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# Research Submission

# No Influence of 5-HTTLPR Gene Polymorphism on Migraine Symptomatology, Comorbid Depression, and Chronification

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**Background.—The serotonergic system is thought to play an important role for mediating susceptibility to migraine and depression, which is frequently found comorbid in migraine. The functional polymorphism in the serotonin transporter gene linked polymorphic region (5-HTTLPR/SLC6A4) was previously associated with attack frequency and, thus, possibly with chronification.**

**Objective.—We hypothesized that patients with the "s" allele have higher attack frequency and, paralleling results in depression research, higher scores of depression.**

**Methods.—Genetic analysis of the SLC6A4 44 bp insertion/deletion polymorphism (5-HTTLPR) was performed in 293 patients with migraine with and without aura. Self-rating questionnaires were used for assessment of depression.**

**Results.—Multinomial logistic regression analysis found no evidence for association of the 5-HTTLPR polymorphism with either depression or migraine attack frequency.**

**Conclusion.—We were not able to demonstrate any influence of the serotonin transporter 5-HTTLPR polymorphism on migraine phenomenology (attack frequency or comorbid depression), thereby excluding this variant to be a common genetic denominator for chronic migraine and depression.**

**Key words: migraine, serotonin transporter, polymorphism, depression, chronification**

**Abbreviations: 5-HT serotonin, 5-HTTLPR serotonin transporter linked polymorphic region, ICHD-II International Classification of Headache Disorders Version II, MA migraine with aura, MO migraine without aura, CM chronic migraine, TTH tension-type headache, DMKG Deutsche Migräne und Kopfschmerzgesellschaft (German Headache Society), BDI Beck Depression Inventory, CES-D Center for Epidemiologic Studies Depression Scale**

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*Conflict of Interest:* None

Serotonin (5-hydroxytryptamine, or 5-HT) and the serotonergic system are believed to play an important role in the pathophysiology in several neuropsychiatric disorders such as anxiety,[1] depression,[2,3] obsessive-compulsive disorder,[4] drug addiction,[5,6] and also headache.[7-9] This is based to a great part on the effect of drugs acting upon this system to modulate the course of disease (selective serotonin reuptake inhibitors and tricyclic antidepressants for depression and 5-HT 1B/1D agonists for migraine) and to a lesser part to experiments using brain imaging techniques[10,11] and animal models.[12]

After release of 5-HT from axonal terminals of serotonergic neurons, its synaptic effects are terminated by reuptake into the nerve endings.[13] This reuptake is mediated by a 5-HT transporter *SLC6A4* (also commonly known as *SERT*, *5HTT*, or *5-HTT*), which is mainly expressed in serotonergic neurons in the raphe nuclei of the pons and upper brainstem,[14] structures that have been shown to be important in migraine pathogenesis.[15-17] Transcriptional activity and, in consequence, reuptake capacity are genetically modified by several positive and negative regulatory elements within the *SLC6A4* promoter region and by a 17 bp variable number tandem repeat element in intron 2.[18-21] Humans carry a common 44-base pair insertion/deletion polymorphism in the promoter region of the *SLC6A4* gene (serotonin transporter linked polymorphic region; 5-HTTLPR) that is found in 2 forms, long (l) and short (s). Individuals with either 1 or 2 copies of the s-allele (40-70% of a given population) appear to have fewer 5-HT transporters than individuals with an l/l genotype. Furthermore, the l/l genotype is associated with a 1.4 to 1.7 times increase in mRNA and an about twofold increase in uptake capacity.[22,23]

Familial hemiplegic migraine, a rare and severe subtype of migraine with aura (MA), is transmitted in an autosomal dominant fashion; however, the common forms of migraine, comprising migraine without aura (MO) and migraine with typical aura (International Classification of Headache Disorders Version II [ICHD-II] 1.1, 1.2.1-3), follow a complex pattern of inheritance and are multifactorial in etiology. Bearing that in mind, it seemed an interesting hypothesis that alterations in 5-HT metabolism might predispose to migraine attacks; studies in migraine patients, however, gave contrasting results.[24-28] It was shown in one study that the s-allele is associated with higher risk to develop an MA phenotype compared with controls,[29] but other studies could not replicate these results.[30,31] Interestingly, although, in the latter study there was significant association with attack frequency.

