Supplementary Material

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

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Contents

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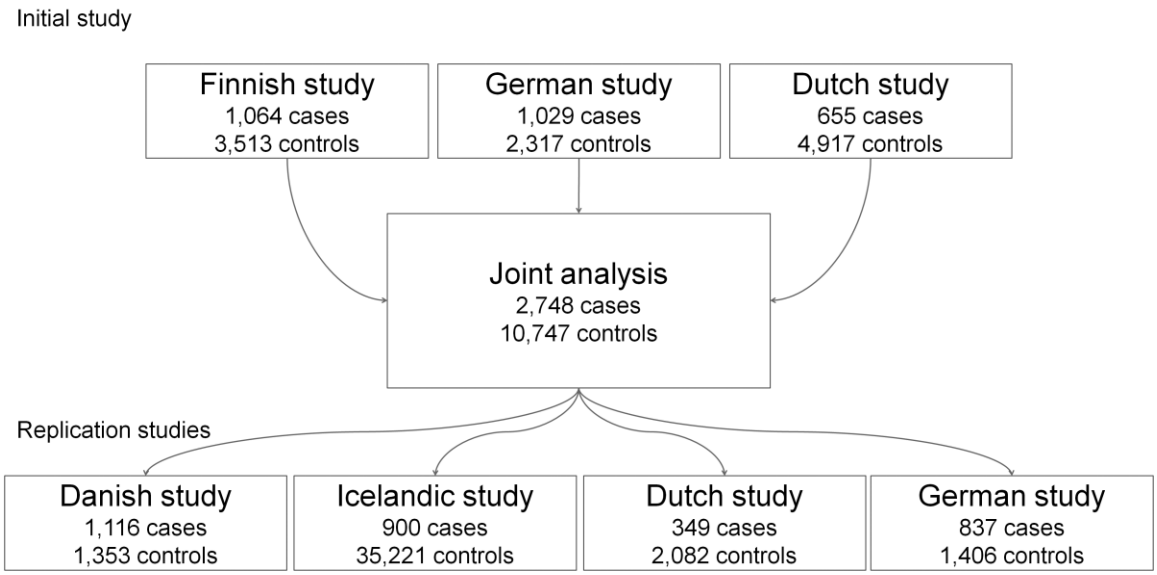
Supplementary Figures and Figure Legends

Supplementary Figure 1. Study design

In the initial study, migraine with aura (MA) patients from three clinic-based collections were analyzed in a joint genome-wide association analysis. The most significant association signal was replicated in an independent Danish clinic-based sample and an Icelandic population-based sample, containing MA and migraine without aura (MO) samples, as well as in a German clinic-based MO-specific sample.



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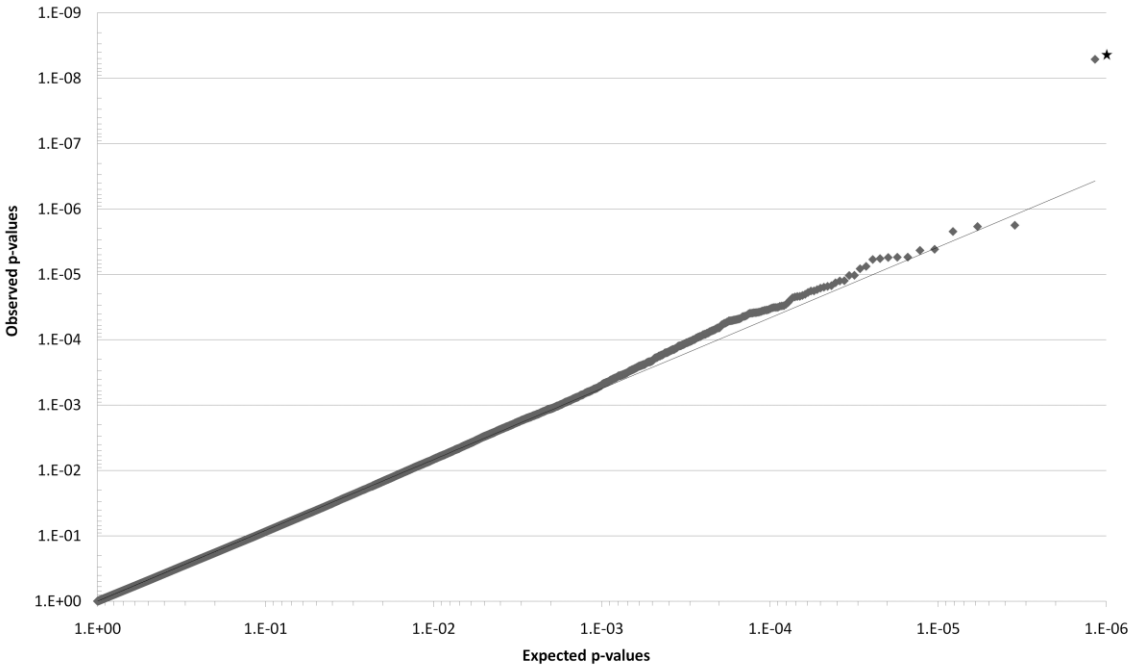
Supplementary Figure 2. Quantile-quantile plot of the results in the Cochran-Mantel-Haenszel analysis

