minor hearing difficulties and was admitted to the paediatrics department of HUH when she was 14 complaining of weakness in the entire right side of her body and displaying signs of right-sided peripheral facial palsy. Serological tests for *B. burgdorferi* were negative, as were tests performed to detect the presence of an array of autoantibodies, including systemic lupus erythematosus (SLE)-associated antinuclear factor. Multiple lesions in both pons and corpus callosum were visualized by MRI, and CSF analysis revealed mononuclear pleocytosis (8.0×10^6 cells per litre) and oligoclonal bands. A subsequent MRI scan a year and a half later uncovered several new supratentorial lesions, whereby the patient was diagnosed with laboratory-supported definite MS.

Genotyping

Blood samples were collected from all five diagnosed patients and four healthy family members: the parents (III:3, III:4) and the spouse (IV:1) of the index case, as well as one of her healthy sisters (IV:6). DNA was extracted using the QIAmp DNA extraction kit (QIAGEN, Hilden, Germany).

Genotyping of microsatellite markers was conducted by deCODE Genetics in Reykjavik, Iceland. In the present framework marker set, the average spacing between markers was approximately 3 cM, with no gaps larger than 10 cM. A fine mapping marker set of 40 markers in a region of interest was later selected. Marker positions were obtained from an in-house high-resolution genetic map.[7] The polymerase chain reaction (PCR) amplifications were set up on Cyberlab C-400 (Cyberlab, Inc., Brookfield, CT, USA), run on MJR PCR machines (MJ Research, Inc., Boston, MA, USA) and pooled on Cyberlab C-400, with a similar protocol for each marker. The reaction volume was 5 νL and for each PCR reaction 15 ng of genomic DNA was amplified in the presence of 2 pmol of each primer, 0.25 U AmpliTaq Gold (Applied Biosystems, Inc., Foster City, CA, USA), 0.2 mM dNTPs and 2.5 mM $MgCl_2$ (buffer was supplied by the manufacturer). Cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 37 cycles of denaturation at 94°C for 15 s, annealing for 30 s at 55°C and extension for one minute at 72°C. An internal size standard was added to the PCR products, and pools were separated and detected on an

Applied Biosystems model 3700 Sequencer. Alleles were called automatically with the DAC (deCODE Allele Caller) program, and the program deCODE GT was used to fractionate allele readings according to quality and to edit the called genotypes.[8]

Statistical analysis

Before linkage analysis, genotyping data were used to verify familial relations, which included calculating identity by state (IBS) sharing between individuals III:3, III:4 and IV:1, and comparing it with IBS sharing distributions of the respective relationships from a set of families genotyped at deCODE Genetics, Iceland.

In the linkage analysis, multipoint, affected-only allele sharing methods were used to assess the evidence for linkage. The linkage results were obtained using the program ALLEGRO.[9] The S-pairs scoring function[10,11] and the exponential allele sharing model[12] were used to generate the relevant test statistics for one degree of freedom. A pointwise P value was computed by comparing the observed LOD score with its complete data sampling distribution under the null hypothesis.[9]

Results

From an original set of 1100 framework and 40 fine-mapping microsatellite markers, for each subject, the total number of successfully genotyped markers was as follows: III:3, 404; III:4, 654; IV:1, 678; IV:2, 676; IV:3, 563; IV:4, 649; IV:5, 498; IV:6, 690; V:1, 664. These markers were all used in the final analysis.

Analysis of the genome-wide screen using the framework markers revealed a suggestive LOD score of 2.29 (P value 0.0009) close to marker D9S1790 (Figure 2), with a one LOD drop between markers D9S175 and D9S261. The maximum possible allele sharing LOD score, if all patients in this family shared two copies of the disease allele by descent, is 3.91.

The 40 fine-mapping markers were added around this peak. Reconstruction of haplotypes showed that four out of five affected family members were identically homozygous for a 20-marker haplotype under this peak, spanning approximately 43 cM, with the remaining affected subject and all of the unaffected genotyped subjects being heterozygous for this haplotype (Figure 3).

