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## Loss-of-function mutations in *SLC30A8* protect against type 2 diabetes

A full list of authors and affiliations appears at the end of the article.

### Abstract

Loss-of-function mutations protective against human disease provide *in vivo* validation of therapeutic targets<sup>1,2,3</sup>, yet none are described for type 2 diabetes (T2D). Through sequencing or genotyping ~150,000 individuals across five ethnicities, we identified 12 rare protein-truncating variants in *SLC30A8*, which encodes an islet zinc transporter (ZnT8)<sup>4</sup> and harbors a common variant (p.Trp325Arg) associated with T2D risk, glucose, and proinsulin levels<sup>5–7</sup>. Collectively,

\*Authors for correspondence: David Altshuler, MD, PhD, Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, MA 02445 USA, T: (617) 730-5977, [altshuler@molbio.mgh.harvard.edu](mailto:altshuler@molbio.mgh.harvard.edu). Kári Stefánsson, deCODE genetics, Sturlugata 8, 101, Reykjavik, Iceland., T: 354-570-1989, [kstefans@decode.is](mailto:kstefans@decode.is).

**Accession codes:** *SLC30A8*: NM\_173851<sup>20</sup>, protein NP\_776250<sup>20</sup>

#### Author Contributions:

This manuscript describes an analysis spanning four initially distinct sequencing studies: a collaborative project among Pfizer/MGH/Broad/Lund entitled “Towards Therapeutic Targets for Type 2 Diabetes and Myocardial Infarction in the Background of Type 2 Diabetes” (PMBL), an effort by deCODE genetics to use whole genome sequencing and imputation to identify and genotype over 35 million variants in up to 370,000 Icelanders<sup>23</sup>, the Genetics of Type 2 Diabetes (GoT2D) project, and the Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multi-Ethnic Samples (T2D-GENES) project; as well as four additional genotyping efforts. The overall study bringing together data from these efforts was coordinated by JF and DA, with final analysis combining data from all variants performed by JF. The manuscript was written by JF, DA, and KS, and all authors reviewed, edited, and approved the manuscript.

Author contributions specific to the sequencing or genotyping studies are as follows:

Pfizer/MGH/Broad/Lund

*Study design:* BFV, FB, SP, NPB, DRC, TR, LG, SK, and DA

*Clinical investigation and sample management:* TForsen, BI, TTuomi, LG, and JKravic

*Sequencing, genotyping, and data processing:* NPB, TFennell, SG; performed at the Broad Institute

*Analysis:* JF (p.Arg138X), AVS (p.Trp325Arg)

*Functional studies:* NLB, SBRJ, ZD (cellular models), FVS, HS, DAD (screen for carrier autoantibodies)

*Leadership and management:* NPB, AMR, JBrosnan, JKT, SK, TR, LG, DRC, DA

deCODE

*Clinical investigation and sample management:* ABH, RB

*Sequencing, genotyping, and data processing:* GT, VS, PS, GM, DFG, AK; performed at deCODE genetics

*Analysis:* GT, VS, PS, GM, DFG, AK

*Leadership and management:* AK, UT, KS

T2D-GENES/Go-T2D

*Clinical investigation and sample management:* GA, JBlangero, DWB, JC, YSC, RD, BG, CH, JKooner, ML, TM, JYL, EST, YYT, JGW

*Sequencing and data processing:* NPB, YF, TFennell, SG; performed at the Broad Institute

*Analysis:* JF (*SLC30A8*), PF, APM, TTeslovich (exome-wide)

*Leadership and management:* MIM, MB, DA

HUNT2 sample management, genotyping, analysis, and leadership: KH, AMolven, SJ, PRN

Danish sample management, genotyping, analysis, and leadership: NG, RRM, MEJ, CC, IB, AL, TJ, TH, OP

PIVUS/ULSAM sample management, genotyping, analysis, and leadership: AMahajan, CML, LL, EI, APM

Finnish sample management, genotyping, analysis, and leadership: CF, HMS, ML, KLM, RR, VSalomaa, JT, MB

GT, VS, PS, GM, DFG, AK, UT, and KS are employed by deCODE genetics/Amgen, Inc. SP, AMR, JBrosnan, JKT, TR, and DRC are employees of Pfizer, Inc. FB is a former employee of Pfizer, Inc. and retains shares in the company.

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protein-truncating variant carriers had 65% reduced T2D risk ( $p=1.7\times 10^{-6}$ ), and non-diabetic Icelandic carriers of a frameshift variant (p.Lys34SerfsX50) demonstrated reduced glucose levels ( $-0.17$  s.d.,  $p=4.6\times 10^{-4}$ ). The two most common protein-truncating variants (p.Arg138X and p.Lys34SerfsX50) individually associate with T2D protection and encode unstable ZnT8 proteins. Previous functional study of *SLC30A8* suggested reduced zinc transport increases T2D risk<sup>8,9</sup>, yet phenotypic heterogeneity was observed in rodent *Slc30a8* knockouts<sup>10–15</sup>. Contrastingly, loss-of-function mutations in humans provide strong evidence that *SLC30A8* haploinsufficiency protects against T2D, proposing ZnT8 inhibition as a therapeutic strategy in T2D prevention.

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Genome-wide association studies (GWAS) have identified 65 genomic loci associated with T2D risk<sup>7</sup>, highlighting previously unidentified pathological pathways. Translation into novel therapeutic targets<sup>16</sup> requires identification of causal mutations and genes, as well as the directional relationship between protein activity and disease risk<sup>17</sup>. Toward this end, loss-of-function (LoF) mutations that protect against disease (without adverse phenotypes) are among the most useful findings from human genetics, suggesting targets that, upon inhibition, may prevent disease in the general population.

