Investigation Between the S377G3 GATA-4 Polymorphism and Migraine

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Abstract: Migraine is a common and painful neurological disorder, with genetic and environmental components. Several conditions have been shown to be comorbid with migraine, notably a cardiac malformation affecting the interatrial septum and leading to patent foramen ovale (PFO). Mutations in the development regulatory gene GATA-4, located on human chromosome 8p23.1-p22, have been found to be responsible for some cases of congenital heart defects including PFO. To determine whether the GATA-4 gene is involved in migraine, the present study performed an association analysis of a common GATA-4 variant that results in a change of amino acid (S377G), in a large case/control population (275 unrelated Caucasian migraineurs versus 275 control individuals). The results showed that there was no significant association for this polymorphism between migraine and controls ($\chi^2 = 0.84, P = 0.66$). Thus it appears that the GATA-4 (S377G) mutation does not play a significant role in common migraine susceptibility.

Keywords: Migraine, genetic association, GATA 4 polymorphism, Heart disorder.

INTRODUCTION

Migraine is a common and debilitating neurological disease that affects a significant proportion of the population, usually affecting 10%-12% of the Western population [1]. Characteristic manifestations of migraine include head pain, nausea, vomiting, photophobia and often severe neurological disturbances [2]. The most common forms of this disorder have been classified as migraine with aura (MA) and migraine without aura (MO) [2]. One of the most important aspects of the pathophysiology of migraine is the inherited nature of the disorder, with twin studies showing the importance of both genetic and environmental factors [3]. Migraine is now usually viewed as a polygenic multifactorial disease, with both environmental and genetic causative factors, and with multiple possibly interacting genes [4]. The age of onset for this disorder also varies, but in females it has been found that it is usually at puberty or shortly after, being less frequent in middle life and developing three times as often in women as in men [5].

Several disorders have been shown to be comorbid with migraine, including epilepsy, asthma, depression, stroke and some congenital heart defects [6]. The cardiovascular association between migraine and ischaemic stroke is not coincidental and it could be due to a common genetic component and to the presence of different mutations in the same gene [7]. One heart disorder that has been associated with migraine is the cardiac malformation, patent foramen ovale (PFO) [6].

Patent foramen ovale results from an incomplete anatomical fusion of the atrial septum primum and secundum, which normally takes place shortly after birth [8]. This condition leads to a persistent connection between the right and left atrium of the heart, by which right-to-left shunt may result [8]. Large shunts more often than small shunts are associated with migraine with aura [9, 10]. PFO is believed to play a role in cryptogenic stroke via presumed paradoxical embolism [11]. The right to left shunt brought about by PFO could enhance migraine by affecting systemic levels of brain platelet neuromediators, like 5-hydroxytryptamine (5-HT), which is normally, inactivated by the pulmonary filter, thus triggering a migraine attack [12, 13].

Consequently, the increased risk of stroke in patients with MA could be explained by an increased propensity to paradoxical embolism [8]. The prevalence of PFO in the healthy population is approximately 20%-25%. A high prevalence of right to left shunt has been observed in patients with MA compared to healthy control subjects with a 41%-48% PFO prevalence in migraine with aura patients [11].

Migraine has been shown to be an independent risk factor for subsequent coronary heart disease events among women in the Women’s Health Study (WHS) and in men in the Physician’s Health Study (PHS) [11]. Recent evidence has shown that migraineurs who experience an aura are twice as likely to have a PFO compared to the general population [14]. Association between PFO and migraine has been found to be stronger in patients with MA than in those without aura [15]. Recent reports have emphasised an association between PFO, MA and stroke with 25 patients (PFO was considered...
to play a causal role in stroke) among 74 consecutive patients with cryptogenic stroke found to have a 52% prevalence of MA [12]. The incidence of PFO in patients with migraine is about 50% if migraine is accompanied by visual aura, versus 20% in the general population [16].