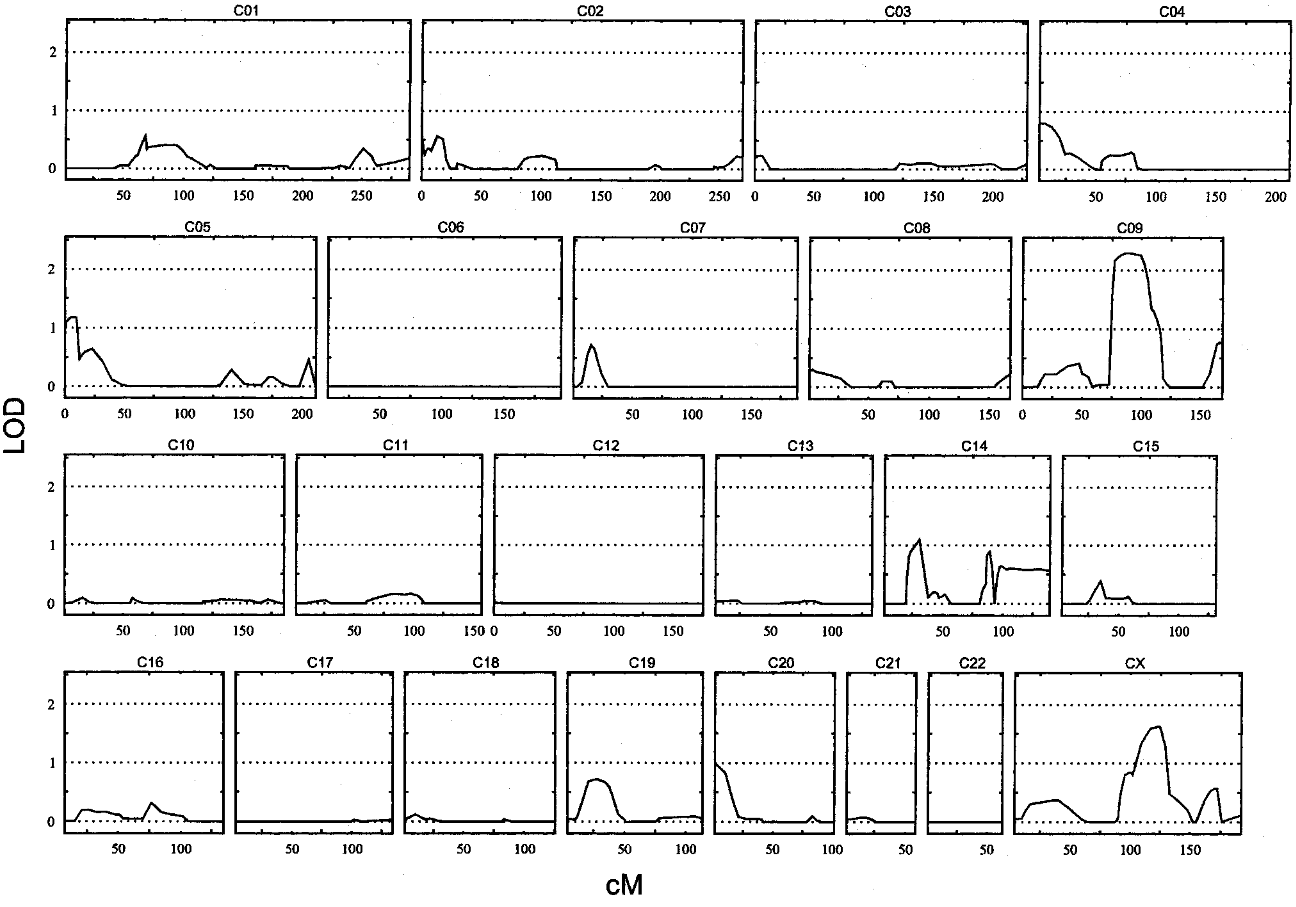

Figure 2 *Nonparametric multipoint LOD scores for all chromosomes. Five affected and four unaffected members of the Huddinge kinship were genotyped for a total of 1140 microsatellite markers (see Results for numbers of successful typings for each subject). The length of each X axis is proportional to the genetic length of the corresponding chromosome.*

