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Investigation of the vitamin D receptor gene (*VDR*) and its interaction with protein tyrosine phosphatase, non-receptor type 2 gene (*PTPN2*) on risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young (DAISY)

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Abstract

The present study investigated the association between variants in the vitamin D receptor gene (*VDR*) and protein tyrosine phosphatase, non-receptor type 2 gene (*PTPN2*), as well as an interaction between *VDR* and *PTPN2* and the risk of islet autoimmunity (IA) and progression to type 1 diabetes (T1D). The Diabetes Autoimmunity Study in the Young (DAISY) has followed children at increased risk of T1D since 1993. Of the 1692 DAISY children genotyped for *VDR* rs1544410, *VDR* rs2228570, *VDR* rs11568820, *PTPN2* rs1893217, and *PTPN2* rs478582, 111 developed IA, defined as positivity for GAD, insulin or IA-2 autoantibodies on 2 or more consecutive visits, and 38 IA positive children progressed to T1D. Proportional hazards regression analyses were conducted.

There was no association between IA development and any of the gene variants, nor was there evidence of a *VDR*PTPN2* interaction. Progression to T1D in IA positive children was associated with the *VDR* rs2228570 GG genotype (HR: 0.49, 95% CI: 0.26–0.92) and there was an interaction between *VDR* rs1544410 and *PTPN2* rs1893217 ($p_{\text{interaction}} = 0.02$). In children with the *PTPN2* rs1893217 AA genotype, the *VDR* rs1544410 AA/AG genotype was associated with a decreased risk of T1D (HR: 0.24, 95% CI: 0.11–0.53, $p = 0.0004$), while in children with the *PTPN2* rs1893217 GG/GA genotype, the *VDR* rs1544410 AA/AG genotype was not associated with T1D (HR: 1.32, 95% CI: 0.43–4.06, $p = 0.62$). These findings should be replicated in larger cohorts for confirmation. The interaction between *VDR* and *PTPN2* polymorphisms in the risk of progression to T1D offers insight concerning the role of vitamin D in the etiology of T1D.

Keywords

Vitamin D; Genetics; Epidemiology; Islet autoimmunity; Type 1 diabetes; Vitamin D receptor

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jsbmb.2012.08.012>.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing beta cells of the pancreas are destroyed. There is typically a preclinical phase of circulating autoantibodies, called islet autoimmunity (IA) that precedes the clinical diagnosis of T1D.

Vitamin D deficiency has been associated with a number of diseases, including multiple sclerosis [1], rheumatoid arthritis, and T1D [2], although not consistently. The mechanism by which vitamin D may exert its effects on these diseases is still not understood completely, particularly with regard to underlying genetic risk. The gene for the vitamin D receptor (*VDR*), through which vitamin D acts, has long been a candidate gene for T1D. Initial small studies found *VDR* polymorphisms to be associated with T1D [3–9]. However, Nejentsev et al. analyzed association of the 98 *VDR* single nucleotide polymorphisms (SNPs) in up to 3763 type 1 diabetic families and found no evidence of association with T1D in the populations tested [10]. Moreover, in a meta-analysis, Guo et al. found no evidence of an association between *VDR* gene polymorphisms (*FokI*, *BsmI*, *ApaI*, and *TaqI*) and T1D risk [11]. Finally, in a recent analysis of 19 genes for association with T1D in the Type 1 Diabetes Genetics Consortium families, none of the forty SNPs genotyped in the *VDR* region were associated with T1D [12]. A recent study [13] showed that the vitamin D receptor binds to a number of genomic positions across the genome, including a novel intronic binding site in the protein tyrosine phosphatase, non-receptor type 2 gene (*PTPN2*), which has also been associated with T1D through a genome-wide association scan in 2007 [14,15]. This suggests the possibility of a more complex relationship in which variation in both *VDR* and *PTPN2* is necessary to have an effect on diabetes risk, which may explain why previous findings regarding *VDR* have been inconsistent.

The Diabetes Autoimmunity Study in the Young (DAISY) has been prospectively following children at increased T1D risk for the development of IA and progression to T1D since 1993. The purpose of this study was to examine the associations between 5 particular *VDR* and *PTPN2* SNPs and the development of IA and progression to T1D in the prospective DAISY cohort. We also aimed to explore a potential gene–gene interaction between *VDR* and *PTPN2* polymorphisms and the risk of IA and progression to T1D.