Results are more consistent regarding the role of this *SLC6A4* polymorphism in depression. There was no association between a certain genotype and depression per se; yet, individuals with a "risk" genotype are clearly more prone to develop depression in response to stressful life events.[23,32-35] This suggests that the presence of additional interacting genetic and/or environmental factors is a prerequisite for the 5-HTTLPR genotype to be effective in the etiology of depression.[36]

Comorbidity of migraine headache and major depression has been reported in up to 80% of patients[37-40] and it contributes significantly to disability for each individual.[40] Studies have suggested a shared common risk factor increasing the probability of acquiring either condition rather than finding a causal relationship between migraine and depression.[37,39,41-43]

The aim of this study was to investigate whether 5-HTTLPR genotype is a genetic factor relating migraine and depression. Our first hypothesis is that, rather than causing migraine itself, the effect of a 5-HTTLPR "risk" genotype would be (1) comorbidity of depression; and (2) higher attack frequency when migraine is present.

As chronification in headache is in contrast to other pain syndromes defined by headache frequency, we then further hypothesize that 5-HTTLPR genotype is a genetic risk for frequent headaches and thus should be associated with headache chronification.

## PATIENTS AND METHODS

A first set of patients was recruited from 3 university-based headache clinics (departments of Neurology of the Universities of Vienna, Münster, and Halle); a second set was included originating from a large, cross-sectional, population-based study conducted by the German Headache Society between

2000 and 2005 to investigate prevalence of headache in Germany (DMGK). Migraine was diagnosed in all patients according to the International Headache Society (IHS) criteria of 2003 (ICHD-II);[44] only patients with MA (IHS 1.2.1-3), MO (IHS 1.1), and chronic migraine (CM, IHS 1.5.1) were included. The study and the use of patients for the present investigation were evaluated and approved by each local ethics committee separately; all patients gave their written informed consent prior to entering the study.

Age- and sex-matched controls were taken from the headache prevalence study and comprised 2 groups (1) of individuals without any kind of headache; and (2) patients with tension-type headache (infrequent, frequent, and chronic) (TTH) (IHS 2.1-3).

In patients, migraine frequency, age at onset, duration and course of disease, migraine characteristics (attack duration, intensity, accompanying symptoms, triggers, type of aura when present), other headaches, and other diseases (diabetes, thyroid, cardiac, etc) were carefully recorded. Family history and medication for headache (type, dosage, prophylaxis) as well as for other conditions were also documented. Patients with concomitant diseases prone to depression (eg, cancer, chronic low back pain) were not included.

To compare our results with those published by Kotani et al,[31] patients were grouped according to attack frequency with group I having less than 2 attacks per month, group II with between 2 and 3.5 attacks per month, and group III with more than 3.5 attacks per month.

**Depression.—**To investigate depression, self-rating questionnaires were used. All patients completed the "Beck Depression Inventory" (BDI) or the "Allgemeine Depressions Skala," the German version of the Center for Epidemiologic Studies Depression Scale (CES-D), which are both validated screening tools for depressive symptoms and consist of items assessing fatigue, withdrawal, hopelessness, and listlessness among others.[45-48]

Patients were grouped in 3 groups regarding depression as set out in the manual for the BDI; this assignment is validated in numerous studies and has been proven useful for pain research and has been adapted for the CES-D. Group I is normal, with no indication of depression (0-10 points BDI, 0-16 CES-D), group II includes patients with mild to moderate depression (11-17 points BDI, 17-23 CES-D), and group III includes patients with clinical relevant depression (>17 points BDI, 24-60 CES-D).[45]

**Genetics.—**Venous blood samples were taken from all participants and genomic DNA extracted according to standard procedures.[49] The *5-HTTLPR* polymorphism is a 44 bp insertion/deletion polymorphism in the untranslated promotor region of the 5-HT transporter gene *SLC6A4* located about 1 kilobase upstream; it can be easily detected by PCR amplification of the region using flanking primers. Extracted DNA was amplified according to the protocol published by Lesch et al.[23] Two fragments are generated according to the presence or absence of the 44 bp long element containing 16 repeats; they have a length of 484 and 528 base pairs (short and long allele), respectively, and were visualized on a 2% agarose gel after ethidium bromide staining.