Mutations in developmental regulatory genes have been found to be responsible for some cases of congenital heart defects [17]. GATA-4 is one such regulatory gene. It is part of the GATA family of zinc finger transcription factors [18], which play important roles in transducing nuclear events that modulate cell lineage differentiation during development and hypertrophy of adult cardiac myocytes [18].

To investigate the potential role of GATA-4 gene in migraine an association study of the S377G polymorphism was conducted in a migraine case and age, sex and ethnicity matched control population. The S377G polymorphism (rs3729856) is located in exon 5 of GATA-4 gene and was genotyped using a new silica bead-based technology. It is a non-synonymous coding polymorphism with an amino acid change (serine to glycine) and unpublished studies (R.H., L.G., unpublished data) have shown a reasonably high frequency of the S377G rarer allele in Caucasian populations (Australian Caucasians (n=400, 13.9%).

MATERIALS AND METHODS

Subjects

This research has been approved by the Griffith University Ethics Committee for experimentation on human subjects; all participants of the study gave consent. The association population has been matched for sex, age (+/- 5 years), and ethnicity. All subjects were of Australian Caucasian origin, interviewed by clinical neurologists and were diagnosed for either migraine without aura (MO) or migraine with aura (MA) according to the criteria of the International Headache Society [2]. None of the subjects were tested for PFO or other heart disorders. The study population comprised 275 migraineurs and 275 unrelated control individuals, with the control group matched for sex, age (+/- 5 years), and ethnicity, to minimize potential bias from population stratification.

Genotyping

In this study we utilized standard polymerase chain reaction (PCR) analysis for the genotyping of the migraine case/control population (275 unrelated Caucasian migraineurs versus 275 control individuals), using forward primer: 5’ TGT CCC CGG CAA ATG TAG ATA AAG’3 and reverse primer: 5’ CAG TCG GCC TCC CCA AAC ACA GC’3, resulting in a 318 base pair fragment following PCR. The thermocycler conditions were 94°C for 15 minutes followed by 40 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. PCR was completed with an extended incubation of 5 minutes at 72°C. The genotyping was undertaken by means of single nucleotide polymorphism (SNP) genotyping, using flow cytometry developed by General Biosystems. This is a silica bead-based SNP genotyping system that discriminates between alleles on the basis of competitive hybridisation between fluorescently labelled allele specific probes. In addition to the forward and reverse primers a mix containing three reagents specific for the SNP detection were used. These reagents consisted of, the AmpaSandTM beads with a covalently bound oligonucleotides which is complementary to the ssDNA, an oligonucleotide probe specific for one allele labelled with a yellow or green dye and an oligonucleotide probe specific for the alternative allele labelled with a red dye. Each reaction was analysed by a FACSArray flow cytometer. Genotypes for all samples were determined by MPlots© software.

Sequencing

The samples were sequenced using ABI Prism 377 dideoxy chain termination reaction. To confirm the results previously obtained by SNP genotyping, 5% of the population was sequenced. The sequences produced were analysed through the computer software CHROMAS.

Statistics

Chi-square analysis was used to determine if the allele frequency of the S377G polymorphism in the GATA-4 gene were associated or not with migraine, and if the allele frequencies were significant (α = 0.05). Chi-square analysis was also used to determine if the obtained genotype frequencies were in Hardy-Weinberg equilibrium.

Ethical approval

This research was reviewed and approved by the Griffith University Human Research Ethics Committee (ethics proto-

| Genotypes | AA | GA | GG | total (n) | A | G | total (n) |
|-----------|----|----|----|---------|---|---|---------|
| Migraine  | 158 (81%) | 34 (18%) | 2 (1%) | 194 | 350 (90.2%) | 38 (9.8%) | 388 |
| FEMALE    | 111 (81%) | 25 (18.2%) | 1 (0.8%) | 137 | 247 (90%) | 27 (10%) | 274 |
| MA        | 47 (82.4%) | 9 (15.8%) | 1 (1.8%) | 57 | 103 (90.4%) | 11 (9.6%) | 114 |
| CONTROL   | 169 (84.1%) | 29 (14.4%) | 3 (1.5%) | 201 | 367 (91.3%) | 35 (8.7%) | 402 |
| FEMALE    | 122 (84.1%) | 20 (13.8%) | 3 (2.1%) | 145 | 264 (91%) | 26 (9%) | 290 |
| MA        | 47 (83.9%) | 9 (16.1%) | 0 (0) | 56 | 103 (92%) | 9 (8%) | 112 |

