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ORIGINAL ARTICLE

Family-based association of 4q27chromosomal region covering IL2-IL21 genes with type 1 diabetes (T1D)—a study of genetic risk factors

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Abstract

Objective Pancreatic beta cell destruction is a hallmark of type 1 diabetes (T1D), a heterogeneous disorder with a wide range of potential causes. T cell activation molecules have been shown to play an important role in the development of T1D, according to the majority of studies. Some autoimmune diseases have been linked to SNPs in the 4q27 region, specifically in the KIAA1109-interleukin 2 (IL2)-IL21 block. The purpose of this research was to look into how certain polymorphic variants in the 4q27 region are linked to T1D.

Methods We investigated whether variants in the 4q27 region could be a causal factor in T1D susceptibility. Polymorphisms of ten single-nucleotide polymorphisms (SNPs) belonging to the KIAA1109/IL21/IL2 block were studied in 255 individuals from 59 families using the Sequenom MassARRAY platform.

Results The IL21/IL2 region was found to have a significant association with T1D in Tunisian cohorts. We found that the T allele of the rs2221903 marker is disproportionately passed down from parents to their children. In addition, haplotype analyses encompassing all of the SNPs under consideration show that the GACAGGA and the shortly TT haplotypes were significantly over-transmitted from parents to their children, suggesting they may be a T1D genetic susceptibility factor in our population.

Conclusion Several autoimmune disorders (ADs) have been linked to the IL2/IL21 genes, suggesting that there is a shared genetic background that confers a common genetic predisposition across ADs. More research into the genetic and functional aspects of the 4q27 region is needed to better explain the role it plays in the risk of ADs.

Keywords 4q27 · T1D · Polymorphism · Genetics · Autoimmunity · IL2/IL21 · Fbat

Introduction

Absolute insufficiency of insulin secretion is the hallmark of T1D, a heterogeneous disorder characterized by the destruction of pancreatic beta cells [1]. Research into the genetics of T1D and how it interacts with specific demographic, clinical, and biologic markers has the potential to enhance current methods of prediction, prevention, and intervention in both individual patients and the population at large.

Research into the genetics of T1D has yielded several candidate gene studies, and efforts are currently underway to identify the risk genes. Several genes, including CTLA-4, IL2RA, PTPN22, and INS-VNTR, have been linked to an increased risk of T1D, in addition to the human leukocyte antigen (HLA) on chromosome 6p21. It is now generally agreed that many different genes play a role in T1D, and that studying these genes may shed light on the disease's complex, multifactorial pathogenesis [2, 3]. T1D has been linked to a number of genes, including PTPN22, IL2RA (CD25), and CD28 [4, 5], and our research confirms the results of many previous studies [6–8]. Recent studies in the UAE, Sudan, and southern India have linked multiple variants in the CTLA-4, PTPN22, INS, and IL2-RA genes to T1D [9–11]. Although variations in these genes are a major

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contributor to the development of T1D, it is possible that other genes play a role in the pathogenesis of the disease as well.

Numerous autoimmune disorders (ADs), including T1D [12–14], ulcerative colitis [15], celiac disease (CD) [16], systemic lupus erythematosus (SLE) [17], and Grave's disease (GD) [18] have been linked to the KIAA1109-IL2-IL21 region at 4q27. Recent research has pinpointed the IL2/IL21 locus on 4q27, which contains genes involved in controlling immune responses, as a potential risk factor for developing coeliac disease [19]. This data supports the hypothesis that the KIAA1109-IL2-IL21 pathway affects the heritability of Alzheimer's disease in humans. The 4q27 locus, which contains the IL2 and IL21 genes, spans about 200 kb. Both of these genes are promising biological candidates for ADs because their encoded cytokines play important roles in T- and B-cell proliferation and distinct immunological activation pathways [20].