**Statistical Analysis.—**The sample size calculation was based on the comparison between 2 complementary genetic groups (either the s/s + s/l vs l/l or s/s vs s/l + l/l) with regard to the migraine attack frequency per month. Assuming a group difference of 1.5 attacks per month, a standard deviation of 3 for the monthly migraine attack frequency, and a minimum sample size fraction of 13% for the homozygotic groups (either s/s or l/l), the *t*-test at level 0.05 would have a power of at least 80% with a total of 285 patients. The assumption of a minimum sample fraction of 13% in the homozygotic groups is in accordance with the frequencies observed in other samples being at least 12.9%.[29] Assuming a dropout rate of 5%, the sample size was fixed at 300 patients.

Using the observed allele frequencies, expected genotype frequencies according to Hardy–Weinberg law were calculated and compared with observed genotype frequencies. Patients from the 3 recruiting centers and the DMKG study cohort were compared with respect to the baseline characteristics (age, sex, and genotype) and the response variables *frequency of migraine attacks per month* and *depression*. Quantitative variables are summarized by means ± standard

deviation, qualitative variables by counts and percents.

The frequency of migraine attacks per month was analyzed on a logarithmic scale by a 2-way analysis of variance with the factors origin and genotype (additive and dominance terms) and the interaction between the factors. Depression was analyzed by a proportional odds logistic regression analysis with the factors genotype and origin, including the interaction term (analysis of deviance). Within the DMKG cohort the association of genotype with migraine vs no migraine, including TTH and migraine vs no migraine excluding TTH were analyzed by logistic regression analyses. The association between genotype and MO as well as between genotype and MA was analyzed by similar logistic regression analyses. *P* values below .05 are considered nominally significant.

Multinomial logistic regression[50] was used to study the cross-sectional association of depression risk and frequency of migraine attacks with the *SLC6A4* 44 bp insertion/deletion polymorphism. In the multinomial logistic regression, the response variable *depression risk* was coded as 1 (normal), 2 (mild to moderate depression), and 3 (clinical relevant depression). The response variable *migraine attack frequency* was coded analogous to Kotani et al (2002): 1 = 0 to <2, 2 = 2 to 3.5, and 3 ≥ 3.5 attacks/month. The stimulus (ie, explanatory) variable *SLC6A4-polymorphism* was coded according to Table 1. In addition, analogous to Kotani et al,[31] the mean *migraine attack frequency* scores between group S (s/s genotype) and group L (s/l, l/l genotype) were compared by a *t*-test. $P < .05$ was considered nominally significant. All statistical analyses were performed using the R statistical package.[51]

**Table 1.—Variable Coding for the 5-HTTLPR Polymorphism**

| Model | s/s | s/l | l/l |
|---|---|---|---|
| Additive effects | –1 | 0 | +1 |
| Dominance effects | –1 | +2 | –1 |
| Dominant MOI for allele s | 1 | 1 | 0 |
| Recessive MOI for allele s | 1 | 0 | 0 |
| Dominant MOI for allele l | 0 | 1 | 1 |
| Recessive MOI for allele l | 0 | 0 | 1 |
| Allelic for allele s | 2 | 1 | 0 |

The allelic model is coded as a numeric variable (ie, integer). All other models are coded as a factor (ie, categorical) variable. Because the dominant MOI for allele s is equivalent to recessive MOI for allele l, and the dominant MOI for allele l is equivalent to recessive MOI for allele s, only results for the dominant and recessive MOI for allele s are reported.
5-HTTLPR = serotonin transporter linked polymorphic region; MOI = mode of inheritance.

## RESULTS

Out of the original 295 study patients, 293 patients were analyzed; 2 patients had to be omitted because of incomplete data sets. Patient characteristics are given in Table 2 for the 4 patient cohorts detailed above.

