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Population-Based Analysis of Cluster Headache-Associated Genetic Polymorphisms

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Abstract

Cluster headache is a disorder with increased hereditary risk. Associations between cluster headache and polymorphism rs2653349 of the *HCRTR2* gene have been demonstrated. The less common allele (A) seems to reduce disease susceptibility. The polymorphism rs5443 of the *GNB3* gene positively influences triptan treatment response. Carriers of the mutated T allele are more likely to respond positively compared to C:C homozygotes, when treated with triptans. DNA was extracted from buccal swabs obtained from 636 non-related Southeastern European Caucasian individuals and was analyzed by real-time PCR. Gene distribution for the rs2653349 was G:G = 79.1%, G:A = 19.2%, and A:A = 1.7%. The frequency of the wild-type G allele was 88.7%. The frequencies for rs5443 were C:C = 44.0%, C:T = 42.6%, and T:T = 13.4%. The frequency of the wild-type C allele was 65.3%. The frequency distribution of rs2653349 in the Southeastern European Caucasian population differs significantly when compared with other European and East Asian populations, and the frequency distribution of rs5443 showed a statistically significant difference between Southeastern European Caucasian and African, South Asian, and East Asian populations. For rs2653349, a marginal statistically significant difference between genders was found ($p = 0.080$) for A:A versus G:G and G:A genotypes (OR = 2.78), indicating a higher representation of male homozygotes for the protective mutant A:A allele than female. No statistically significant difference was observed between genders for rs5443. Cluster headache pathophysiology and pharmacotherapy response may be affected by genetic factors, indicating the significant role of genotyping in the overall treatment effectiveness of cluster headaches.

Keywords *GNB3* · *HCRTR2* · Cluster headache therapy · Southeastern European Caucasians · Pharmacogenomics

Abbreviations

CH	Cluster headache
IHS	International Headache Society
TACs	Trigeminal autonomic cephalalgias
SNP	Single-nucleotide polymorphism
HCRTR2	Hypocretin receptor type 2
5-HT	5-hydroxytryptamine
GNB3	G protein beta-3 subunit
SEC	Southeastern European Caucasians
OR	Odds ratio
CI	Confidence intervals
HWE	Hardy–Weinberg equilibrium

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PET	Positron-emission tomography
CGRP	Calcitonin-gene related peptide
IL-2	Interleukin-2

Background

Cluster headache (CH) is trigeminal autonomic cephalalgia (TAC) according to the Headache Classification Committee of the International Headache Society (Headache Classification Committee of the International Headache Society (IHS) 2018). The periodic occurrence of CH episodes suggests that the pathogenesis of this particular type of headache may include the rhythm regulating centers of the hypothalamus. Several studies support this hypothesis, providing evidence of decreased melatonin levels, as well as melatonin and cortisol circadian secretion disruption during CH episodes and remission periods (Leone et al. 1998; Lucini et al. 1995). Although a direct relation between melatonin, cortisol levels, and CH has not been established, the observed elevation of cortisol after CH episodes may serve as an adaptive response to CH pain (Waldenlind et al. 1987).

Hereditary occurrence of CH proposes that genetic factors may affect disease development, as is the case in other diseases and pathologic conditions (Dardiotis et al. 2013; Dardiotis et al. 2018; Katsarou et al. 2016, 2017; Tsatsakis et al. 2011). According to several genetic epidemiological studies, which aimed to evaluate the disease risk of CH patients' relatives compared to the general population, first-degree relatives had 5–18 times higher risk and second-degree relatives had 1–3 times higher risk (Pinessi et al. 2005). Two G-protein-coupled orexin receptors have been identified, HCRTR1 and HCRTR2. The neuropeptides hypocretin-1 and hypocretin-2 (orexin-A and orexin–B) are processed from a common precursor polypeptide, pre-prohypocretin (or prepro-orexin), with usual proteolytic processing probably by prohormone convertases. Hypocretin-1 and hypocretin-2 neuropeptides are bounded to HCRTR2 (hypocretin receptor 2). This system's peptides influence many physiological and behavioral processes in mammals. Hypocretin systems have a pivotal role important for survival: they sense the body's external and internal environment and they regulate the sleep and wakefulness state (Tsujino and Sakurai 2009).

HCRTR1 and HCRTR2 regulate ion channels, adenylyl cyclase, phospholipases, and protein and lipid kinases, through coupling to several members of heterotrimeric G-protein families and other proteins. In the central nervous system, HCRTRs can presynaptic stimulate the release of neurotransmitters and impact on synaptic plasticity and postsynaptic and excite neurons by depolarization (Haghparast et al. 2017). The hypocretin receptors also influence Ca^{2+} signaling (Nasman et al. 2006).

