Chapter 4

Imputation of SNPs to identify susceptibility loci for migraine

4.1 Introduction

To identify common susceptibility variants for migraine, we carried out a GWAS, described in the previous chapter. This study provided evidence of association for a SNP on chromosome 8q22.1 (rs1835740). Expression quantitative trait (eQTL) analysis revealed this SNP to be a key regulator of astrocyte elevated gene 1 *AEG–1* in lymphoblastoid cell lines [243]. A subsequently published population-based GWAS has identified other three risk loci for migraine (chromosome 1p36.23, chromosome 2q37.1, chromosome 12q13.3) [244].

The hundreds of thousands of SNPs directly assayed represent only a fraction of the millions of SNPs contained in the human genome. Genotype imputation is useful to join together datasets genotyped on different platforms and to evaluate association with a phenotype at variants that are not directly genotyped. The

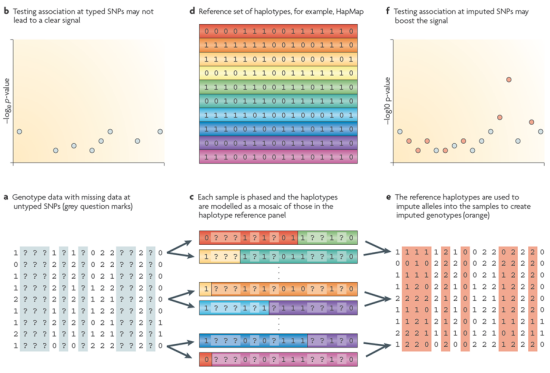

Figure 4.1: **Genotype imputation.** [245]

term imputation means predict genotypes of SNPs, which have not been directly assayed, in a sample using a reference panel of haplotypes including a much larger number of SNPs (Figure 4.1).

Genotype imputation tools involve phasing the typed SNPs in each individual of the study. Then these haplotypes are compared to the haplotypes of the reference panel and missing genotypes are predicted after matching haplotypes with the reference ones. A probability distribution over the possible genotypes is produced for each one of the imputed genotypes [245]. It has been shown that imputation error rate decreases as the minor allele frequency and the size of the reference panel increase [211, 245].

In order to identify novel risk loci for migraine, I have imputed untyped SNPs in migraine cases and population-matched controls from Finland, Germany and the Netherlands, using the 566 haplotypes of 1000 Genomes project (December 2010 release) as reference. The results obtained from the imputed data were replicated in independent migraine case and population-matched controls from Finland, the Netherlands and Spain.

4.2 Results

4.2.1 Initial imputation run

In an initial imputation run, 3279 European individuals affected by migraine with aura (MA) only or by migraine with and without aura (MA/MO) (1124 Finnish, 1276 Germans, and 879 Dutch) and 12369 population-matched controls (Helsinki Birth Cohort study, Health2000 study, KORA study, HNR study, PopGen study, Illumina iControlDB and Rotterdam study I) were included (see Methods).

Study samples had been screened for SNP call rate, presence of population outliers, duplicates and relatedness (see Methods). Overall 2948 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), around 7000000 untyped SNPs were imputed separately in cases and controls of each cohort using the software IMPUTE2 and 1000 Genomes plus HapMap III data as reference [211].

Genotyped and imputed SNPs were tested for association with migraine using a score test, as implemented in SNPTEST v2 [213]. The results of the association tests across the three cohorts (Finnish, German and Dutch) were combined using a fixed effect meta-analysis, as implemented in GWAMA version 2.0.4 [214]. This led to the identification of 62 loci that surpassed the threshold for genome-wide significance ($P = 5 \times 10^{-8}$) (Figure 4.2) [49]. However, quantile-quantile plot of the distribution of the test statistic suggested an overall inflation of P-values (λ= 1.38)(Figure 4.3).

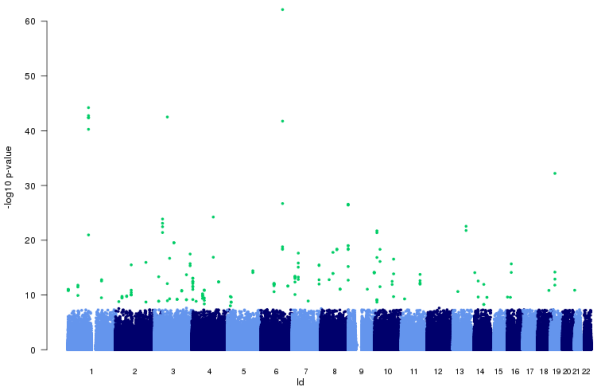

Figure 4.2: **Genome-wide P-values for the initial imputation run.** P-values are log transformed (-log10) (y axis) and plotted against chromosomes (x axis). The signal in green are the ones above the threshold for genome-wide significance.

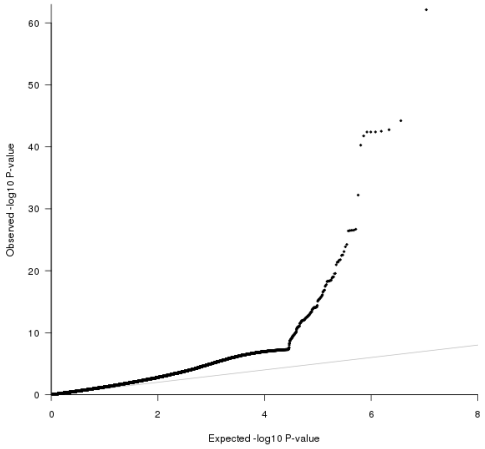

Figure 4.3: **Quantile-quantile plots of the initial imputation run** Plots of the fixed effect meta-analysis results of the initial imputation run.