**Clinical Findings: Headache.—**In total, 253 patients (86%) were female; 58 had MA (20%) and 235 MO; the mean age of participants was 43.3 years. Mean attack frequency per month was 3.7, with 75% having 1 attack per week or less and 17% having between 4 and 10 attacks per month; 7 patients (2%) fulfilled the criteria for CM (more than 15 headache days/month). When grouped according to the Kotani study (see above), 95 patients were in group I (16 MA and 79 MO), 93 in group II (22 MA and 71MO), and 105 in group III (20 MA and 85 MO). Mean attack duration was 33.5 hours; mean attack intensity was 6.9 on the 11-point visual analog scale. Participants had a mean of 10 days per month with headache and a mean disease duration of 25 years. Family history with at least 1 first or second degree relative also suffering from migrainous headache was positive in 123 patients (27 MA and 96 MO).

In 83 (28%) patients, other forms of headache were present in addition to migraine; cervicogenic headache was diagnosed in 3, cluster headache in one of the cases, 60 patients suffered from a infrequent or frequent episodic tension-type headache (eTTH) (IHS 2.1 and 2.2) and 8 from chronic tension-type headache (cTTH) (IHS 2.3). Eleven patients reported overuse of acute relief medications (IHS 8.2).

The samples from the different university clinics did not differ in any of the aspects investigated (Table 2); patients recruited from the epidemiological study were markedly less affected compared with patients recruited in the university outpatient clinics. Accordingly, the factor "origin" was highly significant

**Table 2.—Characterization of the Headache Cohort**

| Origin | DMKG (n = 94) | Halle (n = 53) | Münster (n = 99) | Vienna (n = 47) | All (n = 293) |
|---|---|---|---|---|---|
| Age | 44.78 ± 10.49 | 47.40 ± 11.13 | 40.62 ± 12.30 | 41.85 ± 14.14 | 43.31 ± 12.10 |
| Sex, n (%) | | | | | |
| Female | 80 (85) | 45 (85) | 86 (87) | 423 (89) | 253 (86) |
| Male | 14 (15) | 8 (15) | 13 (13) | 5 (11) | 40 (14) |
| Attacks per month | 2.28 ± 3 .56 | 5.63 ± 4.52 | 3.53 ± 2.60 | 4.87 ± 3.67 | 3.71 ± 3.68 |
| Disease duration | 27.02 ± 11.94 | 20.17 ± 8.31 | 23.60 ± 11.05 | 21.47 ± 16.03 | 25.00 ± 12.98 |

Summary of quantitative characteristics (mean ± standard deviation, percents, and counts in each cohort). Age and disease duration in years. Patients from the different sites did not differ significantly in all aspects relevant for the study except that patients from the epidemiological study had markedly less attacks per month.

($F = 26.19$, d.f.1 = 3, d.f.2 = 280, $P < .0001$) in the 2-way ANOVA for the frequency of migraine attacks.

**Clinical Findings: Depression.—**Depression according to the study criteria outlined above was absent in 185 patients (63%), and mild depression was found in 60 (20%); 51(17%) patients had clinically relevant depression. There were no significant differences regarding depression between university-based outpatient clinics. However, in patients of the epidemiological study, the status "no depression" was found more frequently although the difference between the cohorts is not significant in the proportional odds regression analysis for depression (chi-square test = 6.43, d.f. = 3, $P = .093$). In general, grade of depression increased with attack frequency but not with duration of disease, but failed to reach significance (Figure).

The status "no depression" and "mild depression" was equally distributed over migraine groups with rare attacks (Kotani groups I and II, ie, less than 4 attacks per month); the number of patients with "clinically relevant depression" increased with attack frequency (group I n = 10 [10%], group II n = 16 [17%], and group III n = 25 [24%]). Some 50% of patients with clinically relevant depression were found in the high attack group (Kotani group III). However, the difference failed to reach significance ($\chi^2 = 8.59$, d.f. = 4, $P = .073$).

**Genetic Analysis.—**Overall, the 5-HTTLPR polymorphism genotypes were distributed according to Hardy-Weinberg Law ($\chi^2 = 0.0029$, d.f. = 1, $P = 1$) in controls and patients.

