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ORIGINAL INVESTIGATION

New genetic evidence for involvement of the dopamine system in migraine with aura

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Abstract In order to systematically test the hypothesis that genetic variation in the dopamine system contributes to the susceptibility to migraine with aura (MA), we performed a comprehensive genetic association study of altogether ten genes from the dopaminergic system in a large German migraine with aura case-control sample. Based on the genotyping results of 53 variants across the ten genes in 270 MA cases and 272 controls, three genes—*DBH*, *DRD2* and *SLC6A3*—were chosen to proceed to additional genotyping of 380 MA cases and 378 controls. Four of the 26 genotyped polymorphisms in these three genes displayed nominally significant allelic *P*-values in the sample of 650 MA patients and 650 controls. Three of these SNPs [rs2097629 in *DBH* (uncorrected allelic *P* value = 0.0012, OR = 0.77), rs7131056 in *DRD2* (uncorrected allelic *P* value = 0.0018, OR = 1.28) and rs40184 in *SLC6A3* (uncorrected allelic *P* value = 0.0082, OR = 0.81)] remained significant after gene-wide correction for multiple testing by permutation analysis. Further consideration of imputed genotype data from 2,937 British control individuals did not affirm the association with *DRD2*, but supported the associations with *DBH* and *SLC6A3*. Our data provide new evidence for an involvement of components of the dopaminergic system—in particular the dopamine-beta hydroxylase and dopamine transporter genes—to the pathogenesis of migraine with aura.

Introduction

Migraine is a common and genetically complex disorder. Family and twin studies provide convincing evidence that hereditary factors contribute significantly to its etiology (Palotie and Wessman 2002). The genetic influence seems

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to be stronger in migraine with aura (MA) compared to migraine without aura (MO) (Russell and Olesen 1995). The molecular basis for both migraine forms is largely unknown.

Many migraine candidate genes have been analyzed in case-control or family based association studies during the past 10 years, among these several genes from the dopamine pathway (Asuni et al. 2007; Cevoli et al. 2006; de Sousa et al. 2007; Del Zompo et al. 1998; Dichgans et al. 1998; Lea et al. 2000; Maude et al. 2001; Mochi et al. 2003; Noble 2003; Peroutka et al. 1997, 1998; Rebaudengo et al. 2004; Shepherd et al. 2002; Stochino et al. 2003). However, most of the results of these association studies were negative, and positive findings could very often not be replicated. The dopamine D2 receptor gene is an illustrative case: several groups analyzed different polymorphisms in this gene in patients with MA and/or MO, and significant findings were reported in some of these studies for some of the genotyped polymorphisms (Del Zompo et al. 1998; Peroutka et al. 1997, 1998), but could not be confirmed by other groups (Dichgans et al. 1998; Rebaudengo et al. 2004; Stochino et al. 2003). Diverse reasons may contribute to these inconsistencies: inadequate sample sizes, arbitrarily chosen polymorphisms, existence of different risk alleles in different populations, inadequate corrections for multiple testing, phenotypic differences between study populations (in which e.g., patients with MA, MO, childhood migraine, or any common type of migraine were included), and/or publication bias towards studies with positive results.

In an attempt to overcome these problems, we (1) have studied a large and ethnically homogenous case-control sample, comprising 650 patients and 650 control individuals [all cases were of Caucasian ancestry and uniformly diagnosed with *migraine with aura* according to the revised criteria of the International Headache Society (Headache Classification Subcommittee of the International Headache Society 2004) by experienced physicians], (2) have applied a systematic, haplotype-based strategy, which takes into account the results of the International Hapmap Project (International_HapMap_Consortium 2005) and, in addition to single-marker analyses, compares the frequency of the common haplotypes in the European population between cases and controls, (3) have chosen a system-based approach, i.e., rather than testing single migraine candidate genes, we included a larger number of genes belonging to a plausible biological pathway.

Indeed, there is strong evidence from neuroanatomic, pharmacologic, clinical, and pathophysiologic studies that the dopamine system is involved in the etiology of migraine (reviewed e.g., in Akerman and Goadsby 2007; Del Zompo 2000; Peroutka 1997). Genes encoding proteins of this biological system (e.g., receptors or transporters) can therefore be regarded as promising migraine candidate genes. In total, we analyzed 53 genetic variants (mainly haplotype-tagging SNPs) in ten genes from the dopamine system with a two-step study design: in the first step we screened ten genes from the dopamine system in a subset of our case-control sample. For genes that displayed a nominally significant case-control difference for any SNP, we subsequently genotyped all haplotype-tagging SNPs for the respective gene in the complete case-control sample.

Materials and methods

Patients and control individuals

German patients with MA (650 individuals in total) were recruited at a single tertiary headache centre in Northern Germany (Pain Clinic, Kiel, Germany). They were randomly assigned to one of the two subsamples. All patients were diagnosed as having MA according to the revised criteria of the International Headache Society (Headache Classification Subcommittee of the International Headache Society 2004) by experienced neurologists with a specialization in headache disorders, as described previously (Netzer et al. 2006, 2008a, b; Todt et al. 2006). A total of 33.5% of the patients had pure MA attacks, i.e., an aura was always present during the attacks; 66.5% of patients exhibited both MA and MO attacks, i.e., they had migraine attacks with a preceding or accompanying aura as well as migraine attacks without an aura. The headache phase of these migraine attacks always fulfilled the revised international diagnostic criteria (ICHD-II) for migraine. The detailed migraine anamnesis was obtained either by face-to-face interviews or by telephone interviews. Interviews were standardized in so far as a comprehensive questionnaire had to be filled out. All patients gave their written informed consent for participating in the study. The study was approved by the local university ethics committees. The two patient subsamples and the clinical features of the participants are described in detail in Supplementary Table 1.

The population-based control sample comprised 650 German individuals. They were interviewed by a psychiatrist with a 1-year experience in neurology. In order to assess the number of individuals with a migraine or migraine-like headache disorder (which is highly prevalent in the general population) these healthy control individuals had to answer an extensive questionnaire regarding the cardinal ICHD-II diagnostic criteria for MA and MO. The control sample was matched to the migraine case sample with regard to gender and ethnicity. Individuals with maternal and paternal grandparents of non-German ancestry were excluded.

SNP assortment

Haplotype-tagging (ht-) SNPs were selected based on the HapMap database (http://www.hapmap.org; version September 2004). SNPs were chosen to discriminate between all common haplotypes (i.e., haplotypes with an estimated frequency >5%) within haplotype blocks in the Central European HapMap sample. We used the program hapblock (Zhang et al. 2004) to define haplotype blocks as regions in which >85% of total haplotype diversity is covered by common haplotypes.

Genetic analysis

Genomic DNA was used for all genotyping experiments. SNPs tested in sample 1 were genotyped on customized Illumina™ platforms including altogether 1,948 SNPs (which were analyzed in the course of several migraine studies mainly testing hypotheses on the involvement of genes encoding ion channels and transporters as well as neuronal receptors in disease pathogenesis) according to the manufacturer's protocol. Methods used for SNP genotyping in sample 2 as well as SNP-specific allele call rates are listed in Supplementary Table 2. The microsatellite marker (Porter et al. 1992) (GenBank Accession X63418) and 19 bp insertion/deletion polymorphism (Nahmias et al. 1992) in the promotor region of *DBH* were amplified with fluorescence-labeled primers and genotyped on an ABI sequencer. Primers for the *DBH* microsatellite marker were: 5′-GCAGTCACGCATCCTTATGG and 5′-CAGC TCTGGGCTCATGCTC (Porter et al. 1992). Primers for the adjacent 19 bp insertion/deletion polymorphism were: 5′-AATCAGGCACATGCACCTCC and 5′-GGCCCTGA GGAATCTTACAGG.