4.2.2 Discovery stage

Since the number of genome-wide significant loci seemed excessively high, I thought that bias could have been introduced by imputing, in each population, cases and control separately. Therefore, it was decided to repeat the imputation in merged sets of cases and controls for each population. In the meantime a new release of 566 European haplotypes was released by the 1000 Genomes project and, hence, it was decided to use this new set as reference for the new imputation run, since the higher number of reference haplotypes would have improved the imputation accuracy. Moreover, two other migraine data sets became available, including 2490 migraine without aura cases (MO) (1208 German and 1282 Dutch) and 4580 population-matched controls. Therefore, since the two main types of migraine (MA and MO) seem to share a common genetic component, we decided to include them in the discovery stage of our study, to increase the power of detection of migraine risk loci [69].

The discovery stage included 5403 European individuals affected by migraine, of which 2748 were part of our previous GWAS. Diagnoses were made by headache specialists using a combination of questionnaires and individual interviews according to the ICHD-II guidelines [58]. Population-matched controls (15327) were drawn from previously genotyped population-based cohorts previously genotyped (see Methods). Study samples had been screened for SNP call rate, presence of population outliers, duplicates and relatedness (see Methods).

After excluding SNPs which did not pass the quality control filters (see Methods), around 11000000 untyped SNPs were imputed in each cohort using the software IMPUTE2 and 566 European haplotypes from the 1000 Genomes project

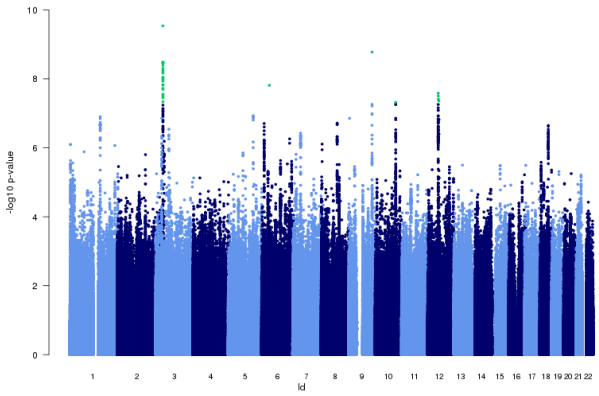

Figure 4.4: **Genome-wide P-values for the discovery phase.** P-values are log transformed (-log10) (y axis) and plotted against chromosomes (x axis). The signal in green are the ones above the threshold for genome-wide significance.

(December 2010 release) as reference [211].

Genotyped and imputed SNPs were tested for association with migraine using a score test, as implemented in SNPTEST v2, to take into account the uncertainty of the imputed genotypes [213]. The results of the association tests across the three cohorts (Finnish, German and Dutch) were combined using a fixed effect meta-analysis, as implemented in GWAMA version 2.0.4 [214].

Six loci that surpassed the threshold for genome-wide significance ($P = 5 \times 10^{-8}$) were identified (Figure 4.4 and Table 4.1) [49]. The genome-wide significant SNPs had the same direction of allelic effect in all the study cohorts. Two were previously identified loci (chromosome 2q37.1 and chromosome 12q13.3) and four were newly identified loci. Quantile-quantile plot of the distribution of the test statistic suggested a modest overall inflation of P-values (λ= 1.09) (Figure 4.5).