Studies have indicated an association between CH and the *HCRTR2* gene, which is located on chromosome 6p12.1. *HCRTR2* consists of seven exons and encodes for a G-protein-coupled receptor, exclusively expressed in the brain (Rainero et al. 2007). A G1246A SNP on the *HCRTR2* gene, rs2653349, which is responsible for an amino acidic substitution (Val308Iso) within the receptor sequence, has been reported to be related to CH (Thompson et al. 2014). CH pathogenesis is associated with the transmission of pain as well as in autonomic and neuroendocrine functions mediated by hypocretins. According to a meta-analysis of three studies which were conducted in Caucasian populations, a relationship between the G1246A polymorphism (rs2653349) in the *HCRTR2* gene and CH was found (fixed effect OR: 1.58 (CI 95% 1.27–1.95), random effect OR:1.55 (CI 95% 1.14–2.12)) (Rainero et al. 2007). The less common allele (A) seems to reduce disease developing the risk and the more common wild-type allele (G) increases the risk (Thompson et al. 2014).

Acute and preventive therapy has been proposed to relieve symptoms and reduce frequency of attacks, respectively (Law, Derry, and Moore 2013). Triptans are G-protein-linked serotonergic (5-HT) receptor selective agonists, commonly used for acute CH therapy as injectable or intranasal preparation (Ashkenazi and Schwedt 2011). The β3-subunit of the 5-HT-linked G protein is involved in various physiological and pathophysiological processes. It is encoded by the gene *GNB3*. In 1998, the rs5443 or C825T variant, which is located in exon 10 of GNB3, was identified. The T allele was associated with alternative splicing and with increased signal transduction in human cells and tissues (Klenke, Kussmann, and Siffert 2011). A study conducted with 231 Caucasian patients showed that rs5443(T) carriers taking triptans as treatment for migraines or CH were ~threefold more likely to respond positively compared to rs5443(C:C) homozygotes (Schürks et al. 2007).

The genotype status of *GNB3* did not affect responses to other acute and preventive therapies with drugs that have no direct effect on G proteins, such as oxygen, verapamil, or corticosteroids (Schürks et al. 2007).

A population-based frequency distribution analysis of the *HCRTR2* gene (rs2653349) and *GNB3* (rs5443) gene polymorphism was performed in an attempt to evaluate potential biomarkers for CH susceptibility and triptans' efficacy in CH, respectively.

Materials and Methods

In total, 636 Southeastern European Caucasians (SEC), who represented a general SEC population, were recruited, including 245 (38.5%) male and 391 (61.5%) female. The

participants' median age was 46 years, ranging from 18 to 93 years. SEC origin and aged > 18 years was considered for inclusion in the study. Volunteers without SEC origin, obese, and aged < 18 years were excluded.

The study was approved by the Ethics Committee of Eginition Hospital, Medical School, and National and Kapodistrian University of Athens, and all participants gave written informed consent for using their data. Epithelial cells were collected and DNA extraction was performed after anonymization and de-identification in all samples (Tissue Nucleospin, Machery-Nagel, Germany).

HCRTR2 and *GNB3* polymorphisms were analyzed using LightSnip kits (TIB MOLBIOL Germany) according to the manufacturer's instructions. The genotypes were classified as homozygote for wild type (G:G or C:C) allele, heterozygote (G:A or C:T), and homozygote mutated (A:A or T:T) for rs2653349 or rs5443 polymorphisms, respectively.

Hardy–Weinberg equilibrium (HWE) was calculated (http://www.oege.org/software/hwe-*mr*-calc.shtml) for the genotypic distribution of each polymorphism. In all cases, the null hypothesis of genotypes and alleles HWE was not rejected. Chi square (Pearson and Fischer exact) tests were used to compare the frequencies of genotypes and alleles in SEC and other populations. For this purpose, contingency 2 × 2 and 2 × 3 tables were designed, and odds ratios (OR) as well as the corresponding confidence intervals (CIs) were calculated. All statistical tests were performed at a significance level of $\alpha = 0.05$. SPSS package (version 21.0 for Windows) and R (3.4.3 version)* were used for statistical analyses.

Results

For polymorphism rs2653349 of the *HCRTR2* gene, 79.1% volunteers were homozygous for the wild-type genotype (G:G); 19.2% were heterozygous (G:A), and 1.7% were homozygous for the rare allele (A:A). The frequency of the wild-type allele (G) was 1128 (88.7%) and the frequency of the rare allele (A) was 144 (11.3%).(Table 1). HWE applied for the *HCRTR2* gene polymorphism rs2653349 in SEC population of this study. The Chi square statistics of 1.27 gives sufficient statistical significance that the population is in HWE for the polymorphism rs2653349 of the *HCRTR2* gene ($p = 0.260$).

