Association of Protein Tyrosine Phosphatase Non-receptor, Type 22 (PTPN22) C1858T Polymorphism with Type 1 Diabetes in North India: A Replication Study

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Abstract

Objective: Type 1 diabetes (T1D) is a multifactorial autoimmune disorder where several genes have been associated with the disease. While the major histocompatibility complex has been shown to be the major locus, contributions of other loci in different combinations seem to have synergistic effects. PTPN22 (Protein tyrosine phosphatase non-receptor, type 22) gene encoding lymphoid tyrosine phosphatase (LYP) is a negative regulator of T cell signalling. A gain of function mutant at nucleotide position 1858 C>T has been associated with diabetes, however, it is reported to be absent in Asians. We have studied PTPN22 C1858T polymorphism in T1D patients from North India since there are no reports from this region.

Methods: PTPN22 C1858T polymorphism was studied in 250 T1D patients and 480 healthy controls using polymerase chain reaction followed by restriction digestion (PCR-RFLP). Alleles of HLA-DRB1 locus were studied using PCR followed by hybridization with sequence specific oligonucleotide probes using a bead based assay on Luminex platform.

Results: In spite of reports of absence of 1858T allele in Asians, we observed this allele to be present in North Indians, albeit with low frequency (1.98%). However, T1D patients from the same ethnic background showed significantly higher frequency of the allele and heterozygous genotype 1858CT as compared to controls. Patients with both 1858CT and 1858CC genotypes had predisposing MHC alleles.

Conclusion: The association of PTPN22 1858CT genotype with Type 1 diabetes was independent of the predisposing Human leukocyte antigen (HLA) alleles DRB1*03:01, DRB1*04:01, DRB1*04:05 in North Indian patients, suggesting their integrated roles in manifestation of T1D. Based on the reported role of PTPN22 1858CT genotype in defective innate immune responses against viral infections, and defects in early T cell signalling, it is tempting to speculate that it may be detrimental for the destruction of pancreatic beta cells in the present scenario.

Keywords: PTPN22; Major histocompatibility complex; Type 1 diabetes

Introduction

Type 1 diabetes (T1D) is a multifactorial autoimmune disorder where insulin producing pancreatic beta cells are lost before the manifestations of the disease. It has an incidence of 10.5/100,000/year in India [1]. Several genetic and environmental factors have been implicated as the causative factors, however, a role of complex interaction of these factors leading to the autoimmunity destruction of the pancreatic beta cells cannot be ruled out [2]. The cell-mediated autoimmune destruction of pancreatic beta cells is brought about by infiltrating macrophages and lymphocytes in the pancreatic islets, the phenomenon known as Insulitis. While the function of most T-cells is to mount host-defense response, regulatory T-cells are programmed to moderate the reactivity of effector T-cells, in order to protect the body from autoimmune responses. It has been proposed that an increase in the ratio of effector T-cells to regulatory T-cells may result in autoimmunity in T1D [3]. Both CD4+ helper T cells and CD8+ Cytotoxic T cells are the effector T cells required to induce beta cell autoimmunity [4]. Defects in T cell signaling may be involved in altering the ratios of effector and regulatory T cells.

Of the 20 genomic intervals implicated in the predisposition for development of T1D [5], we and others have reported association of T1D with the major histocompatibility complex (MHC) alleles on chromosome 6p21 and the 5’ regulatory region of the insulin (INS) gene on 11p15.5 in North Indians and other ethnic groups [6-8].

Besides, we have also reported association and interaction of TNF-α gene with other cytokine genes [9], Vitamin D receptor gene (VDR) and their role in expression of predisposing MHC allele in manifestation of T1D [10]. Interestingly, it seems that several genes have integrated roles in manifestation of the disease since simultaneous presence of different alleles of two or more genes seem to be significantly increased in T1D patients. For instance, simultaneous presence of HLA DRB1*0301, DRB1*0401 and/or DRB1*0405 along with class-I alleles of INS-linked variable number of tandem repeats (VNTR) were very significantly associated with T1D [6]. Similarly an interaction was observed between VDR gene and predisposing HLA alleles, and this interaction of VDR with DRB1*0301 was through the vitamin D response element (VDRE) present in the promoter region of the predisposing HLA allele and was involved in regulating the expression of HLA-DRB1*0301 [10].