To identify T2D-protective LoF variants, in 2009 we sequenced exons of 115 genes near T2D GWAS signals (Supplementary Tables 1–2, Supplementary Fig. 1) in 758 individuals from Finland or Sweden (modeling previous studies<sup>18</sup>). To increase power, we selected individuals at the extremes of T2D risk: 352 young and lean T2D cases and 406 elderly and obese euglycemic controls<sup>19</sup> (Supplementary Table 3). In total, 1,768 non-synonymous variants were identified (1,683 single nucleotide variants [SNVs] and 85 indels), 1,474 (83%) with minor allele frequency (MAF)  $<1\%$  and 1,108 (63%) observed in only one individual. We found no evidence of association with T2D when testing individual variants or a burden of rare variants within genes (Supplementary Fig. 2). Genotyping 71 select SNVs (with nominally significant association or predicted to impact protein structure) in 11,288 additional individuals also yielded results consistent with the null distribution (Supplementary Fig. 3)

To increase power to detect association, we used the Illumina Human Exome Array to further genotype a subset of SNVs in 21,096 Finnish or Swedish individuals (10,534 with diabetes and 10,562 without, a superset of the individuals genotyped for the 71 SNVs, Supplementary Table 4). Analysis focused on variants with clear functional interpretation: nonsense, frameshift, or splice site mutations predicted to cause protein truncation (Supplementary Table 5). Six such variants identified via the sequencing were present on the Exome Array.

Of these variants, only a nonsense SNV (c.412C>T, p.Arg138X) in *SLC30A8* (transcript accession number NM\_173851<sup>20</sup>) showed nominally significant association with T2D (OR=0.46,  $p=0.012$ , Supplementary Table 6). A second *SLC30A8* nonsense variant (c.456G>A, p.Trp152X) was observed in one control (Supplementary Table 5) from sequencing, but was absent from the Exome Array. As a further experiment, we genotyped p.Arg138X in 26,566 additional European individuals (8,210 cases and 18,356 controls; Supplementary Table 7): although only 16 heterozygotes were observed (two cases and 14 controls), the association with T2D risk was directionally consistent (OR=0.56,  $p>0.05$ ).

Based on the combined data, heterozygosity for p.Arg138X was estimated to yield a 53% reduction in T2D risk ( $p=0.0067$ ,  $N=48,115$ ).

*SLC30A8* encodes an islet zinc transporter ZnT8 (NP\_776250<sup>20</sup>), which is necessary for zinc flux into  $\beta$ -cell insulin-secretory granules<sup>4</sup> and subsequent insulin crystallization<sup>10,12</sup>. Upon co-secretion with insulin, zinc also fulfills auto- and paracrine signaling roles<sup>21</sup>. A previously-identified common *SLC30A8* missense variant (rs13266634; c.973T>A, p.Trp325Arg) associates with T2D risk<sup>7,22</sup>, glucose<sup>5</sup>, and proinsulin<sup>6</sup>, at significance levels beyond genome-wide thresholds.

Cellular characterization has suggested that the risk-increasing allele of p.Trp325Arg reduces ZnT8 zinc transport activity<sup>8,9</sup>. In *Slc30a8* knockout mice, however, the phenotype varies with gender and genetic background: observations range from no effect on insulin secretion or glucose homeostasis, to modest hyperglycemia on a high fat diet<sup>8–15</sup>. Furthermore, a recent  $\beta$ -cell-specific *Slc30a8* knockout proposes a multi-organ effect on the resultant mouse phenotype, with circulating zinc shown to influence hepatic insulin clearance<sup>21</sup>. Thus, the directional relationship between perturbed ZnT8 function and whole organism phenotype is uncertain despite much genetic and biological data.

Because the observed protective association between p.Arg138X and T2D risk was statistically modest, we sought additional evidence. Unfortunately, the near absence of p.Arg138X outside Western Finland limited ability to further characterize its effect in other populations (Supplementary Figs 4–5). We thus sought to identify a wider spectrum of protein-truncating variants in *SLC30A8*, through investigation of the catalog of 35 million variants collected by deCODE genetics through whole-genome sequencing<sup>23</sup>. The p.Arg138X variant was not observed in this dataset. However, an independent protein-truncating variant was observed at 0.17% frequency: a deletion (c.101\_107del, p.Lys34SerfsX50; Supplementary Figs 6–7) predicted to cause a frameshift and loss of all six transmembrane domains in the islet specific transcript (NM\_173851) of *SLC30A8*<sup>4</sup>.

Heterozygosity for p.Lys34SerfsX50 was associated with 80% reduced T2D risk, with two observations in 2,953 T2D cases (0.03%) versus 248 observations in 67,919 controls (0.18%, OR=0.18,  $p=0.004$ ; Supplementary Tables 8–9). Based on the ancestral relationship between Norway and Iceland, we genotyped the variant in 5,714 Norwegians (Supplementary Table 8) and observed zero carriers in 1,645 cases versus three carriers in 4,069 controls. Combining the evidence for p.Lys34SerfsX50 and p.Arg138X strengthened the association between *SLC30A8* protein-truncating variants and reduced T2D risk (combined OR=0.32,  $p=2.4 \times 10^{-4}$ ).

Both rare *SLC30A8* variants are bioinformatically predicted to cause ZnT8 truncation and consequently impact activity. To test this prediction, we assessed over-expressed, V5-tagged ZnT8 variants (Trp325, Arg325, X138 [as well as Arg138X], and Ser34fsX50.) in HeLa cells<sup>20</sup> (Fig. 1a). Despite similar RNA transcript levels for all variants (Supplementary Fig. 8), only Trp325- and Arg325-ZnT8 proteins were easily detectable in cells<sup>8</sup>, with Arg138X, X138-, and Ser34fsX50-ZnT8 present at low to undetectable levels (Fig. 1bc). Similar results were obtained using antibodies against the native protein or the V5-tag, via Western

blot (Fig. 1b) and immunofluorescence (Fig. 1c), and in HeLa as well as Ins1 rat insulinoma cells (Fig. 1d). Co-expression of X138- or Ser34fsX50-ZnT8 with Trp325-ZnT8 did not decrease expression of the full-length allele, nor rescue expression of either truncating variant (Supplementary Fig. 9).