Polymorphism rs5443 revealed 44% homozygous wild-type (C:C), 42.6% heterozygous (C:T), and 13.4% homozygous for the rare allele (T:T). The frequency of the wild-type C allele was 831 (65.3%) and that of the mutated T allele was 441 (34.7%). (Table 2) The SEC sample is also in HWE for the polymorphism rs5443 of the *GNB3* gene. For the Chi square statistics of 2.24, the null hypothesis of HWE at the significance level of 5% ($p = 0.118$) was accepted.

To detect possible gender-specific associations, frequency distribution analysis of rs2653349 and rs5443 was calculated. (Tables 3, 4) No association between the polymorphism rs5443 and gender could be established, but for the rs2653349 polymorphism there seems to be a marginally significant 2.8-fold frequency of the A:A genotype appearance probability versus the A:G + G:G genotypes in the male group compared to the female group ($p = 0.080$).

Discussion

CH is a primary ipsilateral cranial autonomic, attack-associated headache featuring nasal congestion, conjunctival injection, forehead and facial sweating, lacrimation, rhinorrhea, meiosis, ptosis, and eyelid edema. The pain recurs at regular intervals and appears almost always in the same area. It lasts from 15 to 180 min and may occur from once every other day to eight times daily (Waldenlind et al. 1987; Matharu 2010). Headaches often begin during sleep, so it can be presumed that disturbed circadian rhythms may be a possible contributor (Weaver-Agostoni 2013; Costa et al. 2015). Neuroendocrine disturbances and circadian biological changes verify the importance of hypothalamic signals in CH. Using functional neuroimaging with PET and anatomical imaging with voxel-based morphometry, the posterior hypothalamic gray matter has been identified as the key area for the basic defect in CH (Goadsby 2002).

Despite circadian effects, vascular dilation and trigeminal nerve stimulation may be mechanisms implicated in the pathophysiology of CH. Further, factors that contribute in the appearance of symptoms include the release of histamine, mast cells increase, genetic factors, and autonomic nervous system activation. Vasodilators such as alcohol and nitroglycerin, inflammatory metabolic intermediaries such as calcitonin gene-related peptide (CGRP), substance P, and somatostatin may also trigger CH (Law et al. 2013; Weaver-Agostoni 2013). In addition, tobacco smoke exposure constitutes a risk factor for developing the disease (Weaver-Agostoni 2013). Immunological processes may also be partially involved in the pathophysiology of this disorder, suppressing CH symptoms, possibly through their anti-inflammatory properties. A possible explanation could be that since interleukin-2 (IL-2) levels, which serve as an indicator of an activated immune system, are elevated in CH-activated periods, corticosteroids work by suppressing IL-2-mediated responses (Steinberg et al. 2011; Empl et al. 2003). Stimulation of trigeminal fibers leads to the release of calcitonin gene-related peptide (CGRP). During a cluster bout, elevated CGRP levels represent a hyperactive state of the trigeminal nervous system and hypothalamic dysfunction (Empl et al. 2003). In a recent study by Neeb et al., it is proposed that these elevated levels can be attenuated by corticosteroids,

Table 1 Comparative allele and genotype distribution frequencies of rs2653349 polymorphism in the African, South Asian, European, East Asian, American, and SEC –male (♂) and female (♀) populations

