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Linkage disequilibrium screening for multiple sclerosis implicates *JAG1* and *POU2AF1* as susceptibility genes in Europeans

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Abstract

By combining all the data available from the Genetic Analysis of Multiple sclerosis in EuropeanS (GAMES) project, we have been able to identify 17 microsatellite markers showing consistent evidence for apparent association. As might be expected five of these markers map within the Major Histocompatibility Complex (MHC) and are in LD with *HLA-DRB1*. Individual genotyping of the 12 non-MHC markers confirmed association for three of them — D11S1986, D19S552 and D20S894. Association mapping across the candidate genes implicated by these markers in 937 UK trio families revealed modestly associated haplotypes in *JAG1* ($p=0.019$) on chromosome 20p12.2 and *POU2AF1* ($p=0.003$) on chromosome 11q23.1.

Keywords: *JAG1*; *POU2AF1*; Linkage disequilibrium; Genome screen; Meta-analysis; Multiple sclerosis

1. Introduction

Epidemiological analysis indicates that multiple sclerosis results from unknown environmental factors acting on genetically susceptible individuals (Hogancamp et al., 1997). Early efforts to identify relevant genes quickly identified association with Major Histocompatibility Complex (MHC) haplotypes — DR15 (*DRB1∗1501-DQB1∗0602*) in Northern Europeans (Compston et al., 1976; Haines et al., 1998; Jersild et al., 1972; Olerup and Hillert, 1991; Stewart et al., 1997; Winchester et al., 1975) along with DR3 (*DRB1∗ 0301-DQA1∗0501-DQB1∗0201*) and DR4 (*DRB1∗0405-DQA1∗0501-DQB1∗0301*) in southern Europeans (Coraddu et al., 1998; Marrosu et al., 1998; Saruhan-Direskeneli et al., 1997). No other candidate gene showing consistent evidence for association has yet been identified. Fortunately developments in the human genome project have now reached the point where systematic whole genome approaches are now becoming possible.

The Genetic Analysis of Multiple sclerosis in EuropeanS (GAMES) collaborative was formed in order to take advantage of these emerging methods (Sawcer and Compston, 2003). In its first project, the 19 research groups each performed a low-resolution genome screen for linkage disequilibrium (LD) using a common panel of microsatellite markers and pooled DNA methodology (Barcellos et al., 1997). However, the additional sources of error introduced by pooling along with the relatively modest number of cases (c200) included in each cohort limits the power of these individual screens (Setakis, 2003). Given that the same markers, and the same alleles at each marker, were considered in each individual GAMES screen, combining the data in a meta-analysis represents an obvious way to reduce these confounding effects. Here, we present a meta-analysis, with individual genotyping of the most promising markers, culminating in detailed association mapping of the candidate genes suggested by the most strongly supported markers.

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2. Materials and methods

2.1. The GAMES screens

In total, the GAMES collaborative screened 19 case-control cohorts and 10 trio family based cohorts (Sawcer and Compston, 2003) as described in the original publications: Australia (Ban et al., 2003), Belgium (Goris et al., 2003), Finland (Laaksonen et al., 2003), France (Alizadeh et al., 2003), Germany1 (Goedde et al., 2002), Germany2 (Weber et al., 2003), Hungary (Rajda et al., 2003), Iceland (Jonasdottir et al., 2003), Ireland (Heggarty et al., 2003), Italy (Liguori et al., 2003), Poland (Bielecki et al., 2003), Portugal1 (Santos et al., 2003), Portugal2 (Martins Silva et al., 2003), Sardinia (Coraddu et al., 2003), Scandinavia (Harbo et al., 2003), Spain (Goertsches et al., 2003), Turkey (Eraksoy et al., 2003) and UK (Sawcer et al., 2002). Table 1 summarizes the number of markers successfully analyzed in each cohort studied, as well as the number of samples in each pool.

2.2. Genotyping of the microsatellites emerging from the meta-analysis of GAMES

Individual genotyping of the samples making up the pools used in the individual GAMES screens was performed using standard methods. This genotyping task was split between Cambridge and deCODE, except for the Sardinian samples, which were analyzed locally. Consistent allele definition was ensured by genotyping a reference individual, CEPH 1346-02.