We hypothesized that decreased expression of these two mutants might be due to protein instability and/or enhanced degradation. Following treatment with chloroquine or MG132 (lysosomal and proteasomal inhibitors respectively<sup>24</sup>), higher X138- and Ser34fsX50-ZnT8 expression was detected via immunofluorescence (but remained undetectable via Western blot; Fig. 1e, Supplementary Fig. 10). These results are consistent with (but do not prove) instability and subsequent degradation of these truncated proteins<sup>25</sup>. Through additional experiments (data not shown), we observed zinc transport in cells expressing Arg325- and Trp325-ZnT8 but not X138- or Ser34fsX50-ZnT8 (expected given the low levels of mutant protein). Further experiments are needed to assess the *in vivo* impact of these variants, including susceptibility to nonsense-mediated decay and potential dominant negative effects on protein oligomerization.

These genetic and functional data suggest *SLC30A8* haploinsufficiency reduces T2D risk. However, confidence would be further increased through observation of multiple, additional, putative LoF variants demonstrating protective effects. As part of the T2D-GENES and GoT2D consortia, we sequenced *SLC30A8* exons in 12,294 individuals spanning multiple ethnicities (Supplementary Table 10). Nine additional protein-truncating variants were identified – two frameshift indels and two nonsense, four splice site, and one initiator codon SNV – in 23 heterozygous individuals from African American, East Asian, and South Asian ancestries (Supplementary Data Set 1). p.Arg138X was seen in three additional carriers (one case and two controls); p.Lys34SerfsX50 was not observed.

In aggregate, carriers of these additional variants exhibited 60% reduced T2D risk (four case versus 18 control observations, OR=0.38, p=0.0025), with similar effects and statistical significance observed upon analysis of only frameshift or nonsense variant carriers (two case versus 13 control observations, OR=0.37, p=0.0027). Combining all data from sequencing and genotyping in 149,134 subjects, heterozygosity for any of the 12 protein-truncating variants was associated with 65% reduced T2D risk (OR=0.34, p=1.7×10<sup>-6</sup>), a statistically significant association even after correction for ~20,000 genes in the human genome (Table 1).

We investigated potential confounding factors for the observed protective association. We first assessed whether the p.Trp325Arg haplotypic background might influence results. While p.Lys34SerfsX50 and p.Met50Ile variants were isolated to the protective common variant haplotype, the remaining variants (including p.Arg138X) were observed on the risk common variant haplotype. Thus, independent protective protein-truncating variants were observed on opposite p.Trp325Arg haplotypic backgrounds. Second, we tested for a survivor effect, where rare variant carriers with diabetes would die at a younger age. However, (a) carrier ages did not significantly differ from non-carrier ages for either p.Arg138X (69.6±8.4 versus 65.5±11.0 for cases [p>0.1], 46.4±15.7 versus 50.3±15.5 for controls [p>0.1]) or p.Lys34SerfsX50 (70.5±4.5 versus 65.6±13.8 for cases [p>0.1],

48.5±20.1 versus 50.0±23.2 for controls [ $p>0.1$ ]), and (b) p.Lys34SerfsX50 association attained equivalent significance even when analysis was restricted to age-matched controls. Finally, we acknowledged the noted challenges to control for population stratification in rare variant association studies<sup>26</sup>. We had insufficient data to perform a family-based transmission disequilibrium test (pedigree information was only available for Icelanders, with three carrier parents all transmitting the risk allele to affected children). However, the consistent association of multiple independent protein-truncating variants across multiple cohorts and ancestries argues against population stratification as entirely responsible for the protective association.

These data thus provide compelling evidence that mutations inactivating one copy of *SLC30A8* reduce T2D risk in humans. In addition to T2D risk, the common *SLC30A8* variant (p.Trp325Arg) is associated with proinsulin and fasting plasma glucose levels at genome-wide significance<sup>5,6</sup>, as well as 2-hr glucose levels post-oral glucose tolerance test (OGTT) at nominal significance (Supplementary Table 11)<sup>27</sup>. We asked whether rare protein-truncating *SLC30A8* variants also affected T2D-related phenotypes, particularly glycemic traits that might be indicative of altered islet function.

Among traits analyzed (Supplementary Table 12), the strongest association was observed in Iceland between p.Lys34SerfsX50 and random (non-fasting) glucose: non-diabetic carriers of the protective allele had lower glucose ( $\beta=-0.17$ s.d.;  $N=182$  carriers;  $p=4.6\times 10^{-4}$ ), with a consistent effect seen in three Norwegian carriers ( $\beta=-0.3$ s.d.,  $p>0.1$ ). Glucose was lower at one hour in the small number of p.Lys34SerfsX50 carriers characterized by OGTT ( $\beta=-0.73$ s.d.;  $N=4$  carriers;  $p=0.05$ ). We did not observe a significant difference in fasting glucose or insulin, although the directions of effect were consistent with the above: lower for fasting glucose (average  $\beta=-0.10$ s.d.;  $N=146$  carriers;  $p>0.1$ ) and higher for fasting insulin ( $\beta=0.24$ s.d.;  $N=52$  carriers;  $p=0.09$ ). The co-directionality of glucose levels and T2D risk parallels the pattern observed for p.Trp325Arg, where the T2D-protective allele also associates with lower glucose (Supplementary Table 9), providing further evidence against a survivor effect or population stratification as driving the protective association.

