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A case-control study of interleukin-12 1188A/C polymorphism in obsessive-compulsive disorder in Chinese population

Liu S, Liu Y, Yin Y, Zhang X, Ma X. A case-control study of interleukin-12 1188A/C polymorphism in obsessive-compulsive disorder in Chinese population.

Objective: Previous studies indicated that the levels of serum interleukin (IL)-12 at baseline were elevated in obsessive-compulsive disorder (OCD) patients compared with control subjects, suggesting that IL-12 maybe involved in the etiopathogenesis of OCD. Therefore, we study the relationship between 1188A/C polymorphism of IL-12(rs3212227) and OCD in Chinese Han population.
Methods: We genotyped 194 OCD patients and 322 healthy individuals by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), then analyzed the data using case-control association test.
Results: No significant differences were found in 1188A/C genotypic and allelic frequencies between OCD cases and controls ($\chi^2 = 2.12$, df = 2, P = 0.35 by genotype; $\chi^2 = 0.005$, df = 1, P = 0.94 by allele).
Conclusions: Our results suggested that there may be lack of an association between OCD and IL-12 1188A/C polymorphism in Chinese Han population.

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Significant outcomes

- One hundred ninety-four OCD patients and 322 OCD-free control were evaluated for the association of IL-12 using case-control design.
- No significant association was found between polymorphism of IL-12 and OCD.
- This is the first report on association of IL-12 and OCD in Chinese population.

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Limitations

- Limited sample sizes.
- Low-level technology.
- Tenuous role of IL-12 to increase the onset risk of OCD.

Introduction

Obsessive-compulsive disorder (OCD), a debilitating chronic psychiatric disorder, whose clinical manifestation is represented as recurrent and intrusive thoughts and repetitive behaviours to reduce distress associated with obsessions. According to population surveys, the lifetime prevalence of OCD in adults is estimated at around 1–4% (1). It has disturbed the normal living, work and sociality badly and brought heavy burden to the society for its high levels of morbidity and disability (2). Over the years, many researchers have made the observation that OCD tends to be associated with immune dysfunction (3), which indicated that proinflammatory cytokines such as interleukin (IL) activities could be involved in the etiopathogenesis of OCD. Several studies have implicated ILs such as IL-6, IL-8, IL-10 and IL-12 (4–7) in mental disorders, including Tourette's syndrome (TS) (7) and Sydenham chorea (SC) (8).

As a member of IL family, IL-12 plays a key role in the modulation of the immune response by activating natural killer (NK) cells and induces CD4 T-cell differentiation to T-helper (Th)1-like cells (9). It is a heterodimer of p35 and p40 subunits and is a key cytokine in promoting anti-viral Th1 responses. Previous studies of adult OCD patients have reported decreased numbers of NK cells in the peripheral blood, as well as decreased NK activity (10); however, Leckman et al. (7) found that the concentrations of serum IL-12 at baseline were elevated in OCD patients compared with control subjects.

From genetic point of view, a single nucleotide polymorphism (A/C) of the gene encoding IL-12 p40 (IL12B) has been identified 11 years ago (11). It is located at position 1188 of IL-12 on 5q31–33 (12) and reported to be associated with susceptibility to multiple sclerosis and insulin-dependent diabetes mellitus (9,13). Moreover, the production of IL-12 could be enhanced by the variant C allele of the 1188A/C polymorphism (14). We have conducted the present study to make clear whether the 1188A/C polymorphism in *IL-12* is association with OCD development in a Chinese Han population.

Materials and methods

Study population

A total of 194 OCD patients and 322 OCD-free control were included in this study from Medical School Hospital of Qingdao University. The affected individuals, including 100 male and 94 female patients, were between the ages of 18 and 65 years. All patients were diagnosed according to Diagnostic and Statistical Manual of mental disorders (DSM-IV) diagnostic criteria by means of the Structured Clinical Interview for DSM-IV, and the severity of the disorder was evaluated with the Yale-Brown Obsessive Compulsive Scale (15) by a trained clinical consultant psychiatrists. The control subjects were selected among healthy volunteers and all had no history of any psychiatric disorder. The patients with OCD and healthy controls were Chinese people from the same geographic region and of the same ethnic origin. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Medical School Hospital of Qingdao University and the National Research Institute for Family Planning.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard methods. The segments of polymorphism were replicated using the primers as follows: IL12B 1188A/C 5′ CTGATCCAGGAT GAAAATTTGG 3′ and 5′ CCCATGGCAACTTGA GAGCTGG 3′; PCR reactions were carried out in a final volume of 20 μl containing 2× PCR MasterMix (New England Biolabs, Beijing, China), 0.4 μmol/l of each primer and 100 ng of genomic DNA. The reaction of IL12B 1188A/C involved a starter step at high temperature of 94 °C for 4 min followed by 35 cycles with the following cycle profile: denaturation at 94 °C for 30 s, annealing temperature 55 °C for 1 min and elongation at 72 °C for 30 s and a final elongation at 72 °C for 5 min. The product, a 227 bp DNA fragment, was digested by the restriction enzyme TaqI (New England Biolabs) at 65 °C for 24 h. The presence of the C allele produces two fragments of 156 and 71 bp, which were separated on agarose gel (2%) and visualised under ultraviolet (UV) light.

