Chapter 3

Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1

3.1 Introduction

Migraine is a multifactorial disorder and therefore, both environmental and genetic factors contribute to its susceptibility [88]. Genes predisposing to common migraine remain unknown, even though several genomic regions have been implicated in linkage studies [162–172], and several genes have been tested in candidate-gene association studies [173–184]. Linkage studies have had limited success in mapping risk loci in complex diseases, such as migraine [31–33]. Compared to linkage studies, association studies have higher statistical power to detect common variants that confer modest disease susceptibility [187]. Risch (2000) has estimated that for variants with modest genotype relative risks (GRR $\leq$ 2) and intermediate allele

frequency (0.05%-0.50%), linkage analysis are not able to provide any statistical evidence, except in unrealistically large samples. By contrast, case-control association studies, even using a stringent significance level as the one used in GWAS ($P = 5 \times 10^{-8}$), have adequate power to detect variants with GRR as low as 1.5 and with an intermediate allele frequency [34]. Candidate-gene association studies have identified several variants associated to migraine. However, most of the associations have not been replicated. The lack of well-replicated findings could be due to a limited number of cases and controls tested in most of the studies performed so far [173–184]. Genome-wide association studies (GWAS) are association studies in which most of the common variations in the human genome are tested for association with the trait of interest [47]. Compared to candidate-gene association studies, they do not require any assumptions about the causal variants [37]. GWAS are an effective approach to identify common genetic variants which confer disease susceptibility [37, 40, 44–50]. GWAS for migraine have not been performed so far.

To identify common susceptibility variants for migraine, a two stage GWAS was carried out. In the discovery stage, 2748 migraineurs from headache clinics and 10747 population-matched controls from Finland, Germany and the Netherlands were analyzed. In the replication stage, further 3202 cases and 40062 population-matched controls from Iceland, Denmark, The Netherlands and Germany were studied. Owing to the overlap between individuals having migraine with aura (MA) and those having migraine without aura (MO), the following diagnostic subgroups were analyzed: (i) 'all migraine', defined as individuals with migraine irrespective of the subtype; (ii) 'migraine with aura only' (MA), defined as individuals who have only attacks where the aura is present; (iii) 'both migraine with

aura and migraine without aura' (MA/MO), defined as individuals having both attacks with and without aura; (iv) 'migraine without aura only' (MO), defined as individuals with only attacks of migraine without aura.

3.2 Results

3.2.1 Discovery stage

In the discovery stage, more than 500000 SNPs across the genome were genotyped in 3279 European individuals affected by migraine with aura (MA and MA/MO) (1124 Finnish, 1276 Germans, and 879 Dutch), recruited in headache specialized clinics. Diagnoses were made by headache specialists using a combination of questionnaires and individual interviews according to the ICHD-II guidelines [58]. In this stage of the study, we focused our attention on MA, since currently the diagnosis of migraine is based only on clinical features and the presence of aura as diagnostic criteria reduces the possibility to include other headache types among the cases. Population-matched controls (12369) were drawn from population-based cohorts previously genotyped (Helsinki Birth Cohort study, Health2000 study, KORA study, HNR study, PopGen study, Illumina iControlDB and Rotterdam study I)(see Methods).

Study samples had been screened for SNP call rate, presence of population outliers, duplicates and relatedness (see Methods). Overall 2748 cases and 10747 controls passed the quality control filters and remained in the study. After excluding SNPs which did not pass the quality control filters (see Methods), 429912

SNPs were available for analysis.

I performed a Cochran-Mantel-Haenszel (CMH) test (1 degree of freedom) for differences in SNP allele frequencies between cases and controls, stratified by the three different European study samples (Finnish, German and Dutch). The strongest association with migraine, above the threshold for genome-wide significance ($P = 5 \times 10^{-8}$) [49], was found for a locus on chromosome 8q22.1, marked by rs1835740. The minor allele of rs1835740 (A) was significantly associated, with a P-value of 5.38×10^{-9} (OR = 1.23, 95% confidence interval = 1.15-1.32) (Table 3.1, Figure 3.1, 3.2 and 3.3). The pattern of association was similar in all three study cohorts and there was no evidence of heterogeneity of the association (Table 3.1).