2. Materials and methods

2.1. Subjects

DAISY is a prospective study composed of two groups of children at increased risk for T1D who were recruited between 1993 and 2004 and are being followed prospectively for the development of IA and T1D. One group is made up of first degree relatives of patients with type 1 diabetes mellitus, recruited between birth and eight years of age. The second group consists of infants born at St. Joseph's Hospital in Denver, Colorado, whose umbilical cord blood was screened for diabetes-susceptibility genotypes in the HLA region. The St. Joseph's Hospital newborn population is representative of the general population of the Denver metropolitan area. Details of the newborn screening, sibling and offspring recruitment, and follow-up of both cohorts have been published previously [16,17]. Cord blood or the first available blood sample (depending on enrolment group) was sent to Roche Molecular Systems, Inc., Alameda, CA, for PCR-based HLA class II typing. All study protocols were approved by the Colorado Multiple Institutional Review Board, and informed consent was given by parents of all participating children.

2.2. Measurement of autoantibodies

Autoantibodies were tested at 9, 15, and 24 months, and annually thereafter, or at their first visit and annually thereafter if the child enrolled after birth. Radio-immunoassays were used to measure serum autoantibodies to insulin, GAD-65, and IA-2 (BDC512), as previously described [18–21], with rigorous confirmation of all positive and a subset of negative results. The cut-off for positivity was established as the 99th percentile of healthy controls. Children who tested autoantibody positive were put on an accelerated testing schedule of every 3–6 months.

Cases of persistent IA were defined as those children positive for at least one islet autoantibody (IAA, GAD-65, IA-2) on two or more consecutive visits. Type 1 diabetes was diagnosed by a physician and defined as random blood glucose >200 mg/dL and/or HbA1c (A1C) >6.2% with clinical symptoms of diabetes.

2.3. VDR and PTPN2 genotyping

DAISY children were genotyped for *VDR* rs1544410 (*BsmI*), *VDR* rs2228570 (*FokI*), *VDR* rs11568820 (*Cdx2*), *PTPN2* rs1893217, and *PTPN2* rs478582. The three *VDR* SNPs were chosen based on previous associations with T1D [3,4,8,9,22] as well as function [10,23–25]. The *VDR* rs1544410 SNP is located at the 3' end of *VDR* and is not known to alter the structure or function of *VDR* [22,26,27]. The *VDR* rs2228570 polymorphism is a T-C transition at the translation initiation codon of *VDR* that results in a shorter protein with increased biological activity [23–25]. *VDR* rs11568820 is in the 5' promoter region of *VDR*, results in reduced transcriptional activity of the promoter, and affects calcium absorption in the intestine [27–30]. The *PTPN2* SNPs, both of which are intronic, were chosen for their association with both T1D and celiac disease [15]. Linkage disequilibrium (LD) between the three *VDR* SNPs and between the two *PTPN2* SNPs was tested in our population using Haploview version 4.2 and none of the SNPs were found to be in LD as measured by r^2 , with $r^2 = 0.005$ for the *VDR* SNPs, and an $r^2 = 0.124$ for the *PTPN2* SNPs.

VDR rs1544410 was genotyped using a linear array method at Roche Molecular Systems, Inc., as described in Mirel et al. [31]. *PTPN2* rs1893217 was genotyped as part of a second project using Illumina 48-plex VeraCode technology following the manufacturer's protocol. Genotyping data analysis and clustering was performed in Illumina's GenomeStudio. Clustering clouds were manually investigated and adjusted if necessary. All plates included one duplicate sample and one positive control. *VDR* rs2228570, *VDR* rs11568820, and *VDR* rs478582 were typed utilizing the Taqman SNP genotype-based OpenArray platform [Applied Biosystems, CA, USA]. Custom designed arrays were loaded using the OpenArray AccuFill system and cycling was performed on a GeneAmp 9700 PCR system [Applied Biosystems, CA, USA], all according to manufacturer's protocol. Alleles were analyzed using the OpenArray SNP genotyping analysis software v.1.0.3 and Taqman Genotyper Software 2.0 [Applied Biosystems, CA, USA].

All five SNPs had a 95% call rate or higher and were in Hardy–Weinberg equilibrium. Each SNP was tested for consistency with Hardy–Weinberg proportions using a 1-degree of freedom χ^2 goodness-of-fit test with a p -value of 0.001 considered as evidence of a departure from Hardy–Weinberg equilibrium.

