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STin2 VNTR polymorphism in the serotonin transporter gene and migraine: pooled and meta-analyses

Markus Schürks · Pamela M. Rist · Tobias Kurth

Abstract Data on the association between the *SLC6A4* STin2 VNTR polymorphism and migraine are conflicting. To perform pooled and meta-analyses, we searched for studies published until September 2009 using electronic databases (MEDLINE, EMBASE, Science Citation Index) and reference lists of studies. Assessment for eligibility and extraction of data was performed by two independent investigators. We extracted allele and genotype frequencies for each study. We then calculated study-specific and pooled odds ratios (OR) and 95% confidence intervals (CI) assuming allele and genotype models. We also calculated pooled ORs and 95% CIs based on study-specific effect estimates for the allele model. We included five studies investigating the association between the STin2 VNTR polymorphism and migraine. Results from the allele model suggested a protective effect against migraine for the STin2.9 and STin2.10 alleles compared to the STin2.12 allele among populations of European descent, which however was not significant. Results from the genotype model indicated a significant ∼25% reduced risk for migraine among carriers of the 10/12 genotype compared with carriers of the 12/12 genotype among all study populations (OR = 0.76, 95% CI 0.60–0.97) for any migraine, which was more pronounced among populations of European descent (OR = 0.68, 95% CI 0.53–0.87). Results for migraine with and without aura were of similar magnitude, but were not statistically significant. Our results suggest a protective effect of non-STin2.12 alleles compared to STin2.12 alleles, respectively, 10/12 and 10/10 genotypes compared to the 12/12 genotype against migraine among populations of European descent. Associations in non-European populations may differ.

Keywords Migraine · Serotonin transporter · *SLC6A4* · STin2 VNTR · Meta-analysis

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Abbreviations

SLC6A4	Serotonin transporter
Bp	Base pair
STin2 VNTR polymorphism	Polymorphism consisting of a 17 bp variable number of tandem repeats in intron 2 of the *SLC6A4* gene
OR	Odds ratio
CI	Confidence interval
MA	Migraine with aura
MO	Migraine without aura

IHS	International Headache Society
df	Degree of freedom
HWE	Hardy–Weinberg equilibrium
SNP	Single nucleotide polymorphism

Introduction

Migraine is a common headache disorder affecting 10–20% of the population, women three to four times more often than men [1]. Up to one-third of migraine patients experience an aura prior to or during the migraine headache.

Migraine is viewed as an inherited brain disorder, characterized by neurotransmitter imbalances leading to neuronal dysfunctions, which then present with recurrent headache attacks and combinations of gastrointestinal and autonomic nervous system symptoms [1]. The neurotransmitter serotonin appears to play a pivotal role and alterations in serotonin metabolism and in the processing of central serotonin-mediated responses have been described as typical for migraineurs [2]. Following axonal release, serotonin action is rapidly terminated by presynaptic re-uptake [3] through the serotonin transporter SLC6A4 [4]. Furthermore, SLC6A4 is predominantly expressed in the brainstem and midbrain [5], areas implicated in migraine pathophysiology.

A polymorphism in intron 2 of the SLC6A4 gene has been described consisting of a 17 bp variable number of tandem repeats (termed STin2 VNTR). This polymorphism involves two major alleles (termed 'STin2.10' and 'STin2.12') corresponding to 10 or 12 repeat units [6]. In addition, low-frequency alleles (termed 'STin2.9' and 'STin2.7') have been described including nine and seven repeat units [7]. While the biological function of this polymorphism is unclear, STin2.12 has been reported to possess greater transcriptional activity than STin2.10 [8].

A number of studies investigated the association between the STin2 VNTR polymorphism and migraine, with varying results. Some reported altered allelic or genotypic distribution among patients with any migraine [9], migraine with aura (MA) [10–12] or migraine without aura (MO) [10]. Others did not find an association [13].

We sought to summarize the current data on the association between the SLC6A4 STin2 VNTR polymorphism and migraine including MA and MO by systematically reviewing the literature and performing pooled and meta-analyses.

Methods

Selection of studies

We followed the guidelines for systematic reviews of genetic association studies [14]. Two investigators (M.S., P.M.R.) independently searched MEDLINE, EMBASE and Science Citation Index from inception to September 2009, combining text words and MESH terms, where appropriate, for serotonin and serotonin transporter ("serotonin plasma membrane transport proteins", "serotonin transporter", "serotonin" or "SLC6A4") with terms for genetic variations ("gene", "polymorphism" or "genetic variation") and terms for headache and migraine ("headache", "headache disorders", "migraine" or "migraine disorders"). The search terms were combined with the "explode" feature where applicable. We considered full articles without language restrictions. In addition, we manually searched the reference list of all primary articles and review articles.

