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Critical Association Study of Olfactory Receptor Gene Polymorphism in Diabetic Complications

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Abstract

Type I diabetes (TID) is an autoimmune disease (AD) known to trigger retinopathy, neuropathy and nephropathy. Recent findings suggest that numerous pathways are activated during the course of T1D and that these pathways individually or collectively influence progression to Diabetic Nephropathy (DN).

A single nucleotide polymorphism (SNP) in the promoter region of the Olfactory Receptor family 14 (OR14) gene, rs9257691, has been shown to be significantly associated with T1D in general. Recent investigations have emphasized on the significance of OR in diabetic complications. In this pilot study, we sought to confirm these findings by investigating the OR14 gene in a group of patients with and without diabetic complications. A hundred patients with (n=75) and without (n=25) different diabetic complications (i.e. Retinopathy (DR), Neuropathy (DNu) and DN) were recruited. Patients with long term ≥ 20 years history of diabetes with no complications were considered as the control group. Case and control subjects were genotyped for OR14 gene adjacent to the Human Leukocyte Antigen (HLA)-F region.

The current results showed that the OR14-CC genotype is more significant in patients with DN, p=0.004, in comparison to groups with other complications. No other statistically significant difference was found among male and female groups. In addition, there was no difference in allelic frequencies of cases compared to control subjects.

This critical analysis indicates that patients with T1D who have the OR14-CC genotype are significantly susceptible to progressive DN. Screening and identifying diabetic patients at risk for future nephropathy would permit better clinical management. However, a large scale association study is recommended to confirm the above findings.

Keywords: Diabetic nephropathy; Olfactory receptor gene; Diabetic complications; MHC-X gene

Introduction

AD is defined when the progression from benign autoimmunity to pathogenic autoimmunity occurs; such as T1D [1]. In the case of T1D secondary diseases are manifested such as DR, DNu and DN [2,3]. The aforementioned secondary complications are also found in type-2-diabetes (T2D), however, they are more serious in T1D [4-6].

T1D is an AD that is caused by the destruction of the insulin-producing pancreatic β-cells. Several factors may contribute to the pathogenesis of T1D; accumulative evidence suggest that both genetic and environmental factors contribute to the etiology of T1D [7]. Genetic susceptibility of T1D has been determined by studying polymorphisms in multiple genes in both human and animal models [7]. In this regard, Genome Wide Association Studies (GWAS) indicate that the highly polymorphic Major Human Complex (MHC), including both MHC class I and class II, contribute to approximately 50% of genetic susceptibility to T1D [8-13].

According to the recent GWAS there are 60 non-MHC genes or loci associated with the disease [12], out of which 45 are immune related genes [13]. We have also reported a significant association of genes, both MHC and MHC-linked (MHC-x), with T1D [14,15].

OR genes are the largest gene family in the human genome comprising ~400 genes and ~600 pseudogenes [16-18]. Ignatieva and coworkers have recently conducted a novel analysis on SNPs using GWAS data from 1000 Genome Projects and revealed an extremely high level of SNPs in the promoter regions of the OR and HLA genes. The extremely high level of SNP content in promoters of OR genes raise the question about the functional significance of such SNPs for olfactory cognition as well as their association with human diseases [19]. The study provided strong genetic confirmation for already existing reports about the unique appearance of OR that is inextricably related to immunological function [20-22]. In addition, it might be an interesting genetic reasoning for the accumulative evidence that OR dysfunction exists in brain diseases such as Multiple Sclerosis [23-29], Alzheimer Disease [27,28,30-32], Parkinson's Disease [27,28,33-37], and depression [38-44].

OR14 gene is located in the telomeric region of HLA-F [14] where an interesting haplotype-specific association between T1D and a SNP in the promoter region of OR14 gene, rs9257691, has been reported [14].

The association of olfaction has been reported in diabetic complications since 1993, and has also been associated with degenerative

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complications of T1D [45,46]. There is accumulating evidence that glucose-induced oxidative stress plays a role in diabetic complications, and that OR plays an important role in their pathogenesis [47-50]. Meanwhile, studies have shown an association between OR impairment in diabetic ketoacidosis and encephalopathy [51-55], neuropathy [54], retinopathy [56-58] and nephropathy [58-61]. Therefore, the present study aims to further our current understanding of the significance of the SNP in the promoter region of the OR14 gene in the pathobiology of T1D and its associated complications.

Materials and Methods

This pilot study comprised of 100 Europid Caucasoid patients with T1D, as defined by the National Diabetes Data Group, and 25 patients of them were considered as Diabetic Control (DC) for the complication study [62-67] (Table 1).