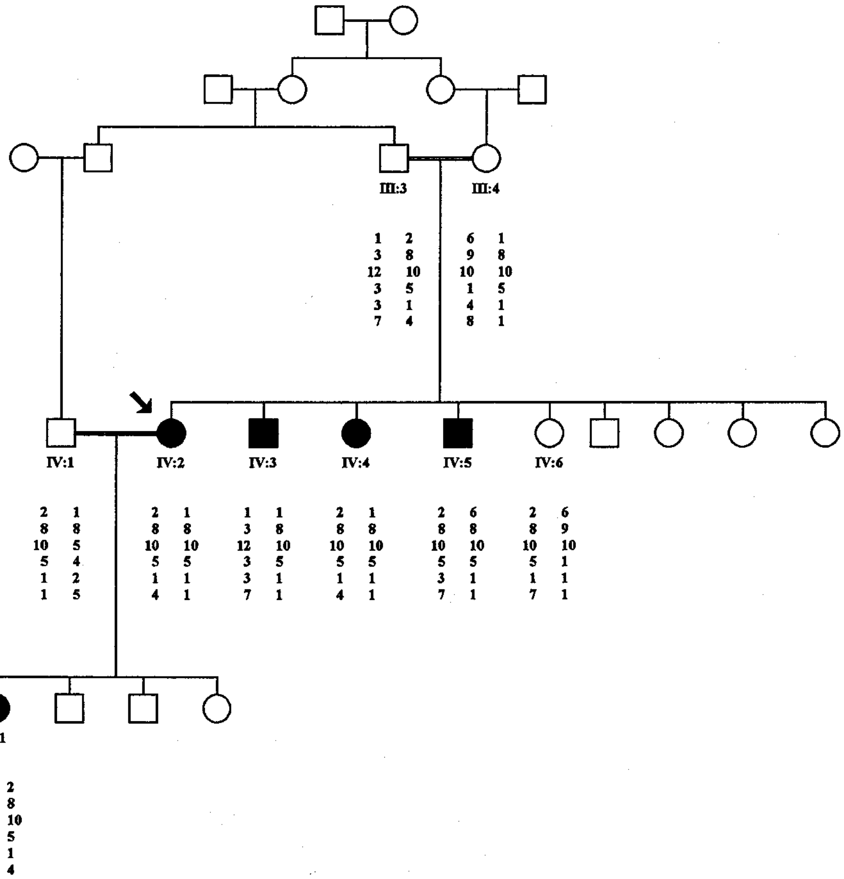

Figure 3 *Haplotype analysis for the long arm of chromosome 9. Allele combinations at the following six markers, out of 32 markers on this area, are indicated: D9S175 (70.3 cM), D9S1843 (80.3 cM), D9S1781 (99.4 cM), D9S910 (104 cM), D9S306 (109.5 cM), D9S1675 (120 cM). Four affected family members are homozygous by descent for the same 20 marker haplotype (of which three markers are shown here), while one affected subject (IV:3) and all unaffected family members are heterozygous for the haplotype.*

Discussion

Like MS, the common neurological disorders PD and AD are considered to be complex traits – i.e., non-Mendelian traits arising from the interaction of one of more genes with environmental factors. However, in both PD and AD, the investigation of rare families in which the disease phenotype is inherited as a highly penetrant Mendelian trait – leading to the identification of causative mutations in specific genes – has helped elucidate molecular pathogenetic pathways that appear to be greatly pertinent to the more common sporadic forms of the disorders as well.

For example, mutations in the gene encoding α-synuclein and in a gene designated *PARKIN*, have been found to cause, respectively, dominantly inherited[13,14] and recessively inherited[15,16] PD in small sets of families. α-Synuclein turns out to be one of the principal components of Lewy bodies, the neuropathological hallmark of classic, sporadic PD,[17] while *PARKIN* encodes an ubiquitin ligase involved in a key intracellular protein degradation pathway.[18] Dominantly inherited AD, which accounts for about 10% of all cases, is caused by mutations in one of three genes,[5] whose protein products – amyloid precursor protein and presenilins 1 and 2 – are all involved in the cascade of molecular events that culminates in the formation of amyloid plaques,[19] the hallmark of sporadic AD.

