Gene 512 (2013) 527–531

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Investigation of lymphotoxin α genetic variants in migraine

Lotta E. Oikari [1], Shani Stuart [1], Rachel K. Okolicsanyi, Hannah C. Cox, Sonum Dixit, Rod A. Lea, Larisa M. Haupt, Lyn R. Griffiths *

Genomics Research Centre, Griffith Health Institute, Griffith University, Gold Coast Campus, Building G05, Griffith University QLD 4222, Australia

ARTICLE INFO

Article history:
Accepted 29 September 2012
Available online 7 October 2012

Keywords:
Lymphotoxin α
Migraine
Cytokines
Sterile inflammation
Tumor necrosis factor α

ABSTRACT

Migraine is a common neurological disease with a genetic basis affecting approximately 12% of the population. Pain during a migraine attack is associated with activation of the trigeminal nerve system, which carries pain signals from the meninges and the blood vessels infusing the meninges to the trigeminal nucleus in the brain stem. The release of inflammatory mediators following cortical spreading depression (CSD) may further promote and sustain the activation and sensitization of meningeal nociceptors, inducing the persistent throbbing headache characterised in migraine. Lymphotoxin α (LTA) is a cytokine secreted by lymphocytes and is a member of the tumour necrosis factor (TNF) family. Genetic variation with the TNF and LTA genes may contribute to threshold brain excitability, propagation of neuronal hyperexcitability and thus initiation and maintenance of a migraine attack. Three LTA variants rs2009658, rs2844482 and rs2229094 were identified in a recent pGWAS study conducted in the Norfolk Island population as being potentially implicated in migraine with nominally significant p values of $p = 0.0093$, $p = 0.0088$ and $p = 0.033$ respectively. To determine whether these SNPs played a role in migraine in a general outbred population these SNPs were gentoyped in a large case control Australian Caucasian population and tested for association with migraine. All three SNPs showed no association in our cohort ($p > 0.05$). Validation of GWAS data in independent case-controls cohorts is essential to establish risk validity within specific population groups. The importance of cytokines in modulating neural inflammation and pain threshold in addition to other studies showing associations between TNF-α and SNPs in the LTA gene with migraine, suggests that LTA could be an important factor contributing to migraine. Although the present study did not support a role for the tested LTA variants in migraine, investigation of other variants within the LTA gene is still warranted.

1. Introduction

Migraine is a common neurological disorder characterized by debilitating head pain and an assortment of additional symptoms, which can include nausea, emesis, photophobia, phonophobia and occasionally visual sensory disturbances. It affects approximately 12% of the population with affected individuals being predominantly female (Lipton and Bigal, 2005). Migraine results in a significant cost to the economy each year mostly because of lost productivity in the work place, but it also has a large personal impact on sufferers. According to classification criteria of the International Headache Society migraine is sub-divided into two major categories namely migraine with (MA) and migraine without (MO) aura (Olesen, 2004). Migraine is a complex disease caused by interplay between predisposing genetic variants and environmental factors (Gervil et al., 1999). Genes involved in neurological, vascular or hormonal pathways have all been implicated to play a role in predisposition towards developing migraine (Maher and Griffiths, 2011).

The neurovascular hypothesis states that migraine is caused through trigeminal nerve activation and that vasodilatation is a secondary response (Levy, 2009). The trigeminovascular system consists of afferent fibres which innervate the proximal parts of the large cerebral vessels, pial vessels, large venous sinuses and the dura mater (Ferrari, 1998). When activated by a stimulus or decreased threshold resulting in cortical spreading depression (CSD), nociceptive information is relayed via the trigeminal nerves to the trigeminal nucleus and then to the cortical pain areas via the thalamus. This in turn stimulates the release of powerful vasoactive peptides resulting in dilation of cerebral and dural blood vessels. Dural vasodilatation is mediated via the release of calcitonin gene-related peptide (CGRP), neurokinin A and substance P (Goadsby, 1997).