Table 4.1: **Summary results of the discovery stage**

				Finnish MA and MA/MO (1064/3513)[a]			Germans MA and MA/MO (1029/2317)[a]			Dutch MA and MA/MO (820/4917)[a]			German MO (1208/2564)[a]			Dutch MO (1282/2016)[a]			Meta-analysis (5403/15327)[a]	
Chr	Position	SNP	Alleles minor/major	MAF cases/controls	OR (95% CI)	P	MAF cases/controls	OR (95% CI)	P	MAF cases/controls	OR (95% CI)	P	MAF cases/controls	OR (95% CI)	P	MAF cases/controls	OR (95% CI)	P	OR (95% CI)	P
1	3319777	rs4471209	A / G	0.36 / 0.34	1.05 (0.95 – 1.16)	3.34×10^{-1}	0.31 / 0.27	1.22 (1.09 – 1.37)	4.10×10^{-4}	0.31 / 0.28	1.13 (1.01 – 1.27)	2.45×10^{-2}	0.30 / 0.27	1.17 (1.05 – 1.30)	2.66×10^{-3}	0.29 / 0.28	1.07 (0.96 – 1.19)	2.16×10^{-1}	1.13 (1.08 – 1.19)	2.93×10^{-6}
1	4185770	rs6679136	T / C	0.33 / 0.34	0.95 (0.86 – 1.06)	3.62×10^{-1}	0.36 / 0.38	0.94 (0.84 – 1.04)	2.05×10^{-1}	0.34 / 0.39	0.79 (0.71 – 0.88)	1.49×10^{-5}	0.34 / 0.38	0.86 (0.78 – 0.95)	1.61×10^{-3}	0.37 / 0.39	0.91 (0.82 – 1.00)	5.27×10^{-2}	0.88 (0.84 – 0.93)	7.98×10^{-7}
1	15532130	rs10927720	T / C	0.20 / 0.19	1.08 (0.95 – 1.22)	2.23×10^{-1}	0.25 / 0.20	1.27 (1.13 – 1.44)	1.27×10^{-4}	0.23 / 0.21	1.15 (1.01 – 1.30)	3.01×10^{-2}	0.24 / 0.21	1.19 (1.06 – 1.34)	2.76×10^{-3}	0.21 / 0.21	1.04 (0.92 – 1.18)	5.01×10^{-1}	1.15 (1.08 – 1.21)	2.75×10^{-6}
1	74118855	rs61767191	C / T	0.06 / 0.04	1.30 (1.05 – 1.62)	8.61×10^{-3}	0.06 / 0.05	1.29 (1.03 – 1.61)	9.11×10^{-3}	0.05 / 0.04	1.16 (0.90 – 1.49)	1.63×10^{-1}	0.07 / 0.05	1.27 (1.04 – 1.55)	4.68×10^{-3}	0.04 / 0.04	1.17 (0.92 – 1.50)	1.43×10^{-1}	1.36 (1.20 – 1.55)	1.31×10^{-6}
1	115677183	rs2078371	C / T	0.15 / 0.13	1.21 (1.06 – 1.39)	5.78×10^{-3}	0.11 / 0.11	0.98 (0.83 – 1.15)	7.66×10^{-1}	0.12 / 0.11	1.12 (0.95 – 1.32)	1.64×10^{-1}	0.12 / 0.11	1.06 (0.91 – 1.23)	4.33×10^{-1}	0.13 / 0.10	1.36 (1.17 – 1.59)	8.60×10^{-5}	1.15 (1.07 – 1.23)	1.54×10^{-4}
1	156456301	rs3790455	C / T	0.43 / 0.42	1.04 (1.15 – 0.94)	4.32×10^{-1}	0.35 / 0.33	1.10 (1.22 – 0.98)	1.01×10^{-1}	0.36 / 0.33	1.11 (1.24 – 1.00)	5.56×10^{-2}	0.38 / 0.33	1.24 (1.37 – 1.12)	3.63×10^{-5}	0.37 / 0.32	1.22 (1.35 – 1.10)	1.72×10^{-4}	1.14 (1.20 – 1.09)	1.26×10^{-7}
1	231361110	rs11590715	T / C	0.07 / 0.06	1.17 (0.97 – 1.42)	7.21×10^{-2}	0.06 / 0.05	1.30 (1.04 – 1.62)	8.55×10^{-3}	0.07 / 0.06	1.21 (0.98 – 1.49)	4.53×10^{-2}	0.07 / 0.05	1.40 (1.15 – 1.71)	1.24×10^{-4}	0.06 / 0.06	1.12 (0.91 – 1.38)	2.26×10^{-1}	1.32 (1.18 – 1.48)	8.58×10^{-7}
2	145222038	rs13403907	G / A	0.18 / 0.15	1.16 (1.02 – 1.32)	1.65×10^{-2}	0.13 / 0.10	1.28 (1.09 – 1.51)	1.04×10^{-3}	0.13 / 0.11	1.17 (1.00 – 1.37)	3.35×10^{-2}	0.12 / 0.11	1.15 (0.99 – 1.34)	4.43×10^{-2}	0.13 / 0.11	1.11 (0.95 – 1.29)	1.59×10^{-1}	1.20 (1.12 – 1.30)	1.58×10^{-6}
2	234821445	rs11892538	C / G	0.11 / 0.14	0.81 (0.70 – 0.94)	3.30×10^{-3}	0.14 / 0.17	0.81 (0.70 – 0.93)	2.86×10^{-3}	0.15 / 0.17	0.85 (0.73 – 0.98)	2.07×10^{-2}	0.14 / 0.17	0.82 (0.71 – 0.94)	2.43×10^{-3}	0.14 / 0.17	0.80 (0.70 – 0.92)	9.92×10^{-4}	0.81 (0.76 – 0.86)	2.92×10^{-10}