Statistics

We compared allele and genotype distributions between patients and control individuals by a χ^2 test with the appropriate degrees of freedom. Hardy–Weinberg disequilibrium (HWD) tests were performed using a χ^2 goodness-of-fit test. Haplotype frequencies between cases and controls were compared with the program cocaphase, which is contained in the unphased package (Dudbridge 2003). Cocaphase estimates maximum-likelihood haplotype frequencies based on an expectation-maximization algorithm and compares haplotype frequencies with a likelihood ratio test. The microsatellite marker was dichotomized for statistical analysis: alleles with 170, 172, and 174 bp were pooled and assigned to a 'Short allele' ('S' in Table 1, allele 1 in Table 2), and alleles with 176, 178, and 180 bp were combined and referred to as the 'Long allele' ('L' in Table 1, allele 2 in Table 2). We obtained gene-wide corrections for multiple testing by randomly permuting the affection status 10,000 times and counting the number of times the test statistic was larger in the permuted than in the actually observed statistic. We applied a logistic regression model to test interactions between SNP markers as implemented by the program PLINK (Purcell et al. 2007). PLINK tests a model based on allele dosage for each SNP, A and B, by fitting the model $Y \sim b0 + b1.A + b2.B + b3.AB + e$. The test for interaction is based on the coefficient b3.

Power calculation

We performed a power analysis with the Genetic Power Calculator (Purcell et al. 2003). For the single-marker analysis (with α of 0.05), we had ~80% power to detect a true difference in allele frequency between cases and controls in the first step of our study. Assumptions for the power calculation were: complete LD between the marker tested and the disease-causing variant, a frequency of the disease-associated allele A of 0.18, a relative risk of 1.5 for genotype Aa and of 2.25 for genotype AA, and a prevalence of MA in the general population of 8%. Using the same parameters, the estimated power increases to >98% in a sample of 650 patients and 650 controls (i.e., in our complete sample).

Results

Single-marker analysis in the German sample

Allele call rates on the Illumina™ platform were >90% for all SNPs. In the first step of our study, comprising 270 MA cases and 272 controls, two of 53 polymorphisms deviated from the Hardy–Weinberg equilibrium in the case sample (see Table 1), an observation that was not significant after Bonferroni correction for multiple testing. Two SNPs in *DBH* (rs2097629 and rs1611131) and one SNP in *DRD2* (rs7131056) displayed significant allelic association with MA (Table 1). The smallest uncorrected *P* value was 0.0009 (OR = 0.66) for rs2097629 in *DBH*. One SNP in *SLC6A3* reached borderline-significance (rs403636, uncorrected allelic *P* value = 0.08).

Based on these results we chose the genes *DBH*, *DRD2*, and *SLC6A3* for analysis in the second step of the study. The complete set of genetic variants analyzed for these genes in the first step (13 polymorphisms for *DBH*, 5 SNPs for *DRD2* and 8 SNPs for *SLC6A3*) was genotyped in an additional 380 MA cases and 378 controls. One SNP in *DBH* (rs2097629), two SNPs in *DRD2* (rs6279 and rs7131056) and one SNP in *SLC6A3* (rs40184) displayed

Table 1 Genotyping results and statistics of stage 1 of the study

Gene	ID	Allele1/2 (strand)	Deviation from HWE (*P* value)		Minor allele frequency (%)		Allelic *P* value	OR (95% CI)
			Controls	Cases	Controls	Cases		
COMT	rs4485648	A/G (−)	0.59	0.62	17.46 (G)	20.82 (G)	0.16	0.8 (0.59–1.09)
COMT	rs933271	A/G (−)	0.66	0.86	26.2 (G)	30.11 (G)	0.15	0.82 (0.63–1.07)
COMT	rs740603	A/G (+)	0.29	0.65	47.98 (A)	44.96 (A)	0.32	0.89 (0.7–1.13)
COMT	rs4680	A/G (+)	0.91	0.66	45.59 (G)	48.7 (G)	0.31	0.88 (0.7–1.12)
COMT	rs4646316	A/G (−)	0.79	0.89	22.06 (A)	22.49 (A)	0.86	1.03 (0.77–1.37)
COMT	rs165774	A/G (+)	0.1	*0.01*	32.66 (A)	34.01 (A)	0.64	1.06 (0.83–1.37)
COMT	rs5993889	A/C (−)	0.98	1.00	0.18 (C)	0 (C)	0.32	N/A
DBH	Microsat.	S/L (+)	0.65	0.69	45.04 (L)	47.91 (L)	0.35	1.12 (0.88–1.43)
DBH	19 bp Indel	S/L (+)	0.81	0.39	49.81 (L)	47.03 (S)	0.30	1.13 (0.89–1.44)
DBH	rs1076153	A/C (−)	0.84	0.55	16.61 (A)	16.54 (A)	0.98	1 (0.72–1.37)
DBH	rs2797849	C/G (–)	0.13	0.27	35.69 (G)	33.14 (G)	0.38	1.12 (0.87–1.44)
DBH	rs3025388	A/G (+)	0.73	0.13	20.04 (G)	20.26 (G)	0.93	0.99 (0.73–1.33)
DBH	rs2007153	A/G (−)	0.11	0.29	40.99 (A)	41.45 (A)	0.88	1.02 (0.8–1.3)
DBH	rs1108581	A/G (+)	0.75	*0.004*	24.26 (G)	24.16 (G)	0.97	0.99 (0.75–1.31)
DBH	rs2873804	A/G (−)	0.1	0.28	41.73 (A)	43.68 (A)	0.52	1.08 (0.85–1.38)
DBH	rs1541332	A/G (+)	0.58	0.47	41.91 (A)	42.19 (A)	0.93	1.01 (0.79–1.29)
DBH	rs2797853	A/G (−)	0.87	0.88	37.68 (A)	33.64 (A)	0.17	0.84 (0.65–1.08)
DBH	**rs2097629**	A/G (+)	0.62	0.73	34.38 (G)	44.22 (G)	*0.0009*	0.66 (0.52–0.84)
DBH	**rs1611131**	A/G (+)	0.85	0.06	26.84 (G)	33.83 (G)	*0.0124*	1.39 (1.07–1.81)
DBH	rs129882	A/G (−)	0.11	0.82	19.3 (A)	16.73 (A)	0.27	0.84 (0.62–1.15)
DDC	rs4947535	A/T (−)	0.9	0.3	29.96 (A)	34.39 (A)	0.12	1.22 (0.95–1.58)
DDC	rs730092	C/G (−)	0.45	0.11	41.54 (G)	44.61 (G)	0.31	0.88 (0.69–1.12)
DDC	rs1451371	A/G (−)	0.08	0.06	46.51 (G)	42.94 (G)	0.24	0.87 (0.68–1.1)
DDC	rs11575404	A/G (+)	0.14	0.66	3.13 (G)	2.6 (G)	0.61	0.83 (0.4–1.7)
DDC	rs4948225	A/G (+)	0.98	1.00	0.18 (G)	0 (G)	0.32	N/A
DDC	rs1470750	C/G (+)	0.84	0.15	43.38 (G)	38.85 (G)	0.13	0.83 (0.65–1.06)
DDC	rs2167363	A/G (+)	0.64	0.68	2.76 (G)	2.42 (G)	0.72	0.87 (0.41–1.85)
DDC	rs998850	C/G (−)	0.79	0.28	45.4 (C)	47.4 (C)	0.51	0.92 (0.73–1.17)
DDC	rs2329371	A/G (+)	0.56	0.81	22.43 (A)	24.16 (A)	0.50	1.1 (0.83–1.46)
DDC	rs2329341	A/C (+)	0.35	0.56	34.01 (C)	34.01 (C)	1.00	1 (0.78–1.29)
DRD1	rs686	A/G (+)	0.64	0.33	41.18 (G)	42.19 (G)	0.73	0.96 (0.75–1.22)
DRD1	rs5326	A/G (−)	0.68	0.76	12.5 (A)	14.18 (A)	0.42	1.16 (0.81–1.64)
DRD2	rs6279	C/G (+)	0.13	0.28	32.35 (G)	29 (G)	0.23	0.85 (0.66–1.11)
DRD2	rs2587548	C/G (+)	0.86	0.88	38.33 (G)	42.94 (G)	0.12	0.83 (0.65–1.05)
DRD2	rs7125415	A/G (−)	0.13	0.25	8.46 (A)	10.04 (A)	0.37	1.21 (0.8–1.82)
DRD2	rs4581480	A/G (−)	0.32	0.17	9.41 (G)	10.41 (G)	0.58	1.12 (0.75–1.67)
DRD2	**rs7131056**	A/C (+)	0.25	0.26	38.6 (A)	47.77 (A)	*0.0023*	1.45 (1.14–1.85)
DRD3	rs963468	A/G (+)	0.80	0.14	45.77 (A)	41.82 (A)	0.19	0.85 (0.67–1.08)
DRD3	rs167770	A/G (+)	0.94	0.68	24.08 (G)	23.79 (G)	0.91	1.02 (0.77–1.34)
DRD3	rs10934256	A/C (+)	0.90	0.42	16.36 (A)	14.5 (A)	0.40	0.87 (0.62–1.21)
DRD4	rs3758653	A/G (−)	0.84	0.06	14.34 (G)	17.79 (G)	0.12	1.29 (0.93–1.79)
DRD5	rs10033951	A/G (−)	0.93	0.81	30.51 (A)	32.9 (A)	0.40	1.12 (0.86–1.44)
SLC6A3	rs40184	A/G (−)	0.41	0.26	46.13 (A)	47.4 (A)	0.68	0.95 (0.75–1.21)
SLC6A3	rs27048	A/G (−)	0.27	0.38	45.59 (A)	47.4 (A)	0.55	1.08 (0.85–1.37)
SLC6A3	rs37022	A/T (−)	0.48	0.25	16.73 (A)	19.22 (A)	0.29	1.18 (0.87–1.62)