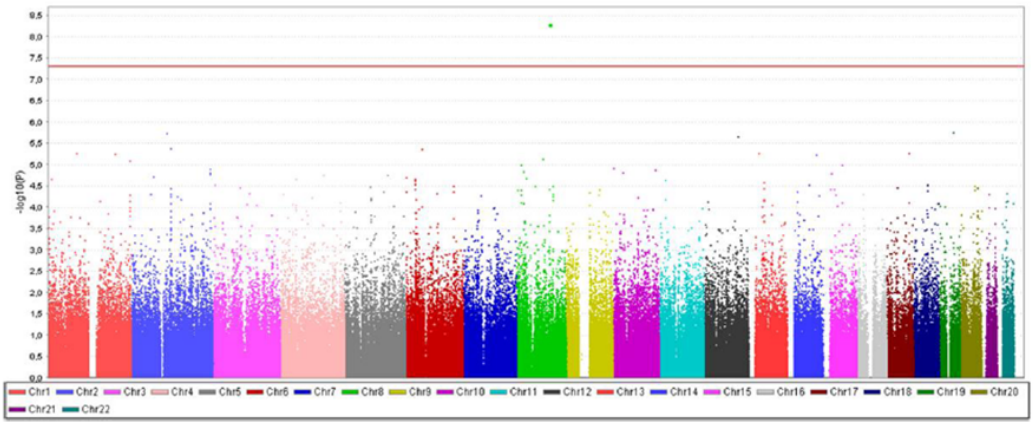

Figure 3.1: **Genome-wide P-values for the discovery phase.** P-values are log transformed (-log10) (y axis) and plotted against chromosomes (x axis). The red line indicates the genome-wide significant threshold. The signal in green above the threshold for genome-wide significance is rs1835740.

Quantile-quantile plot of the distribution of the test statistic for comparison of SNP allele frequencies in cases and controls (1 degree of freedom CMH test) (Figure 3.2) and an estimated genomic inflation factor (λ) of 1.08 suggested a modest overall inflation of P-values.

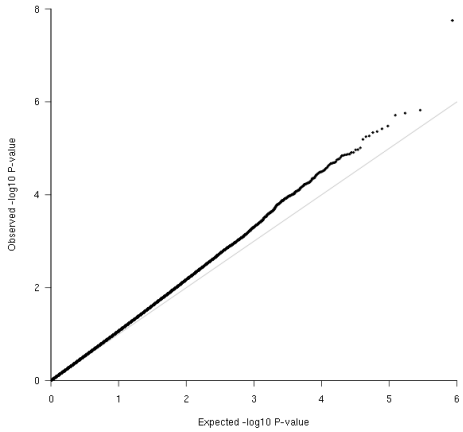

Figure 3.2: **Quantile-quantile plots of the GWAS discovery phase.** Plots of the CMH tests for association between SNP allele frequencies in cases and controls. This shows a slight deviation from the expected distribution (λ= 1.08).

In order to evaluate whether any known untyped marker in the associated region could have explained the signal, the 2-Mb window around rs1835740 was imputed using as reference 1000 Genomes (August 2009 release) and the HapMap3 data. However, no other marker showed evidence of association exceeding the rs1835740 one (Figure 3.3).

Table 3.1: **Summary results of the GWAS discovery phase for the chromosome 8q22.1 locus showing genome-wide significant association with migraine.**