Table 1. Distribution of the S377G Polymorphism in Migraineurs and Controls of Original Sample (MO Migraine without Aura, MA Migraine with Aura)
col number MSC/05/05/HREC) and all subjects participating in the study gave informed consent.

**RESULTS**

To determine whether the *GATA-4* mutation had an important role in migraines, an association analysis of the S377G polymorphism in a large case/control population of migraineurs was performed. Genotypes for 194 cases and 201 controls were determined for the case/control tested population (275/275). The genotype data can be observed in Table 1 as well as the allele frequency for the migraine and control populations. Statistical analysis of the S377G polymorphism revealed that there was no significant difference ($\chi^2 = 0.84, P = 0.66$) between the migraine population and the controls. Moreover there was no significant difference observed in the migraine and control population with regards to female and males for this particular association study. Furthermore no association was found between migraine with aura and controls ($\chi^2 = 0.99, P = 0.61$), nor between migraine without aura and controls ($\chi^2 = 0.2, P = 0.91$). Hardy Weinberg equilibrium was investigated for both migraine cases and controls. It was found that allele frequencies did not deviate from Hardy Weinberg equilibrium in both the case and control groups ($P = 0.91; P = 0.19$).

**DISCUSSION**

Six family members of have been identified in vertebrates to be part of the *GATA* family and have been subdivided in two groups: *GATA-1*, *GATA-2*, and *GATA-3*, which are expressed in the hematopoetic system [19]. *GATA-4*, *GATA-5* and *GATA-6*, which are critical for differentiation and cell-specific gene expression in different endoderm and mesoderm, derived tissues [19]. The human *GATA-4* gene is located in chromosome 8p23.1-p22 and contains 6 exons [20] and regulates its expression through its zinc finger [21]. It is widely expressed in the endoderm, mesoderm, heart, gonads, liver, small intestine and pancreas [19]. Functional studies have shown a role for the *GATA-4* gene in cardiac embryogenesis [21] and mutations in this gene have been found to be associated with congenital heart defects such as atrial septal defect (ASD) and patent foramen ovale (PFO) [17].

Reamon-Buettner et al. (2005) found six mutations in the N-finger of *GATA-4* in patients with PFO. This includes a homozygous deletion (677delC), that would lead to a frameshift mutation affecting critical residues arginine and histidine [21].

A number of studies have found a strong association between PFO and cryptogenic stroke, due to an increase of right heart pressure therefore predisposing to paradoxical embolism [22, 23]. A further association that has been increasingly reported is the one between migraine, particularly migraine with aura, and PFO [24]. A number of studies have shown an increased prevalence of PFO in young patients that present with migraine with aura and cryptogenic stroke [8, 16]. The severity of this disorder varies according to the size of the opening found in the heart of the patient. Family studies suggest that PFO is potentially associated with migraine with aura particularly as a risk factor for stroke in women [25, 26].

The *GATA-4* gene contains a common variation that results in a S377G polymorphism missense mutation with an alternative nucleotide of A/G, changing from the serine amino acid to the glycine amino acid. The variant is prevalent in a number of dispersed Caucasian populations and the allele frequency in Australian Caucasians is 13.9% (R.H. and L.G., unpublished data). It thus could conceivably play a role as a common contributing allele to a disorder like migraine. This study aimed to determine the role of the S377G polymorphism in the *GATA-4* gene in migraine and if there is a significant association between patients with migraine and this mutation. Genotypes were determined for the S377G polymorphism but results showed no association with migraine in the case/control groups studied. Moreover there was no significant association found between migraine with aura and the control population and no significant difference was observed between females with migraine versus control females for the migraine population.