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possibility of developing T1D at a later date after the collection of their
345 men), with a mean age ± SD of 30.16 ± 9.02.

The American Diabetes Association expert committee [26] as reported
Diabetes Clinic' were carefully assessed and categorized as type 1, type
± 7.43), attending 'Juvenile Diabetes Clinic' at the All India Institute
India Institute of Medical Sciences, New Delhi, India. The patient group
Committee's clearance from National Institute of Immunology and All
controls based in Delhi, originally from North Indian states of Uttar
Pradesh, Haryana and Punjab, were studied after obtaining informed
controls and Institutional Human Ethics

The PTPN22 (Protein tyrosine phosphatase non-receptor, type 22) gene codes for lymphoid tyrosine phosphatase (LYP) which is an 807
amino acid residue protein with a molecular weight of 110 kDa and
is involved in negative regulation of T cell signaling [13-15]. A single
nucleotide polymorphism (SNP) in the PTPN22 gene at nucleotide
position 1858 C→T results in the amino acid change from arginine
(R) to tryptophan (W) (CGG to TGG) at codon 620 which has been shown to be a gain-of-function mutant [16]. The negative regulation of
T cells by LYP is brought about by its dissociation from C-terminal Src
kinase (CSK) and recruitment of LYP to the plasma membrane where it
down-regulates TCR signaling. LYP*W620, however, cannot bind CSK
and negatively regulates LCK-mediated phosphorylation of the TCRβ
chain, reduces tyrosine phosphorylation of LAT and reduces activation
of ERK2 as compared to LYP*R620. In essence, LYP*W620 has been
reported to be more efficient at dephosphorylation than LYP*R620 [15,16].
Several autoimmune disorders like systemic lupus erythematosus [17],
rheumatoid arthritis [18,19] and type 1 diabetes [20-22] have been
shown to be associated with the mutant T allele i.e., LYP*W620.

The allele frequency of the minor 1858T allele varies from 0% in
some Asian populations [23] to 8-17% in different ethnic groups of
European origin [17,18,24]. Africans [24] also have a very low
frequency of the minor 1858T allele [10]. Such ethnic differences make
it important to study the role of the minor allele in manifestations of
autoimmune disorders in different populations. While earlier studies
reported absence of 1858T allele in Asia [23], we had reported a very
low frequency (2.03%) of this allele in normal healthy individuals [25].
In spite of this low frequency in the normal population, we observed
a disease specific increase in the frequency of 1858T allele in leprosy
patients. So, we investigated if the same was true for T1D as well, since
several reports have shown association of 1858T allele with Type 1
diabetes in different ethnic groups [4,20-22], however, there are no
studies reported so far from the Indian sub-continent with respect to
PTPN22 polymorphism in T1D, probably due to presumption that the
allele is absent in Asian populations. Here, we report the association of
PTPN22 1858T allele and its association with predisposing MHC class-
II alleles in T1D in patients from North India.

Materials and Methods

Two hundred and Fifty T1D patients and 480 normal healthy controls based in Delhi, originally from North Indian states of Uttar
Pradesh, Haryana and Punjab, were studied after obtaining informed
consent from patients and controls and Institutional Human Ethics
Committee's clearance from National Institute of Immunology and All
India Institute of Medical Sciences, New Delhi, India. The patient group
consisted of 106 females and 144 males with a mean age of 25.96 ± 9.79
and age at onset of diabetes<30 years (Mean age at onset ± SD=15.995
± 7.43), attending 'Juvenile Diabetes Clinic' at the All India Institute
of Medical Sciences, Delhi, India. All the patients attending 'Juvenile
Diabetes Clinic' were carefully assessed and categorized as type 1, type
2 and fibrocysticulous pancreatopathy according to the classification of
the American Diabetes Association expert committee [26] as reported
earlier [9,10]. Controls included 480 healthy subjects (135 women and
345 men), with a mean age ± SD of 30.16 ± 9.02.