rs2653349 (Pearson Chi square test)							
	SEC		African		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1322				
G	1128	88.68%	1180	89.26%	0.943	0.737–1.205	n.s.
A	144	11.32%	142	10.74%			
	SEC		S. Asian		Odds ratio	95% CI	*p*
	n = 1272		*n* = 978				
G	1128	88.68%	847	86.60%	1.212	0.941–1.560	n.s
A	144	11.32%	131	13.40%			
	SEC		European		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1006				
G	1128	88.68%	821	81.61%	1.765	1.395–2.234	< 0.0001
A	144	11.32%	185	18.39%			
	SEC		E. Asian		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1008				
G	1128	88.68%	945	93.75%	0.522	0.384–0.711	< 0.0001
A	144	11.32%	63	6.25%			
	SEC		American		Odds ratio	95% CI	*p*
	n = 1272		*n* = 694				
G	1128	88.68%	608	87.61%	1.108	0.833–1.473	n.s
A	144	11.32%	86	12.39%			
	SEC ♂		SEC ♀		Odds ratio	95% CI	*p*
	n = 490		*n* = 782				
G	437	89.18%	691	88.36%	1.086	0.758–1.555	n.s
A	53	10.82%	91	11.64%			
	SEC		African		*p*		
	n = 636		*n* = 661				
GG	503	79.09%	527	79.73%	n.s		
GA	122	19.18%	126	19.06%			
AA	11	1.73%	8	1.21%			
	SEC		S. Asian		*p*		
	n = 636		*n* = 489				
GG	503	79.09%	369	75.46%	n.s		
GA	122	19.18%	109	22.29%			
AA	11	1.73%	11	2.25%			
	SEC		European		*p*		
	n = 636		*n* = 503				
GG	503	79.09%	336	66.80%	< 0.0001		
GA	122	19.18%	149	29.62%			
AA	11	1.73%	18	3.58%			
	SEC		E. Asian		*p*		
	n = 636		*n* = 504				
GG	503	79.09%	442	87.70%	< 0.0001		
GA	122	19.18%	61	12.10%			
AA	11	1.73%	1	0.20%			
	SEC		American		*p*		
	n = 636		*n* = 347				
GG	503	79.09%	269	77.52%	n.s		
GA	122	19.18%	70	20.17%			
AA	11	1.73%	8	2.31%			
	SEC ♂		SEC ♀		*p*		
	n = 245		*n* = 391				
GG	199	81.22%	304	77.75%	0.068		
GA	39	15.92%	83	21.23%			
AA	7	2.86%	4	1.02%			

(Data for African, South Asian, European, East Asian, and American populations are previously published –http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 6:55141837-55142837;v = rs2653349;vdb = variation;vf = 2007914)

Table 2 Comparative allele and genotype distribution frequencies of rs5443 polymorphism in the African, South Asian, European, East Asian, American, and SEC –male (♂) and female (♀) populations

rs5443 (Pearson Chi square test)							
	SEC		African		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1322				
C	831	65.33%	239	18.08%	8.539	7.121–10.238	< 0.0001
T	441	34.67%	1083	81.92%			
	SEC		S. Asian		Odds ratio	95% CI	*p*
	n = 1272		*n* = 978				
C	831	65.33%	675	69.02%	0.846	0.708–1.011	0.065
T	441	34.67%	303	30.98%			
	SEC		European		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1006				
C	831	65.33%	697	69.28%	0.835	0.700–0.997	0.046
T	441	34.67%	309	30.72%			
	SEC		E. Asian		Odds ratio	95% CI	*p*
	n = 1272		*n* = 1008				
C	831	65.33%	501	49.70%	1.907	1.610–2.258	< 0.0001
T	441	34.67%	507	50.30%			
	SEC		American		Odds ratio	95% CI	*p*
	n = 1272		*n* = 694				
C	831	65.33%	431	62.10%	1.150	0.949–1.393	n.s
T	441	34.67%	263	37.90%			
	SEC ♂		SEC ♀		Odds ratio	95% CI	*p*
	n = 490		*n* = 782				
C	316	64.49%	515	65.86%	0.942	0.743–1.193	n.s
T	174	35.51%	267	34.14%			
	SEC		African		*p*		
	n = 636		*n* = 661				
CC	280	44.03%	24	3.63%	< 0.0001		
CT	271	42.61%	191	28.90%			
TT	85	13.36%	446	67.47%			
	SEC		S. Asian		*p*		
	n = 636		*n* = 489				
CC	280	44.03%	229	46.83%	0.056		
CT	271	42.61%	217	44.38%			
TT	85	13.36%	43	8.79%			
	SEC		European		*p*		
	n = 636		*n* = 503				
CC	280	44.03%	243	48.31%	n.s		
CT	271	42.61%	211	41.95%			
TT	85	13.36%	49	9.74%			
	SEC		E. Asian		*p*		
	n = 636		*n* = 504				
CC	280	44.03%	121	24.01%	< 0.0001		
CT	271	42.61%	259	51.39%			
TT	85	13.36%	124	24.60%			
	SEC		American		*p*		
	n = 636		*n* = 347				
CC	280	44.03%	138	39.77%	n.s		
CT	271	42.61%	155	44.67%			
TT	85	13.36%	54	15.56%			
	SEC ♂		SEC ♀		*p*		
	n = 245		*n* = 391				
CC	103	42.04%	177	45.27%	n.s		
CT	110	44.90%	161	41.18%			
TT	32	13.06%	53	13.55%			

(Data for African, South Asian, European, East Asian, and American populations are previously published –http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 12:6954375-6955375;v = rs5443;vdb = variation;vf = 5200)