Several studies have reported a higher prevalence of migraine in patients suffering from congenital heart disease [24, 26, 27]. Recently, Hirth and collaborators have studied the prevalence between congenital heart disease sufferers with migraine [28]. They reported an increased prevalence of migraine with aura in congenital heart disease more specifically in PFO patients (89% of these patients suffer from migraine) [28].

In addition, Tatdelide et al. has studied the prevalence of PFO in migraine sufferers [29]. They have found percentages of PFO in migraine patients with aura, without aura and

| Table 2. Chi-Squared ($\chi^2$) Analysis of all Migraine Groups Against Controls for the S377G Polymorphism |
|---------------------------------------------------|-------------------|---------|-------------------|-------------------|
| Genotypes                                         | P-value           | Alleles                        | P-value           |
| Migraine vs Control                               | 0.84              | 0.66                            | 0.28              | 0.6               |
| Subtypes                                          |                   |                                 |                   |
| MA vs Control                                     | 0.99              | 0.61                            | 0.29              | 0.59              |
| MO vs Control                                     | 0.2               | 0.91                            | 0.09              | 0.77              |
| Mig Female vs Control Female                      | 1.85              | 0.4                             | 0.13              | 0.72              |
| Mig Male vs Control Male                          | 0.99              | 0.61                            | 0.18              | 0.67              |
the control group were 66.7%, 47.4% and 22.2%, respectively, results suggesting an association between PFO and migraine, especially with aura [29].

CONCLUSIONS

Numerous studies have shown that mutations in the GATA-4 gene have a significative role in cardiogenesis and are associated with congenital heart defects, like PFO. A previous mutation, homozygous deletion 677del C, has been found on the N-terminal zinc finger of GATA-4 gene, and previously reported in PFO patients [28].

Our study has reported a lack of association of the S377G polymorphism in the studied migraine population, which does not support a correlation between this particular gene variant and migraine. It appears that the GATA-4 (S377G) mutation does not play a significant role in common migraine susceptibility. However other variants in this gene may still play a role in migraine susceptibility. Further studies investigating other GATA-4 variants in families and case/control populations are warranted to more clearly determine whether this gene plays a role in migraine. Furthermore an association study investigating individuals that present with both PFO and migraine, particularly migraine with aura, would be very interesting and to this end we are currently collecting subjects with this pertinent information.

