Association of the M315I Variant in the Transient Receptor Potential Vanilloid Receptor-1 (*TRPV1*) Gene with Type 1 Diabetes in an Ashkenazi Jewish Population

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ABSTRACT: **Background:** Type 1 diabetes in humans is an autoimmune disease in which T cells target pancreatic islets of Langerhans, leading to the progressive destruction of the insulin-producing beta cells. Both genetic and environmental factors contribute to the development of autoimmune diabetes. The non-obese diabetic (NOD) mouse model of human type 1 diabetes demonstrates two missense mutations in the transient receptor potential vanilloid receptor-1 (*TRPV1*) gene.

Objectives: To investigate whether polymorphism in the *TRPV1* gene may play a role in the predisposition to human type 1 diabetes.

Methods: We genotyped 146 Ashkenazi Jewish type 1 diabetic patients and 205 Ashkenazi Jewish healthy controls for the rs222747 (M315I), rs224534 (T469I) and rs8065080 (I585V) variants of the *TRPV1* gene.

Results: There was a significant increase in the rs222747 (M315I) variant of the *TRPV1* gene in the type 1 diabetes cohort compared to the control: rs222747 (M315I) homozygous: (61% vs. 48.3%, $P = 0.02$). Logistic regression analysis revealed that type 1 diabetes was significantly associated with rs222747 (M315I), such that having diabetes increased the odds of rs222747 homozygosity (M315I) by 67.2%, odds ratio 1.6, 95% confidence interval 1.08–2.57, $P < 0.02$. No difference was found in the rs224534 (T469I) and rs8065080 (I585V) allelic variants. There was no difference in any of the *TRPV1* variants by gender, age when type 1 diabetes was diagnosed, body mass index, glycemic control, blood pressure, positive autoantibodies (ICA, GAD, IAA), and other autoimmune diseases.

Conclusions: Our study demonstrates that *TRPV1* may be a susceptible gene for type 1 diabetes in an Ashkenazi Jewish population. These results should be replicated in the same ethnic group and in other ethnic groups.

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KEY WORDS: type 1 diabetes, *TRPV1*, polymorphism, association, Ashkenazi Jews

Type 1 diabetes in humans is an autoimmune disease in which T cells target pancreatic islets of Langerhans, leading to the progressive destruction of the insulin-producing beta cells. Although both genetic and environmental factors contribute to the development of autoimmune diabetes, the precise etiology and the initiating factors that trigger the autoimmune response against pancreatic islets are still unknown. Information about genetic factors that modify the risk of type 1 diabetes is believed to lead to a better understanding of the pathogenesis of the preclinical stage of the disease, and may result in preventive treatment [1].

The non-obese diabetic mouse serves as a model of spontaneous type 1 diabetes since it shares many traits with the human disease [2]. Using NOD model mice, Razavi et al. [3] sequenced the *TRPV1* (transient receptor potential vanilloid receptor-1) gene and discovered two unique in-frame amino acid replacements mapping to highly conserved regions among many species. They also showed with electrophysiological studies that the NOD-*TRPV1* is hypofunctional and hyposecretory compared with the wild-type *TRPV1* [3]. They proposed that a defect in sensory neurons innervating the pancreas initiates local inflammation and an autoimmune process in pancreatic islets that lead to local insulin resistance and beta cell destruction. They believe that their data disclose a fundamental role for *TRPV1* function in the pathogenesis of autoimmune diabetes. Their theory and the clues to a linkage between the nervous system and autoimmune diabetes were the subject of reviews and discussions [4-6].

Many previous genetic studies have focused on candidate genes for type 1 diabetes susceptibility. Genome-wide association studies have provided distinct type 1 diabetes loci [7-11]. None of these loci are located near the *TRPV1* gene. Nevertheless, intrigued by the *TRPV1* mutations that

NOD = non-obese diabetic

were found in the NOD mouse, we looked at the possible role of *TRPV1* polymorphism in the predisposition to human type 1 diabetes.

PATIENTS AND METHODS

Our cohort was enrolled by two medical centers: Wolfson in Holon and Hadassah in Jerusalem. The local Helsinki Review Board and the Israel Ministry of Health Genetic Research Helsinki Review Board approved the recruitment protocol. Informed consent was obtained from all parents and patients. Data regarding age at diagnosis, gender, ethnicity, family history of type 1 diabetes and other autoimmune diseases, glycemic control, body mass index and blood pressure were obtained from the patients and the referring endocrinologists and recorded prior to genotyping. All genotyping was performed by a single investigator blinded to phenotypic data (E.L-S). The reference group DNA was obtained from 205 healthy human Ashkenazi Jewish controls, from the National Laboratory for the Genetics of Israeli Populations at Tel Aviv University.