2	241447428	rs4676436	A / C	0.14 / 0.13	1.10 (0.96 – 1.27)	1.71×10^{-1}	0.13 / 0.11	1.24 (1.06 – 1.45)	7.48×10^{-3}	0.12 / 0.11	1.18 (1.00 – 1.38)	4.59×10^{-2}	0.13 / 0.11	1.20 (1.04 – 1.40)	1.42×10^{-2}	0.14 / 0.11	1.27 (1.09 – 1.48)	1.62×10^{-3}	1.20 (1.12 – 1.29)	6.46×10^{-7}
3	30480085	rs6790925	T / C	0.41 / 0.39	1.09 (0.99 – 1.21)	7.48×10^{-2}	0.37 / 0.35	1.11 (0.99 – 1.23)	6.20×10^{-2}	0.40 / 0.38	1.11 (1.00 – 1.24)	5.13×10^{-2}	0.38 / 0.35	1.16 (1.05 – 1.29)	2.88×10^{-3}	0.41 / 0.36	1.20 (1.09 – 1.33)	3.36×10^{-4}	1.14 (1.08 – 1.19)	1.39×10^{-7}
3	67144706	rs4311165	C / G	0.17 / 0.20	0.81 (0.71 – 0.92)	1.35×10^{-3}	0.24 / 0.27	0.87 (0.77 – 0.98)	2.04×10^{-2}	0.25 / 0.26	0.93 (0.82 – 1.05)	2.30×10^{-1}	0.24 / 0.27	0.87 (0.77 – 0.97)	1.01×10^{-2}	0.25 / 0.27	0.87 (0.78 – 0.97)	1.28×10^{-2}	0.87 (0.82 – 0.92)	2.89×10^{-7}
5	75495849	rs6453209	T / A	0.46 / 0.50	0.85 (0.93 – 0.77)	8.84×10^{-4}	0.45 / 0.47	0.91 (1.01 – 0.82)	6.49×10^{-2}	0.47 / 0.48	0.96 (1.06 – 0.86)	3.94×10^{-1}	0.48 / 0.49	0.93 (1.03 – 0.85)	1.30×10^{-1}	0.43 / 0.47	0.85 (0.93 – 0.76)	8.61×10^{-4}	0.89 (0.93 – 0.85)	1.41×10^{-6}
5	127722107	rs77050147	C / G	0.10 / 0.09	1.24 (1.05 – 1.45)	1.11×10^{-2}	0.10 / 0.08	1.27 (1.06 – 1.52)	8.43×10^{-3}	0.12 / 0.10	1.24 (1.05 – 1.46)	9.28×10^{-3}	0.09 / 0.08	1.16 (0.98 – 1.39)	8.78×10^{-2}	0.12 / 0.09	1.25 (1.07 – 1.47)	5.37×10^{-3}	1.25 (1.15 – 1.35)	1.18×10^{-7}
6	12908747	rs13197912	T / A	0.30 / 0.29	1.06 (0.95 – 1.17)	3.09×10^{-1}	0.37 / 0.36	1.08 (0.97 – 1.21)	1.44×10^{-1}	0.37 / 0.35	1.09 (0.97 – 1.21)	1.32×10^{-1}	0.40 / 0.35	1.23 (1.12 – 1.36)	3.73×10^{-5}	0.38 / 0.33	1.21 (1.09 – 1.34)	2.42×10^{-4}	1.14 (1.09 – 1.20)	1.98×10^{-7}
6	39177971	rs873690	C / G	0.09 / 0.07	1.26 (1.05 – 1.50)	3.25×10^{-3}	0.09 / 0.08	1.19 (0.99 – 1.44)	1.50×10^{-2}	0.08 / 0.07	1.10 (0.90 – 1.34)	2.18×10^{-1}	0.09 / 0.08	1.20 (1.01 – 1.42)	1.01×10^{-2}	0.09 / 0.07	1.30 (1.09 – 1.56)	1.81×10^{-4}	1.37 (1.23 – 1.52)	1.54×10^{-8}
6	97039665	rs2971609	G / A	0.20 / 0.18	1.15 (1.02 – 1.30)	2.44×10^{-2}	0.26 / 0.24	1.11 (0.98 – 1.25)	8.59×10^{-2}	0.23 / 0.23	1.01 (0.89 – 1.14)	9.24×10^{-1}	0.27 / 0.24	1.19 (1.07 – 1.33)	1.51×10^{-3}	0.27 / 0.23	1.22 (1.08 – 1.36)	7.17×10^{-4}	1.14 (1.08 – 1.21)	2.34×10^{-6}
6	143288832	rs1041655	A / C	0.41 / 0.42	0.93 (0.84 – 1.02)	1.29×10^{-1}	0.37 / 0.41	0.88 (0.79 – 0.98)	1.58×10^{-2}	0.35 / 0.38	0.87 (0.78 – 0.98)	1.79×10^{-2}	0.37 / 0.41	0.86 (0.78 – 0.95)	1.35×10^{-3}	0.36 / 0.39	0.90 (0.81 – 1.00)	3.04×10^{-2}	0.88 (0.84 – 0.93)	5.51×10^{-7}
6	153997563	rs6937294	C / T	0.43 / 0.45	1.08 (1.19 – 0.98)	1.11×10^{-1}	0.40 / 0.43	1.12 (1.24 – 1.01)	3.11×10^{-2}	0.38 / 0.42	1.20 (1.34 – 1.08)	5.28×10^{-4}	0.41 / 0.43	1.07 (1.18 – 0.97)	1.85×10^{-1}	0.37 / 0.40	1.14 (1.26 – 1.03)	9.10×10^{-3}	1.12 (1.18 – 1.07)	2.46×10^{-6}