The release of inflammatory mediators such as cytokines and mast cells following CSD, may further promote and sustain the activation and sensitisation of meningeal nociceptors, inducing the persistent throbbing headache characterised in migraine (Ferrari, 1998). Mast cells release proteases and inflammatory mediators, including tumour

Abbreviations: CGRP, calcitonin gene-related peptide; CSD, cortical spreading depression; CVD, cardiovascular disease; GWAS, genome wide association; LTA, lymphotoxin α; LTB, lymphotoxin β; MA, migraine with aura; MHC, major histone compatibility; MO, migraine without aura; pGWAS, pedigree based GWAS; SNP, single nucleotide polymorphism; TNF, tumour necrosis factor.

* Corresponding author. Tel.: +61 7 5552 8664; fax: +61 7 5552 9081.
E-mail address: l.griffiths@griffith.edu.au (L.R. Griffiths).

[1] Joint first authors.

0378-1119/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2012.09.116

necrosis factor (TNF)-α and interleukin (IL-6) upon exposure to sensory neuropeptides (Levy, 2009). The tumor necrosis factor (TNF) gene cluster, made up of TNF, lymphotoxin α (LTA) and lymphotoxin β (LTB) has been implicated by a number of studies to influence the intensity and duration of local inflammation. It is thought that sterile inflammation mediated by LTA and TNF contributes to threshold brain excitability, propagation of neuronal hyperexcitability and thus initiation and maintenance of a migraine attack (Aurora and Welch, 2000).

As discussed previously, LTA belongs to the TNF locus. This gene locus is located on the short arm of chromosome 6 and is part of the Major Histone Compatibility (MHC) class III region. The LTA gene consists of 4 exons and 3 introns which undergo alternative splicing to produce at least 7 isoforms (Smirnova et al., 2008). A previous pGWAS study genotyped 620 901 SNPs in 76 migraine affected cases and 209 non-migraine individuals in the Norfolk Island (NI) population. Association testing using measured genotype analysis identified a number of SNPs located within the LTA gene significantly associated with migraine in the NI population (Nam et al., 2011). Norfolk Island is a self-governing Australian territory located in the South Pacific Ocean between New Caledonia, New Zealand, and Australia along the Norfolk Ridge. The majority of current, permanent residents are descended from 9 Isle of Man (Caucasian), 'Bounty' Mutineers and 6 Tahitian (Polynesian) women, and 2 European Whalers (Male) who joined the small colony in the early 19th century. Population structure, pedigree verification and CVD-risk trait molecular genetics in addition to migraine molecular genetics have been well characterized in the Norfolk Island population (Bellis et al., 2005; Cox et al., 2012).

The aim of this study was to determine if the LTA SNPs identified in the pGWAS play a role in migraine in a general outbred population. The NI pGWAS identified five LTA SNPs namely rs2009658, rs2844482, rs1800683, rs2229094, and rs1041981 with strong association with migraine susceptibility, summarised in Table 1 (Cox, 2011). A number of other studies have also investigated variation within the LTA gene and possible associations with migraine. Of particular interest was a study conducted in a Korean population, where rs2844482 was found to be significantly ($p = 0.0003$) associated with migraine (Lee et al., 2007a). Here, we examined three of these SNPs, rs2009658, rs2844482 and rs2229094 in a large Australian case control cohort for association with migraine.

2. Materials and methods

2.1. Norfolk Island pGWAS

Blood samples along with participant phenotype information were collected as described by Bellis et al. (2005). Briefly, following DNA extraction from blood samples using salting out (Miller et al., 1988), a sub-sample of 285 related individuals (135 males; 150 females) descended from the population founders were genotyped for 620,901 genome wide markers (mean spacing 4.7 kb) on an Illumina Infinium High Density (HD) Human610-Quad DNA analysis BeadChip v1. The population included 76 migraine cases (22 males; 54 females). Association between SNPs and migraine was tested using measured genotype analysis (Boerwinkle et al., 1986) embedded in a variance component-based linkage model (Bellis et al., 2005) and annotated using the Whole Genome Association Study Viewer (WAGViewer) program (http://people.genome.duke.edu/~dg48/WGAViewer/) and NCBI Build 37.1.