Statistical analysis

For all data of patients and controls, the Hardy-Weinberg equilibrium of the genotype distribution was tested using the homogeneity chi-squared test.

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Table 1. Genotype distribution and relative allele frequencies of 1188A/C polymorphism of *IL-12* in Chinese with OCD ($n = 194$) and controls ($n = 322$)

Group	Number of participants	Genotype frequency (%)			Allele frequency (%)	
		A/A	A/C	C/C	A	C
OCD	194	60 (30.9)	108 (55.7)	26 (13.4)	228 (58.8)	160 (41.2)
Controls	322	109 (33.9)	159 (49.4)	54 (16.8)	377 (58.5)	267 (41.5)
		$\chi^2 = 2.13$, df $= 2$, $p = 0.35$			$\chi^2 = 0.005$, df $= 1$, $p = 0.94$	

A case-control study was performed using the homogeneity chi-squared test, which was carried out using the Statistical Package for Social Sciences (Version 12.0 for Windows; SPSS, Inc., Chicago, IL, USA) and Microsoft Excel 2000.

Results

A total of 194 OCD patients and 322 OCD-free controls participated in this study. Genotype and allele frequencies of 1188A/C for the case-control study are shown in Table 1. The genotypic distribution was not significantly different from the distribution expected according to Hardy-Weinberg equilibrium in OCD patients ($\chi^2 = 0.05$ with 1 df and $p = 0.81$) and control group ($\chi^2 = 0.09$ with 1 df and $p = 0.76$). No significant differences were found in 1188A/C genotypic and allelic frequencies between OCD cases and controls ($\chi^2 = 2.12$, df $= 2$, $p = 0.35$ by genotype; $\chi^2 = 0.005$, df $= 1$, $p = 0.94$ by allele) (Table 1).

Discussion

In the past few years, the National Institute of Mental Health reported an increasing possibility of an immune-mediated pathophysiology of obsessive-compulsive symptoms in patients with SC (16). Subsequently, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (17), presented as precipitating streptococcal infection in children with sudden onset of obsessive-compulsive symptom (OCS), also involved in immune dysfunction. Therefore, some studies have tried to demonstrate alterations of immune parameters in OCD patients. Konuk et al. (18) found a significant increase in the plasma levels of tumor necrosis factor (TNF)-α and IL-6 in OCD patients compared to healthy controls, and Monteleone et al. (19) observed decreased TNF-α plasma levels in OCD patients. However, Carpenter et al. (20) found no alteration in IL-6 plasma levels in OCD.

IL-12 is secreted by activated monocyte, macrophage and dendritic cells. Its main biological function is accommodating the activation of NK cell, the proliferation of T cell and the production of cytokine and B-cell antibody (9). It can then participate in the progress of anti-infection and inflammatory disease. Previous studies found that IL-12 took part in many kinds of diseases such as psoriasis (21), multiple sclerosis (22), Crohn's disease (23) and so on. In addition, it is of interest that serum levels of IL-12 were found to be increased in a group of 14 patients with acute Sydenham's chorea compared with control serum (8). Its association with other mental disorders such as TS was also been reported (7). With regards directly to OCD, recent studies indicated that cytoactive material such as inflammatory factors may participate in the aetiology of OCD (24). Given that IL-12 is the strongest cytoactive material stimulating cellular immunity to date, IL-12 is worth to be investigated as a candidate gene, which might be related to OCD. However, our results did not confirm an involvement of the 1188A/C polymorphism of IL-12 in conferring susceptibility to OCD in Chinese population. Most likely reason seems to be relatively small number of patients and controls. Also, the role of *IL-12* was so minor to increase the onset risk of OCD that it was below threshold of detectability. As we found no other study addressing this issue, our finding remains to be confirmed by further studies that should consider larger specimens in more area and update method adopting. An additional explanation for the results in the given study is that the selected variant 1188A/C simply is not associated with OCD – at least not in this small Chinese sample. Therefore, it would not be of interest in the future to describe the genetic variation within the gene, systematically using tag single nucleotide polymorphisms (SNPs).