7	17014115	rs17136988	G / T	0.05 / 0.06	0.84 (0.68 – 1.04)	1.03×10^{-1}	0.08 / 0.10	0.75 (0.62 – 0.91)	1.48×10^{-3}	0.07 / 0.08	0.83 (0.67 – 1.01)	4.84×10^{-2}	0.09 / 0.10	0.87 (0.73 – 1.03)	8.08×10^{-2}	0.07 / 0.09	0.80 (0.66 – 0.96)	8.66×10^{-3}	0.80 (0.73 – 0.88)	1.55×10^{-6}
7	40466200	rs4379368	T / C	0.16 / 0.14	1.17 (1.02 – 1.33)	2.14×10^{-2}	0.12 / 0.11	1.15 (0.97 – 1.35)	9.87×10^{-2}	0.12 / 0.11	1.17 (1.00 – 1.38)	5.24×10^{-2}	0.13 / 0.10	1.29 (1.11 – 1.50)	7.58×10^{-4}	0.13 / 0.11	1.20 (1.03 – 1.40)	1.58×10^{-2}	1.20 (1.12 – 1.29)	3.76×10^{-7}
8	4391037	rs17070498	C / T	0.10 / 0.11	0.90 (0.77 – 1.06)	2.01×10^{-1}	0.12 / 0.15	0.79 (0.67 – 0.92)	1.79×10^{-3}	0.13 / 0.15	0.86 (0.74 – 1.01)	5.99×10^{-2}	0.13 / 0.16	0.84 (0.73 – 0.97)	1.59×10^{-2}	0.12 / 0.14	0.82 (0.71 – 0.95)	6.92×10^{-3}	0.84 (0.79 – 0.90)	7.83×10^{-7}
8	81379656	rs368280	A / C	0.19 / 0.23	0.81 (0.72 – 0.92)	8.82×10^{-4}	0.25 / 0.28	0.85 (0.75 – 0.95)	4.89×10^{-3}	0.27 / 0.27	0.96 (0.86 – 1.09)	5.40×10^{-1}	0.25 / 0.28	0.86 (0.77 – 0.96)	5.80×10^{-3}	0.26 / 0.29	0.87 (0.78 – 0.98)	1.68×10^{-2}	0.87 (0.82 – 0.92)	1.93×10^{-7}
9	3943997	rs7024842	T / C	0.26 / 0.24	1.12 (1.01 – 1.26)	3.47×10^{-2}	0.34 / 0.30	1.18 (1.06 – 1.32)	3.02×10^{-3}	0.36 / 0.32	1.18 (1.06 – 1.32)	2.35×10^{-3}	0.33 / 0.30	1.15 (1.04 – 1.27)	9.03×10^{-3}	0.35 / 0.33	1.08 (0.98 – 1.20)	1.26×10^{-1}	1.15 (1.09 – 1.21)	1.40×10^{-7}
9	119252629	rs6478241	A / G	0.39 / 0.37	1.11 (1.22 – 1.00)	4.96×10^{-2}	0.39 / 0.36	1.12 (1.25 – 1.01)	3.55×10^{-2}	0.40 / 0.37	1.14 (1.26 – 1.02)	1.84×10^{-2}	0.42 / 0.36	1.26 (1.39 – 1.14)	5.45×10^{-6}	0.40 / 0.37	1.16 (1.29 – 1.05)	4.11×10^{-3}	1.16 (1.21 – 1.10)	1.68×10^{-9}
10	105039048	rs1163084	T / C	0.45 / 0.47	0.92 (1.02 – 0.84)	1.02×10^{-1}	0.50 / 0.48	0.92 (1.02 – 0.83)	9.48×10^{-2}	0.50 / 0.47	0.89 (0.99 – 0.80)	2.55×10^{-2}	0.51 / 0.47	0.87 (0.96 – 0.79)	3.99×10^{-3}	0.51 / 0.46	0.81 (0.89 – 0.73)	2.93×10^{-5}	0.88 (0.92 – 0.84)	4.79×10^{-8}
12	53831472	rs12820532	A / G	0.15 / 0.14	1.12 (0.98 – 1.29)	7.90×10^{-2}	0.17 / 0.15	1.14 (0.99 – 1.32)	4.16×10^{-2}	0.19 / 0.17	1.16 (1.01 – 1.32)	2.26×10^{-2}	0.18 / 0.14	1.28 (1.13 – 1.46)	6.42×10^{-5}	0.19 / 0.17	1.18 (1.04 – 1.35)	6.73×10^{-3}	1.21 (1.13 – 1.29)	2.61×10^{-8}
12	57527283	rs11172113	C / T	0.37 / 0.40	0.90 (0.82 – 1.00)	4.43×10^{-2}	0.39 / 0.41	0.92 (0.83 – 1.02)	1.20×10^{-1}	0.38 / 0.42	0.85 (0.76 – 0.94)	2.18×10^{-3}	0.36 / 0.40	0.86 (0.77 – 0.95)	1.90×10^{-3}	0.38 / 0.41	0.86 (0.77 – 0.95)	2.68×10^{-3}	0.88 (0.83 – 0.92)	4.38×10^{-8}
18	6767965	rs4798494	T / C	0.33 / 0.36	1.13 (1.25 – 1.02)	1.95×10^{-2}	0.34 / 0.37	1.14 (1.27 – 1.02)	2.21×10^{-2}	0.32 / 0.34	1.07 (1.20 – 0.96)	2.32×10^{-1}	0.33 / 0.36	1.12 (1.24 – 1.01)	2.82×10^{-2}	0.32 / 0.35	1.16 (1.29 – 1.04)	5.52×10^{-3}	1.12 (1.18 – 1.07)	2.64×10^{-6}
18	43706491	rs28532950	T / C	0.40 / 0.37	1.13 (1.02 – 1.25)	1.43×10^{-2}	0.45 / 0.41	1.15 (1.03 – 1.27)	9.51×10^{-3}	0.46 / 0.43	1.14 (1.02 – 1.26)	1.64×10^{-2}	0.46 / 0.42	1.17 (1.06 – 1.29)	1.20×10^{-3}	0.44 / 0.43	1.06 (0.96 – 1.18)	2.14×10^{-1}	1.13 (1.08 – 1.19)	2.26×10^{-7}