Table 3 Comparative genotype distribution frequencies of rs2653349 polymorphism in the African, South Asian, European, East Asian, American, and SEC –male (♂) and female (♀) populations

rs2653349 (Pearson Chi square test)							
	SEC $n = 636$		African $n = 661$		Odds ratio	95% CI	p
GG	503	79.09%	527	79.73%	0.962	0.735–1.259	n.s.
GA and AA	133	20.91%	134	20.27%			
	SEC $n = 636$		S. Asian $n = 489$		Odds ratio	95% CI	p
GG	503	79.09%	369	75.46%	1.230	0.929–1.629	n.s
GA and AA	133	20.91%	120	24.54%			
	SEC $n = 636$		European $n = 503$		Odds ratio	95% CI	p
GG	503	79.09%	336	66.80%	1.880	1.440–2.453	< 0.0001
GA and AA	133	20.91%	167	33.20%			
	SEC $n = 636$		E. Asian $n = 504$		Odds ratio	95% CI	p
GG	503	79.09%	442	87.70%	0.531	0.382–0.736	< 0.0001
GA and AA	133	20.91%	62	12.30%			
	SEC $n = 636$		American $n = 347$		Odds ratio	95% CI	p
GG	503	79.09%	269	77.52%	1.097	0.799–1.505	n.s
GA and AA	133	20.91%	78	22.48%			
	SEC ♂ $n = 245$		SEC ♀ $n = 391$		Odds ratio	95% CI	p
GG	199	81.22%	304	77.75%	1.238	0.830–1.846	n.s
GA and AA	46	18.78%	87	22.25%			
	SEC $n = 636$		African $n = 661$		Odds ratio	95% CI	p
AA	11	1.73%	8	1.21%	1.437	0.574–3.595	n.s.
GG and GA	625	98.27%	653	98.79%			
	SEC $n = 636$		S. Asian $n = 489$		Odds ratio	95% CI	p
AA	11	1.73%	11	2.25%	0.765	0.329–1.779	n.s
GG and GA	625	98.27%	478	97.75%			
	SEC $n = 636$		European $n = 503$		Odds ratio	95% CI	p
AA	11	1.73%	18	3.58%	0.474	0.222–1.013	0.049
GG and GA	625	98.27%	485	96.42%			
	SEC $n = 636$		E. Asian $n = 504$		Odds ratio	95% CI	p
AA	11	1.73%	1	0.20%	8.853	1.139–68.801	0.012
GG and GA	625	98.27%	503	99.80%			
	SEC $n = 636$		American $n = 347$		Odds ratio	95% CI	p
AA	11	1.73%	8	2.30%	0.746	0.297–1.872	n.s
GG and GA	625	98.27%	339	97.70%			
	SEC ♂ $n = 245$		SEC ♀ $n = 391$		Odds ratio	95% CI	p
AA	7	2.86%	4	1.02%	2.777	0.922–8.361	0.080*
GG and GA	238	97.14%	387	98.98%			

(Data for African, South Asian, European, East Asian, and American populations are previously published –http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 6:55141837-55142837;v = rs2653349;vdb = variation;vf = 2,007,914)

*Fisher's exact test (one-sided)

which may act directly on the trigeminal nerve system and the hypothalamus, but it cannot be safely assumed that the changes in CGRP and melatonin levels, observed in this study, are the causes of the reduced attack frequency, rather than a mere consequence (Neeb et al. 2015).

Table 4 Comparative genotype distribution frequencies of rs5443 polymorphism in the African, South Asian, European, East Asian, American, and SEC –male (♂) and female (♀) populations