A priori, we defined the following criteria for inclusion:

1. Studies must have a cross-sectional, case–control or cohort design and must be published as full papers.
2. Authors must investigate patients with migraine, diagnosed according to the criteria of the International Headache Society (IHS) [15, 16] and healthy control subjects.
3. Authors must provide information on genotype frequencies of the SLC6A4 VNTR STin2 polymorphism or sufficient data to calculate these.
4. In studies with overlapping cases and/or controls, the largest study with extractable data was included.

In a first step, two investigators (M.S., T.K.) by consensus identified all studies not meeting any of the pre-specified criteria by screening the title and abstracts. These studies were excluded. In a second step, the same investigators evaluated the remaining studies in their entirety. Studies were excluded if they did not meet all criteria.

Data extraction

Two investigators (M.S., P.M.R.) independently extracted data from the published studies and entered them in a customized database. Disagreements were resolved by consensus. The extracted data included authors and title of study, year of publication, country of origin, ethnicity of population investigated, setting (clinic vs. population), study design, genotyping method, migraine status (any migraine, MA, MO), age range and gender of study participants, study size, allele and genotype frequencies, and information on additional genetic variants as well as gene–gene and gene–environment interactions, if investigated. If

not given, genotype frequencies were calculated where possible. We did not contact the authors to collect further information.

Statistical analysis

In contrast to single nucleotide polymorphisms, which are characterized by two alleles and three genotypes, the *SLC6A4* STin2 VNTR polymorphism is characterized by four alleles [6, 7]. Although studies in migraine have only reported the STin2.9, STin2.10 and STin2.12 alleles, it still precludes employment of simple biological models at the allele or genotype level (additive, dominant, recessive) based on one degree of freedom (*df*) tests. Further, model-free approaches at the genotype level based on 'number of genotypes-1' *df* yield more than one odds ratio (OR) from one model. These cannot easily be combined for meta-analysis to obtain single pooled effect estimates, tests statistics, and measures of heterogeneity. Hence, we used two different, but complementary approaches, to investigate the association of the *SLC6A4* STin2 VNTR polymorphism and migraine.

In the first approach, we investigated an association at the allele level. For each study, we combined the STin2.9 and STin2.10 alleles and compared these to the more frequent STin2.12 alleles, as done in a previous meta-analysis [17]. This approach investigates whether an excess of alleles in one of the two groups alters the risk for migraine. We used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for each study for the association between alleles of the *SLC6A4* STin2 VNTR polymorphism and migraine. We then weighted the log of the ORs by the inverse of their variance to obtain pooled estimates. We ran random effects models, which include assumptions about the variability between studies. We performed the DerSimonian and Laird Q test for heterogeneity and also calculated the I^2 statistic for each analysis [18]. This statistic describes the percentage of total variation across studies that is due to heterogeneity rather than chance (25%: low, 50%: medium, 75%: high heterogeneity). We used Galbraith plots to visually examine the impact of individual studies on the overall homogeneity test statistics. We evaluated potential publication bias visually by examining for possible skewness in funnel plots [19] and statistically with the methods described by Begg and Mazumdar [19] and Egger [20].

The second approach was performed at the genotype level. Since one of the studies [12] only presented combined data for the very infrequent genotypes, we also combined these genotypes in the other studies for further analyses ('other genotypes': STin2.9/STin2.10 and STin2.9/STin2.12). We did not further combine any of the other genotypes. We then pooled genotype data from the studies. Finally, we used logistic regression analysis to evaluate the association between pooled genotypes of the *SLC6A4* STin2 VNTR polymorphism and migraine. We created indicator variables for each genotype; the most frequent genotype STin2.12/STin2.12 served as reference (3 *df* test). We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each of the other observed genotypes.

We also investigated Hardy–Weinberg equilibrium (HWE) in each study and in the pooled data, considering the frequent alleles (STin2.10 and STin2.12).

The genotype-based approach has two advantages. Firstly, it is biologically more meaningful, since the genotype as opposed to the allele is the biological unit characterizing an individual. Secondly, it makes no assumption about the underlying biological model (model-free) allowing for non-linear effects and thus yields effect estimates for each genotype compared to the reference.