2.3. Re-sequencing genes of interest

In order to identify variants not already included in the public databases, we re-sequenced genes of interest in patients with a family history of multiple sclerosis. All sequencing was carried out using Applied Biosystems BigDye® Terminator v3.1 cycle sequencing kit according to the manufacturers recommended conditions. Excess dye-terminators were removed from the sequencing reaction using the Qiagen DyeEx 96 Spin Kit. Electrophoresis was performed on an Applied Biosystems 3700 DNA analyzer and sequence was read using Sequencing Analysis software v3.7 (Applied Biosystems). Resequencing of *POU2AF1* and *AKT2* was performed using the Applied Biosystems VariantSEQr™ Resequencing System. For resequencing of *JAG1* and *MAP3K10*, we designed our own primers (available in supplementary Table S1). In total, resequencing was attempted for four genes. In each case, we aimed to resequence all exons and a variable extent of flanking sequence upstream and downstream (approximately 2 kb for *JAG1* and 0.5 kb for all other genes). We were unable to obtain reliable sequence from exon 1 and the immediate 5′ upstream region of *JAG1* or from 305 bp of exon 9 in *MAP3K10*.

2.4. United Kingdom Trio families used to investigate genes of interest

Variants of interest were tested for evidence of association in a cohort of 937 trio families (an affected individual and their parents) from the United Kingdom. All typed individuals gave informed consent and all affected individuals satisfied Poser criteria for the diagnosis of multiple sclerosis. The demographic features of the affected individuals are unremarkable; average age was 37.8 years, mean duration of disease 11.9 years, mean EDSS 4.3, and sex ratio 3F:M. These 937 trio families include the 745 used in the original United Kingdom GAMES screen (Sawcer et al., 2002). Power calculations indicate that 937 trio families would have 84% power to find association with a nominal (uncorrected) p-value of 0.05 assuming a common risk allele (10% frequency) with an odds ratio of 1.2 (multiplicative model) (Purcell et al., 2003).

2.5. Genotyping variants from genes of interest

All SNPs were genotyped using Applied Biosystems TaqMan methodology according to the manufacturer's recommended conditions. PCR was performed on Applied Biosystems 384 well 9700 Viper PCR machines after which genotypes were called on a 7900 High Throughput Sequence Detection System using SDS software. Where available Applied Biosystems Assay-On-Demand (AoD) products were used ($n=59$); for all other variants, we used the Assay-By-Design (AbD) service. Single nucleotide polymorphisms (SNPs) identified through our own sequencing efforts were supplemented by searching NCBI, the HapMap database, Ensembl and Applied Biosystems own SNP browser database. Indels were genotyped on a 3700 DNA analyzer. Primers, basic marker performance measures and single point transmission disequilibrium test (TDT) results as determined using the TRANSMIT program (Clayton, 1999) are available for each marker in supplementary Table S2.

All assays were first tested against a test panel of 378 samples. No workable assay could be designed for 44 variants and a further 127 (40.8%) were found to be non-polymorphic. Polymorphic working assays were then typed in the remaining United Kingdom trio families. One hundred and sixty three samples were typed in duplicate. Only markers showing ≤ 1 genotyping error (Mendelian inconsistency and/or duplicate genotype discrepancy), genotyping success rate $\geq 90\%$ and minor allele frequencies $>5\%$ were considered in the multipoint analysis.

3. Statistical methods

3.1. Individual microsatellite genotyping

Data from case-control cohorts were analyzed using a chi-squared test, with uncommon alleles grouped together so that

Table 1
Number of samples and successfully typed markers for each cohort studied in GAMES

	Cases (M/F)[c]	Controls (M/F)[c]	Typed[e]	Trios (M/F)[d]	Typed[e]
Cambridge					
Australia[a]	217 (33/184)	188 (75/113)	4346	–	–
Belgium	204 (78/126)	198 (115/83)	4314	131 (50/81)	4359
France	200 (65/135)	200 (66/134)	3998	200 (63/137)	4041
Germany1[ab]	198 (81/117)	198 (138/60)	4227	–	–
Germany2	234 (68/166)	209 (110/99)	4634	68 (16/52)	4664
Ireland[b]	200 (61/139)	200 (91/109)	2537	–	–
Italy	224 (79/145)	231 (96/135)	4192	185 (68/117)	4789
Scandinavia1	199 (73/126)	200 (75/125)	4015	–	–
Scandinavia2	201 (60/141)	200 (60/140)	4204	–	–
Poland	200 (71/129)	200 (93/107)	4219	129 (33/96)	4432
Portugal 1	188 (56/132)	188 (56/132)	4661	–	–
Sardinia	229 (88/141)	264 (126/138)	3291	235 (67/168)	3512
Turkey	200 (81/119)	200 (94/106)	4359	–	–
United Kingdom	216 (64/152)	219 (106/113)	5396	745 (188/557)	5330
Decode					
Finland	195 (63/132)	205 (64/141)	3979	118 (36/82)	2788
Hungary	88 (19/69)	75 (13/62)	3025	–	–
Iceland	200 (59/141)	200 (115/85)	4110	103 (33/70)	3894
Portugal2	200 (68/132)	200 (109/91)	1745	–	–
Spain	200 (67/133)	228 (115/113)	3984	186 (63/123)	2994