In summary, we identified 12 rare, predicted protein-truncating *SLC30A8* variants (Fig. 2). Carriers of these variants had 65% reduced T2D risk at a level of significance adequate to correct for ~20,000 genes in the human genome ( $p=1.7\times 10^{-6}$ ). Non-diabetic Icelandic carriers of p.Lys34SerfsX50 also demonstrated lower glucose levels ( $\beta=-0.17$ s.d.,  $p=4.6\times 10^{-4}$ ). Notably, initial sequencing of 115 genes in 758 extreme individuals produced only two observations of p.Arg138X, without significant evidence of association of low-frequency or rare variants individually or in aggregate for any of the sequenced genes. Rather, establishing the association of *SLC30A8* protein-truncating variants with T2D protection at levels of exome-wide significance (correction for 20,000 genes) required genotyping ~150,000 individuals spanning multiple ethnicities. Detecting similar effects in genes without prior evidence of association may require analysis at a similar or larger scale, for not only T2D but also other complex traits.

Previous modeling of the relationship between ZnT8 activity and T2D risk centered on p.Trp325Arg, where mildly attenuated zinc transport is concomitant with increased T2D

risk<sup>8</sup>, and *Slc30a8* knockout mice, where phenotypic heterogeneity is observed<sup>13,15</sup>. We find a clear and consistent association between putative *SLC30A8* LoF variants and T2D risk, across multiple ethnic backgrounds, demonstrating convincingly that a 50% reduction in gene dosage protects against T2D in humans. These data reject the model that *SLC30A8* LoF is associated with as little as a 1.2-fold increase in T2D risk (similar to that for the common p.Trp325Arg variant) at significance of  $p \approx 10^{-9}$ . Phenotypic interrogation of human mutation carriers is needed to determine the physiological mechanism behind this protective association and establish the effects of *SLC30A8* haploinsufficiency in the pancreas and other tissues<sup>21</sup>.

The observed human genetics data present several implications for *SLC30A8* function in T2D pathophysiology. The identification of multiple disease-associated protein-altering variants in *SLC30A8* unambiguously (albeit unsurprisingly) documents *SLC30A8* as the causal gene behind GWAS association signals. The observation that protein-truncating variants protect against T2D defines the directional relationship between *SLC30A8* activity and T2D risk in humans. The expanded *SLC30A8* allelic series offers a more functionally-informative catalog of variation versus p.Trp325Arg alone, enabling future experiments investigating potential mechanisms. Although significant work is required to understand how reduced *SLC30A8* activity lowers T2D risk, the current observations motivate experiments to test ZnT8 inhibition in T2D treatment in human populations.

## Methods

### Sequencing and genotyping

Individuals were selected for initial sequencing from several population-based cohorts from Finland and Sweden. A custom hybrid selection array was used to target genes, which were sequenced on an Illumina HiSeq 2000. Additional individuals from these same cohorts, as well as other cohorts drawn from different European populations, were genotyped for the *SLC30A8* nonsense SNV p.Arg138X through the Illumina HumanExome v1.1 array. All sequenced individuals were also genotyped, with data showing 100% concordance.

Icelandic individuals were genotyped for the frameshift p.Lys34SerfsX50 variant using a combination of whole-genome sequencing and imputation (either direct imputation based on chip-genotyping, or through familial-based imputation). Sanger sequencing was used to confirm carriers. Norwegian individuals were genotyped with a fragment-length-based method using differentially-labeled fluorescent primers, with Sanger sequencing again used to confirm carriers. Further *SLC30A8* sequencing (aimed at identifying further rare variant carriers) was performed as part of a whole-exome sequencing experiment, with the Agilent SureSelect Human All Exon platform used to capture exons and an Illumina HiSeq 2000 used for sequencing.

These studies were performed using protocols approved by the ethics committees of Helsinki University Hospital, Finland and Lund University, the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland, the Regional Committee for Research Ethics and the Norwegian Data Inspectorate, and the Massachusetts Institute of

Technology Institutional Review Board, as well as with informed consent from all participants.

### Association analysis

Association analysis was performed separately for three groups of variants: p.Arg138X, p.Lys34SerfsX50, and the remaining variants. For p.Arg138X, association analysis was separate for each analyzed cohort, and used a linear mixed model so as to account for sample structure including population stratification and genetic relatedness. Results were combined via a fixed-effects meta-analysis. For p.Lys34SerfsX50, association analysis was performed in Iceland using logistic regression, with controls matched to cases based on how informative the imputed genotypes were, and in Norway using a simple logistic regression with significance calculated via the score statistic. For the remaining variants, all individuals were analyzed jointly via a collapsing method, treating carriers of any variant indistinguishably, and regressing phenotype on the presence of any variant, with a linear mixed model used to account for sample structure. The resulting three association statistics were combined via a random-effects meta-analysis to obtain combined estimates of effect size and statistical significance.

For further details, see Supplementary Information.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