2.4. Analysis population

We obtained genetic data for all five SNPs on 1692 children in the DAISY cohort. A flow-chart showing selection of subjects for the analyses is shown in Fig. 1. Comparisons of the children who have complete genetic data with those who do not are presented in Supplemental Table 1 for the cohort examining risk of IA, and Supplemental Table 2 for the

cohort examining progression to T1D in IA positive children. There were small differences in HLA, first-degree relative status and sex between children with and without complete genetic data in the cohort examining risk of IA, and no differences in the cohort examining progression to T1D in IA positive children. The 1692 children with complete genetic data included 122 children who developed persistent IA, of whom 38 went on to develop T1D. However, 11 IA cases were positive on their first clinic visit, thus considered left censored, and were removed from the development of IA analysis cohort.

2.5. Statistical analyses

SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) statistical software package was used for all statistical analyses. SNPs were analyzed for their association both with development of IA and with progression from IA to T1D. For each model described below, hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression. A clustered time to event analysis was performed treating siblings from the same family as clusters, and robust sandwich variance estimates [32] were used for statistical inference. Analyses of time to development of IA were adjusted for the HLA-DR genotype (HLA-DR3/4, DQB1*0302 vs. other genotypes), presence of a first degree relative with T1D, and self-reported ethnicity (non-Hispanic white vs. other). Analyses of time to progression to T1D were adjusted, in addition, for age at first positive autoantibody. The significance threshold was defined a priori as $\alpha < 0.05$. Because our analyses were based on an a priori hypothesis, *p*-values were not corrected for multiple testing.

Genotype frequencies of the five SNPs are presented in Supplemental Table 3 for the cohort examining risk of IA, and in Supplemental Table 4 for the cohort examining progression to T1D in IA positive children. To avoid sparse cell counts, the genotypes of each SNP were dichotomized in the following manner: *VDR* rs1544410 genotypes were dichotomized as AA/AG vs. GG, *VDR* rs2228570 genotypes as AA/AG vs. GG, *VDR* rs11568820 genotypes as TT/TC vs. CC, *PTPN2* rs1893217 genotypes as GG/GA vs. AA, and the *PTPN2* rs478582 genotypes as CC/CT vs. TT for all analyses.

We analyzed *VDR* and *PTPN2* in separate models before testing the interaction. For the *VDR* gene analysis, all three *VDR* SNPs were included in the same model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, and non-Hispanic white ethnicity. Similarly, for the *PTPN2* gene analysis, the two *PTPN2* SNPs were included in the same model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, and non-Hispanic white ethnicity. These analyses were performed for both the development of IA as well as for progression to T1D in IA positive children. Then, based on the finding by Ramagopalan et al., all possible *VDR***PTPN2* SNP interactions were examined for association with development of IA and progression to T1D [13], with separate models for each interaction tested. For significant interaction terms, we computed HRs and 95% CIs from the coefficient and standard error estimates of the main effect and interaction terms to describe the interaction.

3. Results

3.1. Development of persistent islet autoimmunity

We first examined whether variants in *VDR* were associated with development of persistent islet autoimmunity, and whether these interacted with variants in *PTPN2*. Table 1 describes the children by whether or not they developed persistent IA. Of a total of 122 IA positive children in DAISY, 11 had to be excluded from the subsequent proportional hazards analyses of IA because they tested autoantibody positive on their first study visits (i.e., they were left-censored). The mean age at first IA positive visit was 5.5 years, and the mean age

at last follow-up visit in children who did not develop IA was 9.1 years. IA positive children, were more likely to have the HLA-DR3/4, DQB1 *0302 genotype or a first degree relative with T1D compared to children who did not develop IA.

Table 2 shows the association between the SNPs and the development of IA for the model containing *all* three *VDR* SNPs and the model containing both of the *PTPN2* SNPs, adjusted for HLA DR3/4 status, having a first degree relative with T1D, and ethnicity. There was no evidence of significant association between the three *VDR* SNPs together and development of IA. The two *PTPN2* variants (rs1893217 and rs478582) were also not associated with risk of IA.

We then explored whether variants in *VDR* and *PTPN2* interacted to affect risk of IA. With three *VDR* SNPs and two *PTPN2* SNPs, we tested six potential *VDR*PTPN2* interactions for their associations with risk of IA and found no evidence of *VDR*PTPN2* interactions, adjusting for HLA DR3/4 status, having a first degree relative with T1D, and ethnicity (data not shown).

3.2. Progression to T1D in children with IA

We examined whether variants in *VDR* were associated with progression to T1D in IA positive children, and whether these variants interacted with variants in *PTPN2*. The study population of IA positive children is described in Table 3. Of the 122 IA positive children in DAISY, 38 developed T1D; the mean age at T1D diagnosis was 8.2 years. The mean age at last follow-up visit in non-diabetic children with IA was 12.8 years. Children who develop T1D were younger when they first tested positive for an autoantibody than IA positive children who have not progressed to T1D, 3.6 and 6.1 years, respectively. They were also more likely to have the HLA-DR3/4, DQB1 *0302 genotype compared to children who did not progress to T1D.