**No Association of 5-HTTLPR Genotype With Migraine Status.—**Within the cohort from the epidemiological study, no statistically significant association was found between genotype and migraine ($\chi^2 = 1.27$, d.f. = 2, $P = .53$); this was true also when controls with TTH were excluded (controls without TTH: $\chi^2 = 1.34$, d.f. = 2, $P = .51$, data not shown). Further, no association of genotype was found with migraine subtypes MA ($\chi^2 = 2.28$, d.f. = 2, $P = .32$) or MO ($\chi^2 = 1.24$, d.f. = 2, $P = .54$) (data not shown). To investigate the influence of possible bias introduced by our use of clinic-based controls, only the subset of patients recruited for the epidemiological study (n = 93) were analyzed. This provided a representa-

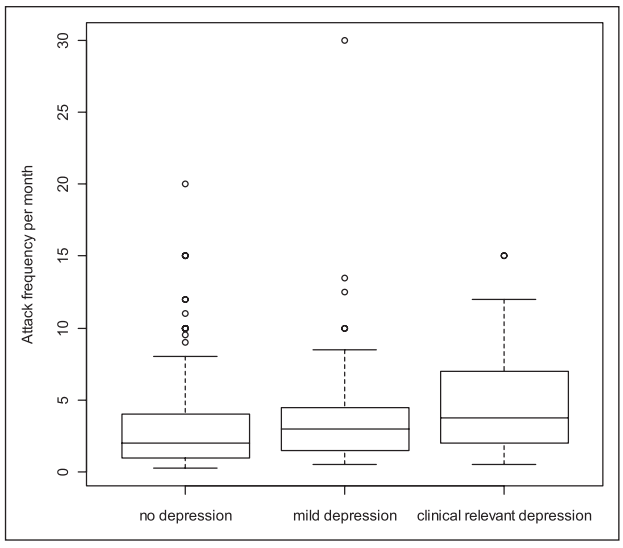


**Figure.—As could be shown in several studies before, depression increases with headache severity.**

**Table 3.—No Association of Any Genotype With Depression or Attack Frequency**

| | s/s (n = 48) | l/s (n = 140) | l/l (n = 104) |
|---|---|---|---|
| Depression, n (%) | | | |
| No depression | 34 (71) | 88 (63) | 61 (59) |
| Mild depression | 7 (15) | 25 (18) | 27 (26) |
| Clinically relevant | 7 (15) | 27 (19) | 16 (15) |
| Attacks per month | 2.81 ± 2.22 | 3.51 ± 3.13 | 4.43 ± 4.68 |

The *P* value in the proportional odds regression analysis is *P* = .53, indicating no statistically significant interaction in the study population between genotype and depression. There was even a negative association between the s/s genotype and attack frequency, but failed to be significant (2-way ANOVA *P* = .07).

tive control group not only matched to ethnicity, age, and sex but also recruited in the identical way (controls n = 244, with n = 118 "no headache" and n = 126 "infrequent or frequent tension-type headache").

**No Association of 5-HTTLPR Genotype With Migraine Attack Frequency or Depression.—**As shown in Table 3, no positive association between any genotype and the frequency of migraine attacks was found; results even indicate a negative association, although this result does surpass our nominal significance threshold in the 2-way ANOVA ($F = 2.65$, d.f.1 = 1, d.f.2 = 280, *P* value = .07; no statistically significant interaction term). The trend toward a negative association was most pronounced in the data of the epidemiological study and stronger in the data from the Viennese outpatient clinic, but was not well or not at all detected in the other 2 university centers (data not shown). There was also no association of any genotype with depression.

**Multinomial Regression Analysis Does Not Identify an Interaction Between 5-HTTLPR Genotype With Depression Risk and Migraine Phenotype.—**Multinomial logistic regression analysis found no evidence for association of the 5-HTTLPR polymorphism with either *depression risk* or *migraine attack frequency* (Kotani groups), with all tests producing *P* values > .05. There was no significant difference in *migraine attack frequency* score between group L (2.05 ± 0.05) and group S (1.94 ± 0.12). The mean score for l/l individuals (2.12 ± 0.08) also did not differ from s/s, s/l individuals (1.98 ± 0.06).

## DISCUSSION

Detecting gene effects with only small possible impact on disease phenotype is still a difficult task. Increasing sample size to increase power also increases genetic heterogeneity, hampering the detection of possible small influence of a certain genetic predisposition. The variety of publications reporting contrasting results from association studies as well as the obvious difficulty in replicating previous findings is visible expressions of that dilemma.