First of all, IL2 is a growth factor, a differentiation factor, and a regulator of cell death, so it is no surprise that it is on the list of promising candidate genes for autoimmunity disease [21]. IL2 is a pleiotropic cytokine, meaning that it not only plays a role in the termination of Tcell responses but also in the promotion and induction of T cell proliferation. Therefore, IL2 can stimulate the development and expansion of natural killer T cells (NKT) and induce immunoglobulin production in B cells [20, 22]. T helper 17 (Th-17) cells have been shown to be the primary producers of the effector cytokine IL-21, which has been shown to have pleiotropic effects on both innate and adaptive immune responses by, for example, stimulating CD8 T cells and NKT to acquire a more potent cytotoxicity and promoting T cell proliferation and differentiation [23, 24]. Because of this, alterations in the expression of these genes may lead to immune regulation disorders that manifest as autoimmunity.

Many autoimmune diseases (ADs) have been linked to genetic variation within the chromosome 4q27 locus, which contains the IL2/IL21 genes [13, 25]. These include T1D, coeliac disease, Graves' disease, and systemic rheumatoid arthritis. Multiple subsequent studies in various populations have confirmed these findings, and the application of this strategy to additional autoimmune diseases (ADs) like inflammatory bowel disease, lupus, and psoriasis has also been demonstrated [26, 27].

T1D susceptibility has been linked to a number of candidate genes over the past decade, but many of these genes still need to be studied in independent populations. Given the significance of the interleukin (IL) 2/interleukin (IL) 21 pathway in immune response, autoimmunity, and the increased efficacy of haplotype approaches, we set out to assess the role of the 4q27 region, which contains the IL2 and IL21 genes, in T1D genetic susceptibility.

Methods and materials

Study design

Blood samples were collected from members of 59 families that including 86 childrens with T1D (mean age, 12 ± 6.36 years with a range of 2–45 years) and 169 of their parents (mean age, 30 ± 10.60 years with a range of 3–57 years) (Table 1). Clinically affected patients and their first-degree relatives were recruited at the pediatric departments of Hedi Chaker Hospital (Sfax, Tunisia). The inclusion criteria for the recruitment of T1D patients were the presence of diabetic ketosis at onset, a dependence on insulin therapy for controlling hyperglycemia, their serum blood contain at least one of the anti-islet auto-antibodies (glutamate decarboxylase (GADA), insulin (IAA), zinc transporter 8 autoantibodies (ZnT8A), islet cell antigen (ICA), and IA2 protein (IA2A)). We excluded studies of patients who have other types of diabetes. The prospective study included patients who were not obese, free of concomitant complications, did not receive any further treatment. All subjects were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

DNA extraction and genotyping

Genomic DNA was purified from whole blood by overnight proteinase K digestion of lysed peripheral lymphocytes followed by phenol/chloroform extraction, according to a previously described protocol [28]. Ten

Table 1 Demographic and clinical characteristics of the sample

Features	T1D patients	Relatives (controls)
Number	86	169
Mean age	12 ± 6.36	30 ± 10.60
Male	49	86
Female	37	83
Origin	South/center	South/center
Serology (anti-GAD, anti-IAA, anti-ZnT8A, anti-ICA, anti-IA2)	Positive for at least one of the auto-antibodies	Negative

single-nucleotide polymorphisms (SNPs) were chosen from the KIAA1109-IL21-IL2 region (Table 2) and tested for their association with T1D genetic risk. SNPs for this study were chosen from HapMap, and mapping data was obtained from the db SNP built 126 database, both of which can be found at http://www.ensembl.org (Table 2). Genomic sequences containing target SNPs were amplified by multiplex polymerase chain reactions (PCR). The amplified product was then cleaned using shrimp alkaline phosphatase and used for allele-specific primer extension reaction. The reaction mixture was then spotted into a SpectroCHIP microarray. The extended products were analyzed by MALDI-TOF (matrix-assisted laser desorption ionization–time-of-flight) mass spectrometry; the time-of-flight is proportional to mass, which allows to determine the size of products generated. Sequenom supplies SpectroTYPER software that automatically translates the mass of observed primers into a genotype for each reaction. SNPs that have a call rate of 80% or higher in Hap Map's control samples and that have been verified to be in Hardy–Weinberg equilibrium in healthy parent samples ($p > 0.05$) have been chosen.