Asterisk denotes marker rs1835740. Black line represents the distribution of p-values under the null given study inflation factor lambda of 1.08.



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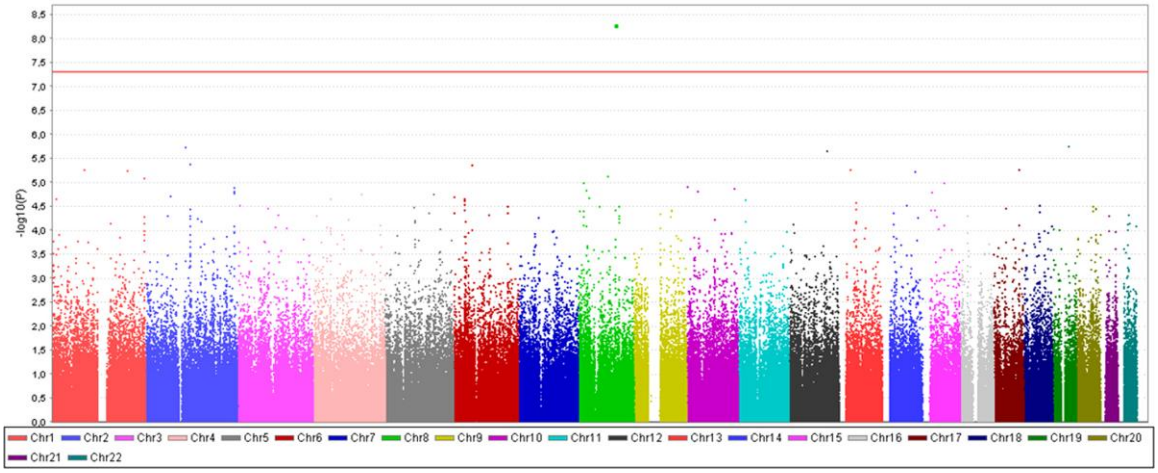
Supplementary Figure 3. Genome-wide Cochran-Mantel-Haenszel results for association between each marker and migraine with aura in the combined analysis of the three initial study populations

Red line denotes the threshold of genome-wide significance ($p \leq 5 \times 10^{-8}$). Only marker rs1835740 on 8q22.1 exceeded this threshold.