Table 4 displays the three *VDR* SNPs modeled together in one model and their association with the progression to T1D in IA positive children, adjusting for HLA DR3/4 status, having a first degree relative with T1D, ethnicity, and age at first IA positive visit. One of the two functional *VDR* SNPs, *VDR* rs2228570, was significantly associated with development of T1D when the three *VDR* SNPs were modeled together. The two *PTPN2* SNPs were analyzed together similarly in a separate model to more completely describe the variation in the *PTPN2* gene, but there was no evidence of significant associations with progression to T1D in IA positive children. We then explored whether variants in *VDR* and *PTPN2* interacted to affect progression to T1D in IA positive children. We found a significant interaction between *VDR* 1544410 and *PTPN2* rs1893217 on progression to T1D, adjusting for HLA DR3/4 status, having a first degree relative with T1D, ethnicity and age at first IA positive visit (Table 4 and Fig. 2).

The association between *VDR* rs1544410 and the progression to T1D in IA positive children differs by *PTPN2* rs1893217 genotype, as shown in Fig. 2. In children with the *PTPN2* rs1893217 AA genotype, the *VDR* rs1544410 AA/AG genotype is associated with a significantly lower risk of progressing to T1D compared to children with the *VDR* rs1544410 GG genotype (HR: 0.24, 95% CI: 0.11–0.53, p -value = 0.0004). In IA positive children with the *PTPN2* rs1893217 GG/GA genotype, the *VDR* rs1544410 AA/AG genotype was not associated with progression to T1D. A subanalysis on non-Hispanic whites only produced similar results to those reported herein for development of IA and for progression to T1D in IA positive children (data not shown).

4. Discussion

In the DAISY population, the three *VDR* SNPs, *VDR* rs1544410, *VDR* rs2228570, and *VDR* rs11568820 we tested were not significantly associated with the appearance of IA. However, *VDR* rs2228570 was found to be associated with progression to T1D in IA positive children, and *VDR* rs1544410 significantly interacted with *PTPN2* rs1893217 on risk of progression to T1D, suggesting that the role of *VDR* in risk of T1D is complex. Besides the aforementioned interaction between *PTPN2* rs1893217 and *VDR* rs1544410, the two *PTPN2* SNPs we tested were not significantly associated with the appearance of IA or progression to T1D as main effects. This is in slight contrast to our recent report in which we found *PTPN2* rs1893217 to be weakly associated with development of islet autoimmunity [33]. This discrepancy can be attributed to the use of different IA case definitions. DAISY uses two definitions of IA, one that defines IA as the presence of at least one islet autoantibody on two consecutive visits (which is the definition used in the present study); and the other that further requires that the children still be autoantibody positive or diabetic on their most recent visits (which is the definition used in the previous study).

The studies that have examined *VDR* variants and the outcome of T1D have been inconsistent, and it is possible that the effects of *VDR* are only important for a faster development of T1D in the presence of IA, but not for the overall risk of T1D among genetically (HLA-DR,DQ) susceptible children. We found the functional *VDR* SNP, *VDR* rs2228570, to be a significant predictor in the progression to development of T1D. It also appears that *VDR* rs1544410, presumed to be non-functional because of its location in an intron, becomes mechanistically important for the progression to T1D in IA positive children when in combination with *PTPN2* rs1893217. This, coupled with the finding that the vitamin D receptor has an intronic binding site in the *PTPN2* gene [13], may provide insight into one of the ways in which the vitamin D receptor exerts its effects on T1D, which currently are still widely unknown. *VDR* rs1544410 has been shown to be in LD with a poly(A) microsatellite located in the 3'-untranslated region of the *VDR* gene, which has been discussed to influence *VDR* mRNA stability [34,35]. Ramagopalan et al. identified two *VDR* binding intervals in the *PTPN2* gene; and the SNP involved in the interaction (*PTPN2* rs1893217) is 5 kb and 58 kb away from these two intervals [13]. Thus, it is possible that this SNP is in LD with a variant in one of the two identified *VDR* binding intervals. This unique finding should be confirmed in other populations.