Migraine, although a unique headache syndrome, displays enormous clinical variability, regarding not only pain intensity, attack duration, and frequency of attacks but particularly its accompanying symptomatology such as premonitory (prodromal) symptoms, trigger factors, drug response rate, aura, and kind of aura, as well as its comorbid traits and states. A number of genetic loci have been implicated on chromosomes 1q31, 4q24, 11q24, 15q11-13, 6p12.2-21.1, 5q21, 10q22-23, 14q21.2-22.3, Xq24-28, and 19p13.3 (for review see the study by Wessman et al[52]), indicating a high degree of genetic heterogeneity. Although genetic association studies are a valid tool to identify disease relevant genes beyond family-based linkage studies in complex genetic, multifactorial, and possibly heterogenic diseases, pronounced genetic heterogeneity might prevent its ability to come up with consistent results.

The present study, using a large cohort of headache patients, failed to confirm the hypothesis, derived from results from earlier reports, that a specific genetic background regarding the serotonergic system is an influencing factor on disease phenotype of either migraine or depression. The functionally relevant 5-HT transporter gene polymorphism was not associated significantly either with migraine attack frequency or with presence or absence of depression in our patients. Sample size reached the numbers as calculated, so that we are confident that the results can be validated as "true," under the given uncertainties, which are commonly accepted for this kind of studies.

**Study Sample and Between Center Variance.—**Increasing sample size is one approach to achieve sufficient power in association studies; to meet that goal, the cooperation of more than 1 center for recruiting patients is often necessary and genetic heterogeneity will be increased. In our study we included patients from different parts of Europe; careful comparison of all clinical and epidemiologic characteristics failed to show differences that one would expect relevant to the analysis. The most striking difference was, not unexpectedly, that patients from the epidemiological study were markedly less affected; this is easily explained because most severely affected patients are more likely to seek help from a highly specialized, university-based outpatient department. Health providers requiring referral from a general practitioner to the university-based clinics add to this bias.

**Migraine and SLC6A4 Polymorphism.—**The central serotonergic system is located in the raphe nuclei and the adjacent reticular formation in the brain stem. It is known to play an important role in pain processing and pain modulation; yet, its exact role in headache and specifically migraine is not fully understood. Intravenous infusions of 5-HT can terminate migraine attacks. A possible way of action is inhibition of trigeminal nucleus activity as could be experimentally shown when this nucleus was activated by electrical stimulation of the sagittal sinus, an experimental model thought to reflect migraine mechanisms closely.[53] Further evidence of the importance of the central serotonergic system for migraine was presented recently by demonstrating, using a highly specific SERT radio ligand in a single photon emission tomography study, that there is a significant increase of brainstem SERT protein availability in migraineurs compared with controls, suggesting a dysregulation of the brain stem serotonergic system in these patients.[54]

However, variation in transport capacity of 5-HT, regulated by the polymorphism in the promoter of the 5-HT transporter gene, does not appear to be a mechanism with relevant consequences regarding the phenotype as more recent, large studies fail to replicate earlier results that had implicated this gene.[30] In accordance with these findings, we were not able to show genetic association of a *SLC6A4* polymorphism with the presence or absence of migraine compared with controls. To avoid bias because of insufficiently matched controls, we selected only probands recruited for the epidemiological study for this subanalysis, accepting a reduced power for this analysis because of the reduced remaining sample size. Thus, we were able to make use of a control group that was carefully matched not only for sex and age, but also with respect to recruitment. Using anonymous controls such as blood donors or other clinical populations, as is often performed, the prevalence of the phenotype in question is unknown, possibly influencing the results. A further strength in our approach is that controls were all carefully phenotyped so that the presence of the disease under investigation was excluded, which is especially important when studying a disease such as migraine with a high prevalence in the general population.

**Depression and the SLC6A4 Polymorphism.—**The SLC6A4 polymorphism was not associated with the presence or absence of depression in our sample confirming results from numerous studies conducted on this topic with mostly negative results.[55-60] As stated in the introduction, however, the presence of the s-allele seems to be a genetic risk for developing depression in response to life events, which is one finding that could be consistently replicated.[23,32-35,61] The probably complex interaction that results in depression in certain individuals with a risk allele in response to life events, is obviously not relevant in chronic pain patients as our results show.