				Finnish			Germans			Dutch			Combined		
Chr	SNP	Position	Alleles minor/major	MAF cases/controls	P	OR	MAF cases/controls	P	OR	MAF cases/controls	P	OR	CMH P	OR	BD P
8	rs2436046	98,232,193	C/T	0.21/0.19	0.0445	1.13	0.22/0.19	0.0005	1.25	0.20/0.18	0.0762	1.14	1.78×10^{-5}	1.18	0.48
8	rs2436047	98,235,049	A/G	0.15/0.16	0.3216	0.93	0.20/0.20	0.8699	0.99	0.22/0.21	0.1793	1.10	0.9529	1.00	0.25
8	rs1835740	98,236,089	A/G	0.26/0.22	0.0005	1.22	0.25/0.22	0.0014	1.20	0.26/0.21	0.0009	1.26	5.38×10^{-9}	1.23	0.79
8	rs982502	98,237,879	T/C	0.24/0.23	0.1085	1.10	0.23/0.20	0.0007	1.24	0.21/0.20	0.1172	1.12	1.34×10^{-4}	1.16	0.33
8	rs1155199	98,246,539	G/A	0.36/0.36	0.9068	1.01	0.42/0.38	0.0079	1.15	0.41/0.39	0.1162	1.10	0.0137	1.08	0.18

Position from human NCBI build 36. MAF, minor allele frequency. OR, Odds ratio. CMH , Cochran-Mantel-Haenszel.
BD, Breslow-Day test for heterogeneous odds ratios between strata.

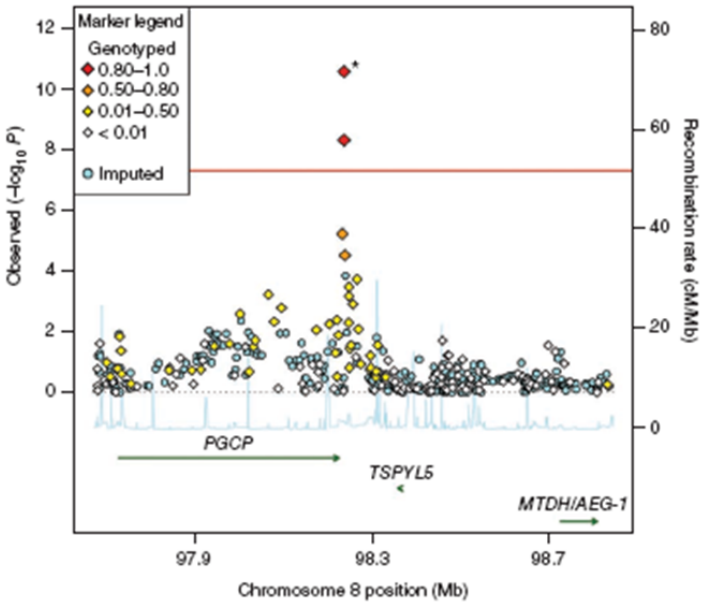

Figure 3.3: **Association signals and recombination rates for the chromosome 8q22.1 locus showing genome-wide significant association with migraine.** The RED line indicates the genome-wide significant threshold ($P = 5{\times}10^{-8}$). Diamonds represent genotyped markers in the region, with colors indicating the extent of linkage disequilibrium (measured in r^2) with the marker rs1835740. Blue circles indicate imputed markers.The signals in red above the threshold for genome-wide significance correspond to rs1835740. For which, P values are shown for both the discovery phase and the meta-analysis of the discovery and replication studies (denoted by asterisk). The blue line shows the recombination rate based on HapMap Phase II data [11] . This figure was generated using the script available at http://www.broadinstitute.org/node/555 modified by Pablo Marin-Garcia and me.

3.3 Conditional and haplotype analysis

According to the HapMap2 recombination maps [11], rs1835740 is located in a region of chromosome 8 between two close recombination hotspots, and an analysis conducted using the ssSNPer program demonstrated that no long range LD to rs1835740 exists within a 5 Mb window [210]. Among the genotyped SNPs in this region, two (rs2436046 and rs982502) showed a P–value $\leq 10^{-3}$ (Table 3.2 and figure 3.4). Based on our data rs2436046 ($r^2 = 0.69$) and rs982502 ($r^2 = 0.59$) are in moderate LD with rs1835740.