[a](cases/controls)

Position from human NCBI build 37. MAF, minor allele frequency. OR, odds ratio for the minor allele. CI, confidence interval.

MA, migraine with aura. MA/MO, migraine with and without aura. MO, migraine without aura.

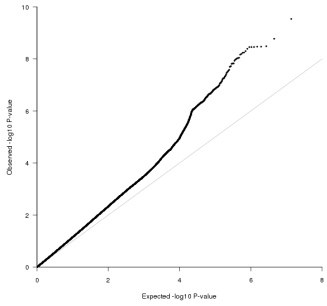

Figure 4.5: **Quantile-quantile plots of the GWAS discovery phase.** Plots of the fixed effect meta-analysis results in the MA discovery phase.

4.3 Replication stage

In the replication stage, SNPs from the top twenty nine loci were genotyped in 3268 migraine case and 2916 control European samples (Finland, The Netherlands and Spain) Of these six had at least one SNP that surpassed the threshold for genome-wide significance ($P = 5 \times 10^{-8}$) and 23 had at least one SNP with a P value lower than 5×10^{-6}.

Among the seventeen SNPs successfully genotyped, three reached the Bonferroni corrected replication threshold ($P \leq 2.94 \times 10^{-3}$): rs11892538 (OR=0.77 , 95% CI = 0.69 – 0.84, $P = 2.74 \times 10^{-5}$) rs4379368 (OR= 1.21, 95% CI = 1.08 – 1.35, $P = 8.68 \times 10^{-4}$) and rs11172113 (OR=0.86 , 95% CI = 0.79 – 0.92, $P = 3.66 \times 10^{-5}$). The effect estimate for rs11892538, rs4379368 and rs11172113 were concordant in direction among all replication cohorts with the discovery cohorts (Table 4.2). All the three SNPs reached genome-wide significance ($P \leq 5 \times 10^{-8}$) in a meta-analysis combining all cohorts (discovery and replication cohorts) (Table 4.3).

Table 4.2: **Summary results of the replication stage**

				Finnish (875/1025)[a]			Dutch (1043/910)[a]			Spanish (1350/981)[a]			Meta-analysis (3268/2916)[a]	
Chr	Position	SNP	Alleles minor/major	MAF cases/controls	OR(95% CI)	P	MAF cases/controls	OR(95% CI)	P	MAF cases/controls	OR(95% CI)	P	OR(95% CI)	P
1	3319777	rs4471209	A / G	0.32 / 0.35	0.88	6.92×10^{-2}	0.29 / 0.28	1.03	0.64	0.30 / 0.29	1.03	0.62	0.98	0.63
1	15532130	rs10927720	T / C	0.20 / 0.20	0.99	0.94	0.23 / 0.22	1.03	0.68	0.22 / 0.23	0.95	0.46	0.99	0.79
2	145222038	rs13403907	G / A	0.17 / 0.16	1.03	0.76	0.13 / 0.13	0.98	0.82	0.13 / 0.12	1.10	0.30	1.04	0.50
2	234821445	rs11892538	C / G	0.13 / 0.16	0.77	5.85×10^{-3}	0.17 / 0.21	0.80	5.20×10^{-3}	0.16 / 0.21	0.74	6.35×10^{-5}	0.77	2.74×10^{-5}
2	241447428	rs4676436	A / C	0.14 / 0.14	1.00	0.99	0.12 / 0.12	0.99	0.94	0.10 / 0.10	1.10	0.35	1.03	0.62
3	67144706	rs4311165	C / G	0.19 / 0.20	0.91	0.24	0.26 / 0.27	0.95	0.48	0.28 / 0.30	0.92	0.18	0.93	0.06
5	127722107	rs77050147	C / G	0.09 / 0.09	1.04	0.72	0.10 / 0.11	0.98	0.86	0.11 / 0.09	1.26	2.48×10^{-2}	1.09	0.14
6	12908747	rs13197912	T / A	0.30 / 0.29	1.02	0.80	0.37 / 0.38	0.97	0.60	0.40 / 0.37	1.13	4.63×10^{-2}	1.04	0.28
6	39177971	rs873690	C / G	0.08 / 0.07	1.03	0.79	0.06 / 0.05	1.05	0.72	0.05 / 0.05	0.95	0.70	1.01	0.89
6	143288832	rs1041655	A / C	0.42 / 0.43	0.97	0.60	0.38 / 0.37	1.07	0.32	0.39 / 0.38	1.05	0.43	1.03	0.46
7	40466200	rs4379368	T / C	0.17 / 0.13	1.31	2.75×10^{-3}	0.13 / 0.11	1.20	0.07	0.09 / 0.09	1.09	0.39	1.21	8.68×10^{-4}
8	4391037	rs17070498	C / T	0.10 / 0.11	0.91	0.38	0.15 / 0.13	1.19	0.06	0.16 / 0.16	1.00	0.96	1.03	0.55
8	81379656	rs368280	A / C	0.20 / 0.20	0.97	0.69	0.28 / 0.25	1.14	0.07	0.35 / 0.36	0.93	0.26	1.00	0.94
9	119252629	rs6478241	A / G	0.41 / 0.36	1.20	6.36×10^{-3}	0.39 / 0.38	1.03	0.69	0.45 / 0.44	1.05	0.38	1.09	2.32×10^{-2}
10	105039048	rs1163084	T / C	0.49 / 0.48	1.02	0.79	0.50 / 0.47	1.12	0.08	0.50 / 0.50	0.99	0.83	0.97	0.33
12	57527283	rs11172113	C / T	0.38 / 0.39	0.97	0.63	0.37 / 0.43	0.77	8×10^{-5}	0.32 / 0.36	0.85	7.61×10^{-3}	0.86	3.66×10^{-5}
18	43706491	rs28532950	T / C	0.38 / 0.38	1.01	0.87	0.45 / 0.42	1.16	2.20×10^{-2}	0.43 / 0.43	0.97	0.61	1.04	0.28

[a](cases/controls)

Position from human NCBI build 37. MAF, minor allele frequency. OR, odds ratio for the minor allele. CI, confidence interval.

MA, migraine with aura. MA/MO, migraine with aura and without aura. MO, migraine without aura.