Variants within the genomic sequence of positive migraine candidate genes identified through a literature review were selected and annotated using WAGViewer. Locality and statistical information were compiled. Haplotypic assessment of potential migraine susceptibility genes was undertaken in the Norfolk pedigree using the program Haploview. A local type I error of $\alpha = 0.05$ was applied. Genome-wide Bonferroni adjustment was not required to protect against type I error inflation as the application of selection criteria for candidate genes negates the global null. Five SNPs within the LTA gene region were found to be significantly associated with migraine. Given the importance that cytokines are known to have in modulating neural inflammation, three of these SNPs were selected for replication in a large Australian Caucasian migraine case–control for genotypic analyses.

2.2. LTA genotyping

2.2.1. Sample selection

Migraine cases and controls were recruited for the local South East Queensland region as previously described (Colson et al., 2004). They were all of Caucasian origin, and diagnosed as having MA or MO based on criteria specified by the International Headache Society. An unaffected control group with no family history of migraine was matched for age (±5 years), sex and ethnicity. Blood samples obtained from patients were collected through the Genomics Research Centre clinic.

2.2.2. Molecular analysis

2.2.2.1. rs2009658. Amplification of a 302 bp target site in the LTA gene was carried out by PCR using forward (5′-CTGGGGAGAGAAGT GGTCTG-3′) and reverse (5′-TTGATGGGGATTTGGTTTGT-3′) primers. The reaction mixture contained buffer, 1.75 mM of $MgCl_2$, 200 μM of dNTPs, 100 nM of each primer, 1 unit of GoTaq® Flexi DNA polymerase (Promega) and 40 ng of genomic DNA in a total reaction volume of 20 μl. PCR was performed in a 96-well thermocycler (Applied Biosystems) with the following conditions: an initial denaturation at 95 °C for 10 min was followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 1 min and a final extension at 72 °C for 7 min. Genotyping was done using restriction fragment length polymorphism (RFLP) analysis. The amplicon product was digested with *NlaIII* which cuts the recognition site CATG. The RFLP reaction mixture contained 8 μl of PCR product, 6 units of *NlaIII*, 1.2 μl of 10× reaction buffer in a total volume of 12 μl. The digest was incubated at 37 °C for 6 hours. Digested products were then separated by electrophoresis on a 4 % agarose gel dyed with ethidium bromide for 1 hour 10 min at 70 V. Different fragment lengths produced by RFLP were used to identify genotypes. A total of 296 cases and 304 cases were genotyped.

2.2.2.2. rs2229094. Foward (5′-TCTCTTTCTCTGCAGGTTCTC-3′) and reverse (5′-CCCAGAAGGAGGAGGTGTAG-3′) primers were used to amplify a 92 base pair region of the LTA gene containing a single SNP (rs2229094). High resolution melt analysis (HRM) was then used to genotype 293 case and 301 control samples. Initially the Rotor-Gene™

Table 1
LTA SNPs associated with migraine in the Norfolk Island pedigree.

Locus	Gene	No. SNPs in Gene	NCBI dbSNP Ref No.	NCBI build 37.1 position (BP)	Function	Minor/major allele	MAF	Beta*	*p* Value
6p21.3	*LTA*	14	rs2009658	31538244	Upstream	G/C	0.211	0.409	0.0093
			rs2844482	31539767	Upstream	A/G	0.214	0.410	0.0088
			rs1800683	31540071	5prime UTR	A/G	0.418	−0271	0.027
			rs2229094	31540556	Non-Synonymous	C/T	0.270	0.302	0.033
			rs1041981	31540784	Non-Synonymous	A/C	0.417	−0.271	0.027