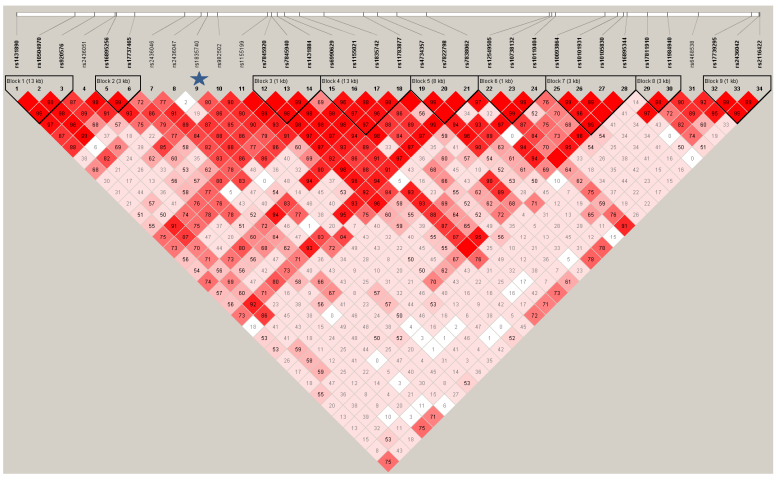

Figure 3.4: **Linkage disequilibrium between pair of SNPs at the chromosome 8q22.1 locus in all subjects included in the discovery phase of the study.** SNPs shown are those which have passed the quality control filters. D′ plot have been produced using Haploview, darker red indicates higher D′ [220]. The most strongly associated SNP (rs1835740) is number 9. The association signal (rs1835740) resides in an intergenic region between two LD blocks (block 2 and 3), defined according to the definition given by Gabriel SB et al. (2002) [221].

To evaluate whether or not the further signals detected in the region were independent from the top SNP association signal, I performed a conditional analysis. The association between SNPs and migraine was tested using the logistic regression with and without conditioning on each one of the SNPs in the associated region with $P \leq 10^{-3}$ (rs2436046, rs1835740 and rs982502). Significant residual association signal was defined as $P \leq 0.05$. Conditioning on rs1835740, no evidence for additional independent signals was found neither for rs2436046 or rs982502 ($P = 0.89$ and $P = 0.47$, respectively) (Table 3.2), suggesting that the moderate association of rs2436046 and rs982502 observed in the CMH test is probably the result of being in LD with rs1835740.

Table 3.2: **Conditional analysis for pair of SNPs.** P-values were calculated using the logistic regression and adjusting for study site.

Chr	SNP A	SNP B	r^2	SNP A P	SNP B P	SNP A given SNP B	SNP B given SNP A
8	rs2436046	rs1835740	0.69	1.78×10^{-5}	5.38×10^{-9}	0.89	1.41×10^{-4}
8	rs982502	rs1835740	0.59	1.34×10^{-4}	5.38×10^{-9}	0.47	4.93×10^{-6}

To determine whether a haplotype-specific effect was present, I inferred haplotypes composed of the five genotyped SNPs in the intergenic region in which rs1835740 resides and tested the association of the inferred haplotypes with migraine before and after conditioning on rs1835740 using the logistic regression. Among the seven haplotypes with frequency higher than 1%, inferred in the region, two showed a modest association signal (haplotype1: $P = 5.72 \times 10^{-5}$, OR=1.18; haplotype7: P = 0.000206, OR = 0.89), which disappeared after conditioning on rs1835740 (haplotype1: P = 0.387, OR = 0.94; haplotype7: P = 0.844, OR = 0.99) (Table 3.3), suggesting that rs1835740 is driving the haplotype association signal.

Table 3.3: **Haplotype frequencies and haplotype-based association test results before and after conditioning for rs1835740**

Haplotype	SNPs					Frequency	OR	P	OR	P
	rs2436046	rs2436047	rs1835740	rs982502	rs1155199				conditioning for rs1835740	
1	C	G	**A**	T	G	0.17	1.18	5.72×10^{-5}	0.94	0.39
2	T	A	G	T	G	0.03	0.88	0.19	0.93	0.45
3	T	A	**A**	C	G	0.04	1.19	0.03	1.01	0.89
4	T	A	G	C	G	0.12	0.98	0.65	1.03	0.64
5	T	G	G	C	G	0.04	0.91	0.27	0.95	0.56
6	C	G	**A**	T	A	0.01	1.24	0.14	1.06	0.72
7	T	G	G	C	A	0.59	0.89	0.0002	0.99	0.84

Marked in bold is the allele more frequently observed in affected individuals compared to controls for the top hit SNP.
Rs2436046, rs2436047, rs1835740, rs982502, rs1155199 are the SNPs forming the haplotypes.
OR, Odds ratio.
P-values were calculated using the logistic regression and adjusting for study site.