Statistics

Using the family based association test "FBAT" software (https://sites.google.com/view/fbat-web-page), we analyzed the genetic association of T1D with a transmission disequilibrium test (TDT). A variety of TDT, including allelic/genotypic and haplotype analyses, were performed by using generalized mixed genetic models, and the best fitting model results were showed. Furthermore, in order to obtain more information and maximize the power analysis of the FBAT test, we include in the study all family members in the pedigree, notably the families with missing parents. For all statistical tests, p value was considered statistically significant if it is less than or equal to 0.05 ($p \leq 0.05$). To estimate the LD between different markers, the haploview programme (http://www.broad.mit.edu/mpg/haploview/) was used to conduct a pairwise LD analysis.

Table 2 Polymorphisms and investigated genes

Genes	Variants	Chromosomes	Location
KIAA1109	rs6534347	4	123,198,435
IL2	rs11575812	4	123,371,049
IL2	rs2069778	4	123,376,135
IL2	rs2069763	4	123,377,482
Upstream IL2	rs2069762	4	123,377,980
Upstream IL2	rs1479924	4	123,387,600
Intergenic	rs6852535	4	123,478,716
Intergenic	rs12642902	4	123,508,501
Intergenic	rs6822844	4	123,509,421
IL21	rs2221903	4	123,538,912
IL21	rs17005931	4	123,545,648

Results

From 10 variants spanning IL21/IL2 region, the risk T allele of rs2221903 was significantly more transmitted from parents to T1D's children ($p = 0.035$, $z = 2.100$). However, the C allele was less transmitted from parents to their affected children ($p = 0.035$, $z = -2.100$). In the genotypic analysis, the homozygous CC genotype was less transmitted than what would be expected by chance from informative parents and seems to be less risk factor for T1D ($p = 0.030$; $z = -2.160$). The remaining SNPs of IL2/IL21 region were not confirmed any association between T1D and genotyped families (Table 3).

FBAT analysis was indicated that T allele (rs6822844) that is located within a noncoding region upstream of IL21 and downstream of IL2 was over-transmitted than what would be expected by chance ($p = 0.057$, $z = 1.897$), but it is still statistically insignificant to conferred a T1D genetic risk (Table 3).

LD analysis revealed two blocks across the IL21-IL2 SNPs. The first little evidence for LD is between rs2221903 and rs6822844, covering 29 kb (Fig. 1). The second block is covering 137 kb that encompassed seven analyzed SNPs (rs6534347, rs11575812, rs2069778, rs2069763, rs2069762, rs1479924, and rs6852535). In contrast to rs6822844 that has been found in complete LD with three SNPs (rs11575812, rs2069778, and rs1479924),whereas SNP (rs2221903) showed a little LD with other variants, indicating a statistical separate association of rs6822844 and rs2221903 with disease risk (Fig. 1). The haplotype association analyses including all studied SNPs for IL21-IL2 genes (block 1 and 2), indicate that GACAGGA haplotype derived from block 1 and TT haplotype derived from block 2, were significantly over-transmitted from parents to affected offspring (Table 4), proving their possible role in T1D genetic risk.

Discussion

In this work, we found a significant and marginal genetic linkage, repectively between rs2221903 and rs6822844 variants located in the IL2/IL21 locus and T1D among some Tunisian families. We found a similar positive association between the IL2/IL21 region and T1D pathology in our previous case–control study [5]. Over the past decade, researchers have found more and more evidence linking the IL2-IL21 region at 4q27 to multiple ADs. Wellcome Trust Case Control Consortium GWAS data have also reported this region in their search for T1D genetic risk [25], which

Table 3 FBAT analysis of SNPs markers in IL21

Marker	Allele	freq	fam#	*S*	*E*(*S*)	*Z*	*p*
rs2221903	C	0.193	25	18.000	25.500	−2.100	0.035
	T	**0.807**	**25**	**56.000**	**48.500**	**2.100**	**0.035**
	CC	**0.000**	**8**	**0.000**	**3.500**	**−2.160**	**0.030**
	CT	0.385	25	18.000	18.500	−0.164	0.869
	TT	0.615	25	19.000	15.000	1.382	0.166
rs6822844	G	0.952	7	12.000	15.000	−1.897	0.057
	T	0.048	7	8.000	5.000	1.897	0.057
	GG	0.905	7	2.000	5.000	−1.897	0.057
	GT	0.095	7	8.000	5.000	1.897	0.057
	TT	0.000	0	*****			

