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ORIGINAL ARTICLE

Association between interleukin-4 (IL-4), gene polymorphisms (C-589T, T + 2979G, and C-33T) and migraine susceptibility in Iranian population: A case–control study

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Abstract *Background:* Migraine is a chronic neurological disease characterized by recurrent moderate to severe headaches commonly in association with neuro-inflammation. Interleukin-4 (IL-4), an anti-inflammatory cytokine, plays an important role in modulating pain threshold and has an essential role in stimulation of pain receptors in the trigeminal nerve fibers.

Aim of the study: The current study aimed to investigate the possible associations between IL-4 single nucleotide polymorphisms (SNPs) and susceptibility to migraine in Iranian patients.

Patients and methods: In a prospective case–control study, we studied blood samples of 190 patients with migraine (migraineurs) and 200 healthy controls (HCs) for analysis of gene variants. Genotyping for the IL-4 SNPs: C-589T (rs2243250), T + 2979G (rs2227284), and C-33T (rs2070874) were performed using PCR-RFLP. Statistical analysis was performed using the SPSS version 21.0 (SPSS, Chicago) and SNPStats version 1.14.0.

Results and conclusion: Among IL-4 SNPs, rs2243250 (TC genotype, OR = 0.25, 95% CI = 0.13–0.50, P = 0.001) and rs2227284 (TG and TT genotypes, OR = 0.44, 95% CI = 0.23–0.92, P = 0.029 and OR = 0.38, 95% CI = 0.18–0.79, P = 0.009 respectively) were significantly

Abbreviations: IL-4, interleukin-4; IL-9, interleukin-9; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; CNS, central nervous system; CCR2, C-C chemokine receptor type 2; TGFB1, transforming growth factor-b1; NOS3, nitric oxide synthase 3; Th2, T helper type 2; SNPs, single nucleotide polymorphisms

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associated with migraine. No significant associations between IL-4 SNP rs2070874 (TC, TT and CC genotypes) and migraine were found. The most frequent genotypes in the migraineurs were CC in both SNPs rs2243250 (79%), and rs2070874 (71.5%), as well as GG for SNP rs2227284 (64%). There was no statistically significant relationship between these SNPs and different subclasses (common, classic and complicated) of migraine. Our findings revealed that in IL-4 rs2243250 and rs2227284 genotypes and allele frequencies have a role in susceptibility to migraine in our population. Therefore, it is suggested that in addition to other factors, IL-4 genetic variations also play a pivotal role in the progress of migraine.

1. Introduction

Migraine is a rigorous and painful headache accompanying with sensory warning and is a public health problem of great impact on both the patient and society [1]. The two major subclasses of migraine are common migraine (without aura) and classic migraine (with aura or neurological symptoms) [2]. Since about half of migraineurs do not pursue medical attention and there is no economic, social or ethnic limitation, it is difficult to precisely determine disease prevalence in the community [3]. It seems that about 15 to 16% of women and 5 to 9% of men are affected with migraine and its prevalence is the highest among the ages of 30–49 worldwide [4]. Migraine etiology is multifactorial, involving both various genetic and environmental factors, but scientists consider three important mechanisms for its pathophysiology including: inflammatory, neurological and cardiovascular impairments [5,6]. Cell and molecular association studies may point to the novel molecules that mediate migraine disorder and enable its management. According to the theory of neuroinflammation, in the migraine, ion channels (Na^{2+}, Ca^{2+}, K^{2+}) and inflammatory mediator (TNF-α and IL-1β) activation in the meninges sensory nerves, stimulates pain receptors in these area [7,8]. In addition to changes in the inflammatory cytokines, variations in the anti-inflammatory mediators in migraine patients have been reported but there are conflicting results on the mechanisms involved [9–14]. The interaction between immune cells is regulated by several mediators, including interleukins and cytokines, which play an essential role in pathobiological processes such as, inflammation, immunity and pain [15]. Widely, interleukins and their receptors are present in both peripheral neurovascular inflammation at meningeal/ganglia level, and essential sensitization processes in central nervous system (CNS) and likely to be involved in pain threshold modulation [16–21]. On the other hand, peripheral blood interleukins operate mainly at a level of areas innervated by trigeminal ganglion neurons related to neurovascular system [22]. The human IL-4 gene is located on chromosome 5q31 and consists of 25 kb. So far, numerous allelic variant polymorphisms (http://www.ncbi.nlm.nih.gov/SNP/) have been found in IL-4 gene, that the important ones are including −590C/T (rs2243250), −33C/T (rs2070874), +3437C/G (rs2227282), and 2979G/T (rs2227284) [23]. To understand the probable role of IL-4, as anti-inflammatory cytokine, in migraine headaches in the leading research we analyzed its imperative polymorphisms in migraineurs with three different subclass of disease and compared them to healthy controls.