3.4 Replication stage

In order to confirm the initial finding, in the replication stage, rs1835740 was analyzed in a further 3202 migraine cases and 40062 population-matched controls from four independent cohorts (Danish, Icelandic, German and Dutch).

In each replication data set the minor allele (A) of rs1835740 was associated with an increased risk of migraine (OR = 1.12–1.23) (Table 3.4). In the overall analysis of the replication stage combined data using the CMH test, the association of rs1853740 was confirmed with a combined P-value of 5.75×10^{-4} (OR = 1.14, 95% confidence interval = 1.06–1.22).

Combining the data of the two stages of the study, convincing evidence for association of rs1835740 with migraine susceptibility was observed, with a combined

P of 1.69×10^{-11} (OR = 1.18, 95% confidence interval = 1.13-1.24). The effect size was similar in the two stages and there was no heterogeneity in the OR estimates among different cohorts in any of the stages (Table 3.4).

3.5 eQTL analysis

Non-coding DNA variants have been shown to modulate gene expression and transcript levels have been correlated to complex traits [222,223]. To evaluate a possible regulatory role of rs1835740 variants on gene expression, Emmanouli Dermitzas' group investigated the effect of this marker genotype on the expression of genes within a 2-Mb window, using a whole-genome gene expression profiling and association analysis with SNPs in three different cell types: fibroblasts, lymphoblastoid cell lines (LCLs) and primary T-cells obtained from the umbilical cord of 75 individuals of Western European origin. These subjects were typed using the Illumina 550K SNP array and transcript abundance for 17945 genes was measured using the Illumina WG-6 v3 expression array [212].

Spearman correlation was used to test for association in cis between SNP genotypes and gene expression levels in each cell type. For each transcript 10000 permutations of expression values relative to SNP genotypes were performed.

This analysis revealed that rs1835740 is an expression quantitative locus (eQTL) for astrocyte elevated gene 1 (*AEG-1*) in LCLs (Table 3.5). At the significance permuted threshold of 0.001, among the 394,651 SNPs tested only rs1835740 was found to be an eQTL for *AEG-1*, with the migraine risk genotype (AA) associated

Table 3.4: **Summary results of rs1835740 in the GWAS discovery and replication phase**