GENETIC ANALYSIS

Genomic DNA was extracted from peripheral blood using the Puregene kit (Gentra, Minneapolis, USA) according to the manufacturer's instructions. In a preliminary study we sequenced the *TRPV1* gene in 12 type 1 diabetes patients and compared the single nucleotide polymorphism frequencies to the published database. Three SNP, which are minor alleles in healthy populations according to the dbSNP database, were found in most of our patients. These SNP were rs222747 (M315I), rs224534 (T469I) and rs8065080 (I585V).We decided to analyze these SNP in larger type 1 diabetes and control cohorts.

The rs222747 C/G (M315I) was genotyped by amplifying exon 4+5: Primers: F:

5'-CACTTTCTGCCCAGTTCCTC-3', R:5'-GAGTCCCGCACACACAGATA-3'. PCR product (815 bp fragment) was digested overnight with DpnII (New England Biolabs, Beverly, MA, USA). Digests were run on a 4% agarose to determine the G or C alleles: 317+334+79+110 bp fragments for the G allele, 317+238+69+79+110 bp fragments for the C allele.

The rs224534 C/T (T469I) was genotyped by amplifying exon 8: F: 5'-CAGGGACTATGGCTTCATGG-3' R: 5'-GAGCGCTGAGGGATCTTCTT-3'. PCR product (253 bp fragment) was digested overnight with BpmI (New England Biolabs). Digests were run on a 4% agarose to determine the C or T alleles: 127+20+106 bp fragments for the C allele, 147+106 bp fragments for the T allele.

The rs8065080 A/G (I585V) was genotyped by amplifying exon 11: F: 5'-CCGTTTCATGTTTGTCTAA-3'R: 5'-TGGCAGCCACAGCACTTC-3'. PCR product (280 bp fragment) was digested overnight with BSrGI (New England Biolabs). Digests were run on a 4% agarose to determine the A or G alleles: 250-+30 bp fragments for the A allele, 250 bp fragments for the G allele.

SNP = single nucleotide polymorphism

DATA ANALYSIS

Analysis of data was carried out using SPSS 19.0 statistical analysis software (IMB Inc., USA). For continuous variables, such as age and anthropometric parameters, descriptive statistics were calculated and reported as mean ± standard deviation as well as median (min-max). Normality of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test (cutoff at $P = 0.01$). Continuous variables were compared by SNP using ANOVA followed by Bonferoni's test. Categorical variables were compared by SNP and, separately, type 1 diabetes, using the chi-square test. Logistic regression analysis was used to model each of the dichotomized SNP, and odds ratios with 95% confidence intervals were calculated. All tests are two-sided and considered significant at $P < 0.05$.

RESULTS

Data were obtained from 146 type 1 diabetic Ashkenazi Jewish patients, 54% female, mean age at diabetes onset 25.4 (SD 14.6), mean HbA1c 7.4% (SD 2.2), mean BMI 26.5 (SD 5.3). Additionally, DNA from 205 Ashkenazi Jewish healthy controls was used for comparison.

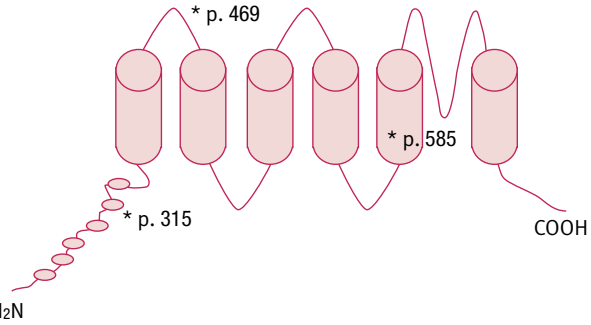
There was a significant increase in the rs222747 (M315I) variant of the *TRPV1* gene in the type 1 diabetes cohort compared to the control: rs222747 (M315I) homozygous (61% vs. 48.3%, $P = 0.02$). Logistic regression analysis revealed that type 1 diabetes was significantly associated with rs222747 (M315I), such that having diabetes increased the odds of rs222747 homozygosity (M315I) by 67%: OR 1.67, 95%CI 1.08–2.57, $P < 0.02$. No difference was found in the rs224534 (T469I) and rs8065080 (I585V) allelic variants. There was no difference in any of the *TRPV1* variants by gender, BMI, HbA1c, blood pressure, positive autoantibodies (ICA, GAD, IAA), or other autoimmune diseases. There was no association of these variants with age at onset of type 1 diabetes or with younger age at diagnosis.

DISCUSSION

Our study identified a significant increase in homozygous allelic variant M315I (rs222747) of the *TRPV1* gene in the type 1 diabetes Jewish-Ashkenazi cohort. This variant is localized

BMI = body mass index
OR = odds ratio
CI = confidence interval

Figure 1. The *TRPV1* protein organization with positions of SNP



to the region of the fifth ankyrin repeat domains, which are postulated to mediate protein-protein interaction [Figure 1].