For controls, we preferred higher age group to rule out their
possibility of developing T1D at a later date after the collection of their
blood samples as normal healthy controls. Controls were asked for
their clinical history and only those without any history of infectious or
autoimmune diseases were included in the study.

DNA was extracted using a standard protocol from 10 ml of
blood from T1D patients and healthy controls from the same ethnic
background after obtaining informed consent and Institutional Ethics
Committee's approval.

PTPN22 Genotyping using PCR-RFLP Polymerase Chain Reaction followed by restriction fragment length polymorphism

The genotyping for PTPN22 single nucleotide polymorphism
at position C1858T in the codon 620 was carried out using PCR-
RFLP methods where standard PCR protocol was followed by
restriction digestion of the PCR amplified product with restriction
enzyme Rsal. Primers used for amplification were forward primer
(5’-ACTGATAATGTTGCTTCAAA-3’) and reverse primer
(5’-TCACCAACCTCTTTACCA-3’), as described by Zheng et al.,
2005 (Gebank accession # AL 137856). Briefly, 100 ng of genomic
DNA (5 μl) was mixed with 2.5 μl of 10X buffer, 0.25 μl of dNTPs (100
mM), 2.5 μl MgCl2, (25 mM), 2.0 μl (5 pm/μl) each of the primers, and
0.125 μl ( 5 U/μl ) of Taq polymerase in 25 μl reaction volume. After
initial denaturation at 94°C for 2 minutes, 35 cycles of denaturation at
94°C, annealing at 60°C and extension at 72°C, each for 30 seconds,
were carried out followed by final extension cycle at 72°C for 7 minutes
in Gene Amp 2700 thermocycler (Applied Biosystems, CA, USA).
After confirmation of the amplified products on 1% Agarose gel, 10 μl of
each of the amplified products were digested with 5 U of Rsal enzyme (New
England Biolabs) overnight at 37°C. The digested PCR products were
run on 2.5% agarose gel in 0.5X TBE buffer along with 50 bp ladder. The
restriction endonuclease Rsal 1 cleaves the DNA strand which contains
the C nucleotide at 1858° position and gives two bands of 176 bp and 42
bp sizes, the mutant 1858T, however, is not digested by Rsal 1 and gives
a single band of 218 bp size (Figure 1).

HLA-DRB1 polymorphism

Alleses of HLA-DRB1 locus were determined for 243 T1D patients

Figure 1: A representative picture of PCR-RFLP of PTPN22 C1858T on 2.5%
Agarose gel: lanes 1-4 and 6-8 show homozygous 1858CT genotype with 218
bp and 176 bp bands. Lane 5 shows heterozygous 1858CT genotype with 218
bp, 176 bp and 42 bp bands, last lane is 50 bp ladder.
and 327 controls for whom PTPN22 C1858T data was available. For 100 patient samples and 94 controls samples, the second exons of the DRB1 genes were PCR amplified using standard conditions and hybridized with 3P-labeled sequence specific oligonucleotide probes (SSOP) as described earlier [6,9]. For additional 143 patients and 233 control samples, Luminex based typing was done using Labtype SSO kit from One Lambda, (Canoga Park, CA, USA) according to the manufacturer's instructions as described earlier [9]. Briefly, 40 ng of DNA was amplified in master mix, primers and 2 μl of Taq DNA polymerase in Gene Amp 2700 thermocycler (Applied Biosystems, CA, USA). After confirmation of amplification, on 1% agarose gel, 5 μl of amplified product was denatured and hybridized with appropriate amount of multipleplex beads conjugated with oligonucleotide probes, in hybridization buffer at 60°C for 15 minutes followed by washings and incubation with 50 μl 1X SAPE solution (Stepavidin Phycoerythrin) at 60°C for 5 minutes. Fluorescence of the labeled beads was acquired on Luminex 2.2 flow cytomter (Luminex corporation, Austin, TX). Acquired data was analysed using Labtype software provided by One Lambda for analysis of MHC alleles. Reproducibility of the Luminex based HLA typing method had been checked by us earlier [9].