Ramagopalan et al. found increased *VDR* binding in intronic and intergenic regions compared with the basal state upon stimulation with calcitriol, the hormonally active form of vitamin D [13]. We were unable to examine what influence calcitriol had on the observed associations between *VDR* and progression to T1D because we do not have measures of 1,25-dihydroxyvitamin D on our children. Circulating 1,25-dihydroxyvitamin D has a short half-life of 15 h, is closely regulated by parathyroid hormone, calcium, and phosphate [36], and measurement requires a large amount of plasma for our pediatric subjects. Therefore, DAISY measures annually only plasma 25[OH]D as an estimate of vitamin D status. Only in situations of severe vitamin D deficiency do levels of 1,25-dihydroxyvitamin D decrease [2,37]. Simpson et al. recently reported no association between 25(OH)D levels and the risk of IA, nor progression to diabetes in IA positive children in DAISY [38].

The observation that *VDR* rs2228570 is associated with progression to T1D but not development of IA suggests that the role of *VDR* may be in the acceleration or deceleration of progression to T1D in autoimmune children. *VDR* 2228570 has been inconsistently associated with T1D [10,22,26,39–45]. *VDR* rs2228570 is a coding non-synonymous SNP located in the translational initiation codon that determines the formation of two protein variants: a longer version of the *VDR* protein (427-amino acids) that corresponds to the A

allele and a form shortened by three amino acids corresponding to the G allele (424-amino acids) [23,46,47]. Functional studies have shown that the shorter version of the protein has greater transcriptional activity and is more effective in trans-activation of the vitamin D signal [23,46–49]. Therefore, the *VDR* rs2228570 minor allele is associated with the production of a longer *VDR* protein that is less transcriptionally active [46,50,51]. In this particular study, we found the *VDR* rs2228570 minor allele (A) to be significantly protective for the development of T1D in IA positive children, which may imply that once autoimmune, less active transcription is beneficial. It is also interesting that *VDR* rs2228570 only becomes significant when adjusting for *VDR* 1544410 and *VDR* rs11568820, which may be an indication of the complexity of the way in which these polymorphisms act together.

The major strength of this study is the prospective long-term follow-up from birth of children at an increased risk for T1D, which allowed us to differentiate between genetic risk factors for the appearance of autoimmunity and the subsequent progression to T1D. However, the cost of assembling and following such a unique cohort has limited the number of IA positive children and children who progress to T1D that we could include in our analysis. While our study had adequate power to detect the novel interaction between variants in *VDR* and *PTPN2* that we present herein, it is possible that we have missed other interactions of smaller magnitude due to limited power. This is the first study to observe this gene–gene interaction in the progression phase to T1D, and it is important that this be investigated in other populations, to rule out type 1 error and lend further evidence to this association.

In conclusion, a functional variant in *VDR*, *VDR* rs2228570 was found to be significantly associated with progression to T1D in IA positive children of the DAISY population. A significant interaction between a non-coding *VDR* variant, *VDR* rs1544410, and a variant in *PTPN2*, *PTPN2* rs1893217, was also found to be associated with progression to T1D in IA positive children. Interestingly, these two *VDR* variants, *VDR* 2228570 and *VDR* 1544410, were not found to be associated with the appearance of IA. These findings should be explored in other prospective cohorts following children with IA for progression to T1D, as they may offer insight concerning the complex role of vitamin D in the etiology of T1D, as well as other autoimmune diseases associated with *PTPN2*, e.g., Crohn's disease and celiac disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

IA	islet autoimmunity
<i>PTPN2</i>	protein tyrosine phosphates, non-receptor type 2 gene
SNP	single nucleotide polymorphism
T1D	type 1 diabetes