Recently, the agonists capsaicin and anandamide were found to elicit a greater maximal response in *TRPV1* I315 and *TRPV1* P91 variants compared to *TRPV1* WT. These two variants demonstrated a markedly increased level of *TRPV1* expression (100% more than the *TRPV1* WT) in whole-cell expression and cell surface expression [12].

The transient receptor potential superfamily is a group of ion channels that share the common feature of six transmembrane domains. They play an important role in sensory physiology [13-17] such as vision, taste, smell, hearing, mechano-sensation, and thermo-sensation. *TRPV1* activation induces a painful burning sensation. *TRPV1* receptors are distributed in the peripheral nervous system and in many other tissues such as the brain, dorsal root ganglia, skin, kidney, bladder and tongue [13-17]. *TRPV1* is also expressed in the network of sensory neurons that innervate the pancreatic islets [3-6]. The islet structure also includes a dense network of nerve terminals that converge at the neuro-insular complex. The nerve endings and ganglia, together with the peri-islet Schwann cells, form a tight envelope that separates exocrine from endocrine tissue [10]. The unique location of pSC places these cells on the frontline facing autoimmune infiltrates. During the pre-diabetes stage, breach of the glial sheath precedes T cell invasion into the islet interior [4]. Although the function of the pSC in the pancreas is unknown, the general physiological characteristics of peripheral Schwann cells and/or central nervous system astrocytes largely apply to pSC. Their functions include neurotropic support for the neurons innervating islet cells as well as support for beta cells themselves in both survival and stress-related processes. Understanding how the pSC might directly contribute to β-cell homeostasis could have important implications for both type 1 and type 2 diabetes.

pSC = peri-islet Schwann cells

Razavi et al. [3] propose that a defect in sensory neurons innervating the pancreas contributes to insulin resistance and β-cell stress in NOD mice by initiating local inflammation and an autoimmune stress on pancreatic islets. These results strongly suggest that autoimmunity, inflammation and the nervous system are linked. Further, two mutations in conserved regions of the *TRPV1* gene in NOD mice were described and *TRPV1* was proposed as a candidate gene for the diabetes susceptibility [3].

It seems that *TRPV1* or downstream *TRPV1*-related pathways are important in the pathogenesis of diabetes. The sensory neurons might be a beta cell-intrinsic phenomenon or reflect an aberrant islet response to stress.

In large genome-wide accociation studies of subjects with type 1 diabetes, many susceptible loci and genes were found – both HLA-associated and non-HLA-associated [1,7-11]. No type 1 diabetes distinct loci have been found in chromosome 17p13.2 (*TRPV1* gene location). The reason for this could be that none of the platforms used for the GWA studies (Affimetrix 500K or Illumina 550K) contains the SNP rs222747. GWA studies in other multifactorial diseases have been shown to be ethnicity dependent, e.g., the Crohn disease-susceptible gene *NOD2*, which was not found in Japanese Crohn disease patients [18]. This suggests that different genetic factors are involved in the pathogenesis of diseases in different populations. Studies in the Jewish Israeli population have demonstrated the association of polymorphisms in the insulin gene in Ashkenazi, Yemenite and Ethiopian Jews as well as the HLA haplotypes with type 1 diabetes [19-21].

The role of *TRPV1* polymorphism in the autoimmune cascade that leads to type 1 diabetes needs further investigation. Our results should be replicated in the same ethnic group and in other ethnic groups.

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References

1. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. *N Engl J Med* 2009; 360: 1646-54.
2. Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol* 2005; 23: 447-85.
3. Razavi R, Chan Y, Afifiyan FN, et al. *TRPV1+* sensory neurons control B cell stress and islet Inflammation in autoimmune diabetes. *Cell* 2006; 127: 1-13.
4. Tsui H, Razavi R, Chan Y, Yantha J, Dosch HM. 'Sensing' autoimmunity in type 1 diabetes. *Trends Mol Med* 2007; 13: 405-13.
5. Bour-Jordan H, Bluestone JA. Sensory neurons link the nervous system and autoimmune diabetes. *Cell* 2006; 127: 1097-9.
6. Suri A, Szallasi A. The emerging role of *TRPV1* in diabetes and obesity.

GWA = genome-wide association

Trends Pharmacol Sci 2008; 29: 29-36.