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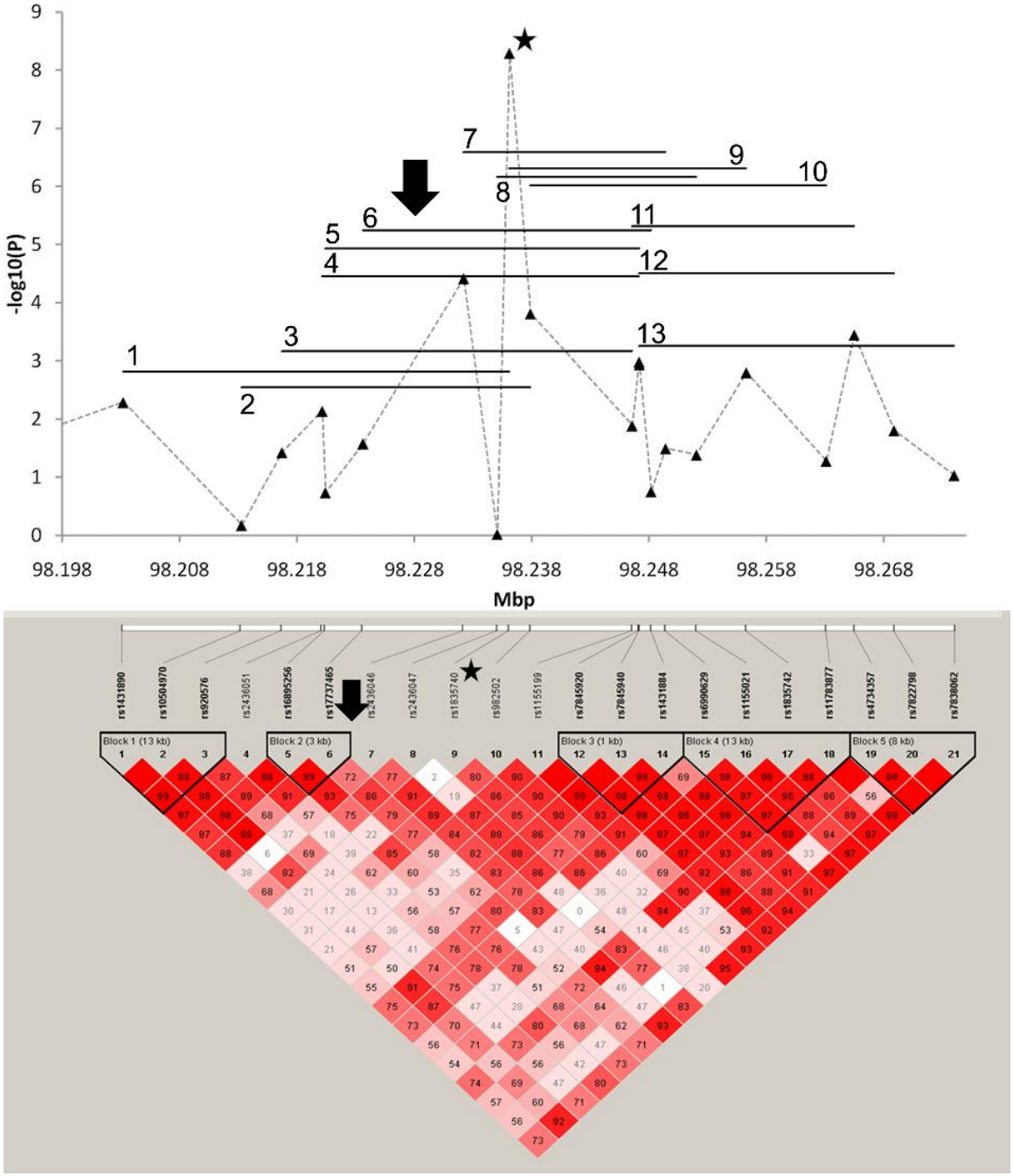
Supplementary Figure 4. Nine SNP sliding window haplotype analysis and local haplotype structure around marker rs1835740 on chromosome 8q22.1

In the upper part of the figure, the black pyramids show single-marker association results for each marker. The horizontal lines show the length and overall p-values for the nine marker sliding windows in the haplotype analysis. The lower part of the figure shows the Haploview D' matrix in the GWA study analysis data, with estimated LD blocks using the Gabriel *et al.* method[1]. Black stars denote the location of rs1835740 and the black arrows denote the 3' end of *PGCP* in either part of the figure.



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Supplementary Tables

Supplementary Table 1. Association signals with $p \leq 5 \times 10^{-5}$ and with multiple nearby associating SNPs

SNP	Chr	Location	p-value	OR	95% CI	Location	Gene
rs12084862	1	244269837	8.20E-06	1.17	1.09-1.25	intragenic	*SMYD3*
rs17528324	2	118572626	4.13E-06	1.27	1.15-1.41	intragenic	*INSIG2*
rs17862920	2	234492734	1.26E-05	0.776	0.693-0.870	intragenic	*TRPM8*
rs2038761	6	2625766	2.02E-05	0.865	0.809-0.925	intragenic	*MYLK4*
rs6456880	6	29071227	2.18E-05	0.873	0.819-0.929	intragenic	*ZNF311*
rs7753655	6	49644523	4.29E-06	0.852	0.796-0.912	intergenic	-
rs10888075	8	13804790	1.04E-05	1.21	1.11-1.31	intergenic	near *SGCZ*
rs10111769	8	21003036	1.49E-05	1.15	1.08-1.23	intergenic	-
rs2042600	11	19709275	2.28E-05	0.876	0.824-0.932	intragenic	*NAV2*
rs3794331	13	44951545	2.70E-05	1.28	1.14-1.43	intragenic	*COG3*
rs473422	15	56453633	1.03E-05	0.864	0.820-0.922	intergenic	near *AQP9*

Footnote: Locations and distances in basepairs, according to NCBI build 36. Only the SNP with the lowest p-value is reported for each locus.

