Contents available at ScienceDirect

Diabetes Research and Clinical Practice

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

International Diabetes Federation

Association of 32 type 1 diabetes risk loci in Pakistani patients

Aysha Karim Kiani [a,*], Peter John [a,*], Attya Bhatti [a], Asima Zia [a], Gulbin Shahid [b], Parveen Akhtar [c], Xingbin Wang [d], F. Yesim Demirci [d], M. Ilyas Kamboh [d]

[a] Department of Healthcare Biotechnology, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan
[b] Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan
[c] Fauji Foundation Hospital, Rawalpindi, Pakistan
[d] Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 27 August 2014
Received in revised form
15 November 2014
Accepted 3 January 2015
Available online 21 January 2015

Keywords:
Diabetes
Type 1 diabetes
Auto-immune disease
Association studies
Diabetes in Pakistan
Insulin dependent diabetes mellitus

ABSTRACT

Aim: To identify risk alleles contributing towards type 1 diabetes in Pakistani patients.
Introduction: Type 1 diabetes (T1D) is an autoimmune disease which is caused by destruction of insulin producing β cells by immune system. Genetic predisposition as well as environmental factors contribute to its etiology. To date more than 40 risk loci have been identified for T1D.
Methodology: A total of 191 family-based and unrelated T1D cases and controls were recruited. DNA was extracted and 32 genome-wide significant single nucleotide polymorphisms (SNPs) previously reported in Europeans were genotyped. Genotyping was performed using TaqMan SNP genotyping assays and the data was analyzed using FamCC software.
Results: Our results showed significant association of 10 single nucleotide polymorphisms (SNPs) with T1D at $p < 0.01$, including *HLA-DQA1/rs9272346*, *ERBB3/rs2292239*, *SIRPG/rs2281808*, *IL2-KIAA1109/rs4505848*, *GLIS3/rs7020673*, *CD226/rs763361*, *PTPN2/rs478582*, *IKZF1/rs10272724*, *BACH2/rs11755527*, *C6orf173/rs9388489*, whereas 5 more SNPs showed their association at $0.01 < p < 0.05$ in Pakistani population.
Conclusion: We have replicated many of the T1D loci established among Europeans in a Pakistani population.

1. Introduction

The hallmark of type 1 diabetes (T1D) is its origin due to autoimmune processes that destroy the insulin producing β cells. Beta cell destruction result in hyperglycemia, which requires exogenous insulin for survival. Hyperglycemia causes long-term clinical problems, including renal failure, retinopathy, neuropathy and heart disease which in turn cause substantial disability and shorten lifespan [1].

Prevalence of T1D in Asian populations is very low (0.4 ± 1.1 cases/year/100,000 individuals) as compared with

* Corresponding authors at: ASAB, National University of Sciences and Technology, Sector H-12, Islamabad, Pakistan. Tel.: +0092 3326983838; fax: +92 51 90851302.
E-mail addresses: ayshakiani@gmail.com (A.K. Kiani), pjohn72@hotmail.com (P. John).
http://dx.doi.org/10.1016/j.diabres.2015.01.022
0168-8227/

Caucasian populations (more than 20 cases/year/100,000 individuals) maybe due to different frequency distribution of HLA alleles in each population. Although many autoimmune diseases affect predominantly women, rate of T1D appears to be similar in both men and women [2,3]. With the collaborated effort of WHO-DAMOND, rate of incidence of T1D in children below the age of 15 years has been registered in different parts of the world [1]. The incidence of T1D has been rising globally during the past decades, and if that trend continues, doubling of new cases of T1D in European children younger than 5 years is predicted between 2005 and 2020 [4].

Type 1 diabetes clusters in families and has a strong genetic basis as reflected by a high sibling recurrence risk ratio (λS) of 15 and a higher prevalence in monozygotic twins than in dizygotic twins [5,6]. Furthermore, siblings of T1D patients develop islet autoantibodies more frequently than their offspring or parents. Both genetic and environmental differences seem to affect the geographic distribution of T1D. Prevalence of T1D is highest in individuals of European background, intermediate in Africans and much lower in East Asians [7].