Statistical analysis

Chi-square test was used to examine the statistically significant differences between patients and controls using Stata 9.2 software. Odds Ratios and 95% confidence Intervals were also calculated using the same software. p values were corrected using Bonferroni's correction for multiple comparisons. Fisher's exact test was used whenever the numbers in any group were less than 5 i.e. in cases or controls for any allele. In such cases, Odds ratios were calculated using Woolf’s method [27] with Haldane's [28] modification as described earlier [29].

Results

We have genotyped 250 T1D patients and 480 healthy controls from the same ethnic background for C1858T alleles of PTPN22 as shown in Figure 1. Table 1 shows that the mutant 1858T allele is significantly increased in patients with T1D as compared to controls (p<0.0004, Odds Ratio=2.82, 95% confidence Interval=1.49-5.4). While homozygous genotype CC was significantly reduced (p<0.0003, Odds Ratio=0.54, 95% confidence Interval=0.18-0.65), heterozygous CT was significantly increased in T1D patients compared to controls (p<0.0003, Odds Ratio=2.93, 95% confidence Interval=1.53-5.71). And these differences remained significant even after Bonferroni’s correction. All genotype frequencies in patients as well as controls were in Hardy Weinberg equilibrium.

To study if there was any gender bias, we stratified the samples for association of 1858CT genotype with gender of the patients. While genotype CT and T allele were significantly increased in both males and females when compared to male and female controls respectively, the association was stronger in females (for CT p<0.002, OR=6.4 and for T p<0.003, OR=6.12) since the frequencies of CT genotype and T allele were much less in female controls (1.5% and 0.75% respectively) compared to male controls (4.9% and 2.51% respectively), although the same were comparable in male and female patients (Table 2).

We further checked whether CT genotype and T allele had any significant association with age at the onset of T1D (Table 3). For this we divided the patients in two groups based on their ages at the onset of the disease i.e., more than (>14) years and less than or equal to (≤14) years. Interestingly, while both age groups had significant association with the predisposing genotype CT and allele T, patients who had early age at onset i.e. ≤14 years showed more significant association (for CT p<0.0007, OR=3.13, for T p<0.0008, OR=3.15).

We had earlier reported HLA-DRB1*03:01, *04:01 and 04:05 to be predisposing for T1D in North Indians [6]. To study the role of predisposing HLA alleles in combination with the PTPN22 alleles, 243 T1D patients and 327 normal healthy controls who were studied for PTPN22 C1858T were also typed for HLA-DRB1 locus alleles. Our results were in accordance with the earlier results i.e. all three alleles, HLA-DRB1*03:01, *04:01 and 04:05 were significantly increased in T1D patients (Table 4) and DRB1*07:01 was significantly reduced in the patients as compared to healthy controls. Simultaneous presence of PTPN22 1858CT and 1858CC along with predisposing HLA alleles was investigated and interestingly both PTPN22 1858CT and 1858CC along with HLA-DRB1*03:01 were significantly increased in the T1D patients.