2. Subjects and methods

2.1. Patients and samples

The study was approved by the ethics in medical research committee at Zahedan University of Medical Sciences, and was conducted with clinical samples from migraine patients ($N = 190$, age: 13 to 66 years, age mean ± SD: 31.72 ± 10.17) who were treated at the Department of Neurology, Ali-ebn Abitaleb Hospital, Zahedan, Iran, from August 2013 to February 2014. Healthy controls (HCs) without any inflammatory, neurological diseases, migraine headache and specific systemic disease ($N = 200$, age: 15 to 75 years, age mean ± SD: 35.1 ± 12.2) from volunteer blood donors were selected during the same time. A diagnosis of migraine was made according to standardized criteria of international headache classification [24]. Patients were excluded if they had a history of inflammatory diseases or received anti-inflammatory medicines. Patients adjusted in three definite groups including common (without aura, $N = 96$, 64 female and 32 male, age mean ± SD: 31.0417 ± 10.457) classic (with aura, $N = 78$, 56 female and 22 male, age mean ± SD: 32.33 ± 10.57) and complicated ($N = 16$, 4 male and 12 female, age mean ± SD: 32.87 ± 6.46) subtypes of migraine. All patients were informed of the study and participated voluntarily and written consents were taken. The work is carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. Blood collection and DNA extraction

Whole blood (10 mL) samples were taken from all subjects and collected in separator tubes (contain EDTA, 0.5 M) and centrifuged for 15 min at 150 g (gravity) at 20 °C and then serum was stored at −20 °C in sterile plastic tubes for DNA extraction. Genomic DNA was extracted from the serum of 190 subjects with migraine headaches and 200 HCs using the DNA extraction kit (DIAtom DNA Prep., GORDIZ, Moscow, Russia) according to the manufacturer's instruction. DNA quality extracts were analyzed by electrophoresis. By NanoDrop DNA concentrations about 60 ng/μl was obtained and ratio of 260/280 nm around 1.7 to 1.9 was accepted [25].

2.3. PCR analysis

Four IL-4 SNPs C-589T (rs2243250), T + 2979G (rs2227284), and C-33T (rs2070874) were analyzed through restriction

fragment length polymorphism–polymerase chain reaction (RFLP–PCR) method [26]. PCR amplifications were performed in a final volume of 20 μl containing, 10 μl master mix (TAKARA, Tokyo, Japan), 0.7 μl (10 pmol) of each primer, 2 μL template DNA, and 6.8 μl DNase-free water was used. For rs2070874 and rs2227284, the amplification was performed with an initial denaturation step at 94 °C for 5 min; followed by 35 cycles at 94 °C for 30 s, 58 °C for 35 s, and 72 °C for 30 s with a final extension at 72 °C for 5 min. For SNP rs2243250, the cycling conditions were as follow: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s with a final extension at 72 °C for 5 min. The PCR product was checked for size and purity by 1.5% agarose gel electrophoresis. The sizes of the fragments were 195bp, 220bp and 223bp for the C-589T, T + 2979G and C-33T regions, respectively (Table 1).

2.4. RFLP analysis

Final volume of 20 μL including 2 μL of 10 × Buffer, 0.5 μL of enzyme, 7 μL of PCR product, 10.5 μL of double distilled water was used for all amplification products overnight at 37 °C, and 10 μL sample loaded for electrophoresis. AvaII (Thermo Scientific) endonuclease digested pattern for rs2243250 amplification product were 177bp and 18bp for the CC, 195bp, 177bp and 18bp for the TC and 195bp for the TT genotypes (Table 1). AluI (Thermo Scientific) endonuclease digested pattern for rs2227284 amplification product were 122bp and 98bp for the GG, 122bp, 98bp, 53bp, and 45bp for the TC and 122bp, 53bp, and 45bp for the TT genotypes (Table 1). BsmAI (Thermo Scientific) endonuclease digested pattern for rs2070874 amplification product were 178bp and 45bp for the CC, 178bp, 140bp, 45bp, and 38bp for the TC and 140bp, 45bp, and 38bp for the TT genotypes (Table 1).