Rs5443 (Pearson Chi square test)							
	SEC $n = 636$		African $n = 661$		Odds ratio	95% CI	p
TT	85	13.36%	446	67.47%	0.074	0.056–0.098	< 0.0001
CT and CC	551	86.64%	215	35.53%			
	SEC $n = 636$		S. Asian $n = 489$		Odds ratio	95% CI	p
TT	85	13.36%	43	8.79%	1.600	1.086–2.357	0.017
CT and CC	551	86.64%	446	91.21%			
	SEC $n = 636$		European $n = 503$		Odds ratio	95% CI	p
TT	85	13.36%	49	9.74%	1.429	0.984–2.075	0.059
CT and CC	551	86.64%	454	90.26%			
	SEC $n = 636$		E. Asian $n = 504$		Odds ratio	95% CI	p
TT	85	13.36%	124	24.60%	0.473	0.348–0.642	< 0.0001
CT and CC	551	86.64%	380	75.40%			
	SEC $n = 636$		American $n = 347$		Odds ratio	95% CI	p
TT	85	13.36%	54	15.56%	0.837	0.579–1.211	n.s
CT and CC	551	86.64%	293	84.44%			
	SEC ♂ $n = 245$		SEC ♀ $n = 391$		Odds ratio	95% CI	p
TT	32	13.06%	53	13.55%	0.958	0.598–1.535	n.s
CT and CC	213	18.78%	338	86.45%			
	SEC $n = 636$		African $n = 661$		Odds ratio	95% CI	p
CC	280	44.02%	24	3.63%	20.875	13.491–32.303	< 0.0001
CT and TT	356	55.98%	637	96.37%			
	SEC $n = 636$		S. Asian $n = 489$		Odds ratio	95% CI	p
CC	280	44.02%	229	46.83%	0.893	0.705–1.132	n.s
CT and TT	356	55.98%	260	53.17%			
	SEC $n = 636$		European $n = 503$		Odds ratio	95% CI	p
CC	280	44.02%	243	48.31%	0.842	0.665–1.064	n.s
CT and TT	356	55.98%	260	51.69%			
	SEC $n = 636$		E. Asian $n = 504$		Odds ratio	95% CI	p
CC	280	44.02%	121	24.01%	2.490	1.924–3.221	< 0.0001
CT and TT	356	55.98%	383	75.99%			
	SEC $n = 636$		American $n = 347$		Odds ratio	95% CI	p
CC	280	44.02%	138	39.77%	1.191	0.913–1.554	n.s
CT and TT	356	55.98%	209	60.23%			
	SEC ♂ $n = 245$		SEC ♀ $n = 391$		Odds ratio	95% CI	p
CC	103	42.04%	177	45.27%	0.877	0.635–1.211	n.s
CT and TT	142	57.96%	214	54.73%			

(Data for African, South Asian, European, East Asian and American populations are previously published –http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 12:6954375-6955375;v = rs5443;vdb = variation;vf = 5200)

The difference in the therapeutic approach between migraines and CH is that in the case of the acute nature of CH episodes, a very rapid pain relief is anticipated. Therefore, the route of administration is of great importance, making the subcutaneous route the most efficient due to its rapid absorption, followed by the intranasal or sublingual routes, where absorption may be partly enteral and partly parenteral. In the acute form of CH, per os administration is not part of first-line

treatment because of its more complex absorption, metabolism (hepatic first-pass metabolism), and bioavailability, limiting the use of triptans in the clinical practice. So far, only sumatriptan is available in the subcutaneous formulation, only zolmitriptan in the intranasal, and only rizatriptan as a melt wafer; and all of them are available as oral tablets (Law et al. 2013).

A comparison of the SEC frequencies for the two polymorphisms versus African population (AFR), South Asian population (SAS), other European populations (EUR), East Asian population (EAS), and American population (AMR) was also performed and visualized by a distance graph (Fig. 1). Possible gender-specific differences were investigated.

The rs2653349 (G1246A) polymorphism of the *HCRTR2* gene reduces the chance of developing CH, meaning that genetic predisposition for developing CH is lower for those individuals carrying the protective rare allele A. The rare A allele occurs in 12.1% of the global population, in 10.7% of the AFR, in 13.4% of the SAS, in 18.4% of the EUR, in 6.2% of the EAS, and in 12.4% of the American population. This study revealed the presence of the A allele in 11.3% of the SEC study population sample. Chi square test was conducted between the AFR, SAS, EUR, EAS, AMR, and SEC populations in order to determine significant differences between the allele frequency distribution regarding the rs2653349 polymorphism. The mutant allele appeared twice as often in our study population as compared to the EAS and 1.7 times more often in the other EUR as compared to SEC population. The allele frequencies of the current SEC study population were significantly different when compared to EAS (OR 0.522, $p < 0.0001$) and other EUR (OR 1.765, $p < 0.0001$). (http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r=6:55141837-55142837;v = rs2653349;vdb = variation;vf = 2,007,914) (Table 1).

The mutant A:A genotype is found in 1.8% of the global population, in 1.2% of the AFR, in 2.2% of the SAS, in 3.6% of the European population, in 0.2% of the East Asian population, in 2.3% of the AMR, and in 1.7% of our study population. Heterozygous individuals, i.e., those carrying the A:G genotype are found in 20.6% of the global population, in 19.1% of the AFR, in 22.3% of the SAS, in 29.6% of the European population, in 12.1% of the East Asian population, in 20.2% of the AMR, and in 19.2% of the SEC population. Homozygous individuals for the wild type G:G are found in 77.6% of the global population, in 79.7% of the AFR, in 75.5% of the SAS, in 66.8% of the European population, in 87.7% of the EAS, in 77.5% of