[a] In these studies, all cases were DR15 positive.
[b] The PCR for these studies was performed in Cambridge using the Cambridge set of primers but the electrophoresis was performed in the respective host institutions.
[c] The number of samples included in each pool is shown, together with the gender breakdown (M:F).
[d] This column indicates the number of index cases from trio families in the index pool (with gender breakdown, Male:Female). The number of parents is not listed since this is = 2 × the number of index cases, with equal numbers of fathers and mothers.
[e] This column indicates the number of markers which generated data analyzable by the method developed by Setakis (2003). There may be some differences from the original publications where different methods of analysis were used.

the number of common alleles (degrees of freedom) was the same for each cohort studied. The number of common alleles selected for each marker was that which grouped all alleles with a frequency of <5% in the United Kingdom. The individual genotyping data from the trio families were analyzed using the AFBAC program (Thomson, 1995). For the X-linked marker (DXS1684) only maternal transmission was considered.

3.2. Data from variants typed in genes of interest

Marker performance was tested using the PEDSTATS program (Wigginton and Abecasis, 2005). No marker showed significant evidence of deviation from Hardy Weinberg equilibrium. Single point TDT was performed using the TRANSMIT program (Clayton, 1999) with significance judged empirically using the programs bootstrap function (10 000 bootstrap replicates were performed in each case).

In multipoint testing, as family data allows phase of transmission to be observed, we first determined Transmitted and Non-transmitted haplotypes using the GENEHUNTER program (Kruglyak et al., 1996). Haplotype pattern mapping was then performed using the Haplotype Pattern Mining

Table 2
Ranking for the robust markers in the respective meta-analyses based on combining empirical *p*-values

Marker	Without[a]		Marker	With[b]	
	Case/Control[c]	Trio[d]		Case/Control[c]	Trio[d]
D1S2141	5	32	D2S2207	21	4
D2S1346	41	21	D5S2115	258	23
D6S1615	7	177	D6S1615	3	59
D6S2444	3	73	D6S2444	2	9
D6S265	63	15	D6S265	14	2
D6S273	81	120	D6S273	16	65
TNFa	6	146	TNFa	1	12
D9S172	253	83	D9S172	204	41
D11S1986	8	51	D18S52	6	24
MYCL1	4	3	D20S894	157	102
D18S52	1	17	DXS1684	18	1
D19S552	30	95			
D21S1270	35	24			
DXS1684	206	2			

[a] These meta-analyses were based on analysis of the raw data without using the adapting factors suggested by Yeo et al. (2003).
[b] These meta-analyses were based on analysis of the raw data using the adapting factors suggested by Yeo et al. (2003).
[c] Only markers providing data from at least 6 cohorts where included in the meta-analysis of the case-control studies (5335 markers in total).
[d] Only markers providing data from at least 4 cohorts where included in the meta-analysis of the trio family studies (5260 markers in total).

software (Toivonen et al., 2000). In this analysis, we considered haplotype patterns involving up to seven consecutive markers and allowed up to two "wildcard" (ambiguous) alleles per pattern. Only haplotype patterns with a frequency of at least 5% were considered. A Bonferroni correction factor was applied to the haplotype specific significance tests based on the total number of haplotype patterns tested in each corresponding analysis. For markers with more than two alleles, rare variants were down grouped until the data were biallelic.