In both approaches, we investigated any migraine, MA or MO. We considered *p* values < 0.05 as statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC) and STATA 10.1 (Stata, College Station, TX, USA). Since we only utilized previously published data, we did not obtain the approval of an ethics committee or a written informed consent.

All authors had access to all of the data.

Results

For ease of readability, genotypes will be referred to by the number of tandem repeats from here on. For example, STin2.12/STin2.12 will be abbreviated as 12/12, STin2.10/STin2.12 as 10/12 and STin2.10/STin2.10 as 10/10.

Figure 1 summarizes the process of identifying eligible studies. After title and abstract evaluation, we were left with 18 studies [9–13, 21–33]. We excluded 13 more studies [21–33] after evaluating the full paper versions of the remaining articles and were left with 5 studies for this analysis [9–13].

Study characteristics

Table 1 summarizes the characteristics of the five included studies. Four studies were performed in populations of European descent [10–13] and one in a Turkish population [9]. Four studies, each, presented results for any migraine [9, 10, 12, 13] and MA [10–13] and three for MO [10, 12, 13]. All studies investigated only mixed female and male populations, had a case–control design and used standard polymerase chain reaction genotyping methods.

Fig. 1 Process of identifying studies
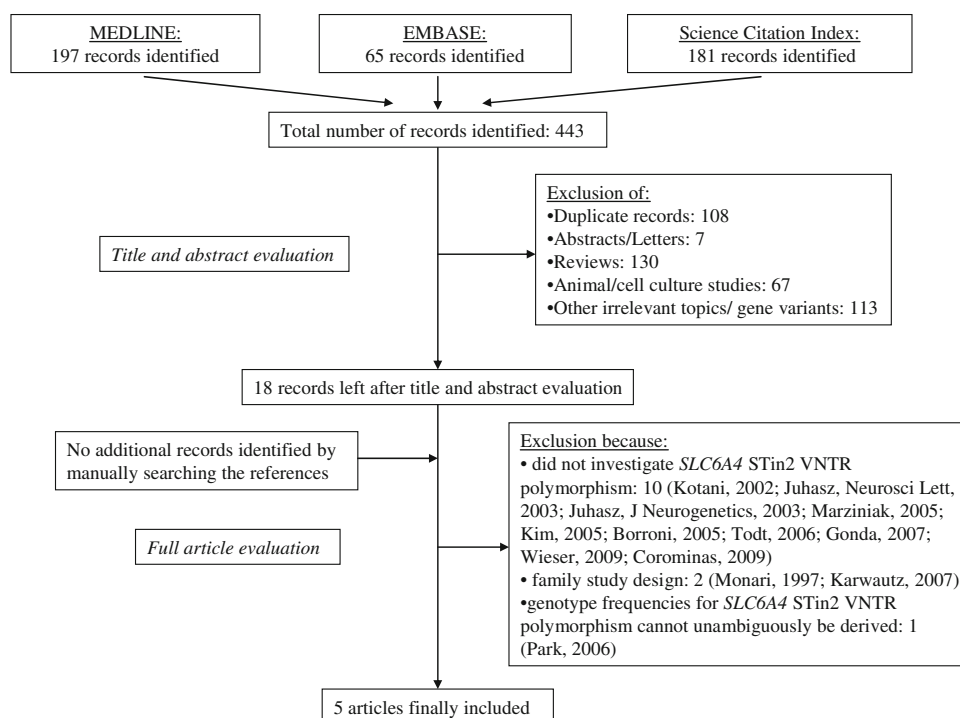
The allele and genotype frequencies for the *SLC6A4* STin2 VNTR polymorphism for migraineurs and controls in each of the included studies are summarized in Table 2.

Table 3 summarizes for each of the studies the ORs (95% CI) for the association between the *SLC6A4* STin2 VNTR polymorphism and migraine assuming allele and genotype models as well as *p* values for the HWE in the controls. Genotype distributions in two control study populations deviated from HWE [9, 13]. Table 4 summarizes results from the pooled and meta-analyses based on an allele model and Table 5 a pooled analysis based on a genotype model.

Association between the *SLC6A4* STin2 VNTR polymorphism and migraine

In the allele model, some of the effect estimates from individual studies suggested a reduced risk for any migraine [10, 12], MA [10–12] or MO [10] among carriers of the STin2.9 and STin2.10 alleles compared to carriers of the STin2.12 allele (Table 3). However, none of the results were statistically significant. In contrast, the study on a Turkish population suggested a significantly increased risk for any migraine (OR = 2.09; 95% CI 1.19–3.67) [9].