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REFERENCES

[1] Breslau N, Rasmussen BK. The impact of migraine: Epidemiology, risk factors, and co-morbidities. Neurology 2001; 56(6 Suppl 1): S4-12.
[2] HICCHS. Headache Classification Committee for the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. 2nd Ed. Cephalgia 2004; 24 (Suppl 1): 1-60.
[3] Gervil M, Ulrich V, Kyvik KO, Olesen J, Russell MB. Migraine without aura: a population-based twin study. Ann Neurol 1999; 46(4): 606-11.
[4] Montagna P. Molecular genetics of migraine headaches: a review. Cephalalgia 2000; 20(1): 3-14.
[5] Bigal ME, Liberman JN, Lipton RB. Age-dependent prevalence and clinical features of migraine. Neurology 2006; 67(2): 246-51.
[6] Scher AI, Bigal ME, Lipton RB. Comorbidity of migraine. Curr Opin Neurol 2005; 18(3): 305-10.
[7] Buzzi MG, Cologno D, Fornirosso R. Migraine disease: evolution and progression. J Headache Pain 2005; 6(4): 304-6.
[8] Diener HC, Weimar C, Katsarava Z. Patent foramen ovale: paradoxical connection to migraine and stroke. Curr Opin Neurol 2005; 18(3): 299-304.
[9] Wilmshurst PT, Pearson MJ, Nightingale S, Walsh KP, Morrison WL. Inheritance of persistent foramen ovale and atrial septal defects and the relation to familial migraine with aura. Heart 2004; 90(11): 1315-20.
[10] Messe SR, Silverman IE, Kizer JR, et al. Practice parameter: recurrent stroke with patent foramen ovale and atrial septal aneurysm: report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2004; 62(7): 1042-50.
[11] Pierangelini G, Cevoli S, Zanigni S, et al. The role of cardiac diseases in the comorbidity between migraine and stroke. Neurol Sci 2004; 25 (Suppl 3): S129-31.
[12] Morandi E, Anzola GP, Angeli S, Melzi G, Onorato E. Transcatheter closure of patent foramen ovale: a new migraine treatment? J Interv Cardiol 2003; 16(1): 39-42.
[13] Wilmshurst P, Nightingale S. The role of cardiac and pulmonary pathology in migraine: a hypothesis. Headache 2006; 46(3): 349-352.
[14] Reisman M, Christofferson RD, Jersurum J, et al. Migraine headache relief after transcatheter closure of patent foramen ovale. J Am Coll Cardiol 2005; 45(4): 493-5.
[15] Dalla Volta G, Guindani D, Zavaripe P, Griffini S, Pizzini A. Prevalence of patent foramen ovale in a large series of patients with migraine with aura, migraine without aura and cluster headache, and relationship with clinical phenotype. J Headache Pain 2005; 6(4): 328-30.
[16] Tobis MJ, Azarbal B. Does patent foramen ovale promote cryptogenic stroke and migraine headache? Tex Heart Inst J 2005; 32(3): 362-5.
[17] Zeisberg EM, Ma Q, Jurasek AL, et al. Morphogenesis of the right ventricle requires myocardial expression of Gata4. J Clin Invest 2005; 115(6): 1522-31.
[18] Suzuki YJ, Nagase H, Day RM, Das DK. GATA-4 regulation of myocardial survival in the preconditioned heart. J Mol Cell Cardiol 2004; 37(6): 1195-203.
[19] Ritz-Laser B, Mamin A, Brun T, Avril I, Schwitzgebel VM, Philippe J. The zinc finger-containing transcription factor Gata-4 is expressed in the developing endocrine pancreas and activates glucon gene expression. Mol Endocrinol 2005; 19(3): 759-70.
[20] Pehlivan T, Pober BR, Brueckner M, et al. GATA4 haploinsufficiency in patients with intrasitial deletion of chromosome region 8p23.1 and congenital heart disease. Am J Med Genet 1999; 83(3): 201-6.
[21] Reamons-Buettner SM, Borkaj J. GATA4 zinc finger mutations as a molecular rationale for septation defects of the human heart. J Med Genet 2005; 42(5): e32.
[22] McGaw D, Harper R. Patent foramen ovale and cryptogenic cerebral infarction. Intern Med J 2001; 31(1): 42-7.
[23] Di Tullio M, Sacco RL, Goyal A, Mohr JP, Homma S. Patent foramen ovale as a risk factor for cryptogenic stroke. Ann Intern Med 1992; 117(6): 461-5.
[24] Tepper SJ, Sheffiel FD, Bigal ME. The patent foramen ovale-migraine question. Neurol Sci 2007; 28 (Suppl 2): S118-23.
[25] Morelli N, Tartaglione A, Gorl S, et al. Migraine with aura and patent foramen ovale: are they related? Headache 2008; 48(4): 637-8.
[26] Diener HC, Kurth T, Dodick D. Patent foramen ovale and migraine. Curr Pain Headache Rep 2007; 11(3): 236-40.
[27] Domiritz I, Mieszkowski J, Kwiecinski H. The prevalence of patent foramen ovale in patients with migraine. J Neuro Neurochir Pol 2004; 38(2): 89-92.
[28] Hirth A, Angeli S, Melzi G, Onorato E. Transcatheter closure of patent foramen ovale in patients with migraine. Neurol Sci 2004; 25 (Suppl 3): S129-31.
[29] Morandi E, Anzola GP, Angeli S, Melzi G, Onorato E. Transcatheter closure of patent foramen ovale: a new migraine treatment? J Interv Cardiol 2003; 16(1): 39-42.