Table 1 continued

Gene	ID	Allele1/2 (strand)	Deviation from HWE (*P* value)		Minor allele frequency (%)		Allelic *P* value	OR (95% CI)
			Controls	Cases	Controls	Cases		
SLC6A3	rs37020	A/C (+)	0.82	0.51	43.75 (C)	45.35 (C)	0.60	1.07 (0.84–1.36)
SLC6A3	rs463379	C/G (+)	0.62	0.23	21.32 (C)	21.46 (C)	0.96	1.01 (0.75–1.35)
SLC6A3	rs403636	A/C (+)	0.38	0.96	11.4 (A)	15.06 (A)	0.08	1.38 (0.97–1.96)
SLC6A3	rs3756450	A/G (+)	0.08	0.76	12.32 (G)	14.18 (G)	0.37	1.18 (0.83–1.67)
SLC6A3	rs2078247	C/G (−)	0.42	0.73	26.84 (G)	24.35 (G)	0.35	0.88 (0.67–1.15)
TH	rs2070762	A/G (+)	0.72	0.90	48.71 (G)	49.81 (G)	0.72	1.05 (0.82–1.33)
TH	rs6357	A/G (−)	0.45	0.38	33.09 (A)	31.27 (A)	0.52	0.92 (0.71–1.19)
TH	rs6356	A/G (−)	0.07	0.77	34.74 (A)	36.19 (A)	0.62	1.07 (0.83–1.37)

Analyzed were 53 polymorphisms located in ten genes of the dopaminergic system. *P* values ≤ 0.05 are italicized

HWE Hardy–Weinberg equilibrium, *OR* odds ratio, *CI* confidence interval, *Microsat. DBH* microsatellite marker (GenBank accession X63418), *Indel* 19 bp insertion/deletion polymorphism in the *DBH* promotor. *S/L* short/long allele of the microsatellite marker after dichotomization for statistical analysis (see "Materials and methods" for details)

Polymorphisms displaying significant allelic association with migraine with aura are given in bold

significant allelic association with MA in the comparison between the complete sample of 650 MA patients and 650 controls (Tables 2, 3, 4). Again, rs2097629 in *DBH* had the smallest uncorrected *P* value ($P = 0.0012$, OR = 0.77). Three of these four SNPs had significant allelic *P* values after gene-wide correction for multiple testing by permutation analysis: rs2097629 in *DBH* (corrected *P* value = 0.0116), rs7131056 in *DRD2* (corrected *P* value = 0.0058) and rs40184 in *SLC6A3* (corrected *P* value = 0.032).

Haplotype analysis

We next compared the estimated frequencies of case and control haplotypes for *DBH*, *DRD2*, and *SLC6A3*. For each of these genes, we looked for differences in the overall haplotype frequency distribution over all possible combinations of up to three markers in the set of 650 cases and 650 controls (Figs. 1, 2, 3). The most prominent global differences in the haplotype frequency distribution resulted in *P* values of 6.8×10^{-5} for *DBH* over the SNPs (rs1076153–rs1541332–rs2097629], 2.6×10^{-4} for *DRD2* over the SNPs [rs6279–rs7125415–rs7131056], and 2.7×10^{-2} for *SLC6A3* over the SNPs [rs27048–rs463379–rs3756450]. These three-marker-haplotypes may indicate the genomic regions where susceptibility variants for MA are most likely to reside. However, it is also important to point out that after correction for multiple testing these haplotype *P* values are weaker than the corrected *P* values from the single marker analysis. Thus, our data do not support disease models that would produce strong haplotype effect, such as the existence of different (untyped) mutations that are in LD to each other.

Genetic interaction analysis

As all genes are coding for components of a system of functionally interacting proteins, we tested all possible pairs of SNPs for evidence of genetic interactions by using the logistic regression option of the PLINK analysis program. In total, 68 of 1,275 tested interactions were nominally significant ($P < 0.05$), which is close to the random expectation (data not shown). The most significant result involved the SNPs rs2797853 in *DBH* (located on chromosome 9) and rs740603 in *COMT* (located on chromosome 22), which attained a *P* value of 0.0003. However, given the number of 1,275 pairwise interaction tests performed, this observation most likely has no biological significance.

Single marker analysis including additional control individuals

We next used the imputated genotype counts for SNPs rs2097629, rs7131056, and rs40184 from the Wellcome Trust Case Control Consortium [WTCCC, 2,937 British control individuals (Wellcome_Trust_Case_Control_Consortium 2007)] that were published online on the WTCCC website (open access at http://www.wtccc.org.uk/info/summary_stats.shtml in June 2008) to increase the statistical power by a substantially enlarged control sample. Comparison of allele and genotype frequencies between the Northern German and British control samples showed no significant differences for rs2097629 in *DBH* and rs40184 in *SLC6A3*. However, frequencies for rs7131056 in *DRD2* differed markedly between the two control samples (borderline significant allelic *P* value of 0.06), with a frequency of the

Table 2 Single marker association results of *DBH* in the two MA sub-samples of our study