Results from the genotype model suggested a reduced risk of the 10/12 and 10/10 genotypes for any migraine [10, 12], MA [10, 12] or MO [10] compared with the 12/12 genotype. However, the results only became statistically significant for all three migraine categories for the 10/12

genotype in one study [10] (Table 2). There was no clear pattern of association of the 'other genotypes' with migraine, likely because they are so rare, which is also reflected by the wide confidence intervals. In contrast, the Turkish study [9] suggested a significantly threefold increased risk of any migraine for carriers of the 10/12 and 10/10 genotypes, which was very similar for both genotypes. One study did not show a clear pattern of association for any of the genotypes with MA [11], likely due to the small sample size. Another study [13] did not show an association of any of the genotypes with any of the migraine categories in either the allele or the genotype model.

The effect estimates based on the allele model from the pooled and the meta-analyses pointed toward a protective effect against any migraine, MA or MO for the STin2.9 and STin2.10 alleles compared to the STin2.12 allele among populations of European descent (Table 4). ORs from both analyses were very similar; however, none of the results was statistically significant. Heterogeneity among all studies investigating any migraine was medium ($I^2 = 70.4\%$). This was attributable to the Turkish study [9]; analyses of studies only among populations of European descent did not indicate any heterogeneity. Begg's test and Egger's test did not indicate publication bias among studies in populations of European descent, while Egger's test suggested that there might be some publication bias among studies investigating MO ($p = 0.01$).

Our pooled analyses based on a genotype model suggest a ∼25% reduced risk for migraine among carriers of the

Table 1 Characteristics of included studies investigating the association between the *SLC6A4* STin2 VNTR polymorphism and migraine

Author and year	Country	Setting	Population	Study size with genotypic information				Comment
				Controls	Any migraine	MA	MO	
Ogilvie [10]	Denmark	Population	Mixed	133	285	94	173	
Lea [13]	Australia	Population	Mixed	141	148	81	67	Other polymorphisms investigated: in dopamine beta-hydroxylase gene (*DBH*) and dopamine receptor 2 gene (*DRD2*).
Yilmaz [9]	Turkey	NS	Mixed	79	37	–	–	Other polymorphisms investigated: *SLC6A4* 5-HTTLPR.
Racchi [11]	Italy	Clinic	Mixed	32	–	44	–	Other polymorphisms investigated: *HTR1B* 261 T > G, *HTR1B* 861 G > C, *HTR2C* 68 G > C.
								MA patients were compared to unaffected family members and unrelated controls. For the present analysis, only data from MA patients and unaffected controls are considered.
Szilagyi [12]	Hungary	Clinic	Mixed	464	87	38	49	Other polymorphisms investigated: *SCL6A4* 5-HTTLPR.
								Cases were children and adolescents; controls were high school and university students.
Total number of subjects				849	557	257	289	