VDR vitamin D receptor gene

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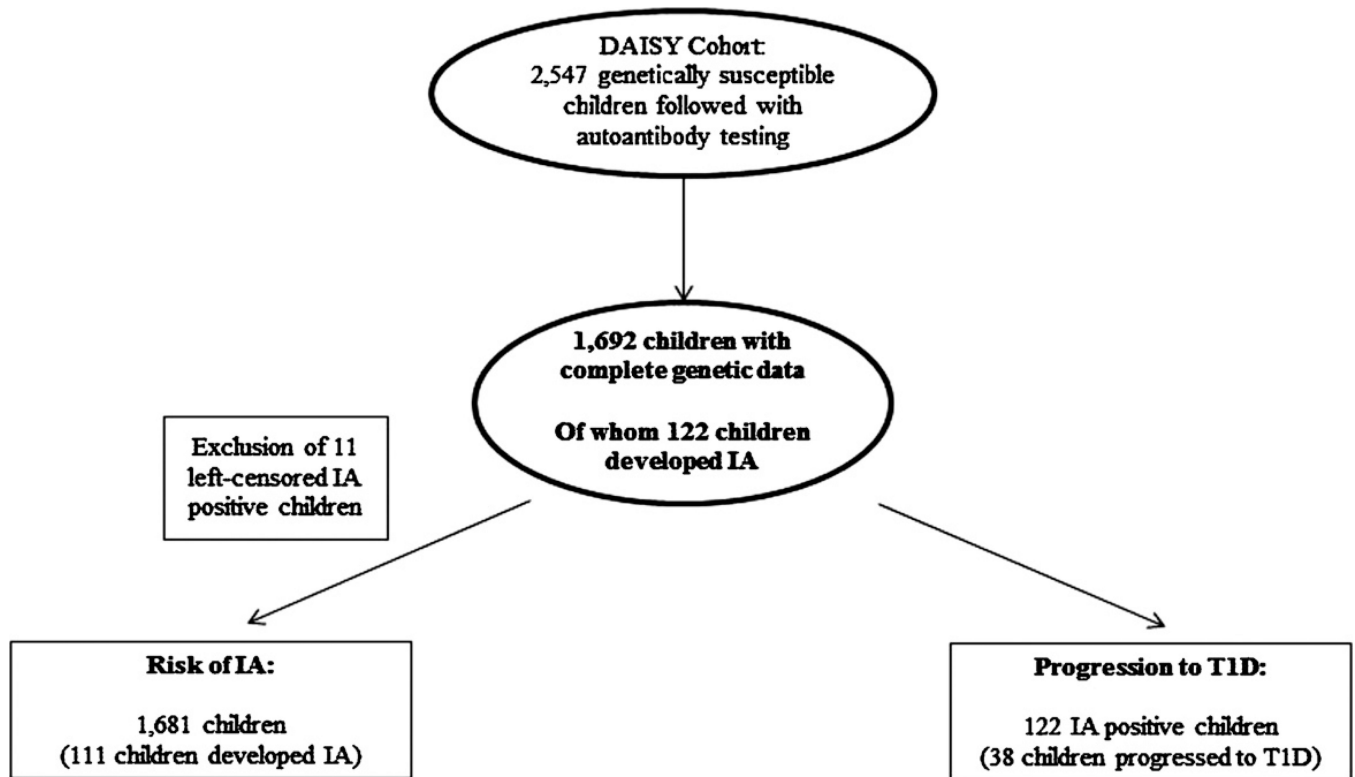


Fig. 1. Flow chart illustrating formation of analysis cohorts from the DAISY study population. IA, islet autoimmunity; T1D, type1 diabetes.