Study group		[a]Cases	[a]Controls	MAF Cases	MAF Controls	OR (95% CI)	P	Heterogeneity P
Discovery phase								
Finnish	Migraine	1064	3513	0.258	0.221	1.22 (1.09–1.37)	0.00045	
Germans	Migraine	1029	2317	0.251	0.216	1.22 (1.08–1.38)	0.00142	
Dutch	Migraine	655	4917	0.255	0.212	1.26 (1.10–1.44)	0.00088	
Combined	Migraine	2748	10747	0.255	0.216	[b]1.23 (1.15–1.32)	[b]5.38×10^{-9}	0.79
	MA only	589	10747	0.267	0.216	[b]1.33 (1.16–1.53)	[b]3.07×10^{-5}	
	MA/MO	2159	10747	0.251	0.216	[b]1.21 (1.12–1.30)	[b]2.69×10^{-6}	
Replication phase								
Danish	Migraine	1116	1353	0.232	0.208	1.15 (0.99–1.34)	0.069	
	MA only	483	1353	0.253	0.208	1.29 (1.05–1.58)	0.015	
	MA/MO	293	1353	0.206	0.208	0.99 (0.79–1.26)	0.951	
	MO only	340	1353	0.225	0.208	1.11 (0.99–1.34)	0.333	
Icelandic	Migraine	900	35,221	0.229	0.202	1.18 (1.04–1.33)	0.010	
	MA only	137	35221	0.255	0.202	1.36 (1.02–1.81)	0.038	
	MA/MO	196	35221	0.209	0.202	1.05 (0.81–1.35)	0.726	
	MO only	567	35221	0.230	0.202	1.18 (1.02–1.38)	0.029	
Dutch	Migraine	349	2082	0.238	0.218	1.12 (0.93–1.35)	0.250	
	MA only	212	2082	0.236	0.218	1.11 (0.87–1.40)	0.406	
	MA/MO	137	2082	0.241	0.218	1.14 (0.85–1.51)	0.382	
Germans	MO	837	1,406	0.240	0.224	1.08 (0.93–1.24)	0.321	
Combined	Migraine	3202	40062	0.234	0.204	[c]1.14 (1.06–1.22)	[c]5.75×10^{-4}	0.82
	MA only	832	38656	0.249	0.203	[c]1.24 (1.08–1.42)	0.002	
	MA/MO	626	38656	0.215	0.203	[c]1.05 (0.90–1.21)	0.533	
	MO only	1744	37980	0.232	0.203	[c]1.12 (1.03–1.23)	0.010	
All combined	Migraine	5950	50809	0.243	0.206	[d]1.18 (1.13–1.24)	[d]1.69×10^{-11}	0.64
	MA only	1421	49403	0.256	0.206	[d]1.29 (1.17–1.41)	[d]6.98×10^{-8}	
	MA/MO	2785	49403	0.243	0.206	[d]1.17 (1.09–1.25)	[d]1.09×10^{-5}	
	MO only	1744	37980	0.232	0.203	[d]1.12 (1.03–1.23)	0.010	

MAF, minor allele frequency. OR, Odds ratio. CMH, Cochran-Mantel-Haenszel.
[a]Number of samples after quality control filtering
[b]OR and P value of CMH test used to combine data sets of the discovery phase
[c]OR and P value of CMH used to combine data sets of the replication phase
[d]OR and P value of the CMH used to combine data sets of the discovery and replication phase

with the highest expression levels (Table 3.5 and figure 3.5).

No correlation between AEG-1 transcripts levels and rs1835740 was found in the other two cell types tested.

These findings suggest that the chromosome 8q22.1 migraine associated SNP is the main genetic determinant of *AEG-1* expression in LCLs. Even if it has been show that peripheral blood can be used for brain-related eQTL mapping further studies will be needed to confirm that rs1835740 is the main *AEG-1* eQTL in the nervous system [224].

None of the other transcripts from the region showed a significant association to rs1835740 in our data set (Table 3.6).

Table 3.5: **SNPs correlated with AEG-1 expression levels.** Correlation between AEG-1 expression and SNP genotypes in lymphoblastoid cell lines (LCLs) has been tested using Sperman rank correlation (SRC). 10000 permutations of AEG-1 expression values relative to SNP genotypes were performed. SNPs for which permuted P-values ≤ 0.01 were obtained are reported. Nominal P-values are shown. Marker rs1835740 is the only significant eQTL detected for AEG-1.

SNP	Gene	SNP position	Gene start	Distance	P
rs11783750	AEG-1	98865219	98725583	-139636	0.00187
rs10105830	AEG-1	98307895	98725583	417688	0.00042
rs1835740	AEG-1	98236089	98725583	489494	0.00004[a]
rs7845920	AEG-1	98247132	98725583	478451	0.00147