MA migraine with aura, *MO* migraine without aura, *NS* not specified

Table 2 Allele and genotype frequencies for the *SLC6A4* STin2 VNTR polymorphism

Author	Disease status	Study size	Allele frequencies, n (%)			Genotype frequencies, n (%)			
			STin2.9	STin2.10	STin2.12	Other[a]	STin2.10/ STin2.10	STin2.10/ STin2.12	STin2.12/ STin2.12
Ogilvie [10]	Controls	133	3 (1.1)	107 (40.2)	156 (58.7)	3 (2.3)	19 (14.3)	68 (51.1)	43 (32.3)
	Any migraine	285	14 (2.5)	187 (32.8)	369 (64.7)	14 (4.9)	38 (13.3)	105 (36.8)	128 (44.9)
	MA	94	6 (3.2)	66 (35.1)	116 (61.7)	6 (6.4)	15 (16.0)	32 (34.0)	41 (43.6)
	MO	173	6 (1.7)	113 (32.7)	227 (65.6)	6 (3.5)	22 (12.7)	68 (39.3)	77 (44.5)
Lea [13]	Controls	141	4 (1.4)	112 (39.7)	166 (58.9)	4 (2.8)	33 (23.4)	45 (31.9)	59 (41.8)
	Any migraine	148	5 (1.7)	116 (39.2)	175 (59.1)	5 (3.4)	34 (23.0)	46 (31.1)	63 (42.6)
	MA	81	3 (1.9)	64 (39.5)	95 (58.6)	3 (3.7)	19 (23.5)	25 (30.9)	34 (42.0)
	MO	67	2 (1.5)	52 (38.8)	80 (59.7)	2 (3.0)	15 (22.4)	21 (31.3)	29 (43.3)
Yilmaz [9]	Controls	79	0 (0.0)	53 (33.5)	105 (66.5)	0 (0.0)	14 (17.7)	25 (31.6)	40 (50.6)
	Any migraine	37	0 (0.0)	38 (51.4)	36 (48.6)	0 (0.0)	10 (27.0)	18 (48.6)	9 (24.3)
Racchi [11]	Controls	32	2 (3.1)	30 (46.9)	32 (50.0)	2 (6.3)	9 (28.1)	12 (37.5)	9 (28.1)
	MA	44	0 (0.0)	35 (39.8)	53 (60.2)	0 (0.0)	3 (6.8)	29 (65.9)	12 (27.3)
Szilagyi [12]	Controls	464	13 (1.4)	340 (36.6)	575 (62.0)	13 (2.8)	59 (12.7)	220 (47.4)	172 (37.1)
	Any migraine	87	3 (1.7)	55 (31.6)	116 (66.7)	3 (3.4)	8 (9.2)	38 (43.7)	38 (43.7)
	MA	38	2 (2.6)	19 (25.0)	55 (72.4)	2 (5.3)	3 (7.9)	13 (34.2)	20 (52.6)
	MO	49	1 (1.0)	36 (36.7)	61 (62.2)	1 (2.0)	5 (10.2)	25 (51.0)	18 (36.7)

MA migraine with aura, *MO* migraine without aura

[a] STin2.9/STin2.10 and STin2.9/STin2.12

10/12 genotype compared with carriers of the 12/12 genotype (Table 5). The pooled ORs (95% CI) among all study populations were 0.76 (0.60–0.97) for any migraine.

When we confined our analysis to populations of European descent, this effect appeared more pronounced (OR = 0.68, 95% CI 0.53–0.87). The results for MA

Table 3 Odds ratios (95% confidence intervals) and Hardy–Weinberg equilibrium for the association between the *SLC6A4* STin2 VNTR polymorphism and migraine

Author	Disease status	Study size	Allele model[a]		Genotype model[b]							
			OR (95% CI)	p value	HWE	STin2.10/STin2.12		STin2.10/STin2.10		Other[c]	
						OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Ogilvie [10]	Controls	133	Referent		0.48	Referent		Referent		Referent	
	Any migraine	285	0.77 (0.57–1.04)	0.09	–	0.52 (0.33–0.82)	0.005	0.67 (0.35–1.29)	0.23	1.57 (0.43–5.72)	0.50
	MA	94	0.88 (0.60–1.29)	0.51	–	0.49 (0.27–0.90)	0.02	0.83 (0.37–1.84)	0.64	2.10 (0.50–8.95)	0.32
	MO	173	0.74 (0.54–1.03)	0.08	–	0.56 (0.34–0.92)	0.02	0.65 (0.32–1.33)	0.23	1.12 (0.27–4.70)	0.88
Lea [13]	Controls	141	Referent		0.0001	Referent		Referent		Referent	
	Any migraine	148	0.99 (0.71–1.38)	0.95	–	0.96 (0.56–1.65)	0.87	0.97 (0.53–1.75)	0.91	1.17 (0.30–4.57)	0.82
	MA	81	1.01 (0.68–1.49)	0.96	–	0.96 (0.51–1.84)	0.91	1.00 (0.50–2.02)	1.00	1.30 (0.28–6.16)	0.74
	MO	67	0.97 (0.64–1.47)	0.87	–	0.95 (0.48–1.88)	0.88	0.93 (0.44–1.97)	0.84	1.02 (0.18–5.88)	0.98
Yilmaz [9]	Controls	79	Referent		0.01	Referent		Referent		Referent	
	Any migraine	37	2.09 (1.19–3.67)	0.01	–	3.20 (1.25–8.22)	0.02	3.18 (1.07–9.41)	0.04	–[d]	–[d]
Racchi [11]	Controls	32	Referent		0.28	Referent		Referent		Referent	
	MA	44	0.66 (0.35–1.27)	0.21	–	1.81 (0.61–5.42)	0.29	0.25 (0.05–1.20)	0.08	–[d]	–[d]
Szilagyi [12]	Controls	464	Referent		0.42	Referent		Referent		Referent	
	Any migraine	87	0.81 (0.58–1.15)	0.24	–	0.78 (0.48–1.28)	0.33	0.61 (0.27–1.39)	0.24	1.05 (0.28–3.85)	0.95
	MA	38	0.62 (0.37–1.05)	0.07	–	0.51 (0.25–1.05)	0.07	0.44 (0.13–1.53)	0.19	1.32 (0.28–6.29)	0.72
	MO	49	0.99 (0.64–1.52)	0.96	–	1.09 (0.57–2.06)	0.80	0.81 (0.29–2.28)	0.69	0.74 (0.09–5.95)	0.77