Supplementary Table 2. Conditional analyses for the two SNPs with moderate linkage disequilibrium to rs1835740 in chromosome 8q22.1

Chr	SNP A	SNP B	r^2	SNP A p-value	SNP B p-value	SNP B given A
8	rs1835740	rs2436046	0.69	5.12×10^{-9}	1.78×10^{-5}	0.892
8	rs1835740	rs982502	0.59	5.12×10^{-9}	1.34×10^{-4}	0.466

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Supplementary Table 3. Nine SNP sliding window haplotype analysis on the chromosome 8q22.1 associated region from Supplementary Figure 2

Haplotype	First SNP	Last SNP	Chi-sq.	D.f.	Overall p-value
1	rs1431890	rs1835740	43.07	16	2.730E-04
2	rs10504970	rs982502	43.10	17	4.643E-04
3	rs920576	rs1155199	41.37	13	8.291E-05
4	rs2436051	rs7845920	48.48	14	1.093E-05
5	rs16895256	rs7845940	47.68	12	3.553E-06
6	rs17737465	rs1431884	46.52	10	1.156E-06
7	**rs2436046**	**rs6990629**	**51.62**	**9**	**5.327E-08**
8	rs2436047	rs1155021	48.93	10	4.196E-07
9	rs1835740	rs1835742	53.46	10	6.107E-08
10	rs982502	rs11783877	45.23	8	3.327E-07
11	rs1155199	rs4734357	41.91	8	1.410E-06
12	rs7845920	rs7822798	39.34	9	9.995E-06
13	rs7845940	rs7838062	32.98	8	6.208E-05

The nine SNP window in bold is the one referred to in the text. N.B. haplotype value shown in text is for the single haplotype, above values for the association of the whole haplotype distribution.

Supplementary Table 4. SNPs with nominal or higher p-values for association with expression levels of *MTDH/AEG-1*

SNP	Gene	SNP coordinate	Gene start	Distance	SRC p-value
rs11783750	*MTDH/AEG-1*	98 865 219	98 725 583	139 636	0.0018741
rs10105830	*MTDH/AEG-1*	98 307 895	98 725 583	417 688	0.0004235
rs1835740	*MTDH/AEG-1*	98 236 089	98 725 583	489 494	**0.0000396***
rs7845920	*MTDH/AEG-1*	98 247 132	98 725 583	478 451	0.0014652

Footnote: * indicates surpassing the significance threshold 7.7×10^{-5} (corresponding to a 0.001 permutation threshold after 10,000 permutations). SRC = Spearman rank correlation. Locations and distances in basepairs, according to NCBI build 36. Numbers in bold are statistically significant.

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Supplementary Note: Clinical subject ascertainment and control samples

Ethical aspects
Written informed consent was obtained from all participants, and the study was approved by the respective local research ethics committees of the Helsinki University Central Hospital, Pain Clinic Kiel in Kiel, the Department of Neurology at Klinikum Großhadern, Ludwig-Maximilians-University in Munich, and the University of Leiden Medical Centre. Informed consent was obtained from all patients.

Initial study
The initial genome-wide association study consisted of three patient samples, collected from headache clinics in Finland, Germany and the Netherlands.

In Finland, 1,124 Finnish migraine with aura (MA, and MA/MO) patients were recruited. Each patient belongs to a multigenerational family with at least three family members with migraine. Patients were examined by a neurologist, and fulfilled the validated Finnish Migraine Specific Questionnaire for Family Studies ($FMSQ_{FS}$[2]). In cases of insufficient or conflicting information, a follow-up interview was conducted by telephone. All patients were diagnosed by the same headache specialist (M. Kallela) according to the current International Headache Society diagnostic criteria (ICHD-II)[3].

In Germany, patient recruitment was done at two sites, in Kiel and in Munich. At the Pain Clinic in Kiel, a total of 994 German MA and MA/MO patients were recruited to a patient collection maintained at the Universities of Bonn and Cologne. All patients were diagnosed according to the ICHD-II[3] by headache specialists[4]. The detailed migraine anamnesis was obtained either by face-to-face interviews or by telephone interviews standardized by using a comprehensive migraine questionnaire. The second German set of 282 MA and MA/MO cases were recruited and examined by a headache specialist at the Klinikum Großhadern of the Ludwig-Maximilians-University, Munich. Phenotyping was based on a German translation of the $FMSQ_{FS}$[2]. Whenever the information was insufficient or conflicting, an additional telephone interview was performed. Information was obtained on all aspects of the ICHD-II[3] criteria as well as on other aspects (such as age at onset, prodromal symptoms, triggers, acute and prophylactic medication, family history, general past medical history, co-morbidity and place of birth).