ID	Sample	Controls (*n*)	MA patients (*n*)	Genotype frequency controls (*n*)			Genotype frequency MA patients (*n*)			Test for allelic association	
				11	12	22	11	12	22	*P* value	OR (95% CI)
MS	Sample 1	272	263	0.309 (84)	0.482 (131)	0.210 (57)	0.278 (73)	0.487 (128)	0.236 (62)	0.35	1.12 (0.88–1.43)
	Sample 2	368	363	0.302 (111)	0.514 (189)	0.185 (68)	0.311 (113)	0.504 (183)	0.185 (67)	0.85	0.98 (0.8–1.21)
	Sample 1 + 2	640	626	0.305 (195)	0.500 (320)	0.195 (125)	0.297 (186)	0.497 (311)	0.206 (129)	0.64	1.04 (0.89–1.21)
19 bp INDEL	Sample 1	270	269	0.256 (69)	0.493 (133)	0.252 (68)	0.234 (63)	0.472 (127)	0.294 (79)	0.30	1.13 (0.89–1.44)
	Sample 2	369	367	0.187 (69)	0.534 (197)	0.279 (103)	0.207 (76)	0.501 (184)	0.292 (107)	0.88	1.02 (0.83–1.25)
	Sample 1 + 2	639	636	0.216 (138)	0.516 (330)	0.268 (171)	0.219 (139)	0.489 (311)	0.292 (186)	0.57	0.96 (0.82–1.12)
rs1076153	Sample 1	271	269	0.0267)	0.280 (76)	0.694 (188)	0.022 (6)	0.286 (77)	0.691 (186)	0.98	1 (0.72–1.37)
	Sample 2	376	365	0.013 (5)	0.290 (109)	0.697 (262)	0.030 (11)	0.312 (114)	0.658 (240)	0.15	1.22 (0.93–1.6)
	Sample 1 + 2	647	634	0.019 (12)	0.286 (185)	0.696 (450)	0.027 (17)	0.301 (191)	0.672 (426)	0.28	1.12 (0.91–1.38)
rs2797849	Sample 1	269	264	0.435 (117)	0.416 (112)	0.149 (40)	0.432 (114)	0.473 (125)	0.095 (25)	0.38	1.12 (0.87–1.44)
	Sample 2	375	367	0.139 (52)	0.445 (167)	0.416 (156)	0.095 (35)	0.488 (179)	0.417 (153)	0.37	0.91 (0.73–1.12)
	Sample 1 + 2	644[a]	631	0.262 (169)	0.433 (279)	0.304 (196)	0.236 (149)	0.482 (304)	0.282 (178)	0.92	0.99 (0.85–1.16)
rs3025388	Sample 1	272	269	0.636 (173)	0.327 (89)	0.037 (10)	0.621 (167)	0.353 (95)	0.026 (7)	0.93	0.99 (0.73–1.33)
	Sample 2	377	369	0.724 (273)	0.255 (96)	0.021 (8)	0.675 (249)	0.304 (112)	0.022 (8)	0.19	0.83 (0.63–1.1)
	Sample 1 + 2	649	638	0.687 (446)	0.285 (185)	0.028 (18)	0.652 (416)	0.324 (207)	0.024 (15)	0.30	0.9 (0.73–1.1)
rs2007153	Sample 1	272	269	0.191 (52)	0.438 (119)	0.371 (101)	0.156 (42)	0.517 (139)	0.327 (88)	0.88	1.02 (0.8–1.3)
	Sample 2	376	369	0.138 (52)	0.476 (179)	0.386 (145)	0.149 (55)	0.466 (172)	0.385 (142)	0.82	1.02 (0.83–1.26)
	Sample 1 + 2	648	638	0.160 (104)	0.460 (298)	0.380 (246)	0.152 (97)	0.487 (311)	0.361 (230)	0.78	1.02 (0.87–1. 2)
rs1108581	Sample 1	272	269[a]	0.577 (157)	0.360 (98)	0.063 (17)	0.543 (146)	0.431 (116)	0.026 (7)	0.97	0.99 (0.75–1.31)
	Sample 2	378	371	0.653 (247)	0.312 (118)	0.034(13)	0.606 (225)	0.364 (135)	0.030 (11)	0.31	1.14 (0.89–1.47)
	Sample1 + 2	650	640[a]	0.622 (404)	0.332(216)	0.046 (30)	0.580 (371)	0.392 (251)	0.028 (18)	0.46	1.07 (0.89–1.29)
rs2873804	Sample 1	272	269	0.199 (54)	0.438 (119)	0.364 (99)	0.175 (47)	0.524 (141)	0.301 (81)	0.52	1.08 (0.85–1.38)
	Sample 2	365	358	0.211 (77)	0.510 (186)	0.279 (102)	0.193 (69)	0.534 (191)	0.274 (98)	0.81	0.98 (0.79–1.2)
	Sample 1 + 2	637	627	0.206 (131)	0.479 (305)	0.316 (201)	0.185 (116)	0.530 (332)	0.285 (179)	0.81	1.02 (0.87–1.19)
rs1541332	Sample 1	272	269	0.184 (50)	0.471 (128)	0.346 (94)	0.167 (45)	0.509 (137)	0.323 (87)	0.93	1.01 (0.79–1.29)
	Sample 2	360	348	0.208 (75)	0.450 (162)	0.342 (123)	0.204 (71)	0.477 (166)	0.319 (111)	0.73	1.04 (0.84–1.28)
	Sample 1 + 2	632	617	0.198 (125)	0.459 (290)	0.343 (217)	0.188 (116)	0.491 (303)	0.321 (198)	0.75	1.03 (0.88–1.2)
rs2797853	Sample 1	272	269	0.140 (38)	0.474 (129)	0.386 (105)	0.115 (31)	0.442 (119)	0.442 (119)	0.17	0.84 (0.65–1.08)
	Sample 2	–	–	–	–	–	–	–	–	–	–
	Sample 1 + 2	–	–	–	–	–	–	–	–	–	–
rs2097629	Sample 1	272	268	0.437 (119)	0.437 (119)	0.125 (34)	0.306 (82)	0.504 (135)	0.190 (51)	*0.0009*	0.66 (0.52–0.84)
	Sample 2	367	365	0.406 (149)	0.485 (178)	0.109 (40)	0.370 (135)	0.485 (177)	0.145 (53)	0.15	0.86 (0.69–1.06)
	Sample 1 + 2	639	633	0.419 (268)	0.465 (297)	0.116 (74)	0.343 (217)	0.493 (312)	0.164 (104)	*0.0012*	0.77 (0.65–0.9)

Table 2 continued

ID	Sample	Controls (*n*)	MA patients (*n*)	Genotype frequency controls (*n*)			Genotype frequency MA patients (*n*)			Test for allelic association	
				11	12	22	11	12	22	*P* value	OR (95% CI)
rs1611131	Sample 1	272	269	0.533 (145)	0.397 (108)	0.070 (19)	0.413 (111)	0.498 (134)	0.089 (24)	*0.0124*	1.39 (1.07–1.81)
	Sample 2	377	366	0.523 (197)	0.416 (157)	0.061 (23)	0.555 (203)	0.380 (139)	0.066 (24)	0.55	0.93 (0.74–1.17)
	Sample 1 + 2	649	635	0.527 (342)	0.408 (265)	0.065 (42)	0.494 (314)	0.430 (273)	0.076 (48)	0.22	1.11 (0.94–1.32)
rs129882	Sample 1	272	269	0.022 (6)	0.342 (93)	0.636 (173)	0.026 (7)	0.283 (76)	0.691 (186)	0.27	0.84 (0.62–1.15)
	Sample 2	377	363	0.037 (14)	0.340 (128)	0.623 (235)	0.030 (11)	0.328 (119)	0.642 (233)	0.54	0.92 (0.72–1.19)
	Sample 1 + 2	649	632	0.031 (20)	0.341 (221)	0.629 (408)	0.028 (18)	0.309 (195)	0.663 (419)	0.24	0.89 (0.73–1.08)

Genotype frequencies for cases and controls and allelic *P* values are given (genotyping of rs2797853 in subsample 2 technically failed). *P* values ≤ 0.05 are italicized. SNPs displaying significant allelic association with migraine with aura are depicted in bold

OR odds ratio, *CI* confidence interval

[a] Sub-samples with significant deviations from HWE

WTCCC controls that was in-between the German case and the German control sample (frequency of allele 1 was 0.42 in German controls, 0.44 in British controls, and 0.48 in MA patients). Accordingly, the differences between the British controls and our MA sample were just borderline significant for rs7131056 (allelic *P* value = 0.034). In contrast, highly significant differences exist between our Migraine cases and the WTCCC controls for rs2097629 (P value $= 5.57 \times 10^{-8}$) and for rs40184 (P value $= 6.36 \times 10^{-7}$).

To quantify the potential impact of a systematic bias, we next calculated the χ^2 inflation factor lambda-GC (Devlin and Roeder 1999) for the allelic comparison of our control sample and the WTCCC sample. Across all SNPs from the first step of our study, we found a lambda-GC of 1.67, indicating a moderate inflation of the test statistic. When we further restricted the analysis to those SNPs that not only passed stringent quality control in our sample, but also were flagged with a high imputation quality score in the WTCCC data, lambda-GC decreased to 1.1, which is close to the expectation for the absence of any systematic bias. This shows that the British WTCCC genotypes can be used to enlarge our Northern German control sample, although attention has to be given to the quality scores of the respective imputation data. Of note, the above two SNPs rs2097629 and rs40184 did not belong to the set of markers that are marked by high quality imputation scores. To interpret this results it might help that a recent study showed that imputation increases power to detect associations, even when accuracy is poor (Guan and Stephens 2008). However, it also reported evidence of biased effect sizes for SNPs with lower imputation quality, which may be optimally handled by the usage of Bayes-Factors instead of *P* values. Therefore, these *P* values that are based on imputed WTCCC genotypes may increase the confidence in our above genotyping results, but they should not be interpreted as error probabilities in the traditional sense.