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References

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2005;**62**:593–602.

https://doi.org/10.1111/j.1601-5215.2011.00616.x Published online by Cambridge University Press

2. Bobes J, Gonzalez MP, Bascaran MT, Arango C, Saiz PA, Bousono M. Quality of life and disability in patients with obsessive–compulsive disorder. Eur Psychiatry 2001; **16**:239–245.
3. Brambilla F, Perna G, Bellodi L, et al. Decreased TNF-α and NK activity in obsessive-compulsive disorder. Psychoneuroendocrinology 2004;**29**:945–952.
4. Konuk N, Tekin IO, Ozturk U et al. Plasma levels of tumor necrosis factor-alpha and interleukin-6 in obsessive compulsive disorder. Mediators Inflamm 2007;**2007**: 65704.
5. Rovin BH, Lu L, Zhang X. A novel interleukin-8 polymorphism is associated with severe systemic lupus erythematosus nephritis. Kidney Int 2002;**62**:261–265.
6. Gasche C, Grundtner P, Zwirn P et al. Novel variants of the IL-10 receptor 1 affect inhibition of monocyte TNF-α production. J Immunol 2003;**170**:5578–5582.
7. Leckman JF, Katsovich L, Kawikova I et al. Increased serum levels of interleukin-12 and tumor necrosis factor-alpha in Tourette's syndrome. Biol Psychiatry 2005;**57**: 667–673.
8. Church AJ, Dale RC, Cardoso F et al. CSF and serum immune parameters in Sydenham's chorea: evidence of an autoimmune syndrome?. J Neuroimmunol 2003;**136**: 149–153.
9. Morahan G, Huang D, Ymer SI et al. Linkage disequilibrium of a type 1 diabetes susceptibility locus with a regulatory IL-12B allele. Nat Genet 2001;**27**:218–221.
10. Denys D, Fluitman S, Kavelaars A, Heijnen K, Westenberg H. Decreased TNF-a and NK activity in obsessive–compulsive disorder. Psychoneuroendocrinology 2004;**29**:945–952.
11. Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localisation and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. Genes Immun 2000;**1**:515–520.
12. Sieburth D, Jabs EW, Warrington JA et al. Assignment of genes encoding a unique cytokine (IL-12) composed of two unrelated subunits to chromosomes 3 and 5. Genomics 1992;**14**:59–62.
13. van Veen T, Crusius JB, Schrijver HM et al. Interleukin-12p40 genotype plays a role in the susceptibility to multiple sclerosis. Ann Neurol 2001;**50**:275–279.
14. Seegers D, Zwiers A, Strober W, Pena AS, Bouma G. A Taq1 polymorphism in the 3′UTR of the Il-12 p40 gene correlates with increased Il-12 secretion. Genes Immun 2002;**3**:419–423.
15. Arnold PD, Rosenberg DR, Mundo E, Tharmalingam S, Kennedy JL, Richter MA. Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: a preliminary study. Psychopharmacology (Berl) 2004;**174**:530–538.
16. da Roch FF, Correa H, Teixeira AL. Obsessive–compulsive disorder and immunology: a review. Biol Psychiatry 2008;**32**:1139–1146.
17. Stein DJ. Obsessive–compulsive disorder. Lancet 2002; **360**:397–405.
18. Konuk N, Tekin IO, Ozturk U et al. Plasma levels of tumor necrosis factor-alpha and interleukin-6 in obsessive compulsive disorder. Psychiatr Clin North Am 2006;**29**: 445–469.
19. Monteleone P, Catapano F, Fabrazzo M, Tortorella A, Maj M. Decreased blood levels of tumor necrosis factor-alpha in patients with obsessive-compulsive disorder. Neuropsychobiology 1998;**37**:182–185.
20. Carpenter LL, Heninger GR, McDougle CJ, Tyrka AR, Epperson CN, Price LH. Cerebrospinal fluid interleukin-6 in obsessive-compulsive disorder and trichotillomania. Psychiatry Res 2002;**112**:257–262.
21. Kruager GG, Langley RG, Leonardi C et al. A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis [J]. N Engl J Med 2007;**356**:580–592.
22. Rohowsky-koehan C, Molinaro D, Choudhry A. Impaired interleukin-12 production in multiple sclerosis [J]. Mult Scler 1999;**5**:327–334.
23. Neurath MF. IL-23:a master regulator in Crohn disease [J]. Nat Med 2007;**13**:26–28.
24. Kuroda S, Puri P. Lack of association of IL8 gene polymorphisms with familial vesico-ureteral reflux. Pediatr Surg Int 2007;**23**:441–445.

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