7. Cooper JD, Smyth DJ, Smiles AM, et al. Meta-analysis of genome-wide association study data identifies additional type 1 diabetes risk loci. *Nat Genet* 2008; 40: 1399-401.
8. Barrett JC, Clayton DG, Concannon P, et al. Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 2009; 41: 703-7.
9. Grant SF, Qu HQ, Bradfield JP, et al. Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. *Diabetes* 2009; 58: 290-5.
10. Hakonarson H, Grant SF. Genome-wide association studies in type 1 diabetes, inflammatory bowel disease and other immune-mediated disorders. *Semin Immunol* 2009; 21: 355-62.
11. Baker PR 2nd, Steck AK. The past, present, and future of genetic associations in type 1 diabetes. *Curr Diab Rep* 2011; 1: 445-53.
12. Xu H, Tian W, Fu Y, Oyama TT, Anderson S, Cohen DM. Functional effects of nonsynonymous polymorphisms in the human *TRPV1* gene. *Am J Physiol Renal Physiol* 2007; 293: 1865-76.
13. Montell C. The TRP superfamily of cation channels. *Sci STKE* 2005; 272: re3
14. Dhaka A, Viswanath V, Patapoutian A. TRP ion channels and temperature sensation. *Annu Rev Neurosci* 2006; 29: 135-61.
15. Tsui H, Winer S, Jakowsky G, Dosch HM. Neuronal elements in the pathogenesis of type 1 diabetes. *Rev Endocr Metab Disord* 2003; 4: 301-10.
16. Szolcsanyi J. Forty years in capsaicin research for sensory pharmacology and physiology. *Neuropeptides* 2004; 38: 377-84.
17. Gram DX, Ahren B, Nagy I, et al. Capsaicin-sensitive sensory fibers in the islets of Langerhans contribute to defective insulin secretion in Zucker diabetic rat, an animal model for some aspects of human type 2 diabetes. *Eur J Neurosci* 2007; 25: 213-23.
18. Inoue N, Tamura K, Kinouchi Y, et al. Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 2002; 123: 86-91.
19. Benedek G, Brautbar C, Vardi P, et al. Effect of polymorphism in insulin locus and HLA on type 1 diabetes in four ethnic groups in Israel. *Tissue Antigens* 2009; 73: 33-8.
20. Weintrob N, Sprecher E, Israel S, et al. Type 1 diabetes environmental factors and correspondence analysis of HLA class II genes in the Yemenite Jewish community in Israel. *Diabetes Care* 2001; 24: 650-3.
21. Kwon OJ, Brautbar C, Weintrob N, et al. Immunogenetics of HLA class II in Israeli Ashkenazi Jewish, Israeli non-Ashkenazi Jewish, and in Israeli Arab IDDM patients. *Hum Immunol* 2001; 62: 85-91.

Capsule

Macitentan and morbidity and mortality in pulmonary arterial hypertension

Current therapies for pulmonary arterial hypertension have been adopted on the basis of short-term trials with exercise capacity as the primary end-point. Pulido et al. assessed the efficacy of macitentan, a new dual endothelin receptor antagonist, using a primary end-point of morbidity and mortality in a long-term trial. A total of 250 patients were randomly assigned to placebo, 250 to the 3 mg macitentan dose, and 242 to the 10 mg macitentan dose. The primary end-point occurred in 46.4%, 38.0% and 31.4% of the patients in these groups, respectively. The hazard ratio for the 3 mg macitentan dose as compared with placebo was 0.70 (97.5% confidence interval 0.52–0.96, $P = 0.01$), and the hazard ratio for the 10 mg macitentan dose as compared with placebo was 0.55 (97.5%CI 0.39–0.76, $P < 0.001$). Worsening of pulmonary arterial hypertension was the most frequent primary end-point event. The effect of macitentan on this end-point was observed regardless of whether the patient was receiving therapy for pulmonary arterial hypertension at baseline. Adverse events more frequently associated with macitentan than with placebo were headache, nasopharyngitis, and anemia.

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Capsule

Next generation gene therapy

Few disciplines in contemporary clinical research have experienced the high expectations directed at the gene therapy field. However, gene therapy has been challenging to translate to the clinic, often because the therapeutic gene is expressed at insufficient levels in the patient or because the gene delivery vector integrates near proto-oncogenes, which can cause leukemia. Biffi et al.(*Science* 2013:341;1233158, published online 11 July) and Aiuti et al. (1233151; published online 11 July) report progress on both fronts in gene therapy trials of three patients with metachromatic leukodystrophy (MLD), a neurodegenerative disorder, and three patients with Wiskott-Aldrich syndrome (WAS), an immunodeficiency disorder. Optimized lentiviral vectors were used to introduce functional *MLD* or *WAS* genes into the patients' hematopoietic stem cells (HSCs) ex vivo, and the transduced cells were then infused back into the patients, who were then monitored for up to 2 years. In both trials, the patients showed stable engraftment of the transduced HSC and high expression levels of functional *MLD* or *WAS* genes. Encouragingly, there was no evidence of lentiviral vector integration near proto-oncogenes, and the gene therapy treatment halted disease progression in most patients. A longer follow-up period will be needed to further validate efficacy and safety.

Eitan Israeli

"As I grow to understand life less and less, I learn to live it more and more"

Jules Renard (1864-1910), French author