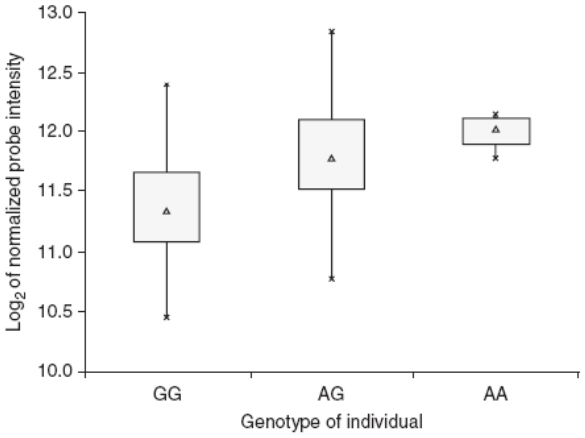

Figure 3.5: **Box-plot of the expression values for AEG1/MTDH based on the rs1835740 genotype** Normalized expression levels of AEG1/MTDH in lymphoblastoid cell lines are shown. In each group, the small pyramid indicates the median, the shaded area represents the lower and upper quartiles, and the crosses show the minimum and maximum values in the expression data

Table 3.6: **Correlation between rs1835740 and gene expression levels.**

SNP	Gene	Strand	SNP position	Gene start	Distance	P
rs1835740	UQCRB	-	98236089	97311911	-924178	0.00132
rs1835740	AEG-1	+	98236089	98725583	489494	0.00004[a]
rs1835740	HRSP12	-	98236089	99183743	947654	0.00287

3.6 Discussion

In the first GWAS for migraine, an associated locus was identified on chromosome 8q22.1. The most significantly associated marker rs1835740 maps to an intergenic region less than 500 kb away from: plasma glutamate carboxypeptidase precursor (*PGCP*), testis-specific Y-encoded-like protein 5 (*TSPY-like protein 5*) and metadherin/astrocyte elevated gene-1 protein (*MTDH/AEG-1*).

Given the available biological data, even though limited, two of these genes (*PGCP* and *MTDH/AEG-1*) seem good candidate genes for migraine. *PGCP* encodes a protein with significant homology to human members of the peptidase family M28, such as glutamate carboxypeptidase II (GCPII) and as other members of the family, it has been shown to have glutamate carboxypeptidase activity. In humans, *PGCP* has been detected principally in blood plasma, where its role has not been identified yet. However, given its observed activity, it has been suggested that PGCP could be involved in the hydrolysis of glutamate from circulating peptides, leading to an increase of plasma glutamate [225]. Several evidences, such as higher concentrations of plasma glutamate in migraine patients compared to controls [226–229] and the induction of migraine through excessive oral intake of glutamate in susceptible individuals [230], suggest a possible role of peripheral glutamate in migraine pathophysiology. These observations make *PGCP* an interesting potential candidate gene for migraine.

MTDH/AEG-1 was originally identified as a HIV-1 and TNF-α inducible gene in primary human foetal astrocytes [231–233]. But for a role in development and progression of different types of cancer, it has been shown that ectopic expression of *MTDH/AEG-1* inhibits excitatory amino acid transporter 2 (*EAAT2/GLT1*) pro-

moter activity [231]. EAATs are trans-membrane proteins involved in the clearance of glutamate from the synaptic cleft. *EAAT2*, expressed by astrocytes, is responsible for most of the glutamate clearance in the brain [234–236]. Several evidences support a role of glutamate in the initiation and propagation of CSD, which is considered to be the most likely pathophysiologic mechanism underlying migraine [82, 86, 237, 238]. MTDH/AEG-1 could increase CSD susceptibility, reducing the glutamate clearance at the synapses via the inhibition of *EAAT2* expression. Using a whole-genome expression profiling assay, we have demonstrated that the migraine associated SNP (rs1835740) is an eQTL for *AEG-1*, with the migraine risk genotype (AA) associated with highest *AEG-1* expression levels. Therefore, it can be hypothesized that the rs1835740 migraine risk variant (A) influences migraine susceptibility increasing *AEG-1* expression and consequently leading to a reduction in *EAAT2* expression, an accumulation of glutamate in the synaptic cleft and finally to an increase in CSD susceptibility. It is worth noting that mutations in the functionally related EAAT1 transporter have been identified in other paroxismal disorders of the nervous system, such as episodic ataxia 6, providing further support for the likely existence of a link between EAAT transporters and episodic disorders of the nervous system [150, 239].