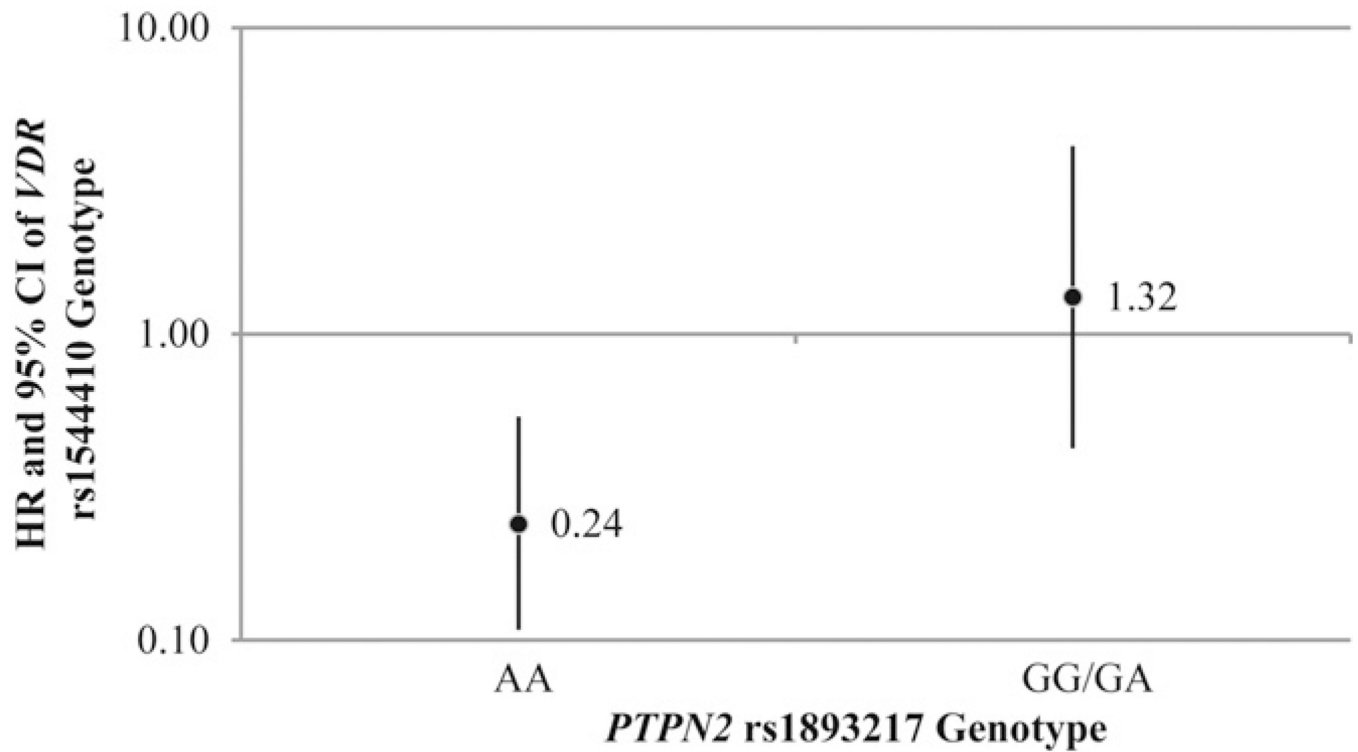


Fig. 2. The association between *VDR* rs1544410 and the risk of progression to T1D in IA positive children differs by *PTPN2* rs1893217 genotype. Black dots represent the hazard ratio for the *VDR* rs1544410 AA/AG genotype (with GG genotype as the referent). The solid lines represent the 95% confidence intervals for the hazard ratios. In children with the *PTPN2* rs1893217 AA genotype ($n = 79$), the *VDR* rs1544410 AA/AG genotype ($n = 54$) was associated with a significantly lower risk of progressing to T1D compared to children with the *VDR* rs1544410 GG genotype ($n = 25$) (HR: 0.24, 95% CI: 0.11–0.53, p -value = 0.0004). In IA positive children with the *PTPN2* rs1893217 GG/GA genotype ($n = 43$), the *VDR* rs1544410 AA/AG genotype ($n = 27$) was not associated with progression to T1D (HR: 1.32, 95% CI: 0.43, 4.06, p -value = 0.62).

Table 1

Characteristics of the analysis population by islet autoimmunity (IA) status.

Characteristic	Children positive for IA <i>n</i> = 111	Children negative for IA <i>n</i> = 1570	Univariate HR and 95% CI	<i>p</i> -Value
Mean age ^a (years)	5.5 (3.8)	9.1 (5.6)	N/A	N/A
HLA-DR3/4, DQB1*0302	34 (30.6%)	274 (17.5%)	2.18 (1.45, 3.26)	0.0002
First degree relative with type 1 diabetes	67 (60.4%)	652 (41.5%)	1.66 (1.13, 2.43)	0.01
Ethnicity (non-Hispanic white)	90 (81.1%)	1120 (71.3%)	1.38 (0.85, 2.25)	0.20
Sex (female)	53 (47.8%)	730 (46.5%)	1.07 (0.75, 1.54)	0.71
<i>VDR</i> rs1544410 (AA/AG)	76 (68.5%)	959 (61.1%)	1.32 (0.90, 1.95)	0.16
<i>VDR</i> rs2228570 (AA/AG)	69 (62.2%)	955 (60.8%)	0.99 (0.68, 1.45)	0.98
<i>VDR</i> rs11568820 (TT/TC)	36 (32.4%)	630 (40.1%)	0.72 (0.49, 1.07)	0.11
<i>PTPN2</i> rs1893217 (GG/GA)	39 (35.1%)	438 (27.9%)	1.34 (0.90, 1.99)	0.15
<i>PTPN2</i> rs478582 (CC/CT)	78 (70.3%)	1077 (68.6%)	1.08 (0.72, 1.62)	0.72

^aAge at first IA positive visit in autoantibody positive children or age at last follow-up in autoantibody negative children.

Table 2

Association between variants in *VDR* and *PTPN2* and risk of islet autoimmunity ($n = 111$ affecteds and 1570 unaffecteds).