2.5. Statistical analysis

SPSS version 21.0 (SPSS, Chicago) and SNPStats version 1.14.0 were used for all the statistical analyses. The association between genotypes and IL-4 was estimated using the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. The Hardy–Weinberg equilibrium (HWE) was tested with the X2 test for any of the SNPs under consideration. The significance level was set at $P \leqslant 0.05$ for all the tests.

3. Results

3.1. Association of IL-4 SNP (rs2070874 C/T) and migraine

The C/C, T/C and T/T genotypes of −33 C/T were found in 74%, 19% and 7% in HCs, in comparison with 79%, 15% and 6% in migraineurs, respectively. The allele frequency of IL-4 rs2070874 (C/T) were 83.5% (C), 16.5% (T) in HCs and 86% (C), 14% (T) in migraineurs, respectively. Distributions of IL-4 polymorphisms in rs2070874 (C/T) were not significantly different between patients and controls for TC (OR = 0.727, $P = 0.412$), and TT (OR = 0.847, $P = 0.773$) genotypes and also C (OR = 1.246, $P = 0.438$) and T (OR = 0.8, $P = 0.434$) alleles (Table 2). Similarly, there were no associations with migraine classic, common and complicated subtypes and IL-4 rs2070874 (G/T) SNP in this population (Table 3).

3.2. Association of IL-4 SNP (rs2243250 C/T) and migraine

The C/C, T/C and T/T genotypes of −589 C/T were found in 45%, 44% and 11% in HCs, in comparison with 71.5%, 18% and 10.5% in migraineurs, respectively. The allele frequency of IL-4 rs2243250 (C/T) were 67% (C), 33% (T) in HCs and 80.5% (C), 19.5% (T) in migraineurs, respectively. There were significant associations between TC (OR = 0.256, $P = 0.001$) and CC (OR = 0.386, $P = 0.00$) genotype and also C (OR = 2.036, $P = 0.002$) and T (OR = 0.428, $P = 0.000$) alleles of IL-4 rs2243250 SNP and migraine (Table 2). TC genotype could be considered as protective and CC genotype could be considered as risk factor in migraine headaches. There were no significant differences between patients and controls for the TT (OR = 0.602, $P = 0.287$) genotype (Table 2). There were no associations with migraine subtypes and IL-4 SNP (rs2243250 C/T) in this population (Table 3).

3.3. Association of IL-4 SNP (rs2227284 T/G) and migraine

The G/G, T/G and T/T genotypes of + 2979 T/G were found in 27%, 38% and 35% in HCs, in comparison with 46.5%,

Table 1 Primer sequences and restriction enzymes used for detection of IL-4 gene polymorphisms.

SNPs (rs number)	Sequence (5′ → 3′) F: Forward R: Reverse	Digestion pieces (restriction enzyme)	Amplicon size
C-33T (rs2070874)	F: CAA GTT ACT GAC AAT CTG GTG T R: CGG CAC ATG CTA GCA GGA A	Allele C: 178, 45 (BsmAI) Allele T: 140, 45, 38 (BsmAI)	223 bp
C-589T (rs2243250)	F: TAA ACT TGG GAG AAC ATG GT R: TGG GGA AAG ATA GAG TAA TA	Allele C: 177, 18 (*AvaII*) Allele T:195 (*AvaII*)	195 bp
T + 2979G (rs2227284)	F: CTA CTC TTG GCA GTT GCT GGA A R: GGA ACT CTC TGT AGA ATT ATG AAC TTT AGG TC	Allele T: 122, 53, 45 (AluI) Allele G: 122, 98 (AluI)	220 bp

Table 2 Genotype and allelic frequencies of IL-4, SNPs in patients and control subjects.

SNP (rs number)	Genotypes and Alleles	Patient *n* (%)	Control *n* (%)	[a]OR (95% CI)	*P*-value
C-33T (rs2070874)	CC	150(79%)	148(74%)	1.00	–
	TC	28(15%)	38(19%)	0.727 (0.340–1.557)	0.412
	TT	12(6%)	14(7%)	0.846 (0.271–2.636)	0.773
	TC + TT	40(21%)	52(26%)	0.759(0.390–1.477)	0.417
	C	164(86%)	167(83.5%)	1.246 (0.712–2.176)	0.438
	T	26(14%)	33(16.5%)	0.8 (0.4–1.4)	0.434
C-589T (rs2243250)	CC	136(71.5%)	90(45%)	1.00	–
	TC	34(18%)	88(44%)	0.256 (0.13–0.502)	0.001***
	TT	20(10.5%)	22(11%)	0.602 (0.236–1.533)	0.287
	TC + TT	54(28.5%)	110(55%)	0.325(0.179–0.589)	0.000***
	C	153(80.5%)	134(67%)	2.036 (1.279–3.241)	0.002**
	T	37(19.5%)	66(33%)	0.428 (0.263–0.696)	0.000***
T + 2979G (rs2227284)	GG	88(46%)	54(27%)	1.00	-
	TG	58(30.5%)	76(38%)	0.448 (0.237–0.925)	0.029*
	TT	44(23.5%)	70(35%)	0.386 (0.188–0.790)	0.009**
	GG + TG	102(54%)	146(73%)	0.429(0.236–0.780)	0.005**
	G	117(62%)	92(46%)	1.881 (1.25–2.816)	0.002**
	T	73(38%)	108(54%)	0.581 (0.386–0.873)	0.008**