HWE p value for Hardy–Weinberg equilibrium, *MA* migraine with aura, *MO* migraine without aura

[a] Compares STin2.9 and STin2.10 versus STin2.12 alleles

[b] Compares each of the genotypes versus the STin2.12/STin2.12 genotype

[c] STin2.9/STin2.10 and STin2.9/STin2.12

[d] In some groups there were no other genotypes

Table 4 Allele model: pooled and meta-analyses of the association between the *SLC6A4* STin2 VNTR polymorphism and migraine

Migraine category	Study populations	No. of studies	Pooled analysis	Meta-analysis						
			Odds ratio[a] (95% CI)	Odds ratio[a] (95% CI)	Heterogeneity				Publication bias	
					Q	df	p value	I^2 in %	p value, Begg	p value, Egger
Any migraine	All [9, 10, 12, 13]	4	0.95 (0.81–1.11)	1.00 (0.72–1.41)	10.1	3	0.02	70.4	0.17	0.045
	European descent [10, 12, 13]	3	0.89 (0.76–1.05)	0.85 (0.70–1.02)	1.3	2	0.54	0	0.60	0.63
MA	European descent [10–13][b]	4	0.93 (0.76–1.14)	0.83 (0.66–1.05)	2.7	3	0.44	0	0.50	0.17
MO	European descent [10, 12, 13][b]	3	0.88 (0.72–1.08)	0.86 (0.69–1.08)	1.5	2	0.48	0	0.12	0.01

MA migraine with aura, *MO* migraine without aura

[a] STin2.9 and STin2.10 alleles were grouped together and compared to STin2.12 as the reference

[b] There are no studies available for MA and MO among populations of non-European descent

Table 5 Genotype model: pooled analysis of the association between the *SLC6A4* STin2 VNTR polymorphism and migraine

Migraine category	Study populations	No of studies	HWE	10/12		10/10		Other[a]	
				Odds ratio[b] (95% CI)	p value	Odds ratio[b] (95% CI)	p value	Odds ratio[b] (95% CI)	p value
Any migraine	All [9, 10, 12, 13]	4	0.18	0.76 (0.60–0.97)	0.03	0.95 (0.69–1.31)	0.75	1.45 (0.77–2.72)	0.25
	European descent [10, 12, 13]	3	0.58	0.68 (0.53–0.87)	0.002	0.86 (0.62–1.21)	0.39	1.32 (0.70–2.47)	0.39
MA	European descent [10–13][c]	4	0.40	0.76 (0.55–1.04)	0.09	0.88 (0.58–1.34)	0.56	1.32 (0.62–2.82)	0.47
MO	European descent [10, 12, 13][c]	3	0.58	0.76 (0.56–1.02)	0.07	0.84 (0.55–1.26)	0.40	0.99 (0.44–2.25)	0.99

HWE p value for Hardy–Weinberg equilibrium, *MA* migraine with aura, *MO* migraine without aura

[a] STin2.9/STin2.10 and STin2.9/STin2.12

[b] Genotype 12/12 was used as the reference

[c] There are no studies available for MA and MO among populations of non-European descent

(OR = 0.76; 95% CI 0.55–1.04) and MO (OR = 0.76; 95% CI 0.56–1.02) were of similar magnitude, but did not reach statistical significance. While the ORs for the 10/10 genotype also pointed toward a protective effect, they were larger and not statistically significant. In contrast, the 'other genotypes' appeared to increase the risk for any migraine and MA, but not MO.

Sensitivity analyses

We performed two kinds of sensitivity analyses.

Firstly, we created Galbraith plots based on the ORs from the allele models (Table 3). The plot for studies investigating any migraine identified the Turkish study [9] as an important source of heterogeneity, since the OR fell outside the margin set by the z score ± 2 standard deviations. An analysis excluding that study was identical to our analysis among populations of European descent. The plots for MA and MO did not identify any of the studies as significant sources of heterogeneity.