The most recently reported meta-analysis identified more than 40 T1D loci, including 18 new regions and confirming regions shared by more than one disease [7]. Candidate gene study and linkage analysis have established that HLA is the most strongly linked locus with T1D and is responsible for almost half of the relative risk for T1D [6]. Four non-HLA loci have also been identified to be associated with the risk for T1D by different candidate gene studies including INS, CTLA4, PTPN22 and IL2RA [6]. Application of genome-wide association studies (GWAS) has revealed several new genes playing role in T1D. These studies have the advantage of implementing large sample sets to increase the power to detect common variation affecting the risk. To date, most GWAS for T1D are conducted on individuals of European ancestry from the United Kingdom and North America [8,9].

Little is known about the genetic background and risk alleles of T1D in the Pakistani population. We hypothesized that there may be some sharing of susceptibility genes among different populations. Therefore, we genotyped 32 genome-wide significant single-nucleotide polymorphisms (SNPs) reported in Caucasians in a Pakistani population to determine whether some of the susceptibility genes are shared between these two populations.

2. Materials and methods

2.1. Subjects

A total of 191 family-based and unrelated cases and controls with type 1 DM diagnosed by clinical findings and hyperglycemia and confirmed with positive antibodies against glutamic acid decarboxylase were recruited (Table 1). Family-based samples were taken from 10 different families with more than two affected individuals.

All cases were clinically diagnosed and blood samples were collected with the collaboration of Pakistan Institute of Medical Sciences (PIMS) Islamabad and Fuji Foundation Hospital Rawalpindi. Healthy controls without any previous T1D history were recruited from Islamabad (Table 1). The study was approved by Institutional Review Board (IRB) ASAB NUST (Pakistan) and University of Pittsburgh Institutional Review Board (USA), and all participants provided written informed consent.

Table 1 – Characteristics of Type 1 Diabetes related and unrelated samples.

	Related individuals ($n = 62$)	Unrelated individuals ($n = 129$)
Cases (n)	23	68
Controls (n)	39	61
Mean age (±SD)	20.91 ± 15.49	14.34 ± 5.56
Female (%)	36	87

SD: standard deviation.

2.2. Genotyping

DNA was extracted from whole blood using either a phenol chloroform based method or using the Fermantas Whole Blood Genomic DNA Purification kit and then quantified using Quant-iT™ PicoGreen® ds-DNA assay kit (Life Technologies, NY, USA).

Genotyping was performed using TaqMan SNP genotyping assays (Life Technologies) following manufacturer's protocol. PCR amplification was performed in 384 well plates on dual-block Gene-Amp® PCR system 9700 (Life Technologies) and end-point readings were performed on ABI Prism 7900HT sequences detection system instrument (Life Technologies). A total of 32 SNPs were selected for genotyping based on their reported genome-wide significant P-values and frequencies in Caucasians, which is considered as closest to the Pakistani population [10] (Table 2).

2.3. Statistical analysis

For the calculation of allele and genotype frequencies in the unrelated sample, the allele counting method was used. Similarly chi-squared (χ^2) goodness-of-fit test was used to check deviation from Hardy–Weinberg equilibrium. PedCheck [11] program was used to check Mendelian inconsistencies in the pedigree data of family-based samples (http:/Watson.hgen.pitt.edu).

Association of the selected and already established SNPs with T1D was examined using family case control (FamCC) software Ver 1.0 [12]. FamCC is a software designed to check the association by combining the family dataset and case/control dataset together, but can also analyze each dataset independently [12]. Briefly, the FamCC performs three sequential steps. First, principal components are generated from the genotype data. Next, multiple linear regression on the top 10 principal components is performed for both the phenotypes and markers for the unrelated individuals, respectively. Finally, the residuals of the phenotypes and the markers are calculated based on the estimated coefficients in the linear