Microvascular complications included Diabetic Neuropathy [DNe (nerve damage)], Diabetic Retinopathy [DR (e.g. glaucoma, cataract, and corneal disease)], and Diabetic Nephropathy [DN (kidney disease)]. Patients with overt DNe were identified by the presence of ankle jerk loss, sensations of pain, foot ulcer, and/or autonomic neuropathy. Patients with retinopathy were identified as having more than five blots per eye: hard or soft exudates, new vessels or flourescein angiographic evidence of maculopathy, or previous laser treatment for preproliferative or proliferative retinopathy. Patients with nephropathy were identified as having had diabetes for >10 years with persistent proteinuria over a 12 month period in the absence of hematuria or infection. Patients who have had diabetes for at least 20 years but remain free of retinopathy and nephropathy were considered as Diabetic Control (DC) as previously explained [65]. Although cardiovascular disease is more prevalent among patients with T1D (as well as type 2 diabetes) than those without; in this study, cardiovascular disease was not considered as a diabetes-specific complication [68-71].

The case and control subjects were genotyped for the OR14 gene polymorphism using Taqman SNP genotyping (Applied Biosystems) as discussed previously [14].

The frequency of the OR14 genotypes met the Hardy-Weinberg principle. The Fisher's 2-sided exact test was used to compare allele and genotype frequencies of A-OR14-C in the entire group of cases (n=75) against diabetic controls (n=25).

Results

There was a critical and significant association among the OR14- CC genotype in patients who developed DN, p=0.004, but no statistically significant differences were noted in cases of DR, p=0.23, or DNu, p=0.09, compared to DC (Table 2).

There were no other significant differences among gender of patients with diabetic complications compared to DC, and no significant differences in allelic comparisons among diabetics with complications compared to DC groups.

Conclusion

Complications of T1D are secondary conditions: DR, DNu and DN are disorders commonly associated with the disease and affect large populations worldwide [66].

The current findings suggest that OR14-CC genotype of A-OR14-C SNP (rs9257691), in the telomeric region of HLA-F, is significantly associated with DN but not DR or DNu in comparison to DC.

Emerging evidence indicate that different genetic and environmental factors play a role in the regulation of inflammatory and profibrotic genes in renal and vascular cells under diabetic conditions which lead to DN [66]. Further, accumulative evidence state that DN develops in 20-25% of patients with T1D [69].

Recently, Gascón et al., have reported an interesting finding that the olfactory function test is an indicator of early microvascular complications in diabetic patients [61]. On the other hand, Pluznick et al. have clearly stated that the olfactory system may play a physiologically critical role in regulating fundamental aspects of renal function [59]. Further, Ignatieva et al. findings of SNPs in promoter regions of ORs raised the question about the functional significance of coding SNPs for OR as well as about their association with human diseases [19]. The current finding provides genetic back up for the above investigations; that OR14-CC could be used as a valuable marker to indicate the presence of mechanisms that play a role in the progression of DN in T1D. T1D and DN still present considerable challenge globally with DN being the most frequent reason for dialysis in many Western countries [67]. Therefore, the detection of a DN marker in the early onset of T1D might be of therapeutic value, thereby postponing and/or preventing the need for renal replacement therapy. Our results confirm the novel findings of Gascón et al. who have recently addressed the usefulness of smell test renal dysfunction [61]. Therefore, it is important to further this study in a large multicenter investigation of diabetic complications. Any screening test as such needs to be rigorously studied in both adult and pediatric populations before any universal screening is recommended.

Take-home Messages

The critical association of the OR14-CC gene with DN patients might be a good marker for early prognoses of DN during early onset of T1D

Detection of a DN marker in the early onset of T1D might be of

Diabetic Complications	Number of Patients	
	Male	Female
Retinopathy (DR)	14	13
Neuropathy (DNu)	9	11
Nephropathy (DN)	13	15
Control (DC)	12	13

Table 1: Distribution of cases and control subjects enrolled in the study.

DR=patients with retinopathy; identified as having more than 5 blots per eye: hard or soft exudates, new vessels or flourescein angiographic evidence of maculopathy or previous laser treatment for preproliferative or proliferative retinopathy.

DNu= patients with overt neuropathy; identified by the presence of ankle jerk loss, sensations of pain, foot ulcer and/or autonomic neuropathy.

DN= patients with nephropathy; identified as having had diabetes for more than 10 years with persistent proteinuria over 12 months in the absence of hematuria or infection.

DC= patients who have had diabetes for at least 20 years but remain free of retinopathy and proteinuria.

Diabetic Complications	p-value
Retinopathy	0.23
Neuropathy	0.09
Nephropathy	0.004

Table 2: Association of the OR SNP among diabetic complication groups.

A comparison between the OR-CC among all diabetic complication groups (ie DR, DNU as well as DN) showed that OR-CC is critically associated with DN, p=0.004. However, neither of DR or DNu cases meet statistical significance, p=0.23 and p=0.09 respectively. The OR-CC distribution among male and female groups also did not show any significant difference.

therapeutic value, thereby postponing and/or preventing the need for renal replacement therapy

This study indicates that patients with T1D who have the OR14-CC genotype are significantly susceptible to progressive DN

It is recommended to run confirmatory multicenter investigation for the OR-CC association with DN before establishing screening test

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