4. Results

4.1. Meta-analysis of GAMES screens

To identify those markers showing the most consistent evidence for association across the various European populations studied, marker specific empirical *p*-values from the individual GAMES studies were combined using the Fisher's method. The case-control ($n=19$) and trio family ($n=10$) studies were meta-analyzed separately and the resulting rankings compared. The raw data for markers appearing in the top 5% of both meta-analyses were inspected to exclude markers that ranked highly due to poor signal quality or genotyping error in one or more populations. The robust markers that emerged from this analysis are listed in Table 2. As many of the markers included in the GAMES project are known to be heavily influenced by pooling related artefacts, this meta-analysis process was repeated using the empirical *p*-values from the individual GAMES studies generated after applying the adapting factors proposed by Yeo et al. (2003). Robust markers appearing in the top 5% of the repeat meta-analysis are also shown in Table 2. A total of 17 independent markers were identified as showing consistent evidence of association across the GAMES screens. Five of these lie in the MHC region (D6S1615, D6S2444, D6S265, D6S273 and TNFa) and show association secondary to LD with the multiple sclerosis associated DR15 haplotype.

Table 4
Meta-analysis *p*-values determined by the Fisher's method for the six markers considered to be validated

	Case-control	Trio families
D11S1986	0.07	0.58
D19S552	0.09	0.001
D20S894	0.04	0.56 [a]
D6S265	1.16E-05	5.93E-06 [a]
D6S273	4.02E-16	3.50E-08 [a]
TNFa	2.78E-21	1.52E-12

[a] These results are based on the analysis of a single data set (the UK trio families).

4.2. Individual genotyping of the GAMES microsatellites

To validate the potential associations identified in the meta-analysis, we individually genotyped each of the twelve non-MHC markers, together with three of the MHC markers (D6S265, D6S273 and TNFa), in as many of the samples used to construct the original pools as were available. The average number of samples from each original pool in which

Table 3
The average number of samples from the original pools in which individual typing was attempted, together with the average corresponding genotyping success rates

Population	Cases/n (%) [a]	Controls/n (%) [a]	GSR [b] Cases/%	GSR [b] Controls/%	Trios/n (%) [a]	GSR [b] Trios/%
Australia [c]	215 (99)	184 (98)	98.1	96.6	–	–
Belgium	202 (99)	193 (97)	93.2	95.6	130 (99)	90.8
France	180 (90)	90 (45)	97.4	95.7	200 (100)	85.5
Germany1	190 (96)	190 (96)	94.5	95.0	–	–
Germany2	217 (93)	180 (86)	95.5	95.8	67 (98)	95.5
Ireland	196 (98)	197 (99)	94.3	95.4	–	–
Italy	198 (88)	177 (77)	78.1	66.3	135 (73)	80.0
Scandinavia1	182 (91)	186 (93)	92.1	94.1	–	–
Scandinavia2	192 (96)	192 (96)	93.3	94.2	–	–
Poland	182 (91)	185 (93)	87.5	93.1	87 (67)	92.0
Portugal1	187 (99)	187 (99)	95.5	97.2	–	–
Sardinia	205 (90)	264 (100)	91.6	98.4	232 (99)	91.4
Turkey	192 (96)	192 (96)	70.9	62.1	–	–
United Kingdom	207 (96)	160 (73)	92.5	82.9	745 (100)	90.1
Finland	169 (87)	180 (88)	95.0	95.9	–	–
Hungary	NA	NA	NA	NA	–	–
Iceland	182 (91)	176 (88)	99.9	99.2	–	–
Portugal2	175 (88)	181 (91)	96.4	98.1	–	–
Spain	176 (88)	178 (78)	95.2	96.3	–	–

The Hungarian samples were not employed in the individual genotyping stage of the experiment.
[a] % indicates the proportion of the samples making up the original pool that were employed in the individual typing stage of the experiment.
[b] The Genotyping Success Rate (GSR) indicates the proportion of typed samples for which individual genotypes were successfully determined.
[c] As the case pool from this population only included DR15 positive individuals, an additional 155 DR15 negative cases were also typed for the HLA markers.

typing was attempted, together with the average genotyping success rate within these samples are shown in Table 3. In order to mirror the meta-analysis identifying these markers, we combined the observed *p*-values using Fisher's method and considered a marker as validated if it continued to show evidence of association (at $p<0.1$) in either the case-control or trio family cohorts. Association was validated for the three MHC markers and also for three non-MHC markers. Table 4 summarizes the meta-analysis results of the individual genotyping data generated for these six markers (results for the individual populations are available in supplementary Table S3).