Secondly, we re-ran all pooled and meta-analyses by excluding studies where the genotype distribution deviated from HWE in the control group [9, 13]. The effect estimates for the allele model from the pooled and the meta-analyses were all slightly lower. Results from the pooled analyses remained insignificant; the ORs (95% CI) for any migraine were 0.84 (0.70–1.02), for MA 0.88 (0.69–1.13), and for MO 0.86 (0.68–1.07). Results from the meta-analysis were significant for any migraine (OR = 0.79, 95% CI 0.63–0.99) and marginally significant for MA (OR = 0.76, 95% CI 0.57–1.00; $p = 0.05$). The OR (95% CI) for MO was 0.83 (0.63–1.09). The results from the genotype model remained very similar. For example, the OR (95% CI) for the 10/12 genotype was 0.64 (0.48–0.86) for any migraine, 0.76 (0.53–1.09) for MA and 0.73 (0.52–1.02) for MO compared to the 12/12 genotype.

Discussion

The main results from these pooled and meta-analyses on the association between the *SLC6A4* STin2 VNTR polymorphism and migraine are that non-STin2.12 alleles compared to STin2.12 alleles and 10/12 and 10/10 genotypes compared to the 12/12 genotype appear to protect against migraine among populations of European descent.

Results from the allele analyses were not statistically significant. Results from genotype analyses suggested the smallest effect estimates for the 10/12 genotype translating into a ∼25% reduced risk and became statistically significant for the largest group of patients with any migraine. The effect estimates were similar for any migraine, MA and MO. In contrast, associations may differ in non-European populations, as suggested by a Turkish study [9] showing a threefold increased risk for any migraine among carriers of the 10/12 and 10/10 genotypes.

Migraine has a clear genetic component with a heritability as high as 50% [34]. However, identifying genetic markers for common forms of migraine has been difficult [35].

Serotonin plays a pivotal role in migraine [2] and a dysfunctional serotonergic system agrees with pathophysiological models of a cortical dysbalance [36] and treatment success of triptans [37]. Among the main features in migraineurs are changes in serotonin metabolism and in the processing of central serotonin-mediated responses [2]. Synaptic serotonin is moved to the intracellular space by the serotonin transporter SLC6A4 and two polymorphisms in the SLC6A4 gene have been investigated with regard to their association with migraine: the 5-HTTLPR and the STin2 VNTR polymorphisms. In a recent meta-analysis on the association of the 5-HTTLPR polymorphism and migraine, we did not find an overall association among Europeans and Asians; however, gender and migraine aura status may have modifying roles among Europeans [38]. Similar to studies on the 5-HTTLPR polymorphism, studies on the association between the STin2 VNTR polymorphism and migraine came to conflicting results. Some have reported an altered allelic or genotypic distribution among patients with any migraine [9], MA [10–12] or MO [10], while others did not find an association [13]. This may in part be due to differences in sample size and study characteristics. In addition, it is unclear whether only a specific combination of alleles, i.e., a single genotype, or the simple presence of one allele, i.e., multiple genotypes, might alter the risk for migraine.

Results from our analyses indicating a protective role against migraine for non-STin2.12 alleles, respectively, for 10/12 and 10/10 genotypes of the STin2 VNTR polymorphism in populations of European descent became only statistically significant for the association between the 10/12 genotype and any migraine. However, we are confident that this reflects a true association and is not a false positive signal for two reasons. Firstly, results from the allele and genotype models agree by pointing toward the same direction of a protective effect of non-STin2.12 alleles/non-12/12 genotypes. Second, our sensitivity analyses suggest similar or even smaller effect estimates and p values.

Employing a genotype model in addition to an allele model yields further valuable information. Firstly, it indicated that both the 10/12 and 10/10 genotypes reduced the risk for migraine compared to the 12/12 genotype. This allows for two possible interpretations. On the one hand, the STin2.10 allele may exert a dominant effect over the STin2.12 allele. The higher ORs and non-significant p values for the 10/10 genotype may be due to the smaller number of 10/10 than 10/12 genotypes (Table 2). Alternatively, the protective effect may only occur with the 10/12 genotype, as suggested by the statistical significance of the results. It may be speculated that an unequal number of tandem repeats on the two chromosomes (heterozygotes) impairs transporter function, which does not occur for homozygotes. Examples from other gene variants exist, for example the GNB3 825C>T polymorphism [39], where the largest association is seen among carriers of heterozygous genotypes. Secondly, providing estimates for genotypes, as opposed to alleles, is biologically more meaningful, since the genotype is the genetic unit characterizing each individual. Thirdly, the ∼25% reduced risk for migraine of the 10/12 genotype appears moderate; however, it is in keeping with migraine being a complex disorder. This means that multiple genetic (and environmental) factors with small to moderate effects determine the susceptibility for migraine.