This table contains only the associated markers

FBAT family-based association test, *freq* allelic and genotypic frequencies, *Fam#* number of informative families, *S* test statistics for the observed number of transmitted alleles, *E(S)* expected value of S under the null hypothesis (i.e., no linkage or association)

Significant *p* values ($p < 0.05$) are in boldface

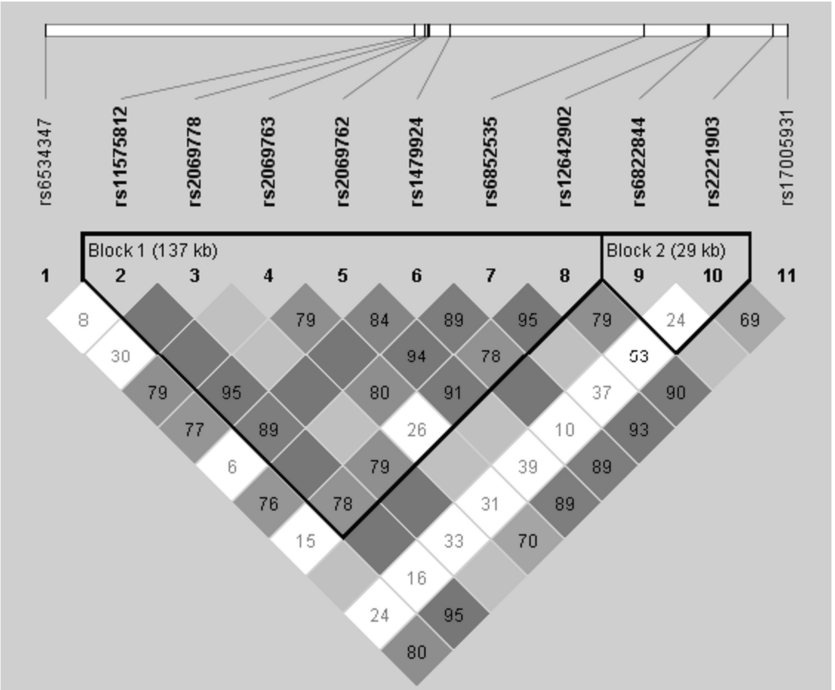

Fig. 1 Haploview analysis for LD (D') measures between SNPs genotyped in *IL2/IL2*. The blocks generated (blocks 1 and 2) under confidence interval algorithm of HAPLOVIEW are marked. Block 1 is constituted by seven SNPs: 5 variants from IL2 (rs11575812, rs2069778, rs2069763, and rs2069762) and two variants from IL21 (rs6852535 and rs12642902). Block 2 is generated between two SNPs from IL21 gene

is consistent with our findings [25]. Studies that followed GWAS included a meta-analysis of 305,090 SNPs from three GWAS [14]. This meta-analysis, along with others found that the 4q27 region was strongly associated with an increased risk of T1D [13, 26, 29]. KIAA1109/Tenr/IL2/IL21 of 4q27 has been previously linked to type T1D risk, and our study confirms this association in the Tunisian population, suggesting that some genetic risk factors are shared by people of different racial and ethnic backgrounds. A better understanding of T1D and its treatment will result from research into the similarities and differences in genetic susceptibility across populations.

4q27 is a long region with a large block (480 kb) of linkage disequilibrium known KIAA1109/Tenr/IL2/IL21 [16] that contain several gene including the KIAA1109 gene that encoding a protein of unknown function, the Testis Nuclear RNA-binding protein (TENR), and the IL2 and IL21 genes, which are both plausible functional candidate genes risk for T1D. As far as we are aware, no single variant can trigger the autoimmune response on its own. The causal variant(s)

Table 4 Results of haplotype association analysis for the SNPs with linkage disequilibrium