4.3. Further analysis of D20S894

Marker D20S894 maps 46.3 kb from the Jagged1 gene (*JAG1*), immediately under one of the strongest linkage signals identified in the recently completed high density multiple sclerosis linkage screen (Sawcer et al., 2005). In order to explore this logical candidate further, we re-sequenced the gene in 20 patients (see Materials and methods) identifying 12 novel variants (9 SNPs, two indels and one microsatellite). Database searching of the 56 kb interval containing the gene identified a further 186 SNPs. Initial screening of these variants left 85 polymorphic variants with working assays which were then genotyped in the 937 UK trio families. Four of the 85 markers showed nominally significant (uncorrected) evidence for association when analyzed individually as shown in Table 5. However, after Bonferroni correction, none of the markers tested showed evidence for association (see Table S2).

Amongst the 85 genotyped markers, 55 passed stringent quality thresholds and were mined for associated haplotype patterns (see Materials and methods). The most strongly associated haplotype pattern was rs7271215(G)–rs6077870(A/G)–rs2235810(C)–rs6040073(A)–rs910119(T)–rs910118(G)–rs6040079(G). This association remains statistically significant even after full Bonferroni correction for the 8780 haplotype patterns considered (corrected *p*-value 0.018). As this haplotype lies at the extreme edge of the set of markers considered, a further five markers centromeric to this associated haplotype were also genotyped in the 937 UK trio families. Three of these markers passed quality standards and were combined with the existing data so that haplotype pattern mining could be repeated. None of these additional markers were found to be part of the most associated haplotype. Repeat haplotype pattern mining considered 9404 possible haplotype patterns. After Bonferroni correction, the association remained significant with a corrected *p*-value of 0.019. Permutation analysis indicates that the susceptibility effect maps to the interval between markers rs910118 and rs6040079.

Table 5
Markers showing nominally significant TDT results

Gene	Marker	*p*-value
JAG1	rs6040069	0.0325
JAG1	ss48400486	0.0367
JAG1	ss48400481	0.037
JAG1	rs6040060	0.0406
POU2AF1	rs4514461	0.012
POU2AF1	rs6589224	0.015
POU2AF1	rs10789825	0.018
POU2AF1	rs1986838	0.026
POU2AF1	rs4622303	0.03
POU2AF1	rs4245182	0.041
POU2AF1	rs1815945	0.044

4.4. Further analysis of D11S1986

Marker D11S1986 lies in the 3′ untranslated region of the POU domain, class 2, associating factor 1 gene (*POU2AF1)*. In order to explore the relevance of this gene we identified SNPs from the HapMap project database and all SNPs with available AoD products from a 110 kb interval including the gene. Re-sequencing of the gene in five individuals was also performed but did not identify any additional variants. Workable assays were designed for 33 of the 35 identified SNPs but initial screening showed that five of these were non-polymorphic in our families. The remaining 28 SNPs were typed in the 937 UK trio families. After Bonferroni correction none of these markers showed evidence of association when analyzed individually (see Table S2). The seven markers showing nominally significant (uncorrected) evidence for association are listed in Table 5. Of the 28 typed markers, 26 met the stringent quality threshold for multipoint testing. Haplotype pattern mining revealed that the most strongly associated haplotype pattern was rs7952176(A)–rs1123066(A/G)–rs4127504(G)–rs883326(C/T)–rs10789825(G)–rs4622303(G)–rs6589224(A). After Bonferroni correction for the 3484 haplotype patterns considered, this association remains statistically significant (corrected *p*-value 0.003). Permutation analysis indicates that the susceptibility effect maps to the interval between markers rs7952176 and rs1123066.

4.5. Further analysis of D19S552

Ten genes lie within 250 kb of the D19S552 marker. We chose to focus our efforts on the two plausible candidate genes with known function lying closest to the D19S552 marker — *MAP3K10* (mitogen activated kinase kinase kinase 10) and *AKT2* (v-akt murine thymoma viral oncogene homolog 2). Re-sequencing in five individuals revealed one novel SNP in *MAP3K10* and three in *AKT2*. An additional 115 SNPs were identified from the 300 kb interval surrounding the D19S552 marker that included all coding SNP variants, SNPs with available AoD products, SNPs from the HapMap project database as well as all SNPs from the Ensembl database lying within 10 kb of the D19S552 marker itself even though this interval did not include a gene.