### Authors

Jason Flannick<sup>1,2,3</sup>, Gudmar Thorleifsson<sup>4</sup>, Nicola L. Beer<sup>1,5</sup>, Suzanne B. R. Jacobs<sup>1</sup>, Niels Grarup<sup>6</sup>, Noël P. Burt<sup>1</sup>, Anubha Mahajan<sup>7</sup>, Christian Fuchsberger<sup>8</sup>, Gil Atzmon<sup>9,10</sup>, Rafn Benediktsson<sup>11</sup>, John Blangero<sup>12</sup>, Don W. Bowden<sup>13,14,15,16</sup>, Ivan Brandslund<sup>17,18</sup>, Julia Brosnan<sup>19</sup>, Frank Burslem<sup>20</sup>, John Chambers<sup>21,22,23</sup>, Yoon Shin Cho<sup>24</sup>, Cramer Christensen<sup>25</sup>, Desirée A. Douglas<sup>26</sup>, Ravindranath Duggirala<sup>12</sup>, Zachary Dymek<sup>1</sup>, Yossi Farjoun<sup>1</sup>, Timothy Fennell<sup>1</sup>, Pierre Fontanillas<sup>1</sup>, Tom Forsén<sup>27,28</sup>, Stacey Gabriel<sup>1</sup>, Benjamin Glaser<sup>29,30</sup>, Daniel F. Gudbjartsson<sup>4</sup>, Craig Hanis<sup>31</sup>, Torben Hansen<sup>6,32</sup>, Astradur B. Hreidarsson<sup>11</sup>, Kristian Hveem<sup>33</sup>, Erik Ingelsson<sup>7,34</sup>, Bo Isomaa<sup>35,36</sup>, Stefan Johansson<sup>37,38,39</sup>, Torben Jørgensen<sup>40,41,42</sup>, Marit Eika Jørgensen<sup>43</sup>, Sekar Kathiresan<sup>1,44,45,46</sup>, Augustine Kong<sup>4</sup>, Jaspal Kooner<sup>22,23,47</sup>, Jasmina Kravic<sup>48</sup>, Markku Laakso<sup>49</sup>, Jong-Young Lee<sup>50</sup>, Lars Lind<sup>51</sup>, Cecilia M Lindgren<sup>1,7</sup>, Allan Linneberg<sup>40,41,52</sup>, Gisli Masson<sup>4</sup>, Thomas Meitinger<sup>53</sup>, Karen L Mohlke<sup>54</sup>, Anders Molven<sup>37,55,56</sup>, Andrew P. Morris<sup>7,57</sup>, Shobha Potluri<sup>58</sup>, Rainer Rauramaa<sup>59,60</sup>, Rasmus Ribel-Madsen<sup>6</sup>, Ann-Marie Richard<sup>19</sup>, Tim Rolph<sup>19</sup>, Veikko Salomaa<sup>61</sup>, Ayellet V. Segrè<sup>1,2</sup>, Hanna Skärstrand<sup>26</sup>, Valgerdur Steinthorsdottir<sup>4</sup>, Heather M. Stringham<sup>8</sup>, Patrick Sulem<sup>4</sup>, E Shyong Tai<sup>62,63,64</sup>, Yik Ying Teo<sup>62,65,66,67,68</sup>, Tanya Teslovich<sup>8</sup>, Unnur Thorsteinsdottir<sup>4,69</sup>, Jeff K. Trimmer<sup>19</sup>, Tiinamaija Tuomi<sup>35,70</sup>, Jaakko Tuomilehto<sup>71,72,73</sup>, Fariba Vaziri-Sani<sup>26</sup>, Benjamin F. Voight<sup>1,74,75</sup>, James G. Wilson<sup>76</sup>, Michael Boehnke<sup>8</sup>, Mark I. McCarthy<sup>7,77,78</sup>, Pål R. Njølstad<sup>1,37,79</sup>, Oluf

Pedersen<sup>6</sup>, the Go-T2D consortium<sup>80</sup>, the T2D-GENES consortium<sup>81</sup>, Leif Groop<sup>48,82</sup>, David R. Cox<sup>58</sup>, Kari Stefansson<sup>4,69,\*</sup>, and David Altshuler<sup>1,2,3,44,45,83,84,\*</sup>

## Affiliations

<sup>1</sup>Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA <sup>2</sup>Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA <sup>3</sup>Diabetes Unit, Massachusetts General Hospital, Boston, MA, USA <sup>4</sup>deCODE genetics/Amgen, Inc., Reykjavik, Iceland <sup>5</sup>Oxford Centre for Diabetes, Endocrinology, and Metabolism, University of Oxford, Oxford, U.K <sup>6</sup>The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark <sup>7</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK <sup>8</sup>Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA <sup>9</sup>Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA <sup>10</sup>Department of Genetics, Albert Einstein College of Medicine, Bronx, NY, USA <sup>11</sup>Department of Endocrinology and Metabolism, Landspítali-University Hospital, Reykjavik, Iceland <sup>12</sup>Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas, USA <sup>13</sup>Center for Genomics and Personalized Medicine Research, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA <sup>14</sup>Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA <sup>15</sup>Department of Biochemistry, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA <sup>16</sup>Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA <sup>17</sup>Department of Clinical Biochemistry, Vejle Hospital, Vejle, Denmark <sup>18</sup>Institute of Regional Health Research, University of Southern Denmark, Odense, Denmark <sup>19</sup>Cardiovascular & Metabolic Diseases Research Unit, Pfizer Inc., Cambridge, MA, USA <sup>20</sup>Cardiovascular and Metabolic Diseases Practice, Prescient Life Sciences, London, UK <sup>21</sup>Department of Epidemiology and Biostatistics, Imperial College London, London, UK <sup>22</sup>Imperial College Healthcare NHS Trust, London, UK <sup>23</sup>Ealing Hospital National Health Service (NHS) Trust, Middlesex, UK <sup>24</sup>Department of Biomedical Science, Hallym University, Chuncheon, Gangwon-do, Korea <sup>25</sup>Department of Internal Medicine and Endocrinology, Vejle Hospital, Vejle, Denmark <sup>26</sup>Department of Clinical Sciences, Unit of Diabetes and Celiac Diseases, Lund University, Malmö, Sweden <sup>27</sup>University of Helsinki, Department of General Practice and Primary Health Care, Finland <sup>28</sup>Vaasa Health Care Centre, Diabetes Care Unit, Vaasa, Finland <sup>29</sup>Endocrinology and Metabolism Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel <sup>30</sup>Israel Diabetes Research Group (IDRG), Israel <sup>31</sup>Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX, USA <sup>32</sup>Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark <sup>33</sup>Department of Public Health, Faculty of Medicine, Norwegian University of Science and Technology, Levanger, Norway <sup>34</sup>Molecular Epidemiology and Science for Life Laboratory, Department of Medical Sciences, Uppsala University, Uppsala, Sweden <sup>35</sup>Folkhalsan Research Centre, Helsinki, Finland <sup>36</sup>The Department of Social