These findings could have important clinical impact, since they support a role of glutamate in migraine pathogenesis and glutamate receptors activity can be inhibited by antagonists. A recent study by Peeters et al. (2007) showed that a glutamate receptor blocker, approved for clinical use, memantine, was able to decrease CSD events induced by potassium chloride in rats [240]. Moreover, pilot clinical studies conducted to evaluate the efficacy of memantine, as preventive therapy in patients with frequent and refractory migraine, obtained promising re-

sults [241,242]. Therefore, memantine could be a potential new agent for migraine preventive treatment.

It is likely that more loci, which confer susceptibility to migraine have to be found. The proportion of genetic variance explained by the rs1835740 variant was estimated to be between 1.5% and 2.5%, depending on the heritability estimate used, and the population attributable risk was estimated to be 10.7% [34]. After exclusion of SNPs at the top associated region, several SNPs showed a $P \leq 10^{-5}$ (Table 3.7). It is likely that some of these will be identified as true associated variants analyzing larger data sets.

In conclusion, in this first GWAS a locus on chromosome 8q22.1 associated with migraine susceptibility was identified and it was shown that the most strongly associated SNP has an allelic-specific effect on *AEG-1* expression. Understanding the mechanisms through which this variant increases migraine susceptibility could improve our knowledge of migraine pathogenesis and could lead to new approaches to treat and prevent the disease. Since the cases enroled in this study were mainly selected from specialized headache clinics, subsequent studies are needed to evaluate the contribution of rs1835740 in population-based migraine cohorts. In these cohorts the migraine spectrum is more heterogeneous and possibly explained by a different combination of genetic susceptibility variants. Even if in the present study, the effect of rs1835740 was found to be larger in individuals with migraine with aura (MA and MA/MO) than in those with migraine without aura (MO), further studies are needed to confirm the role of the variant in different migraine subgroups. Since the identified variant explains only a small fraction of the overall migraine genetic variance, further GWAS, perhaps with different ascertainment schemes, will be needed to identify additional loci.

Table 3.7: **Summary results of the GWAS discovery phase for the regions of the genome showing P-value $\leq 5 \times 10^{-5}$**

Chr	SNP	Position	Alleles minor/major	MAF	P	OR (95% CI)	Location	GENE
1	rs12084862	244269837	A/G	0.27	8.20×10^{-6}	1.17 (1.09–1.25)	intergenic	*SMYD3*
2	rs17528324	118572626	A/G	0.09	4.13×10^{-6}	1.27 (1.15–1.41)	intergenic	*INSIG2*
2	rs17862920	234492734	T/C	0.09	1.26×10^{-5}	0.78 (0.69–0.87)	intergenic	*TRPM8*
6	rs2038761	2625766	A/G	0.31	2.02×10^{-5}	0.87 (0.81–0.93)	intergenic	*MYLK4*
6	rs6456880	29071227	G/T	0.39	2.18×10^{-5}	0.87 (0.82–0.93)	intergenic	*ZNF311*
6	rs7753655	49644523	G/A	0.30	4.29×10^{-6}	0.85 (0.80–0.91)	intergenic	*AL590244.2*
8	rs10888075	13804790	A/C	0.15	1.04×10^{-5}	1.21 (1.11–1.31)	intergenic	*SGCZ*
8	rs10111769	21003036	G/A	0.31	1.49×10^{-5}	1.15 (1.08–1.23)	intergenic	
11	rs2042600	19709275	T/G	0.48	2.28×10^{-5}	0.88 (0.82–0.93)	intergenic	*NAV2*
13	rs3794331	44951545	C/A	0.07	2.7×10^{-5}	1.28 (1.14–1.43)	intergenic	*COG3*
15	rs473422	56453633	A/G	0.35	1.03×10^{-5}	0.86 (0.82–0.92)	intergenic	*AQP9*

For each region the SNP with the strongest signal has been reported. P-values were obtained using the Cochran-Mantel-Haenszel test. Position from human NCBI build 36. MAF, minor allele frequency. OR, Odds ratio. CI, confidence interval.