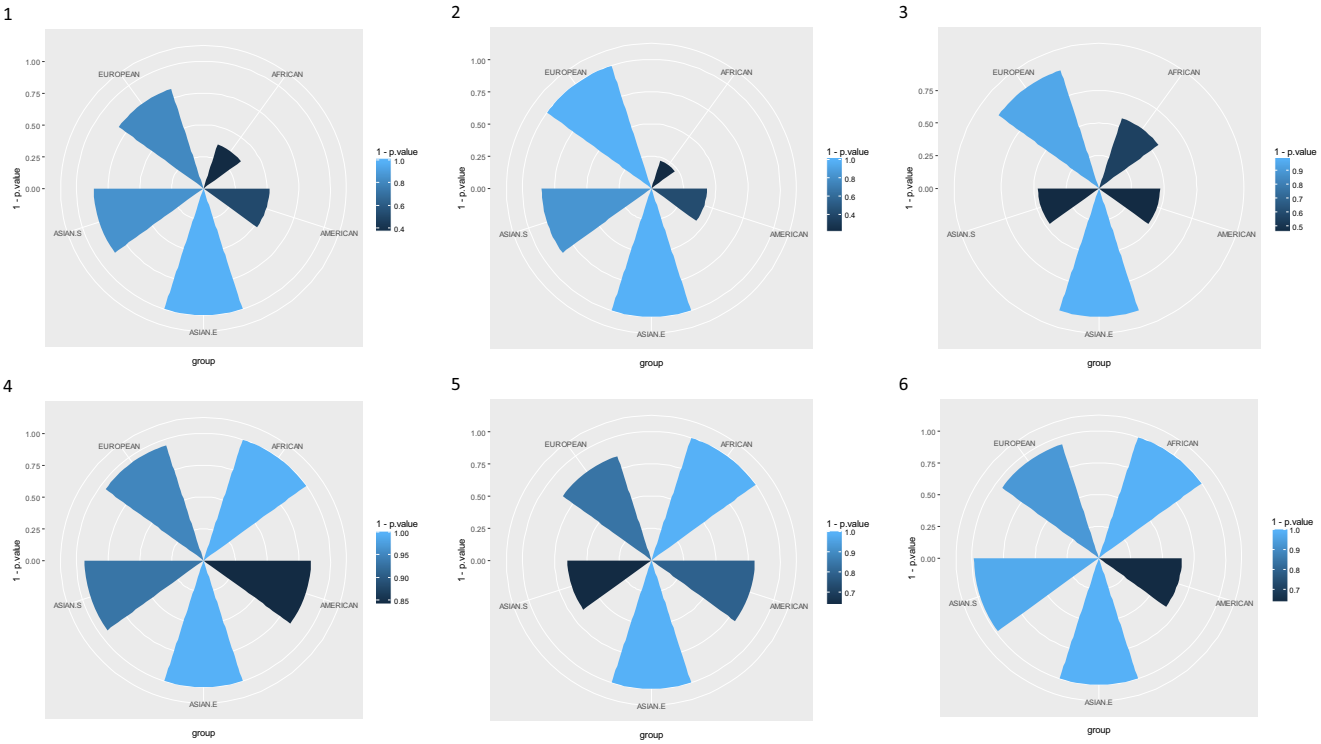

Fig. 1 Distance graphs (determined by 1 minus *p* value of Pearson Chi square test) of (1) similarities/differences between Southeastern European Caucasian and other populations for the frequencies of the *HCRTR2* polymorphism rs2653349 wild-type G allele and the rare A allele, (2) similarities/differences between Southeastern European Caucasian and other populations for the frequencies of the wild-type rs2653349 G:G genotype versus the G:A + A:A genotypes, (3) similarities/differences between Southeastern European Caucasian and other populations for the frequencies of the rs2653349 A:A genotype versus the G:A + G:G genotypes, (4) similarities/differences between Southeastern European Caucasian and other populations for the frequencies of the *GNB3* polymorphism rs5443 wild-type C allele and the rare T allele, (5) similarities/differences between Southeastern European Caucasian and other populations for the frequencies of the rs5443 wild-type C:C genotype versus the C:T + T:T genotype, and (6) similarities/differences between and other populations for the frequencies of the rare rs5443 genotype T:T versus the C:T + C:C genotypes

the AMR, and in 79.1% of the SEC population. According to the Chi square test between the AFR, SAS, EUR, EAS, AMR, and SEC populations, the genotype frequencies of the current SEC study population were significantly different when compared to the EUR and EAS ($p < 0.0001$). (Table 1) The A:A genotype versus the G:G + G:A genotypes appeared twice as often in the EUR population compared to the SEC population (OR 0.474, $p = 0.049$) and 8.9 times more often in the SEC population compared to EAS (OR 8.853, $p = 0.012$) (Table 3).