Table 4.3: **Summary results of the discovery and replication stages**

				Discovery stage (5403/1537)[a]		Replication stage (3268/2916)[a]		Discovery and replication stages (8671/18243)[a]		
Chr	Position	SNP	Alleles minor/major	Meta-analysis OR (95% CI)	P	Meta-analysis OR (95% CI)	P	Meta-analysis OR (95% CI)	P	Gene
2	234821445	rs11892538	C / G	0.81 (0.76 – 0.86)	2.92×10^{-10}	0.77 (0.69 – 0.84)	2.74×10^{-5}	0.80 (0.76 – 0.84)	3.67×10^{-17}	*TRPM8*
7	40466200	rs4379368	T / C	1.20 (1.12 – 1.29)	3.76×10^{-7}	1.21 (1.08 – 1.35)	8.68×10^{-4}	1.20 (1.13 – 1.28)	1.36×10^{-9}	*C7orf10*
12	57527283	rs11172113	C / T	0.88 (0.83 – 0.92)	4.38×10^{-8}	0.86 (0.79 – 0.92)	3.66×10^{-5}	0.87 (0.84 – 0.91)	5.06×10^{-10}	*LRP1*

[a](cases/controls)
Position from human NCBI build 37.
MAF, minor allele frequency.
OR, odds ratio for the minor allele.
CI, confidence interval.

4.3.1 Discussion

In the first GWAS of imputed SNPs for migraine three loci associated with migraine were identified: one on chromosome 2q37.1 (rs11892538), one on chromosome 7p14.1 (rs4379368) and one on chromosome 12q13.3 (rs11172113) (Figure 4.6, 4.7 and 4.8). Two of the three loci (chromosome 2q37.1 and chromosome 12q13.3) had been already identified as associated with migraine in a previous study [244].

On chromosome 2q37.1, the most significantly associated marker rs11892538 maps to an intergenic region less than 5 kb away from the transient receptor potential cation channel 8 gene *TRPM8*. The second closest gene, encoding for Holliday junction recognition protein *HJURP*, maps 58.2 kb away from rs11892538. Given the current knowledge, *TRPM8* could be involved in migraine pathogenesis. *TRPM8* is a cold and menthol modulated ion channel with a role in the detection of cold in the mammals [246]. *TRPM8* is expressed in subpopulations of sensory

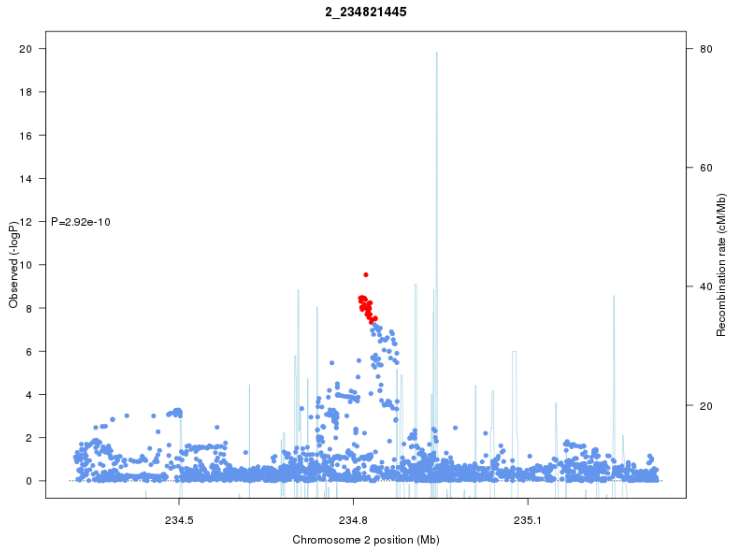

Figure 4.6: **Locus specific association plot: chromosome 2q37.1** The region +/- 500 kb around the most strongly associated SNP is shown. The diamond represents the most strongly associated SNP. P values are shown for the discovery stage. The blue line shows the recombination rate based on HapMap Phase II data. SNP and gene position are based on built 37.

neurons [247]. There is evidence suggesting that *TRPM8* may play a role in inflammatory and neuropathic pain [248]. Given that the migraine headache has some features in common with inflammatory and neuropathic pain, it is possible that *TRPM8* may play a role in its pathogenesis [249]. There is evidence suggesting that the in vivo antagonism of TRPM8 constitutes a possible strategy for treating neuropathic pain [250].

On chromosome 12q13.3, the most significantly associated marker rs11172113 maps to the first intron of low density lipoprotein receptor-related protein gene *LRP1*. *LRP1* is a cell surface receptor member of the low-density lipoprotein (LDL)-receptor family [251,252]. It is expressed in the vasculature, central nervous system, macrophages and adipocytes [253]. *LRP1* seems to play a role in various biological processes including lipoprotein metabolism [253]. Boucher et al. (2003)

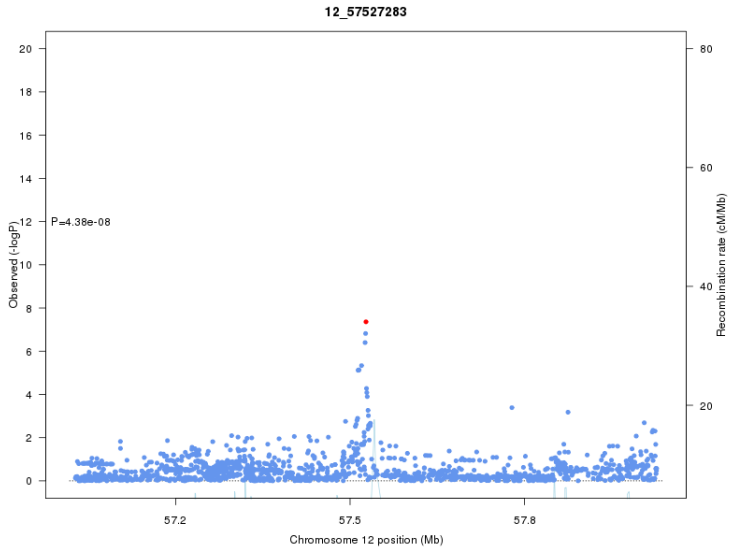

Figure 4.7: **Locus specific association plot: chromosome 12q13.3** The region +/- 500 kb around the most strongly associated SNP is shown. The diamond represents the most strongly associated SNP. P values are shown for the discovery stage. The blue line shows the recombination rate based on HapMap Phase II data. SNP and gene position are based on built 37.