Discussion

For more than 30 years, an implication of the dopamine system in the pathophysiology of migraine has been discussed (Sicuteri 1977). However, genetic studies aiming to substantiate the dopamine hypothesis often yielded inconclusive results. With this study we intended to systematize the genetic approach by performing an analysis of a large number of genes from the dopamine system with an adequately sized case-control sample for migraine with aura. Haplotype-based analyses can theoretically provide a higher sensitivity in detecting associations between genetic markers and diseases, and they offer the additional advantage that the boundaries of the risk haplotypes can be used

Table 3 Single marker association results of *DRD2* in the two MA sub-samples of our study

SNP rs-ID	Sample	Controls (*n*)	MA patients (*n*)	Genotype frequency controls (*n*)			Genotype frequency MA patients (*n*)			Test for allelic association	
				11	12	22	11	12	22	*P* value	OR (95% CI)
rs6279	Sample 1	272	269	0.478 (130)	0.397 (108)	0.125 (34)	0.491 (132)	0.439 (118)	0.071 (19)	0.23	0.85 (0.66–1.11)
	Sample 2	371	365	0.464 (172)	0.461 (171)	0.075 (28)	0.532 (194)	0.400 (146)	0.068 (25)	0.11	0.83 (0.66–1.04)
	Sample 1 + 2	643	634	0.470 (302)	0.434 (279)	0.096 (62)	0.514 (326)	0.416 (264)	0.069 (44)	*0.05*	0.84 (0.71–1.0)
rs2587548	Sample 1	270	269	0.378 (102)	0.478 (129)	0.144 (39)	0.323 (87)	0.494 (133)	0.182 (49)	0.12	0.83 (0.65–1.05)
	Sample 2	373	357	0.332 (124)	0.523 (195)	0.145 (54)	0.347 (124)	0.501 (179)	0.151 (54)	0.87	1.02 (0.83–1.25)
	Sample 1 + 2	643	626	0.351 (226)	0.504 (324)	0.145 (93)	0.337 (211)	0.498 (312)	0.165 (103)	0.38	0.93 (0.79–1.09)
rs7125415	Sample 1	272	269	0 (0)	0.169 (46)	0.831 (226)	0.004 (1)	0.193 (52)	0.803 (216)	0.37	1.21 (0.8–1.82)
	Sample 2	378	372	0.008 (3)	0.175 (66)	0.817 (309)	0.005 (2)	0.148 (55)	0.847 (315)	0.27	0.82 (0.57–1.17)
	Sample 1 + 2	650	641	0.005 (3)	0.172 (112)	0.823 (535)	0.005 (3)	0.167 (107)	0.828 (531)	0.82	0.97 (0.74–1.27)
rs4581480	Sample 1	271	269	0.815 (221)	0.181 (49)	0.004 (1)	0.810 (218)	0.171 (46)	0.019 (5)	0.58	1.12 (0.75–1.67)
	Sample 2	377	371	0.806 (304)	0.178 (67)	0.016 (6)	0.827 (307)	0.167 (62)	0.005 (2)	0.30	0.83 (0.59–1.18)
	Sample 1 + 2	648	640	0.810 (525)	0.179 (116)	0.011 (7)	0.820 (525)	0.169 (108)	0.011 (7)	0.67	0.94 (0.73–1.23)
rs7131056	Sample 1	272	269	0.132 (36)	0.507 (138)	0.360 (98)	0.245 (66)	0.465 (125)	0.290 (78)	*0.0023*	1.45 (1.14–1.85)
	Sample 2	377	369	0.186 (70)	0.501 (189)	0.313 (118)	0.238 (88)	0.474 (175)	0.287 (106)	0.13	1.17 (0.96–1.44)
	Sample 1 + 2	649	638	0.163 (106)	0.504 (327)	0.333 (216)	0.241 (154)	0.470 (300)	0.288 (184)	*0.0018*	1.28 (1.1–1.5)

Genotype frequencies for cases and controls and allelic *P*-values are given. *P*-values ≤ 0.05 are italicized. SNPs displaying significant allelic association with migraine with aura are depicted in bold

OR odds ratio, *CI* confidence interval

Table 4 Single marker association results of *SLC6A3* in the two MA sub-samples of our study

SNP rs-ID	Sample	Controls (*n*)	MA patients (*n*)	Genotype frequency controls (*n*)			Genotype frequency MA patients (*n*)			Allele 1 versus 2	
				11	12	22	11	12	22	*P* value	OR (95% CI)
rs40184	Sample 1	271	269	0.225 (61)	0.472 (128)	0.303 (82)	0.242 (65)	0.465 (125)	0.294 (79)	0.68	0.95 (0.75–1.21)
	Sample 2	372	367[a]	0.210 (78)	0.495 (184)	0.296 (110)	0.316 (116)	0.444 (163)	0.240 (88)	*0.0018*	0.72 (0.59–0.89)
	Sample 1 + 2	643	636[a]	0.216 (139)	0.485 (312)	0.299 (192)	0.285 (181)	0.453 (288)	0.263 (167)	*0.0082*	0.81 (0.69–0.95)
rs27048	Sample 1	272	269	0.191 (52)	0.529 (144)	0.279 (76)	0.238 (64)	0.472 (127)	0.290 (78)	0.55	1.08 (0.85–1.37)
	Sample 2	367	367	0.215 (79)	0.523 (192)	0.262 (96)	0.226 (83)	0.504 (185)	0.270 (99)	0.96	1.01 (0.82–1.23)
	Sample 1 + 2	639	636	0.205 (131)	0.526 (336)	0.269 (172)	0.231 (147)	0.491 (312)	0.278 (177)	0.67	1.03 (0.89–1.21)
rs37022	Sample 1	272	268	0.022 (6)	0.290 (79)	0.688 (187)	0.026 (7)	0.332 (89)	0.642 (172)	0.29	1.18 (0.87–1.62)
	Sample 2	374	360	0.029 (11)	0.278 (104)	0.693 (259)	0.031 (11)	0.247 (89)	0.722 (260)	0.46	0.9 (0.68–1.19)
	Sample 1 + 2	646	628	0.026 (17)	0.283 (183)	0.690 (446)	0.029 (18)	0.283 (178)	0.688 (432)	0.87	1.02 (0.83–1.25)
rs37020	Sample 1	272	269	0.320 (87)	0.485 (132)	0.195 (53)	0.309 (83)	0.476 (128)	0.216 (58)	0.60	1.07 (0.84–1.36)
	Sample 2	366[a]	343	0.281 (103)	0.549 (201)	0.169 (62)	0.341 (117)	0.469 (161)	0.190 (65)	0.45	0.92 (0.75–1.14)
	Sample 1 + 2	638	612	0.298 (190)	0.522 (333)	0.180 (115)	0.327 (200)	0.472 (289)	0.201 (123)	0.84	0.98 (0.84–1.15)
rs463379	Sample 1	272	268	0.040[11)	0.346 (94)	0.614 (167)	0.034 (9)	0.362 (97)	0.604 (162)	0.96	1.01 (0.75–1.35)
	Sample 2	378	371	0.029 (11)	0.347 (131)	0.624 (236)	0.035 (13)	0.313 (116)	0.652 (242)	0.59	0.93 (0.72–1.2)
	Sample 1 + 2	650	639	0.034 (22)	0.346 (225)	0.620 (403)	0.034 (22)	0.333 (213)	0.632 (404)	0.71	0.96 (0.8–1.17)
rs403636	Sample 1	272	269	0.018 (5)	0.191 (52)	0.790 (215)	0.022 (6)	0.257 (69)	0.721 (194)	0.08	1.38 (0.97–1.96)
	Sample 2	374	364	0.019 (7)	0.230 (86)	0.751 (281)	0.019 (7)	0.245 (89)	0.736 (268)	0.66	1.07 (0.79–1.44)
	Sample 1 + 2	646	633	0.019 (12)	0.214 (138)	0.768 (496)	0.021 (13)	0.250 (158)	0.730 (462)	0.14	1.19 (0.95–1.49)
rs3756450	Sample 1	272	268	0.757 (206)	0.239 (65)	0.004 (1)	0.739 (198)	0.239 (64)	0.022 (6)	0.37	1.18 (0.83–1.67)
	Sample 2	377	372	0.788 (297)	0.202 (76)	0.011 (4)	0.761 (283)	0.226 (84)	0.013 (5)	0.37	1.15 (0.84–1.58)
	Sample 1 + 2	649	640	0.775 (503)	0.217 (141)	0.008 (5)	0.752 (481)	0.231 (148)	0.017 (11)	0.21	1.16 (0.92–1.47)
rs2078247	Sample 1	272	269	0.526 (143)	0.412 (112)	0.063 (17)	0.576 (155)	0.361 (97)	0.063 (17)	0.35	0.88 (0.67–1.15)
	Sample 2	377	368	0.536 (202)	0.406 (153)	0.058 (22)	0.557 (205)	0.375 (138)	0.068 (25)	0.80	0.97 (0.77–1.22)
	Sample 1 + 2	649	637	0.532 (345)	0.408 (265)	0.060 (39)	0.565 (360)	0.369 (235)	0.066 (42)	0.42	0.93 (0.78–1.11)