Results for the 'other genotypes' (9/10 and 9/12) suggested that the risk for migraine may be increased for any migraine and MA (Table 5). However, caution is warranted since the frequencies of the 'other genotypes' are very rare rendering the results susceptible to minor alterations in genotype distribution.

The functional implications of the STin2 VNTR polymorphism are not well understood. Expression studies have shown that the STin2.10 and STin2.12 alleles enhance transcriptional activity, Stin2.10 significantly less than STin2.12 [8]. We may speculate that STin2.10 leads to a lower number of SLC6A4 transporters, hence less serotonin reuptake and more synaptic (maybe 'normal') serotonin activity compared to the STin2.12 allele. This may reduce the risk for migraine as also suggested by our analyses. However, functional investigations of the platelet serotonin transporter do not agree, since they show that serotonin uptake is unaltered by the STin2 VNTR polymorphism [40].

The following aspects of our meta-analysis need to be considered. First, migraine is a heterogeneous disorder with a wide clinical spectrum [35]. Patients with different clinical phenotypes have been diagnosed as having migraine, and even strict classification according to IHS criteria [15, 16] may not sufficiently capture this variability and may be a source of misclassification in all studies. Second, the total number of studies identified was five, which limits the statistical power. Hence, a larger sample size than available from all the studies combined might be necessary to

improve precision of our results. While the systematic review part allows for an overview of the available studies including individual results, the meta-analytic part also facilitates evaluation of magnitude and direction of pooled effect estimates including sources of heterogeneity. Third, *SLC6A4* is a large polymorphic gene comprising 37,810 bp organized into 15 exons and 14 introns with 380 single nucleotide polymorphisms (SNPs) reported [41, 42]. Some of these SNPs may be associated with migraine [43]. Hence, in addition to the *SLC6A4* STin2 VNTR and 5-HTTLPR polymorphisms, these SNPs may also affect transporter production and function. Fourth, serotonin's role in migraine is not just determined by transporter activity, but also by its functional effect at the receptor level. Serotonin receptor genes are likewise polymorphic and some of these variants may be associated with migraine [22]. Fifth, in addition to serotonin, other neurotransmitters such as dopamine, orexin and glutamate are important in migraine pathophysiology [44]. Hence, migraine susceptibility is a function of the interaction between various neurotransmitters, which again is determined by the interaction of various genetic variants. Sixth, migraine susceptibility is different between women and men, as indicated by the higher prevalence among women [45]. However, we had no means to investigate a gender differential genetic effect of the STin2 VNTR polymorphism, since all studies were performed in mixed female and male populations and gender-stratified genotypic information was unavailable. Seventh, associations with migraine may differ by ethnicity, especially since the genotype distribution of the STin2 VNTR polymorphism shows ethnic differences [46, 47]. The available studies provide limited indication for that. While the 10/12 and 10/10 genotypes appeared to protect against migraine in populations of European descent, one Turkish study [9] reported an increased risk. Eighth, genotypes deviated from HWE in two studies [9, 13]. However, sensitivity analyses after excluding these studies did not alter our results. Finally, we only used extractable data from the papers. One paper did not unequivocally indicate genotype frequencies for each of the STin2 VNTR genotypes [31]. The study was excluded. However, the authors only investigated MO; hence, this study would not have altered our results for any migraine and MA. In addition, the authors reported a higher frequency of the 12/12 genotype in patients with MO, which corresponds with our results. Thus, including this study would likely not have changed our results for MO either.

Conclusion

The 10/12 genotype of the *SLC6A4* STin2 VNTR polymorphism translates into a $\sim 25\%$ reduced risk for any migraine. However, additional studies with large sample sizes of migraineurs and controls seem warranted. Studies should not just be carried out in populations of European descent, but also in non-European populations. In addition to overall results, results should also be presented stratified by gender and migraine aura status. Finally, analyses should focus on main gene effects and gene–gene interactions investigated only if the sample size is reasonably large.

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