The degree of depression increased with "headache load" (ie, headache days and intensity/month) in our sample. This is in line with previous findings, showing association of "severe" headache (including CM[62-64]) and major depression (with severe headache being defined as high average pain intensity and long duration [total lost time of activity] independent of headache diagnosis). While this association is primarily in only one direction, from severe headache to major depression,[42,65] when looking at migraine alone, it was shown that there is no direct *causal relation* of migraine and depression but there seems to be a *bidirectional association*.[37,41]

The mechanism behind this bidirectional association might be a genetic predisposition, for which the

5-HTTLPR is a plausible candidate. However, our results do not support the 5-HTTLPR polymorphism as a genetic factor acting to explain the presence or absence of depression.

We further investigated an interrelation of occurrence and/or degree of depression and migraine attack frequency depending on a specific genetic background, postulating that co-occurrence of depression in patients with a high headache load could be caused by a common genetic denominator; this model would explain the high comorbidity of both disorders without a direct causal relationship. Despite the serotonergic system being a most attractive candidate,[66] however, no such interaction could be shown in the sample used in the present study.

**Risk Factors for Chronification of Pain or Migraine.—**The processes behind chronification of pain in general and in migraine are not well understood. Some risk factors for chronification have been indentified such as obesity, hypertension, and stressful life events; psychological factors like chronic distress in daily life, depression, pain-related cognition, and coping behavior seem to play an important role, too.[67-69] It is quite likely that genetic factors are also involved in pain chronification. Heightened pain sensitivity because of altered pain-related neurotransmission pathways, differing sensitivity to drugs, comorbidity, and behavioral peculiarities regarding drug intake, drug dependence, and possibly pain coping can be genetically modified. Research to date has focused on genes involved in serotonergic neurotransmission like *SLC6A4*, *COMT*, *MAOA*, and 5-HT receptors, and also on dopamine, inflammatory mediators, and opioid receptors, and deals largely with generalized pain syndromes like chronic widespread pain and fibromyalgia. Although results in some studies were significant, it could often not be confirmed and no single gene gave convincing evidence for a significant role in pain chronification.[70]

It was on this background that we were intrigued to see Kotani reporting in their study of Japanese migraine patients that there was also no association of the 5-HTTLPR polymorphism with the presence of migraine itself but that a certain genotype was associated with high migraine attack frequency. In contrast to other pain disorders, such as chronic back pain, chronification in headache is defined not merely by disease duration but by the frequency of the occurrence of headache (headache days per month). Our results from the present study fail to show that a certain genetic background acts on migraine attack frequency (expressed as headache days or attack frequency), and that the 5-HTTLPR polymorphism is not a genetic risk factor for migraine chronification. One reason for us not being able to replicate these results might be that distribution of alleles in the particular Japanese sample was skewed in comparison with our data with 60% of the s/s genotype present, in contrast to our (16%) and other European samples (11-33%).[24,25,28,29,31,71,72]

## CONCLUSION

Genetically and clinically, migraine is a highly complex syndromatic disease, with comorbid neuropsychiatric symptoms playing an important part not only for the amount of the individual patients suffering but also for therapeutic strategies and, eventually, therapeutic success. Comorbid depression and anxiety disorders are detected in up to 80% of migraineurs; unravelling the complex interaction of the two would improve patient care enormously. Among many neurotransmitters in the brain, the serotonergic system has been most convincingly implicated in migraine and depression pathophysiology; 5-HT neurotransmission is genetically regulated at the level of both its biosynthesis and reuptake.[66] Numerous studies indicated a role of the 5-HTTLPR polymorphism in the pathophysiology of migraine.[24,25,29,71] However, in our large sample of migraineurs, we could demonstrate that the 5-HT transporter 5-HTTLPR functional polymorphism does not play a significant role in this system influencing migraine phenomenology. Further studies using large and homogeneous samples are required to provide evidence for other genetic factors, possibly other genes involved in neurotransmitter metabolism and regulation, playing a role both in migraine, its comorbidity, and chronification.

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