Block	Haplotype	Freq	*T*: *U*	Chi square	*p* value
Block 1	AGCCAAG	0.369	23.0: 28.0	0.49	0.4841
	GGCAGGA	0.217	22.9: 20.7	0.112	0.7383
	AGCAAGG	0.123	13.1: 13.6	0.009	0.9262
	AGAAAGG	0.102	11.5: 10.4	0.051	0.8207
	GGCAGGG	0.028	3.8: 2.6	0.207	0.6494
	GACAGGA	**0.024**	**7.0: 1.0**	**4.5**	**0.0339**
	AGAAAGA	0.020	3.0: 2.0	0.2	0.6544
	AGCAAAG	0.019	3.0: 5.0	0.497	0.4809
	GGCAAGG	0.017	1.6: 0.5	0.556	0.4557
	AGCCGAG	0.011	1.2: 2.3	0.304	0.5813
	AGACAAG	0.010	1.0: 2.0	0.309	0.5783
	AGCAGGA	0.010	1.0: 1.1	0.001	0.9783
Block 2	GT	0.784	34.2: 26.2	1.059	0.3035
	GC	**0.184**	**19.2: 33.2**	**3.736**	**0.0532**
	TT	**0.028**	**8.0: 1.0**	**5.444**	**0.0196**

that may correlate with mRNA translation or regulate the expression or function of the gene may be in LD with the associated SNP. In our study, we found that the GACAGGA and TT haplotypes for the KIAA1109/Tenr/IL2/IL21 SNPs were significantly associated with T1D. The IL2/IL21 genes are both plausible functional candidates as genetic modifiers of autoimmunity [13], and extensive LD within this block means that none of these genes can be ruled out as the causal one.

IL21 is a cytokine that has potent immunomodulatory activity and predominantly produced by Th17 and NKT cells [30]. This cyokine stimulates T cell proliferation, increases cytotoxicity of two cytotoxic lymphocyte subsets; CD8 + T cells and NKT cells, and enhances the naïve B cell differentiation [31]. A previous study showed that increased IL-21 production, elevated IL21R expression, and polymorphisms in either gene have been documented [32, 33]. While increased IL-21/IL21R signaling has been described before as a driver of autoantibody generation [34]. The combination of anti-IL-21 and the glucagon-like peptide-1 receptor antagonist Liraglutide was able to reverse T1D in NOD mice, and histological sections of the pancreata have a visually smaller CD8 T cell infiltrate into the islets [35]. The fact that IL21 exhibits these features raises the possibility that this cytokine has a wide range of effects on both the innate and adaptive immune systems [36]. Any change in IL21 production leads to defective IL-21 signalling in T cells, suggesting that IL21 has a different biological effect on different lymphoid cells. IL21 activates the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, in particular Jak1 and Jak3 and STAT1 and STAT3 [23, 37, 38].

By downregulating FOXP3 expression in CD4 + T cells [39] and making CD4 + CD25-T cells resistant to regulatory T-cell-mediated suppression [40], IL-21 plays a crucial role in Th17 differentiation [41].

High levels of IL-21 expression have been found in Th-17-related autoimmune diseases (ADs), such as multiple sclerosis [42], inflammatory bowel disease [43], and rheumatoid arthritis [44]. Rosanne et al. conducted an analysis of IL-21's role in the onset of diabetes in NOD mice and found that blocking IL-21 signalling completely prevented the disease from manifesting itself [45]. Animal models in which IL2 and IL21 cytokine genes were deleted showed an increased risk of developing ADs, demonstrating the early importance of these cytokines in the immune response [46]. By the same, it has been found that mice deficient in IL2, show hyperativation and uncontrolled proliferation of T cells leading to rapid lethal autoimmunity. A defect in Treg cells due to reduced IL-2 levels coincided with the IL-21-driven expansion of diabetogenic T cells, which provides a scenario by which researchers can strengthen connections between the roles of both IL-2 and IL-21 in T1D [47]. Indeed, another study showed that depletion of IL-2-dependent Treg cells improved the efficacy of IL-21 to mediate supression of FoxP3 + Treg cells [39] argues that these two cytokines work toguether and they need to communicate with each other.

Conclusion

In this study, we confirm that IL2/IL21 region is associated with T1D genetic risk. This association has been found with several ADs, suggests that there is a common genetic background bconfering a genetic predisposition across the different ADs. Much more genetic and functional works is required before researchers can obtain more precise details to explain the role of 4q27 region in the risk to ADs.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All subjects were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

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