<table>
<thead>
<tr>
<th>Allele/Genotype</th>
<th>T1D Patients N=250</th>
<th>Controls N=480</th>
<th>T1D vs. Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>p Odds Ratio 95% C.I.</td>
</tr>
<tr>
<td>Allele1858C</td>
<td>473 (94.6)</td>
<td>941 (98.02)</td>
<td>0.0004 0.35 0.18-0.67</td>
</tr>
<tr>
<td>Allele1858T</td>
<td>27 (5.4)</td>
<td>19 (1.98)</td>
<td>0.0004 2.82 1.49-5.4</td>
</tr>
<tr>
<td>Genotype CC</td>
<td>223 (89.2)</td>
<td>461 (96.04)</td>
<td>0.0003 0.34 0.18-0.65</td>
</tr>
<tr>
<td>Genotype CT</td>
<td>27 (10.8)</td>
<td>19 (3.96)</td>
<td>0.0003 2.93 1.53-5.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele/Genotype</th>
<th>T1D Male N=144</th>
<th>T1D Female N=106</th>
<th>Controls Male N=345</th>
<th>Controls Female N=135</th>
<th>Male T1D vs. Male Controls</th>
<th>Female T1D vs. Female Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>p value Odds Ratio 95% C.I.</td>
<td>p value Odds Ratio 95% C.I.</td>
</tr>
<tr>
<td>Alleles1858C</td>
<td>272 (94.4)</td>
<td>201 (94.8)</td>
<td>673 (97.5)</td>
<td>268 (99.25)</td>
<td>0.015 0.43 0.2-0.9</td>
<td>0.003 0.16” 0.06-0.43</td>
</tr>
<tr>
<td>Allele1858T</td>
<td>16 (5.6)</td>
<td>11 (5.2)</td>
<td>17 (2.5)</td>
<td>2 (0.75)</td>
<td>0.015 2.33 1.08-4.97</td>
<td>0.003 6.12” 2.3-16.26</td>
</tr>
<tr>
<td>Genotype CC</td>
<td>128 (88.9)</td>
<td>95 (89.6)</td>
<td>328 (96.1)</td>
<td>133 (98.5)</td>
<td>0.013 0.41 0.19-0.9</td>
<td>0.002 0.15” 0.05-0.42</td>
</tr>
<tr>
<td>Genotype CT</td>
<td>16 (11.1)</td>
<td>11 (10.4)</td>
<td>17 (4.9)</td>
<td>2 (1.5)</td>
<td>0.013 2.4 1.1-5.2</td>
<td>0.002 6.4” 2.4-17.2</td>
</tr>
</tbody>
</table>

*Fisher’s exact test was used to calculate p values.
*Haldane’s modification was used to calculate the Odds Ratio.

Table 1: Allele and genotype frequencies of PTPN22 C1858T in T1D as compared to normal healthy controls.

Table 2: Gender-wise distribution of allele and genotype frequencies of PTPN22 C1858T in T1D as compared to matched normal healthy controls (07.01.14).
on regulatory T cells [34,35] and defect in CTLA-4 expression may be roles in negative T cell signaling. CTLA-4 is constitutively expressed cells [32,33]. Similarly, the products of [32,33] which may be responsible for poor thymic education for insulin Class-III insulin in thymii of fetuses as compared to those with alleles VNTR class-I alleles have shown to have poor expression of implicated genes have some functional relevance. For example, since it is a very complex autoimmune disorder where most of the inherited risk [8], an integrated role of other genes like (VDR), Vitamin D receptor, CTLA-4

Discussion
Several genes have been implicated for predisposition to develop T1D. While the MHC region has been shown to account for 50% of the inherited risk [8], an integrated role of other genes like INS-VNTR, CTLA-4, Vitamin D receptor (VDR), cytokine genes and PTPN22 amongst a number of other genes cannot be ruled out [6,9,10,30,31] since it is a very complex autoimmune disorder where most of the implicated genes have some functional relevance. For example, INS-VNTR class-I alleles have been shown to have poor expression of insulin in thymii of fetuses as compared to those with Class-III alleles [32,33] which may be responsible for poor thymic education for insulin resulting in autoimmunity against insulin producing pancreatic beta cells [32,33]. Similarly, the products of CTLA-4 and PTPN22 genes have roles in negative T cell signaling. CTLA-4 is constitutively expressed on regulatory T cells [34,35] and defect in CTLA-4 expression may be detrimental for the manifestations of autoimmunity since autoimmune responses may not be regulated in these cases [35].

The other negative regulator of T cell signaling, PTPN22 with SNP C1858T, has been implicated in many autoimmune disorders [18,19,36] and was first reported by Bottini et al. in Type 1 diabetes patients from two independent populations [4,37], followed by several reports where the associations were reported with either T1D per se, or with female gender or with beta cell dysfunction or autoantibodies and even with early age at onset in children fed with cow milk formula, in different populations [20-22,38-41]. Our observations replicated earlier reports, and show a significantly higher number of T1D patients from North India to have 1858CT genotype and T allele as compared to controls [37,39,42,43]. Fedetz et al. [42], observed a significantly higher number of T1D patients from Ukraine to have 1858CT allele as compared to controls [37,39,42,43].

as compared to controls (Table 5), suggesting that while predisposing HLA alleles have a dominant effect, association of PTPN22 C1858T genotype is independent of MHC associations.