Services and Health Care, Jakobstad, Finland <sup>37</sup>KG Jebsen Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway <sup>38</sup>Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway <sup>39</sup>Department of Biomedicine, University of Bergen, Bergen, Norway <sup>40</sup>Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark <sup>41</sup>Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark <sup>42</sup>Faculty of Medicine, University of Aalborg, Aalborg, Denmark <sup>43</sup>Steno Diabetes Center, Gentofte, Denmark <sup>44</sup>Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA <sup>45</sup>Cardiovascular Research Center, Cardiology Division, Massachusetts General Hospital, Boston, MA, USA <sup>46</sup>Department of Medicine, Harvard Medical School, Boston, MA, USA <sup>47</sup>National Heart and Lung Institute (NHLI), Imperial College London, Hammersmith Hospital, London, UK <sup>48</sup>Department of Clinical Sciences, Diabetes and Endocrinology, Lund University and Lund University Diabetes Centre, Malmö, Sweden <sup>49</sup>Department of Medicine, University of Eastern Finland, Kuopio Campus and Kuopio University Hospital, Kuopio, Finland <sup>50</sup>Center for Genome Science, National Institute of Health, Osong Health Technology Administration Complex, Chungcheongbuk-do, Cheongwon-gun, Gangoe-myeon, Yeonje-ri, Korea <sup>51</sup>Department of Medical Sciences, Uppsala University, Uppsala, Sweden <sup>52</sup>Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark <sup>53</sup>Institute of Human Genetics, Technical University Munich, Munich, Germany <sup>54</sup>Department of Genetics, University of North Carolina, Chapel Hill, NC, USA <sup>55</sup>The Gade Laboratory for Pathology, Department of Clinical Medicine, University of Bergen, Bergen, Norway <sup>56</sup>Department of Pathology, Haukeland University Hospital, Bergen, Norway <sup>57</sup>Department of Biostatistics, University of Liverpool, Liverpool, UK <sup>58</sup>Applied Quantitative Genotherapeutics, Pfizer Inc., South San Francisco, CA, USA <sup>59</sup>Kuopio Research Institute of Exercise Medicine, Kuopio, Finland <sup>60</sup>Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland <sup>61</sup>THL-National Institute for Health and Welfare, Helsinki, Finland <sup>62</sup>Saw Swee Hock School of Public Health, National University of Singapore, National University Health System, Singapore, Singapore <sup>63</sup>Department of Medicine, National University of Singapore, National University Health System, Singapore, Singapore <sup>64</sup>Duke-National University of Singapore Graduate Medical School, Singapore, Singapore <sup>65</sup>Centre for Molecular Epidemiology, National University of Singapore, Singapore, Singapore <sup>66</sup>Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore <sup>67</sup>Graduate School for Integrative Science and Engineering, National University of Singapore, Singapore, Singapore <sup>68</sup>Department of Statistics and Applied Probability, National University of Singapore, Singapore, Singapore <sup>69</sup>Faculty of Medicine, University of Iceland, Reykjavík, Iceland <sup>70</sup>Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland <sup>71</sup>Centre for Vascular Prevention, Danube-University Krems, Krems, Austria <sup>72</sup>Diabetes Prevention Unit, National Institute for Health and Welfare, Helsinki, Finland <sup>73</sup>King Abdulaziz University, Jeddah, Saudi Arabia <sup>74</sup>Department of

Pharmacology, The University of Pennsylvania - Perelman School of Medicine, Philadelphia, PA, USA <sup>75</sup>Department of Genetics, The University of Pennsylvania - Perelman School of Medicine, Philadelphia, PA, USA <sup>76</sup>Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA <sup>77</sup>Oxford Centre for Diabetes, Endocrinology, and Metabolism, Churchill Hospital, University of Oxford, Oxford, UK <sup>78</sup>Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK <sup>79</sup>Department of Pediatrics, Haukeland University Hospital, Bergen, Norway <sup>80</sup>The Go-T2D Consortium; Full lists of members and affiliations are provided in the Supplementary Note <sup>81</sup>The T2D-GENES Consortium; Full lists of members and affiliations are provided in the Supplementary Note <sup>82</sup>Finnish Institute for Molecular Medicine (FIMM), Helsinki University, Helsinki, Finland <sup>83</sup>Department of Genetics, Harvard Medical School, Boston, MA, USA <sup>84</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA

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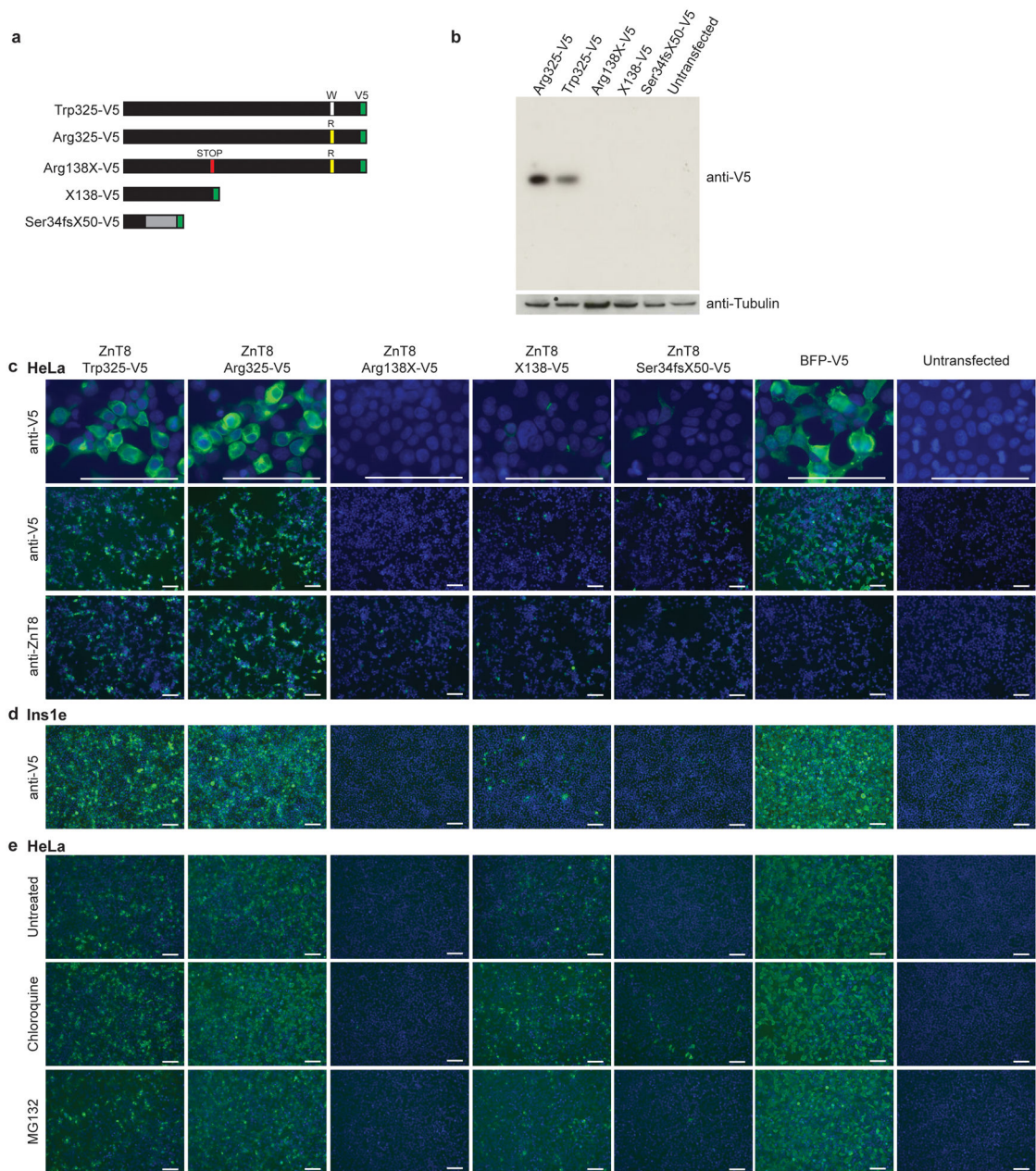
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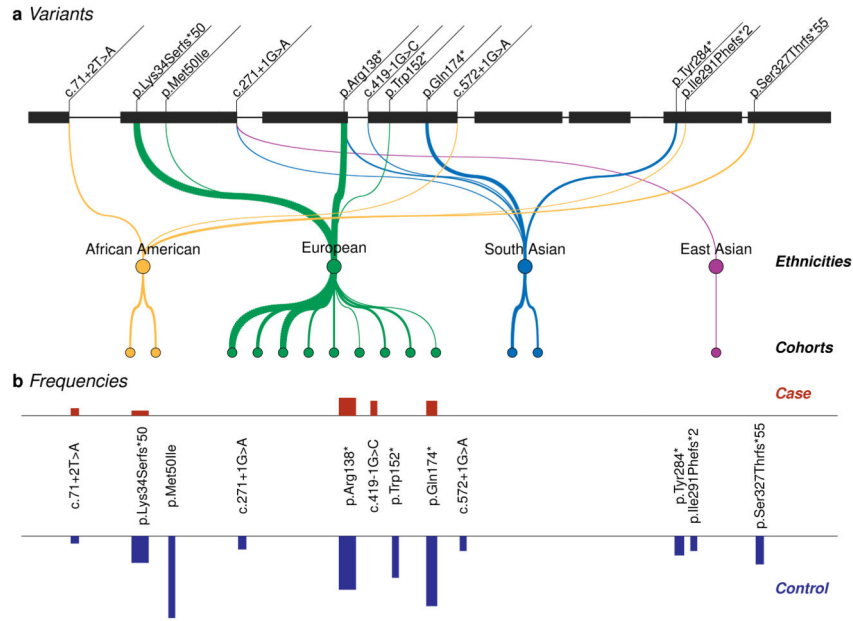
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### Figure 1. Over-expression of p.Arg138X- and p. Ser34fsX50-ZnT8 in HeLa cells

We sought to experimentally evaluate whether the p.Arg138X or p. Ser34fsX50 ZnT8 variants resulted in decreased ZnT8 expression and/or activity. **(a)** Depiction of *SLC30A8* open reading frames in C-terminal V5-tagged constructs (tag highlighted in green). **(b)** Western blot of HeLa lysates following transient over-expression of V5-tagged ZnT8 variants (anti-V5-tag). Antibody against tubulin was used as a loading control for each sample, and untransfected cell lysate was used to demonstrate specificity of anti-V5 antibody. **(c, d)** Immunofluorescent staining of ZnT8 variant expression in **(c)** HeLa and **(d)** Ins1e cells. ZnT8 was detected using antibodies against the C-terminal V5-tag (anti-V5) or the N-terminus of the endogenous protein (anti-ZnT8), as indicated. BFP-V5 and

untransfected HeLa cells serve as controls. Cells were co-stained with Hoechst-33342 to mark nuclei. Within each row of images for the indicated antibody and objective, identical exposure times were used across all proteins. **(e)** ZnT8 variant expression, as detected by anti-V5 immunostaining, following 4hr treatment with inhibitors of the lysosome (chloroquine, 100  $\mu$ M) or the proteasome (MG132, 10  $\mu$ M). Images were acquired using a 10x objective and identical exposure times. Scale bars, 100  $\mu$ M.