Workable assays were designed for 92 SNPs, of these 23 were found to be monomorphic. The remaining 69 SNPs were typed in the 937 UK trio families. None of these markers showed even nominally significant evidence for association when tested individually for TDT (see Table S2). The most associated marker was rs3746006, with a nominal *p*-value of 0.06. Forty-six markers passed the quality standards for multipoint analysis. However the most associated haplotype pattern (involving the seven markers between rs339512 and rs2304186) had only a nominal *p*-value of 0.002 and none of the 4992 haplotype patterns considered (see Materials and methods) survived Bonferroni correction.

5. Discussion

In our meta-analysis of the GAMES project (altogether involving 13,896 individuals), we identified 12 non-MHC markers potentially associated with multiple sclerosis. Individual genotyping in a significant proportion of these samples confirmed the pooling results for three of these markers (D11S1986, D19S552 and D20S894). Association mapping in the candidate genes suggested by these markers indicates that variation in *JAG1* and *POU2AF1* may influence susceptibility to multiple sclerosis.

The power of the individual GAMES screens is limited by several factors, the most significant of which are undoubtedly the modest density of markers employed, the limited number of samples in each cohort and the confounding additional sources of variance introduced by DNA pooling (Sawcer and Compston, 2003). Meta-analysis of these accumulated data is a logical next step. When these experiments were conceived it was anticipated that LD might typically extend for 250 kb; based on this estimate 6000 markers were deemed sufficient to screen the genome (Barcellos et al., 1997). These estimates are now known to be overly optimistic (Kruglyak, 1999) with the result that 6000 markers will only have sensitively examined a fraction of the genome (probably <1%), reducing the possibility that relevant genes are in LD with markers studied. DNA pooling further reduces power by introducing additional sources of error into the analysis. These pooling induced errors become increasingly relevant as the size of a DNA pool is increased (Barratt et al., 2002). It is thus expected that a proportion of apparent associations will be artefactual. Consequently associations demonstrated in the current study need to be replicated in additional cohorts. As initial findings tend to overestimate the size of an effect, follow-up studies will generally require larger numbers (Colhoun et al., 2003).

A meta-analysis of the type presented here tacitly assumes that the same loci are involved in determining genetic susceptibility in the various populations considered. Fortunately the fact that different alleles of a given susceptibility locus may confer susceptibility in different populations is not likely to impact on the power since Fisher's method of combining the evidence for association is allele independent. On the other hand under the 'common disease–common variant' hypothesis, it might be argued that risk alleles at a given locus are likely to share a single common founder (identical by descent: IBD), even across populations. A microsatellite in LD with such a susceptibility gene would show a consistent distortion in the allele frequency distribution in each constituent population even though the distortion may not be significant in every population. We reasoned that such microsatellites may not appear highly ranked in all component populations or associated in any one, but might nevertheless emerge in a meta-analysis that combines allele frequency estimates from all the studies. This analysis did not identify any additional markers of interest (data not shown).

The multipoint analysis we performed revealed significantly associated haplotype patterns in both the JAG1 and POU2AF1 genes, even after Bonferroni correction. Both *JAG1* and *POU2AF1* are potential candidate susceptibility genes. Jagged 1 is known to be involved in the differentiation of oligodendrocyte precursors (Wang et al., 1998) and has been shown to be involved in determining the degree of remyelination occurring in the lesions of multiple sclerosis (John et al., 2002). *POU2AF1* is a transcriptional co-activator involved in the regulation of IgG gene expression in B-cells. As several lines of evidence support the interpretation that intrathecal production of antibody in multiple sclerosis renders the myelin sheath susceptible to immune attack, this is also a plausible candidate gene. In considering these haplotype results, it is important to remember that the *p*-values reported for these most associated haplotypes are heavily influenced by a number of rather arbitrary choices made in performing our haplotype pattern mining.

In summary, we provide detailed analysis of the most promising candidate genes identified through an international collaborative effort — the GAMES project. Two potentially associated haplotypes, one in *JAG1* and a second in *POU2AF1*, are identified but more detailed analysis of these regions is required to validate the associations.

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Appendix A. Web Resources

The GAMES collaborative, http://www-gene.cimr.cam.ac.uk/MSgenetics/GAMES.

Newly described single nucleotide polymorphisms were submitted to the National Center for Biotechnology Information — ss48400478, ss48400479, ss48400480, ss48400481, ss48400482, ss48400483, ss48400484, ss48400485, ss48400486, ss48400487, ss48400488, ss48400489, ss48400490, ss48400491, ss48400492, http://www.ncbi.nlm.nih.gov/SNP.

Appendix B. Contributors by nation

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Appendix C. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.jneuroim.2006.06.003.

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