Genotype frequencies for cases and controls and allelic *P* values are given. *P* values ≤ 0.05 are italicized. SNPs displaying significant allelic association with migraine with aura are depicted in bold

OR odds ratio, *CI* confidence interval

[a] Sub-samples with significant deviations from HWE

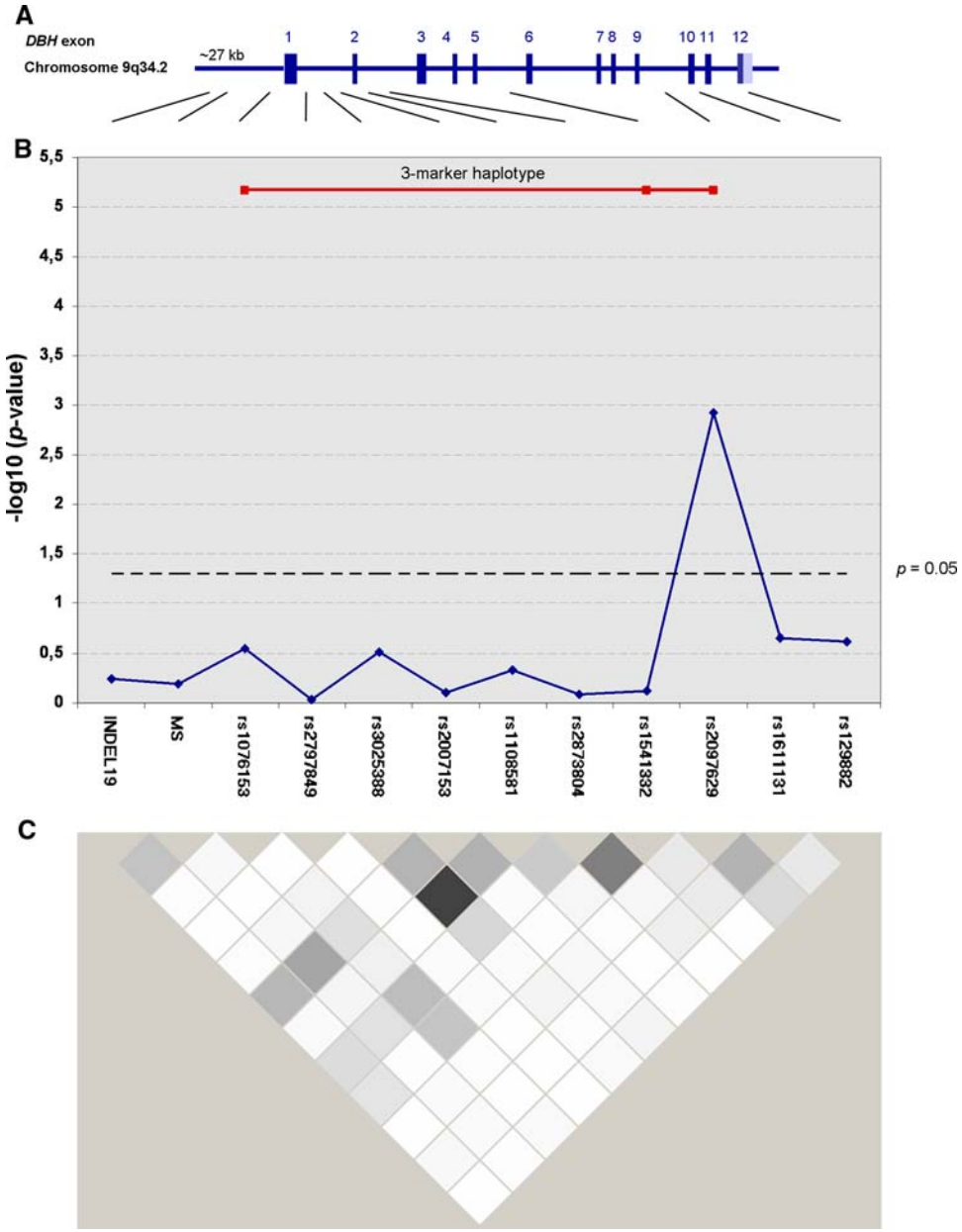

Fig. 1 **a** Schematic illustration of the genomic structure of *DBH*, with coding exons depicted in *dark shading* and UTR regions in *lighter shading*. The exon number is given above the *vertical bars*. The genomic position of each of the genotyped polymorphisms is indicated by the *fan-shaped bars* below the scheme. **b** Results of the association study and haplotype analysis with *DBH*. The −log10 of the allelic *P* values (*y*-axis) are plotted against the respective polymorphisms (*x*-axis). The horizontal *line* indicates the global *P* value of the most significant 3-marker haplotype. **c** Linkage disequilibrium structure of the *DBH* region in our sample. Pairwise r^2-values between markers are shown as calculated by the program haploview (*black* denotes complete LD with $r^2 = 1$, *white* denotes no LD with $r^2 = 0$, *gray* denotes intermediate LD with r^2 between 0 and 1)

to narrow down the genomic interval in which a causal susceptibility variant most likely resides (Cardon and Abecasis 2003; Schaid 2004). Our single-marker analysis in the German samples displayed solid evidence of association for three of 53 markers: one SNP in *DBH* (located in intron 9), one SNP in *DRD2* (located in intron 1) and one SNP in *SLC6A3* (located in intron 14). The association of the latter SNP was identified by genotyping the larger sample 2, highlighting the fact that we had considerably less power to detect a true difference in the first step as compared to step 2 (~80% vs. >98%, see Power Calculation in "Materials and methods"). These associations in three genes from the dopamine system remained significant after gene-wide correction for multiple testing by permutation analysis. However, for none of these SNPs a functional consequence is obvious from the genomic context, with the only currently known mechanisms by which deeply intronic sequence alterations could directly influence the function of the respective gene product being an effect on mRNA-splicing (via altering binding sites for splicing co-factors or by creating novel splice sites) or on binding of transcriptional enhancer/suppressor elements. Thus, the question whether a putative effect on migraine susceptibility would be mediated by these SNPs themselves or by sequence alterations (which could be common as well as rare variants) on the respective risk haplotypes remains unresolved. To answer it, extensive re-sequencing of the critical regions, best delimited by the haplotypes with the most significant global