(http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 6:55141837-55142837;v = rs2653349;vdb = variation;vf = 2007914)

According to the results for polymorphism rs5443, the mutant allele (T) occurs in the 34.7% of the SEC population, in 49.2% of the global population, in 81.9% of the AFR, in 31.0% of the SAS, in 30.7% of the EUR, in 50.3% of the EAS, and in 37.9% of the AMR. The Chi square test was conducted between the SEC and the other populations in order to determine the existence of a significant difference between the allele frequency distribution regarding the rs5443 polymorphism. The allele frequencies of the current study population were significantly different when compared to the EUR ($p = 0.046$), AFR ($p < 0.0001$), and EAS ($p < 0.0001$) populations. The mutated T allele has 8.6 times more frequent appearance probability in the AFR (OR 8.539, $p < 0.0001$) and twice as often in EAS (OR:1.907, $p < 0.0001$) compared to the SEC population and 1.2 times more frequent appearance probability in the SEC population compared to the EUR (OR 0.835, $p = 0.046$). (Table 2).

(http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 12:6954375-6955375;v = rs5443;vdb = variation;vf = 5200)

Homozygotes for the wild-type allele appear in 30.2% of the global population, in 3.6% of the AFR, in 46.8% of the SAS, in 48.3% of the EUR, in 24.0% of the EAS, in 39.8% of the AMR, and in 44.0% of our study population. The heterozygous genotypes appear in the 41.3% of the global population, in 28.9% of the AFR, in 44.4% of the SAS, in 41.9% of the EUR, in 51.4% of the EAS, in 44.7% of the AMR, and in 42.6% of the SEC population. Homozygosity for the mutant allele appears in 28.6% of the global population, in 67.5% of the AFR, in 8.8% of the SAS, in 9.7% of the EUR, in 24.6% of the EAS, in 15.6% of the AMR, and in 13.4% of our study population. The Chi square test showed statistically significant differences between the genotype frequencies of SEC population compared to AFR ($p < 0.0001$) and EAS ($p < 0.0001$) populations (Table 3). There seems to be a 1.6 and 1.4 more frequent T:T genotype appearance probability versus the T:C + C:C genotypes in the SEC population of this study compared to the SAS (OR 1.600, $p = 0.017$) and EUR (OR 1.429, $p = 0.059$) populations, respectively. Furthermore, Chi square test showed a 13.5 more frequent T:T genotype appearance probability versus T:C + C:C genotypes in the AFR (OR 0.074, $p < 0.0001$) and twice as often in the EAS (OR 0.473, $p < 0.0001$) compared to the SEC population (Table 4).

(http://grch37.ensembl.org/Homo_sapiens/Variation/Population?r = 12:6954375-6955375;v = rs5443;vdb = variation;vf = 5200)

The percentage of CH prevalence is calculated to be 0.02 to 0.4, with a male preponderance (Law et al. 2013). The almost threefold higher frequency of the rs2653349 A:A genotype in the male group of the study compared to the female group (OR = 2.78, $p = 0.080$) observed in this study (Table 3), denotes that SEC men may have better odds to be protected against CH, a result that is in contrast to recent literature, according to which the prevalence of CH has a male preponderance (Law et al. 2013). Respectively, SEC women appear to have a 2.8 less frequent probability to be protected while carrying the protective A allele in homozygosity (Table 3). Obviously, the pathophysiology of CH is affected by multiple factors, other than the genetic background. Based on the above, SEC men generally seem to be more "armored" regarding the genetic predisposition to present with CH.

The greater percentage of the rs5443 polymorphism T allele is attributed to the heterozygous genotypes, enhancing a hypothesis that the T allele may be the dominant one, due to the fact that it appears more frequently than expected in the gene pool of this study, especially in the case of heterozygosity.

In conclusion, CH pathophysiology and pharmacotherapy response may be affected by genetic factors. Significant genetic differences of rs2653349 and rs5443 exist between SEC population and other populations. SEC men may have better odds to be protected against CH while SEC women appear a less frequent probability to be protected while carrying the protective A allele of *HCRTR2* gene polymorphism in homozygosity. SEC men generally seem to be more "armored" regarding the genetic predisposition to present with CH. Genotyping potentially enhances diagnosis susceptibility and positively affects the overall treatment effectiveness of CH.

Limitations of the Study

Further, larger scale investigations may be needed, in order to precisely determine CH-associated polymorphism incidence rates. Furthermore, novel CH-associated gene polymorphisms may be necessary in order to form an algorithm capable to reveal CH susceptibility as well as to predict individual drug response. This would enable accurate prediction of CH developing prevalence to serve as individualized prevention tool.

The present study was conducted in a CH-free general population. Further studies should be initialized to investigate the frequency distribution of these polymorphisms in patients experiencing CH.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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