have shown that inactivation of *LRP1* in vascular smooth muscle cells of mice leads to marked susceptibility to cholesterol-induced atherosclerosis [254]. Liu et al. (2010), performing neuronal lrp1 knockout in mice, have shown that the levels of glutamate receptors are reduced in lrp1 knockout neurons and that they are partially rescued by restoring neuronal cholesterol [255]. Glutamate is the main excitatory neurotransmitter in the central nervous system. Data from animal and human studies support a role of glutamate in the pathophysiology of migraine [256]. The second closest gene in the region, signal transducer and activator of transcription 6 (*STAT6*), maps 23.1 kb away from rs11172113. *STAT6* is a member of the STAT family of transcription factors, which plays a role in differentiation and function of T helper 2 (Th2) cells [257].

On chromosome 7p14.1, the most significantly associated marker rs4379368

maps to the dermal papilla derived protein 13 gene *C7orf10*. *C7orf10* is a peroxisomal glutaryl-CoA oxidase [258]. Mutations in this gene have been associated with glutaric aciduria type III, characterized by abnormal amounts of urinary glutaric acid [258]. Bennett et al. (1991) described a lack of peroxisomal glutaryl-CoA oxidase activity in a 1-year-old girl with failure to thrive and hematologic evidence of thalassemia. Sherman et al. (2008) reported three children homozygous for a nonsynonymous variant in C7orf10, who excreted large quantities of glutarate in the urine and remained healthy during a 15 years follow-up period [259]. The second closest gene to rs4379368 , encoding for cell division cycle 2-like 5 *CDC2L5*, maps 331 kb away. This gene encodes for a member of the cyclin-dependent serine/threonine protein kinase family. Members of cyclin-dependent serine/threonine protein kinase family have an important role in cell cycle control. The exact function of the protein encoded by *CDC2L5* has not been defined yet, but it has been suggested that it may have a role in mRNA splicing regulation [260].

In conclusion, in this first GWAS of imputed SNPs for migraine, three loci (one on chromosome 2q37.1, one on chromosome 7p14.1 and one on chromosome 12q13.3), associated with migraine were identified. Two of these three loci (one on chromosome 2q37.1 and chromosome 12q13.3) had already been found associated with migraine in a recent population based study [244]. The most significantly associated marker on chromosome 2q37.1 (rs11892538), maps 5 kb away from the *TRPM8* gene, which encodes for a ion channel with a role in pain pathogenesis [248]. On chromosome 12q13.3, the most significantly associated marker (rs11172113) maps to the first intron of the *LRP1* gene. *LRP1* has been shown to modulate the neural glutamate receptor levels, and therefore, the association of

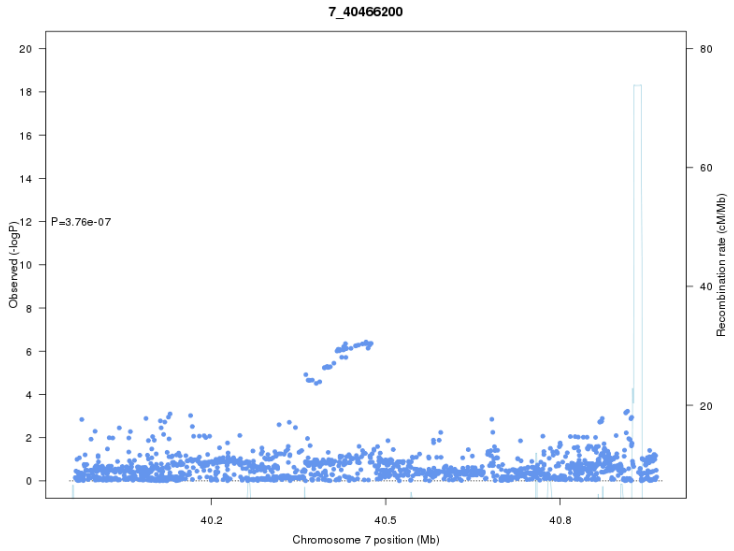

Figure 4.8: **Locus specific association plot: chromosome 7p14.1** The region +/- 500 kb around the most strongly associated SNP is shown. The diamond represents the most strongly associated SNP. P values are shown for the discovery stage. The blue line shows the recombination rate based on HapMap Phase II data. SNP and gene position are based on built 37.

LRP1 with migraine provides further support to the role of glutamate in migraine pathogenesis [255]. The functional role of the new third locus on chromosome 7p14.1 is not currently definable. Future functional studies on the role of genes present in the locus (*C7orf10* and *CDC2L5*) will be provide an understanding of its functional link with migraine. Larger GWAS, currently underway, will allow the identification of further genetic variants underlying the pathophysiology of migraine.