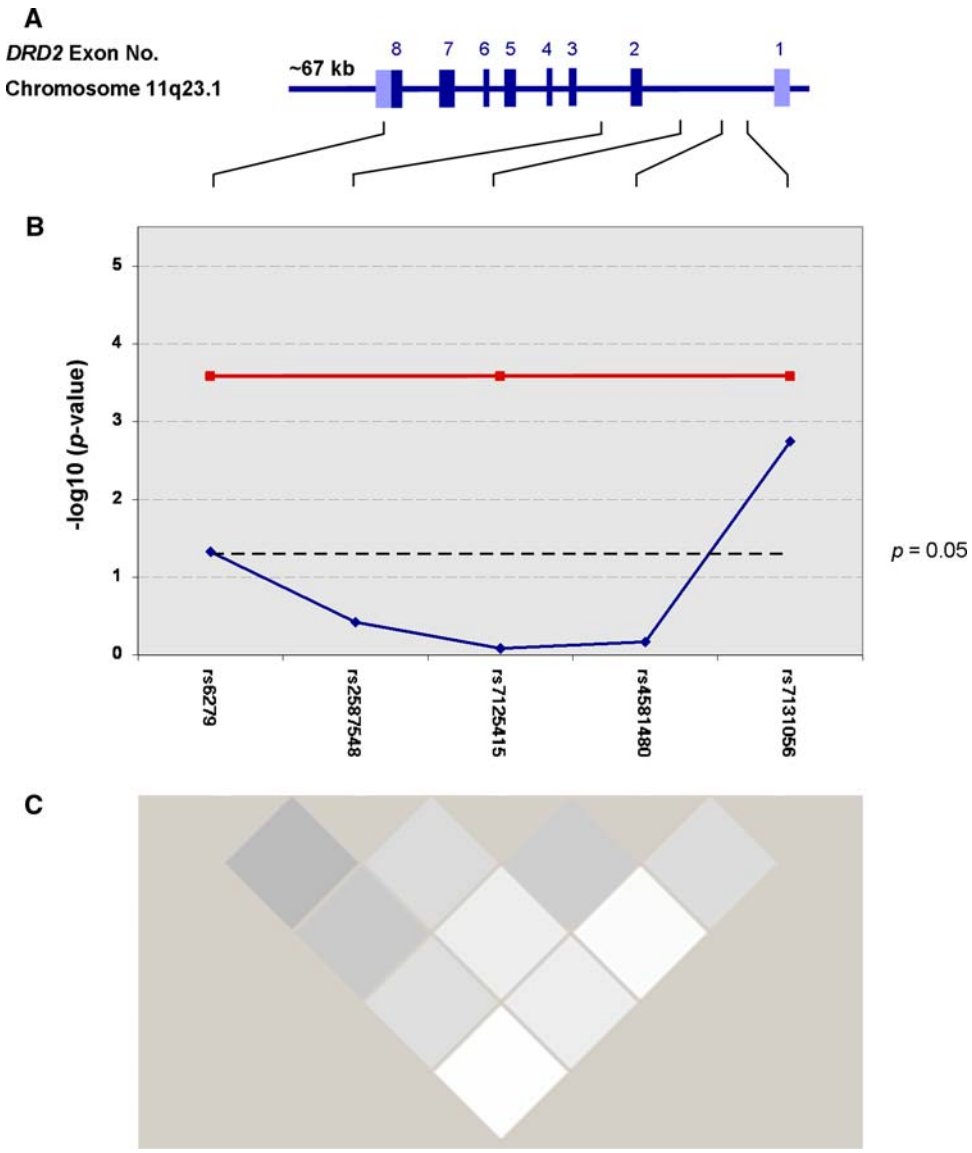

Fig. 2 **a** Schematic illustration of the genomic structure of *DRD2*, with coding exons depicted in *dark shading* and UTR regions in *lighter shading*. The exon number is given above the *vertical bars*. The genomic position of each of the genotyped polymorphisms is indicated by the *fan-shaped bars* below the scheme. **b** Results of the association study and haplotype analysis with *DRD2*. The −log10 of the allelic *P* values (*y*-axis) are plotted against the respective polymorphisms (*x*-axis). The *horizontal line* indicates the global *P* value of the most significant 3-marker haplotype. **c** Linkage disequilibrium structure of the *DRD2* region in our sample. Pairwise r^2-values between markers are shown as calculated by the program haploview (*black* denotes complete LD with $r^2 = 1$; *white* denotes no LD with $r^2 = 0$; *gray* denotes intermediate LD with r^2 between 0 and 1)

P values in our study, in a large number of MA patients will be required. Of note, there are reports on functional polymorphisms at all three gene loci (D'Souza and Craig 2006): the *DRD2* promotor polymorphism-141C Ins/Del (which was not included in this study, see below) affects a putative binding site for the transcription factor Sp1 and is associated with lower transcriptional activity in in vitro reporter assays (Arinami et al. 1997). A 40 bp VNTR polymorphism in the 3′ untranslated region of *SLC6A3* exon 15 (not genotyped in this analysis) has been implicated in regulating expression of the gene in several studies (Fuke et al. 2001; Greenwood and Kelsoe 2003; Michelhaugh et al. 2001; Mill et al. 2005; Miller and Madras 2002). And for DBH, a SNP in the 5′ flanking region of the gene (c.-1021C > T, rs16111115; not included in this study) accounting for 35–52% of the variation in plasma-DBH activity in various human populations was identified through a quantitative-trait analysis (Zabetian et al. 2001). A non-synonymous SNP in exon 11 of the gene (rs6271) appears to independently account for additional variance in plasma-DBH activity (Tang et al. 2006). Furthermore, a (GT)*n* dinucleotide repeat located 4,610 bp upstream from the first ATG of the gene (GenBank Accession X63418; included in this study) has been associated with activity of this enzyme (Wei et al. 1997).

We also performed an additional level of analysis by including genotype data from a large control sample from Great Britain. Recent studies demonstrated the existence of clear genetic differences between European populations (Lao et al. 2008), but the actual difference between the Northern German and the British population seems to be small, which is supported by the χ^2 inflation factor of the comparison of our controls to the WTCCC controls. Therefore, we consider this to be a reasonable approach to