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$: significant *p*-value.
[a] Adjusted for sex and age.

30.5% and 23.5% in migraineurs, respectively. The allele frequency of IL-4 rs2243250 (T/G) were 46% (G), 54% (T) in HCs and 61% (C), 39% (T) in migraineurs, respectively. There were significant associations between TG (OR = 0.448, $P = 0.029$) and TT (OR = 0.386, $P = 0.009$) genotypes and also G (OR = 1.881, $P = 0.002$) and T (OR = 0.581, $P = 0.008$) alleles of IL-4 rs2227284 SNP and migraine (Table 2). T allele could be considered as protective and C allele could be considered as risk factor in migraine headaches. There were no associations with migraine subtypes and IL-4 rs2227284 SNP in this population (Table 3).

4. Discussion

Migraine is a severe neurological disorder that causes a strong throbbing or pulsating pain in one area of the head and can be accompanied by nausea, vomiting and extreme photophobia [2]. Several studies used a candidate gene approach to elucidate genetic contribution to neuropathic pain phenotypes; however, the data are limited and inconsistent [27]. The genetic background of migraine consists of common or overlapped pathways and the responsible genes may provide insight regarding the pathophysiological mechanisms that can explain their comorbidity with migraine [28,29]. Cytokines, small protein molecules secreted in response to immune stimuli, are involved in signaling that activates CNS glial cells and this activation is part of a poorly understood interaction between immune challenge and host that can lead to the development or facilitation of pathologic pain [30]. Data from the large cohort of Caucasian women ($n = 25{,}713$) in 77 different SNPs suggested that there is an association between variants in some inflammatory mediators including TNF-α rs673 (OR = 0.52, 95% CI = 0.30–0.89, $p = 0.017$), CCR2 rs1799864 (OR = 1.12, 95% CI = 1.03–1.21, $p = 0.007$), TGFB1 rs1800469 (OR = 0.93, 95% CI = 0.89–0.89, $p = 0.009$), NOS3 rs3918226 (OR = 1.13, 95% CI = 1.01–1.27, $p = 0.04$), and IL-9 rs2069885 (OR = 1.12, 95% CI = 1.02–1.24, $p = 0.02$) with migraine [31]. In this study, for the first time we focused on anti-inflammatory cytokine, IL-4 in patients with migraine headaches to examine the hypothesis that say migraine headaches could be caused by change in immune system [32]. It has been characterized that IL-4 is required for the generation of the Th2-derived cytokines and several reports indicate a reduced level of IL-4 during migraine attack [33–36]. Perini et al. have found no significant differences between IL-4 plasma levels in migraine patients with and without aura ($p = 0.07$); as well as patients outside and during the attacks ($p = 0.06$) and also between HCs and patients ($p = 0.06$) [16]. Conversely, Sarchielli et al. reported that levels of IL-4 were reduced at the time of migraine attack ($p < 0.004$) and its levels at the end of the attack returned to those detected at attack onset [35]. Moreover, Munno et al. suggested that IL-4 plasma levels were decreased during attacks and undetectable in 62.5% of patients with migraine headaches without aura [37]. In other study, although no significant fluctuations of the IL-4 plasma levels during the headache-free period in children with migraine and tension-type headaches have been found, immune dysfunction (abnormal changes in pro- and anti-inflammatory cytokines) in migraineurs could not be totally excluded [38]. Such controversies may be due to different population studies, long medical history of migraineurs and frequent intake of prophylactic drugs or analgesic treatments. In relation to adult glioma, there were close association between IL4 (rs2243248, T-1098G), IL6 (rs1800795, G-174C) and overall risk of glioma [39]. Another study demonstrated no association between IL-4 rs2227284 SNP and rheumatoid arthritis (RA) that is the

Table 3 Genotype and allelic frequencies of IL-4, SNPs in deferent subclasses of migraine.