**Figure 2. Protein-truncating variants identified in *SLC30A8***

Through sequencing and genotyping of nearly 150,000 individuals across 5 ethnicities, we identified 12 *SLC30A8* variants – each rare and predicted to cause premature protein truncation. **(a)** Shown is the position of each variant on the islet-specific *SLC30A8* transcript (NM\_173851<sup>20</sup>). p.Met50Ile is predicted to alter the initiator codon in other transcripts of *SLC30A8*. Lines are drawn from each variant to ethnicities for which carriers were observed, with greater widths corresponding to ethnicities with more observations. Lines are further drawn from each ethnicity to the populations (cohorts) from which carriers were identified. From left, cohorts are: JHS, WFS, Botnia, Danish, deCODE, Finnish, HUNT2, KORA, Malmo, PIVUS/ULSAM, WTCCC, LOLIPOP, Singapore Indians, and KARE (cohort information in Supplementary Information). Ethnicities or cohorts with no observations are not shown. **(b)** Graphical representation of the case and control frequencies for each observed variant; case frequencies in red (above) and control frequencies in blue (below). Wider bars correspond to variants with more observations. A quantitative and complete representation of these data is given in Table 1.

**Table 1**

**Association of *SLC30A8* variants with type 2 diabetes**

Through sequencing and genotyping of ~150,000 individuals across five ethnicities, a spectrum of 12 rare, predicted protein-truncating variants were identified in *SLC30A8*. Shown for each variant are: ethnicity, cohort, number of genotyped cases and controls (N), number of cases and controls observed to carry a variant (Carriers), and observed allele frequencies in cases and controls (Allele Frequency). Odds ratios (OR) and p-values were computed separately for three groups of variants: p.Arg138X, p.Lys34SerfsX50, and the remaining variants. For p.Arg138X and p.Lys34SerfsX50, for which more than ten carriers were observed, statistics were computed separately for each cohort (see **Methods**, Supplementary Information) and then combined via a fixed-effects meta-analysis. For the remaining variants, an score of association was computed by comparing the aggregate frequency of variant carriers between cases and controls. These three statistics were combined via a random-effects meta-analysis to produce combined estimates of risk and statistical significance (bottom row). Variant counts and frequencies were computed based on all studied individuals, while OR and p-values were computed with correction for sample structure (population stratification and genetic relatedness; see Supplementary Information); thus, displayed ORs differ from those computed solely from frequency estimates.

Variant	Ethnicity	Country	Cohort	N		Carriers		Allele Frequency		OR (95% CI)	P
				Case	Ctrl	Case	Ctrl	Case	Ctrl		
p-Arg138X	European	Finland	Botnia	3,727	5,440	9	39	0.12%	0.36%	0.47 (0.27-0.81)	0.0067
	European	Sweden	Malmo	6,960	5,480	2	3	0.014%	0.027%		
	European	Sweden	PIVUS/ULSAM	270	1,734	1	3	0.19%	0.087%		
	European	Denmark	Danish	3,889	7,869	0	9	0.0%	0.057%		
	European	Finland	Finnish	4,050	8,696	1	2	0.012%	0.011%		
p-Lys34SerfsX50	South Asian	Singapore	Singapore Indians	562	585	1	1	0.089%	0.085%	0.17 (0.05-0.52)	0.0019
	European	UK	UKT2D	321	319	0	1	0.0%	0.16%		
c.71+2T>A	European	Iceland	deCODE	2,953	67,919	2	248	0.034%	0.18%	0.30 (0.14-0.64)	0.0021
	European	Norway	HUNT2	1,645	4,069	0	3	0.0%	0.037%		
p-Met50Ile	African-American	USA	WFS	501	527	1	0	0.1%	0.0%	0.094%	0.55%
	African-American	USA	JHS	530	533	0	1	0.0%	0.094%		
c.271+G>A	European	Germany	KORA	97	91	0	1	0.0%	0.55%	0.091%	0.085%
	East Asian	Korea	KARE	520	551	0	1	0.0%	0.091%		
c.419-1G>C	South Asian	Singapore	Singapore Indians	562	585	0	1	0.0%	0.085%	0.0%	0.28%
	South Asian	UK	LOLIPOP	530	537	1	0	0.094%	0.0%		
p-Trp152X	European	Finland	Botnia	134	180	0	1	0.0%	0.28%		



Variant	Ethnicity	Country	Cohort	N		Carriers		Allele Frequency		OR (95% CI)	P
				Case	Ctrl	Case	Ctrl	Case	Ctrl		
p.Arg138X	European	Finland	Botnia	3,727	5,440	9	39	0.12%	0.36%	0.47 (0.27-0.81)	0.0067
	European	Sweden	Malmö	6,960	5,480	2	3	0.014%	0.027%		
	European	Sweden	PIVUS/ULSAM	270	1,734	1	3	0.19%	0.087%		
	European	Denmark	Danish	3,889	7,869	0	9	0.0%	0.057%		
	European	Finland	Finnish	4,050	8,696	1	2	0.012%	0.011%		
p.Gln174X	South Asian	Singapore	Singapore Indians	562	585	1	1	0.089%	0.085%	0.16%	
	European	UK	UKT2D	321	319	0	1	0.0%	0.47%		
c.572+1G>A	South Asian	UK	LOLIPOP	530	537	1	5	0.094%	0.094%	0.19%	
	African-American	USA	JHS	530	533	0	1	0.0%	0.085%		
p.Tyr284X	South Asian	UK	LOLIPOP	530	537	0	2	0.0%	0.19%	0.085%	
	South Asian	Singapore	Singapore Indians	562	585	0	1	0.0%	0.085%		
p.Ile291PhefsX2	African-American	USA	JHS	530	533	0	1	0.0%	0.094%	0.19%	
p.Ser327ThrfsX5	African-American	USA	WFS	501	527	0	2	0.0%	0.19%		
<b>Combined</b>	-	-	-	<b>30,433</b>	<b>118,701</b>	<b>19</b>	<b>326</b>	<b>-</b>	<b>-</b>	<b>0.34 (0.21-0.53)</b>	<b>1.7 × 10<sup>-6</sup></b>

CI=confidence interval; Ctrl=Control.