Table 3: Genotype frequencies of PTPN22 C1858T in T1D based on age at onset as compared to normal healthy controls (07.01.14).

<table>
<thead>
<tr>
<th>Allele/Genotype</th>
<th>T1D Patients N=243</th>
<th>Controls N=327</th>
<th>p</th>
<th>Odds Ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele1858C</td>
<td>n (%)</td>
<td>n (%)</td>
<td>p  value</td>
<td>Odds Ratio</td>
<td>95% C.I.</td>
</tr>
<tr>
<td>253 (95.1)</td>
<td>220 (94.0)</td>
<td>941 (98.02)</td>
<td>0.008</td>
<td>0.39</td>
<td>0.18-0.88</td>
</tr>
<tr>
<td>13 (4.9)</td>
<td>14 (5.98)</td>
<td>19 (1.98)</td>
<td>0.002</td>
<td>2.54</td>
<td>1.1-5.5</td>
</tr>
<tr>
<td>Genotype CC</td>
<td>120 (90.2)</td>
<td>103 (88.1)</td>
<td>0.0076</td>
<td>0.38</td>
<td>0.17-0.86</td>
</tr>
<tr>
<td>Genotype CT</td>
<td>13 (9.8)</td>
<td>14 (11.9)</td>
<td>0.0076</td>
<td>2.62</td>
<td>1.1-6.8</td>
</tr>
</tbody>
</table>

Table 4: Frequencies of predisposing HLA-DRB1 alleles in T1D patients and controls typed for PTPN22 C1858T.

<table>
<thead>
<tr>
<th>PTPN22 CC/CT with predisposing and protective HLA alleles</th>
<th>T1D N=243</th>
<th>Controls N=327</th>
<th>p</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC- HLA-DRB1’03/01</td>
<td>24</td>
<td>9.9</td>
<td>0.91</td>
<td>6.34 x 10^{-11}</td>
<td>10.34 x 10^{-11}</td>
</tr>
<tr>
<td>CC- HLA-DRB1’03/01</td>
<td>164</td>
<td>67.9</td>
<td>49</td>
<td>14.98</td>
<td>1.36 x 10^{-17}</td>
</tr>
<tr>
<td>CT- HLA-DRB1’04/01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.453</td>
<td>0.00003</td>
</tr>
<tr>
<td>CT- HLA-DRB1’04/03</td>
<td>26</td>
<td>10.7</td>
<td>8</td>
<td>2.453</td>
<td>0.00003</td>
</tr>
<tr>
<td>CT- HLA-DRB1’04/03</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>CC- HLA-DRB1’04/03</td>
<td>8</td>
<td>3.3</td>
<td>29</td>
<td>8.86</td>
<td>0.0075</td>
</tr>
<tr>
<td>CT- HLA-DRB1’04/05</td>
<td>2</td>
<td>0.82</td>
<td>0</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>CC- HLA-DRB1’04/05</td>
<td>20</td>
<td>8.23</td>
<td>1</td>
<td>0.31</td>
<td>3.17 x 10^{-17}</td>
</tr>
<tr>
<td>CT- HLA-DRB1’07/01</td>
<td>3</td>
<td>1.23</td>
<td>5</td>
<td>1.53</td>
<td>0.53</td>
</tr>
<tr>
<td>CC- HLA-DRB1’07/01</td>
<td>27</td>
<td>11.11</td>
<td>98</td>
<td>29.97</td>
<td>7.39 x 10^{-8}</td>
</tr>
</tbody>
</table>

Table 5: Association of predisposing PTPN22 C1858T allele with predisposing HLA alleles.

PTPN22 CC/CT with predisposing and protective HLA alleles

PTPN22 C1858T
While we do find a significant increase of T allele and CT genotype in T1D patients, total absence of homozygous 1858TT genotype was surprising. Under normal Mendelian inheritance pattern, one would expect some homozygous TTs as well since 10.8% of the patients had heterozygous CT genotype. However, since the frequency of the T allele is very low in healthy controls, the chances of getting homozygous TT would probably be very low. While no association of 1858T allele was observed with T1D in Japanese population, another SNP in the region, rs1310182, was significantly associated in them. In fact, this nucleotide position C1858T was reported to be non-polymorphic in Japanese [44]. Frequency of 1858T alleles varies in different populations. While we observed a very low frequency of 1858T allele in North Indians [25], earlier studies reported a total absence of T allele in Asian populations [23]. Our study suggests that Asian populations cannot be generalized as one ethnic group since the earlier reports of absence of the T allele have been from Asian populations from Japan, Korea and China, which being Mongoloid in origin, are ethnically different from Indians who are basically Caucasoid with racial admixture of Mongoloid and Negroid elements [45].