In the Netherlands, 879 MA and MA/MO patients were available from the clinic-based Leiden University Migraine Neuro Analysis (LUMINA) study. Self-reported migraineurs were recruited via the project's website. A set of screening questions validated previously in a population-based study[5] was used first. Participants fulfilling the screening criteria completed then the extended questionnaire focusing on signs and symptoms of migraine headache and aura as outlined in ICHD-II[3]. Individual diagnoses were made using an algorithm based on these criteria. The algorithm diagnosis was validated by a semi-

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structured telephone interview performed by experienced study physicians or by well-trained medical students. Specific attention was paid to migraine aura. A subset of the patients was asked to participate upon visiting the outpatient clinic.

Replication studies

The replication phase of the study consisted of four separately recruited migraine patient samples from Denmark, Iceland, the Netherlands and Germany.

The Danish replication sample comprised 825 MA subjects of which 776 were successfully genotyped. Of these, 483 patients suffered from only MA attacks and 293 from both MA and MO attacks. Patients were selected from the Danish National Patient Register and from case files from neurological clinics, 1,365 took part in a screening telephone interview. If the proband was diagnosed with MA, the proband and selected relatives were diagnosed according to the ICHD-I[6] in a validated telephone interview (M. Kirchmann or A.H.). 305 Danish MO patients were selected from case files at the Danish Headache Center and diagnosed as mentioned above (ICHD-II[3]) in an extensive semi-structured telephone interview performed by trained physicians. In addition 81 MO subjects were identified during recruitment of the MA families. Thus, 386 MO patients were recruited and 340 successfully genotyped.

The Icelandic replication samples were recruited from three sources: first, a list of patients provided by two neurologists (401 potential participants), second, responses to an advertisement in the newsletter of the Icelandic Migraine Society (137 participants), and third, responses to a brief screening questionnaire mailed to a random sample of 20,000 Icelanders, aged 18–50 years and living in the Reykjavik area. All Icelandic recruits were asked to answer the comprehensive validated deCODE Migraine Questionnaire 2 or 3 (DMQ2 or DMQ3[7]). The questionnaire was designed based on ICHD-II[3]. The reliability of the MA and MO diagnoses based on the DMQ3 was assessed using a physician-conducted interview as an empirical index of validity. In total 1,612 subjects reporting five or more headache attacks were genotyped. Of them, 712 subjects reported atypical symptoms, preventing reliable IHS classification through questionnaire data only, and were excluded from the analysis. In total, the Icelandic sample consists of 567 MO patients, and 333 MA patients either with or without the MO attacks.

The German replication cohort includes 837 MO cases from the Department of Neurology of the Ludwig-Maximilians-University, Munich, Germany. Phenotyping followed the same protocol as described for the Munich patient sample.

The Dutch replication sample includes 356 Dutch MA or MA/MO patients that were recently recruited through the clinic-based Leiden University Migraine Neuro Analysis (LUMINA) study. The diagnosis and classification followed the same procedure as in the initial Dutch sample.

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Control samples

Population-matched control samples were obtained from previously genotyped studies (for links to studies, see URL section of Online Methods). 1,881 Finnish controls originated from the Helsinki Birth Cohort study[8] and 2,173 controls from the Health2000 study, genotyped on the Illumina 660K or 610K platforms. 840 German controls were obtained from the KORA S4/F4 study[9], 380 controls from the HNR study[10] and 677 from PopGen study[11], all genotyped on the Illumina 550K platform. In addition, 444 controls were obtained from Illumina iControlDB by querying all Caucasian samples genotyped on the Illumina 550K platform on June 30th, 2008 and filtering these samples based on stratification as observed from multidimensional scaling plots of all existing German samples, and keeping only those identified as being of German descent. 974 Dutch controls were obtained from the Rotterdam study I[12], genotyped on the Illumina 550K platform and imputed to cover all markers on the 610K platform. For each replication study, the group providing a replication dataset supplied a matched control cohort; the controls for the Danish and Icelandic replications were provided by deCODE, and German controls were obtained from the MARS study[13] and from GlaxoSmithKline[14] and Rotterdam study III.

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