SNP (rs number)	Genotypesand Alleles	Common *n* (%)	Classic *n* (%)	Complicated n (%)	[a]OR (95% CI)	*P*-value
C-33T	CC	74(77%)	67(85.5%)	8(50%)	1.00	-
(rs2070874)	TC	12(13%)	11(14.5%)	4(25%)	1.397(0.567–3.44)	0.467
	TT	10(10%)	0	4(25%)	1.035(0.271–3.954)	0.959
	TC + TT	22(23%)	11(14.5%)	8(50%)	1.279(0.584–2.801)	0.538
	C	80(83%)	73(93%)	10(62.5%)	0.706(0.307–1.623)	0.411
	T	16(17%)	5(7%)	6(37.5%)	1.41(0.616–3.251)	0.411
C-589T (rs2243250)	CC	68(71%)	60(77%)	8(50%)	1.00	-
	TC	13(14%)	15(19%)	4(25%)	1.298(0.559–3.014)	0.544
	TT	15(15%)	3(4%)	4(25%)	0.849(0.285–2.528)	0.768
	TC + TT	28(29%)	18(23%)	8(50%)	1.114(0.546–2.271)	0.767
	C	84(87%)	67(86%)	10(62.5%)	0.58(0.309–1.093)	0.090
	T	12(13%)	11(14%)	6(37.5%)	1.82(0.95–3.459)	0.064
T + 2979G (rs2227284)	GG	44(46%)	37(48%)	6(37%)	1.00	-
	TG	25(26%)	28(35%)	4(26%)	1.181(0.558–2.498)	0.664
	TT	27(28%)	13(17%)	6(37%)	0.942(0.411–2.160)	0.888
	TG + TT	52(54%)	41(52%)	10(63%)	1.073(0.561–2.050)	0.832
	G	57(59%)	51(66%)	8(50%)	0.526(0.274–1.006)	0.050
	T	39(41%)	27(34%)	8(50%)	1.901(0.993–3.638)	0.050

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$: significant *p*-value.
[a] Adjusted for sex and age.

commonest autoimmune disease [40]. It has been confirmed that IL-4 gene polymorphisms may influence the function of mononuclear cells to produce not only IL-4 but also other cytokines [41]. In the Pakistani cohort study, it has been reported that the most frequent genotypes of IL-4 in the asthma and allergic rhinitis groups were TT for SNP rs2243250, and GG for SNP rs2227284 [42]. As well as, IL-4 rs2070874 was not found to be associated with either asthma or allergic rhinitis in the Pakistani cohort [42]. Recently Shang et al. have demonstrated that IL-4 rs2243250 SNP may be associated with high levels of serum IL-4, which may increase the risk of atopic dermatitis in children [43]. Lu et al. findings suggest that SNP in IL-4 rs2070874 (OR: 3.438, 95% CI: 1.032–11.458, $P = 0.044$) may be a risk factor for hepatocellular carcinoma in Chinese males [44]. It is notable that, there are not available any data from previous epidemiological studies exploring theses IL-4 SNPs in migraineurs population all over the word. Therefore, our results for the first time provided evidence that enhance our understanding of how migraine may relate to, an anti-inflammatory cytokine, IL-4 gene variation. Mentioned reports are relatively conflicting with our recent leading study (but in different population and disease) which revealed that the IL-4 rs2243250 SNP in the genotype TC and also rs2227284 SNP in the genotypes TG and TT playa protective role. These polymorphisms could resulting to changing in Th2 release IL-4, or affecting IL-4 affinity to their cell targets and consequently unbalance between Th1/Th2 cytokines may possibly influence the spreading of pain producing processes in migraine. IL-4 appears to be a prospective target for future development of migraine-specific preventive therapies. The data presented here must be viewed with caution due to the relatively small number of patients enrolled and therefore these results should be taken as preliminary. Similar studies enrolling greater sample sizes and composed of other ethnic groups from different countries may contribute to confirming our findings.

5. Conclusion

In conclusion, our results implicate that the IL-4 rs2243250 (TC) and rs2227284 (TG and TT) SNPs have a protective role in susceptibility to migraine disease in Iranian patients. No significant associations between IL-4 SNP rs2070874 (TC, TT and CC genotypes) and migraine were found. There was no statistically significant relationship between these SNPs and different subclasses (common, classic and complicated) of migraine.

Conflict of interest

All the authors declare that they do not have financial disclosure or conflicts of interest.

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