Our data further suggests that association of PTPN22 1858CT genotype was independent of the predisposing MHC alleles, suggesting that the primary association is with DRB1*03:01 which was observed in 77.4% of T1D patients, compared to 10.8% of the patients having PTPN22 1858CT genotype. Recently several papers have been published on functional relevance of the PTPN22 1858T or LYP*W620 allele, where PTPN22 encoded LYP has been shown to have roles in both innate and adaptive immune responses. Expression of LYP*W620 has been associated with reduced TLR signaling resulting in defective type 1 IFN-mediated host response to infection which may have role in initiation of T1D that is associated with viral infection [46]. Also, LYP negatively regulates T cell functions by dissociating from C-terminal Src tyrosine kinase (CSK) which is essential for recruitment of LYP to the lipid rafts where it regulates TCR mediated signaling. And the disease associated LYP*W620 allele was shown to partition into lipid rafts more efficiently than LYP*R620 due to its inefficiency to bind CSK and was thus shown to be more potent inhibitor of TCR induced responses [15].

Type 1 diabetes is a multifactorial disease with several components like a jigsaw puzzle with several genomic loci implicated in the manifestation of the disease. We have been trying to put some of the pieces of the puzzle together where some components are inter-related while others are independently affecting the manifestations in the presence of other predisposing factors. We have reported earlier that simultaneous presence of DRBI*03:01 along with homozygous INS-VNTR class-I alleles was significantly increased (p < 10^-6) in T1D patients, giving a relative risk of 70.81 [6]. We also provided evidence of statistically significant interaction between the predisposing HLA allele and high producer alleles of VDR which may be detrimental for the manifestation of T1D in the absence of 1.25-(OH)2D in early childhood and/or in-utero. Our studies on HLA-DRBI*03:01 homozygous cell lines showed enhanced expression of HLA-DR on the B-LCLs stimulated with 100 nM calcitriol as compared to the unstimulated ones confirming that indeed the interaction of VDR with HLA-DRBI*03:01 is occurring through the VDRE present in the promoter region of the gene [10]. Based on these studies we speculated that in the absence of required amount of Vitamin D in early life in the predisposed individuals with HLA-DRBI*03:01, the expression of the allele may be impaired in the thymus [47,48] resulting in escape from thymic deletion of autoreactive T cells leading to T1D manifestations. Poor thymic education for insulin and HLA-DRBI*03:01 proteins along with environmental factors like viral infections, vitamin D deficiency and some milk proteins may be involved in initiation of the autoimmune responses against the pancreatic beta cells. While predisposing HLA class-II molecules may be involved in auto-antigen presentation to T helper cells, higher producing genotypes of pro-inflammatory cytokines like IFN-gamma and TNF-alpha may be involved in enhancing the cell mediated immune responses through proliferation of CD4+ and CD8+ T cells, and higher producing genotypes of IL-10 and TGF-beta may have a role in recruitment of these autoreactive T cells in the pancreas through ICAM-1 and Interleukin dependent pathways [9]. Additionally, PTPN22 1858CT genotype may have a role in defective innate immune responses against viral infections that may trigger islet cell autoimmunity [46] combined with defects in early T cell signaling due to which autoreactive T cells may not be deleted and may lead to insufficient activity of regulatory T cells, thus leading to autoimmunity [4,16] destruction of pancreatic beta cells which may occur through CD4+ and CD8+ T cells and nitric oxide production.

Author Contribution
RR conceived the project and critically evaluated the data, NI, AK SV and JS were involved in generating the Type1 diabetes and control data for PTPN22 and HLA. RR analysed the data and wrote the manuscript.

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