Variable	HR	95% CI	<i>p</i> -Value
Model of <i>VDR</i> Variants			
<i>VDR</i> rs1544410 (AA/AG vs. GG)	1.39	0.94, 2.07	0.10
<i>VDR</i> rs2228570 (AA/AG vs. GG)	0.99	0.68, 1.45	0.96
<i>VDR</i> rs11568820 (TT/TC vs. CC)	0.73	0.49, 1.10	0.13
Model of <i>PTPN2</i> Variants			
<i>PTPN2</i> rs1893217 (GG/GA)	1.36	0.90, 2.04	0.15
<i>PTPN2</i> rs478582 (CC/CT)	1.23	0.80, 1.87	0.35

In the Model of *VDR* variants, the three *VDR* SNPs were included in a single model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, and non-Hispanic white ethnicity. Likewise, in the Model of *PTPN2* variants, the two *PTPN2* SNPs were included in a single model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, and non-Hispanic white ethnicity.

Table 3

Characteristics of islet autoantibody (IA) positive subjects by type 1 diabetes (T1D) status.

Characteristic	IA positive children who progressed to T1D <i>n</i> = 38	IA positive children who have not progressed to T1D <i>n</i> = 84	Univariate HR and 95% CI	<i>p</i> -Value
Mean age ^a (years)	8.2 (4.0)	12.8 (4.6)	N/A	N/A
Mean age at first IA positive visit (years)	3.6 (2.9)	6.1 (3.8)	0.88 (0.78, 0.98)	0.03
HLA-DR3/4, DQB1*0302	23 (60.5%)	14 (16.7%)	3.98 (2.13, 7.41)	<.0001
First degree relative with T1D	26 (68.4%)	52 (61.9%)	1.11 (0.57, 2.17)	0.76
Ethnicity (non-Hispanic white)	35 (92.1%)	66 (78.6%)	1.95 (0.57, 6.62)	0.29
Sex (female)	20 (52.6%)	37 (44.1%)	1.47 (0.76, 2.85)	0.26
<i>VDR</i> rs1544410 (AA/AG)	24 (63.2%)	57 (67.9%)	0.67 (0.34, 1.29)	0.23
<i>VDR</i> rs2228570 (AA/AG)	18 (47.4%)	56 (66.7%)	0.63 (0.34, 1.17)	0.14
<i>VDR</i> rs11568820 (TT/TC)	14 (36.8%)	30 (35.7%)	1.37 (0.70, 2.71)	0.36
<i>PTPN2</i> rs1893217 (GG/GA)	12 (31.6%)	31 (36.9%)	0.77 (0.40, 1.48)	0.43
<i>PTPN2</i> rs478582 (CC/CT)	28 (73.7%)	57 (67.9%)	1.23 (0.61, 2.45)	0.57

^aAge at T1D diagnosis in diabetic children or age at last follow-up in non-diabetic children.

Table 4

Association between variants in *VDR* and *PTPN2* and risk of progression to type 1 diabetes in IA positive children ($n = 38$ affecteds and 84 unaffecteds).

Variable	HR	95% CI	<i>p</i> -Value
Model of <i>VDR</i> variants			
<i>VDR</i> rs1544410 (AA/AG)	0.61	0.31, 1.21	0.16
<i>VDR</i> rs2228570 (AA/AG)	0.50	0.26, 0.95	0.03
<i>VDR</i> rs11568820 (TT/TC)	1.87	0.93, 3.74	0.08
Model of <i>PTPN2</i> variants			
<i>PTPN2</i> rs1893217 (GG/GA)	0.65	0.27, 1.60	0.35
<i>PTPN2</i> rs478582 (CC/CT)	1.07	0.43, 2.67	0.89
<i>VDR</i> model with interaction term			
<i>VDR</i> rs1544410* <i>PTPN2</i> rs1893217 interaction	*	*	0.02
<i>PTPN2</i> rs1893217	*	*	0.007
<i>VDR</i> rs1544410 (AA/AG)	*	*	0.0004
<i>VDR</i> rs2228570 (AA/AG)	0.49	0.26, 0.92	0.03
<i>VDR</i> rs11568820 (TT/TC)	1.82	0.90, 3.66	0.10

In the model of *VDR* variants, the three *VDR* SNPs were included in a single model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, non-Hispanic white ethnicity, and age at first IA positive visit. Likewise, in the Model of *PTPN2* variants, the two *PTPN2* SNPs were included in a single model, adjusting for HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, non-Hispanic white ethnicity, and age at first IA positive visit. In the *VDR* model with interaction term, the variables included were *VDR* rs1544410, *VDR* rs2228570, *VDR* rs11568820, *PTPN2* rs1893217, the *VDR* rs1544410**PTPN2* rs1893217 interaction term, HLA-DR3/4, DQB1*0302 genotype, first degree relative with type 1 diabetes, non-Hispanic white ethnicity, and age at first IA positive visit.