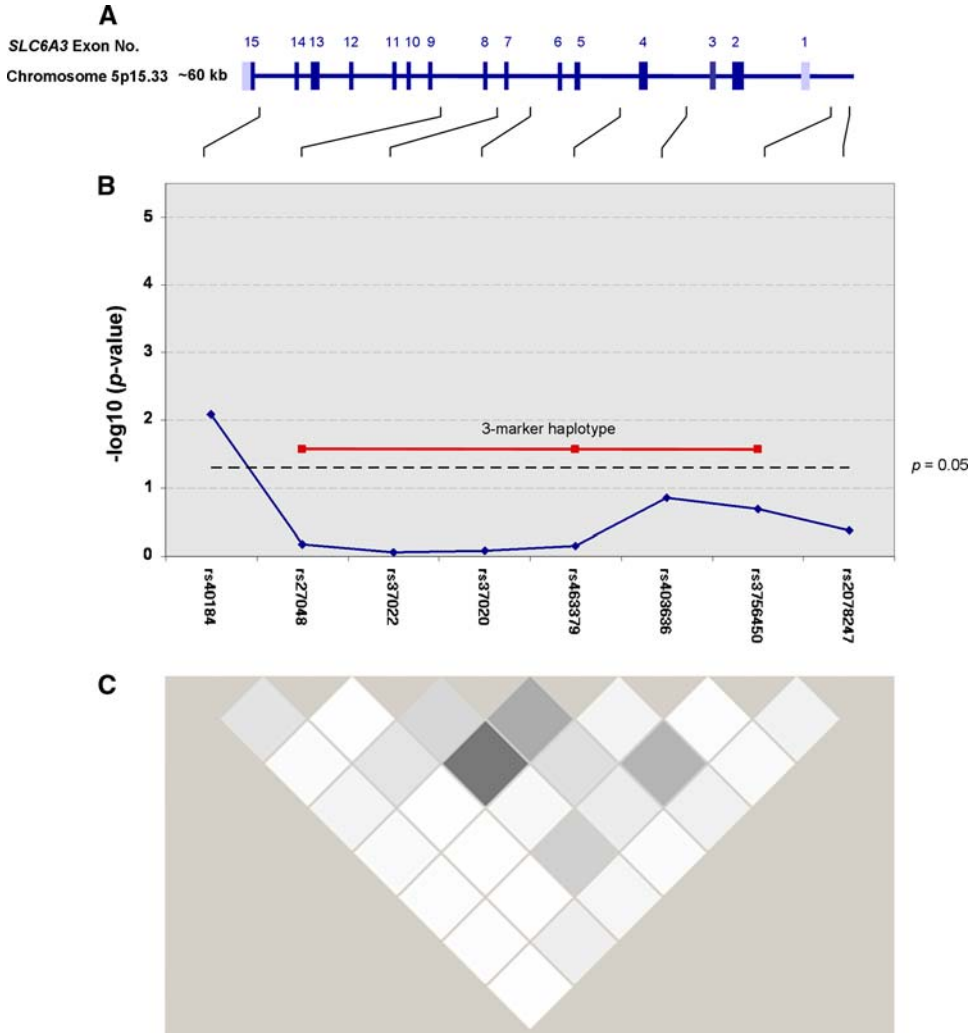

Fig. 3 **a** Schematic illustration of the genomic structure of *SLC6A3*, with coding exons depicted in *dark shading* and UTR regions in *lighter shading*. The exon number is given above the *vertical bars*. The genomic position of each of the genotyped polymorphisms is indicated by the *fan-shaped bars* below the scheme. **b** Results of the association study and haplotype analysis with *SLC6A3*. The −log10 of the allelic *P* values (*y*-axis) are plotted against the respective polymorphisms (*x*-axis). The *horizontal line* indicates the global *P* value of the most significant 3-marker haplotype. **c** Linkage disequilibrium structure of the *SLC6A3* region in our sample. Pairwise r^2-values between markers are shown as calculated by the program haploview (*black* denotes complete LD with $r^2 = 1$, *white* denotes no LD with $r^2 = 0$, *gray* denotes intermediate LD with r^2 between 0 and 1)

possibly affirm or mitigate our findings. Indeed, we could show that allele frequencies between the two control samples were not significantly different for the two SNPs in *DBH* and *SLC6A3*. Interestingly, for both SNPs the allele frequency differences between controls and cases were even larger for the British controls than for the German ones (frequency of allele 1 of rs2097629 was 0.65 in German controls, 0.67 in British controls, and 0.59 in MA patients; frequency of allele 1 of rs40184 was 0.54 in German controls, 0.57 in British controls, and 0.49 in MA patients), which provides an affirmation of our initial findings. Consequently, if we performed a conservative Bonferroni correction of the *P* values obtained from these genotype data—not just for the SNPs genotyped under the dopamine system hypothesis, but also for all other SNPs that we have genotyped so far in our Migraine case-control sample under different hypotheses (we altogether have tested 2,015 SNPs so far, with various genotyping platforms)—the results for *DBH* and *SLC6A3* would still be significant, supporting the role of genetic variation in the dopamine system for MA etiology.

On the contrary, the possible association between MA and the D2 dopamine receptor gene (*DRD2*) SNP rs7131056 was not affirmed by the enlargement of the control sample. Previous genetic studies had reported inconsistent results for several variants at the *DRD2* locus (a silent change at amino acid position His313 of *DRD2* named "NcoI polymorphism" (Dichgans et al. 1998; Peroutka et al. 1997, 1998; Rebaudengo et al. 2004; Stochino et al. 2003), with the underlying SNP meanwhile termed rs61689984; a (possibly functional) insertion/deletion polymorphism in the promotor region designated "-141C Ins/Del" (Maude et al. 2001); and an intronic dinucleotide repeat (Del Zompo et al. 1998; Stochino et al. 2003). None of these markers was included in the HapMap project and information on possible linkage disequilibrium (LD) between these previously genotyped polymorphisms and the haplotype-tagging SNPs tested in our study are not available. However, an association between *DRD2* and MA seems less likely in our opinion, and our association of *DRD2* might be better explained as a false positive result due to random fluctuations in our German control sample.

The dopamine β-hydroxylase gene (*DBH*) on chromosome 9q34 encodes the enzyme that catalyzes the conversion of dopamine to norepinephrine. It has been associated with migraine previously in a Caucasian sample from Australia (Fernandez et al. 2006; Lea et al. 2000). However, both genetic variants that were tested positive in these earlier studies (a possibly functional dinucleotide microsatellite marker and an adjacent 19-bp deletion/insertion polymorphism in the promotor region of *DBH*) did not display significant differences in allele distribution in our study. The association of the dinucleotide microsatellite marker could also not be replicated in an Italian migraine sample (Mochi et al. 2003). The *DBH* SNP rs2097629 with strongest evidence for association in our analysis was not included in any of these studies. Whether these discrepancies between the different samples with respect to the two *DBH* promotor polymorphisms reflect e.g., differences in the genetic architecture of the disease between the study populations or should be interpreted as a hint for false-positive results in the previous studies is currently unclear. Of note, the number of participants was by far highest in our association study. Based on the Hapmap Phase II data, no significant LD exists between rs2097629 and the putatively functional *DBH* SNP rs1611115 ($D' = 0.15$ and $r^2 = 0.01$), whereas rs6271 (the second *DBH* SNP with earlier evidence for a functional relevance) is in LD with rs2097629 as measured by D', but shows no allelic correlation with rs2097629 ($D' = 1$, $r^2 = 0.04$). This suggests that the MA association of rs2097629 is not directly mediated by rs1611115 or rs6271.

Our finding that the dopamine transporter gene *SLC6A3* (also named *DAT1*) is significantly associated with MA is in contrast to previous genetic association studies on this gene (Karwautz et al. 2008; McCallum et al. 2007; Mochi et al. 2003). However, these studies tested VNTR polymorphisms (among these the 3′ UTR polymorphism with evidence for an effect on gene expression), which can only partially tag SNPs (Payseur et al. 2008). These negative reports are therefore not in contradiction to the results of our study. Furthermore, the previous studies were based on case-control samples recruited in Italy, Austria, and Australia, raising the possibility that population-specific differences in disease etiology account for the discordant results. SLC6A3 mediates the active reuptake of dopamine from the synapse and is a major regulator of dopaminergic neurotransmission. Disturbances of this transport mechanism are well compatible with current models on migraine pathophysiology (Akerman and Goadsby 2007).

In the last 5 years, several genome-wide linkage studies for complex forms of migraine or trait components were performed which resulted in the identification of putative susceptibility loci on chromosomes 1, 3, 4, 5, 6, 8, 10, 11, 13, 14, 15, 17, and 18. For none of the loci the causative gene or genetic variant has been identified, and only the loci on 4q21-q31, 10q22-q23, 15q11-q13 and 18q12 have been replicated in independent analyses in different family samples. The two genes of the dopamine system with strongest evidence for association in our study (i.e., *SLC6A3* on 5p15 and *DBH* on 9q34) are not located near any of the putative linkage loci. Also *DRD2* on 11q23 is located outside the 1-LOD interval of the putative and non-replicated locus on 11q24 (Cader et al. 2003), making an involvement of these genes as candidates for known linkage loci unlikely.

In conclusion, we provide genetic evidence for an association of two genes of the dopamine system with migraine with aura. The design of our study and the magnitude of the association are well compatible with real disease susceptibility. Moreover, an important advantage of our haplotype-based approach is that the results of this study can easily be incorporated into the analysis of future genome-wide association studies for migraine, as many SNPs on current DNA-Chip-platforms are haplotype-tagging SNPs and will therefore overlap with our SNP selection or will be in linkage disequilibrium with these SNPs. Indeed, two of the three SNPs displaying evidence for association with MA in our initial study (rs7131056 and rs40184) have been integrated into Affymetrix's Genome-Wide Human SNP Array 6.0 and Illumina's Human 1 M-Duo chip, and the third one, rs2097629, is in complete LD ($r^2 = 1$) with rs2097628 on Illumina's genotyping platform, allowing a future joint analysis of data.

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