This study reports the results of a genome-wide screen of a kinship displaying clustering of MS cases. Although the risk of MS in siblings to MS patients is about 20 times greater than the risk in the general population, the occurrence of the disorder in more than two siblings from the same family is extremely rare.[20] The fact that MS-afflicted subjects in the Huddinge kinship were in each case the offspring of pairs of first cousins was the basis of the original hypothesis – namely, that the mode of inheritance of MS in the pedigree would prove to be autosomal recessive. Lander and Botstein[21] have described the rationale behind 'homozygosity mapping' and underscored its power to detect disease loci responsible for recessive traits in inbred kinships: each affected offspring of a consanguineous marriage should be homozygous by descent for a chromosomal region harbouring the disease locus.

In the present analysis, however, no region was found for which all affected family members were homozygous by descent. Assuming other, seemingly less probable patterns of inheritance – either Mendelian or non-Mendelian – the number of informative meioses in the pedigree was too small to allow detection of linkage. Yet there are indications that MS may not be a straightforward autosomal recessive trait in the Huddinge pedigree: the only observed instance of affected-to-affected transmission (i.e., from the female index case to her daughter) is reminiscent of the matrilineal inheritance of a mitochondrial DNA mutation; the ages of onset of the index case and her siblings were between 26 and 34 years, while the index case's daughter developed MS at the age of 14, consistent with a dominant trait and anticipation; and a LOD score of 1.7 was found on the X chromosome (Figure 2), suggestive of an X-linked trait partially penetrant in females. At the same time, consanguinity is known to increase the likelihood that non-Mendelian, oligogenic traits will occur multiple times within the same family.[22] In a recent genome-wide screen of 16 members of a large inbred Amish kinship, seven of whom had MS, Vitale and colleagues[23] reported a maximum LOD score of 2.7, conditional on the presence of the HLA-DR15 haplotype, for a locus on chromosome 12p12, suggesting a two-locus inheritance model in the pedigree. (HLA class II-conditioned inheritance was not apparent in the current pedigree, in which the following serology resolution HLA-DRB1 genotypes were found: III:3, DR1/DR13; III:4, DR4/DR15; IV:2, DR1/DR15; IV:3, DR4/DR13; IV:4, DR1/DR15; IV:5, DR4/DR13; and V:1, DR1/DR11.)

Natowicz and Bejjani[24] have inventoried the various Mendelian disorders that can 'masquerade' as MS; they include X-linked adrenoleukodystrophy (which is symptomatic in over 10% of female heterozygotes), matrilineally transmitted Leber's hereditary optic neuropathy and the autosomal dominant cerebellar ataxias. Although no formal effort was made to rule out these diagnoses, the authors are nonetheless convinced that the neurological signs and symptoms of the five affected members of the Huddinge kinship are compatible with conventional conceptions of the (admittedly heterogeneous) nosological entity MS. In addition to information gleaned from clinical histories and examinations – which in three cases sufficed for the diagnosis of definite MS (Table 1) – all five had both IEF-detected CSF oligoclonal bands (a highly sensitive finding in MS[25]) and at least one MRI lesion either abutting a lateral ventricle or in the corpus callosum (localizations highly specific to MS[26]).

All five affected family members genotyped for this study emigrated from the Middle East, an area of low MS prevalence, to Sweden, an area of high prevalence,[27] before the onset of neurological disease. Four of them emigrated before the age of 15 – i.e., before the purportedly protective effect of growing up in an area of low MS prevalence had been established.[28] Moreover, intriguingly, despite ages at onset spanning more than two decades (from 14 to 34 years), the five affected family members all developed MS within a single decade of each other. Post-emigration disease onset – as well as the tendency towards date-of-onset rather than age-at-onset clustering – could be consistent, ironically, with involvement of environmental, possibly infectious, factors[29] (with or without heritable ones) in the aetiology of MS in the Huddinge kinship.

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