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

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Loss-of-function mutations protective against human disease provide *in vivo* validation of therapeutic targets[1–3], but none have yet been described for type 2 diabetes (T2D). Through sequencing or genotyping of ~150,000 individuals across 5 ancestry groups, we identified 12 rare protein-truncating variants in *SLC30A8*, which encodes an islet zinc transporter (ZnT8)[4] and harbors a common variant (p.Trp325Arg) associated with T2D risk and glucose and proinsulin levels[5–7]. Collectively, carriers of protein-truncating variants had 65% reduced T2D risk ($P = 1.7 \times 10^{-6}$), and non-diabetic Icelandic carriers of a frameshift variant (p.Lys34Serfs*50) demonstrated reduced glucose levels (–0.17 s.d., $P = 4.6 \times 10^{-4}$). The two most common protein-truncating variants (p.Arg138* and p.Lys34Serfs*50) individually associate with T2D protection and encode unstable ZnT8 proteins. Previous functional study of *SLC30A8* suggested that reduced zinc transport increases T2D risk[8,9], and phenotypic heterogeneity was observed in mouse *Slc30a8* knockouts[10–15]. In contrast, loss-of-function mutations in humans provide strong evidence that *SLC30A8* haploinsufficiency protects against T2D, suggesting ZnT8 inhibition as a therapeutic strategy in T2D prevention.

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

To identify loss-of-function variants protective against T2D, in 2009, a collaboration of Pfizer, Inc., Massachusetts General Hospital, the Broad Institute and Lund University sequenced the exons of 115 genes near T2D association signals identified by GWAS (**Supplementary Fig. 1** and **Supplementary Tables 1** and **2**) in 758 individuals from Finland or Sweden (modeling previous studies[18]). To increase power, we selected individuals at the extremes of T2D risk, including 352 young and lean T2D cases and 406 elderly and obese euglycemic controls[19] (**Supplementary Table 3**). In total, we identified 1,768 nonsynonymous variants (1,683 single-nucleotide variants (SNVs) and 85 indels), 1,474 (83%) with minor allele frequency (MAF) of <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 of 71 select SNVs (showing nominally significant association ($P < 0.05$) or predicted to affect

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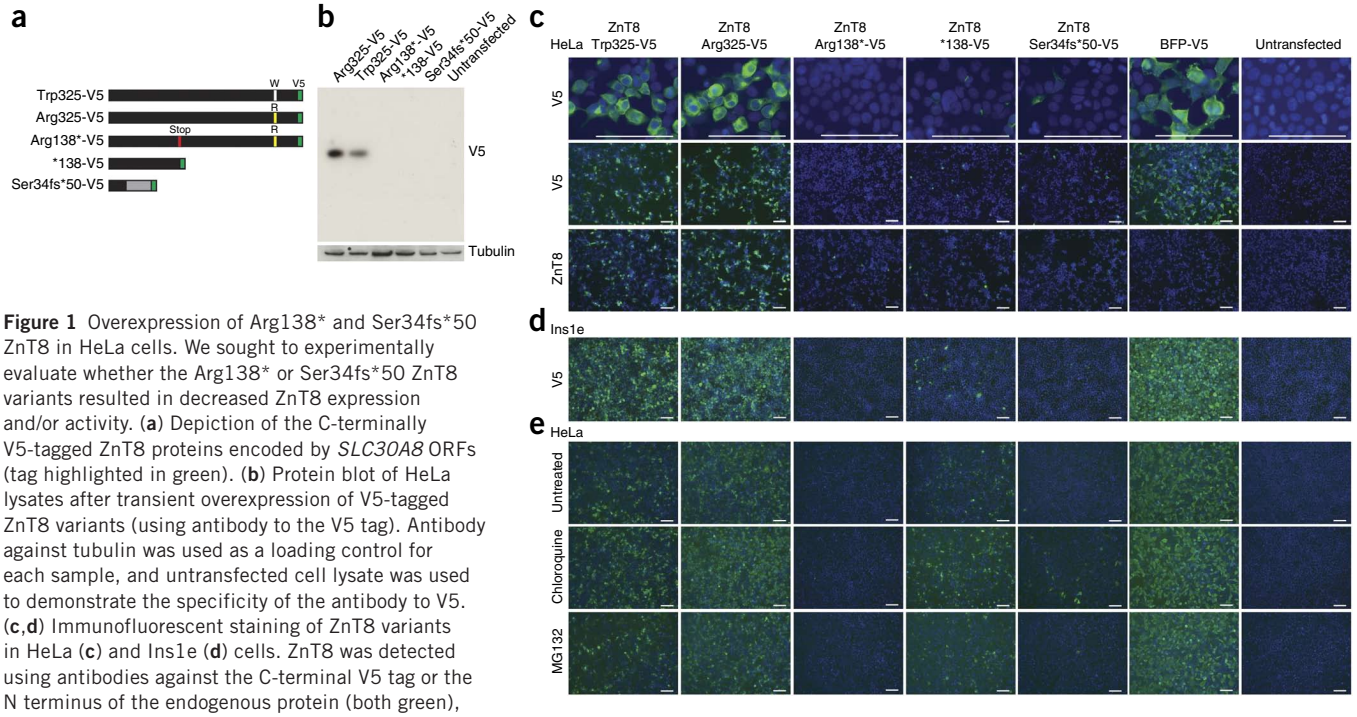

Figure 1 Overexpression of Arg138* and Ser34fs*50 ZnT8 in HeLa cells. We sought to experimentally evaluate whether the Arg138* or Ser34fs*50 ZnT8 variants resulted in decreased ZnT8 expression and/or activity. (**a**) Depiction of the C-terminally V5-tagged ZnT8 proteins encoded by *SLC30A8* ORFs (tag highlighted in green). (**b**) Protein blot of HeLa lysates after transient overexpression of V5-tagged ZnT8 variants (using antibody to the V5 tag). Antibody against tubulin was used as a loading control for each sample, and untransfected cell lysate was used to demonstrate the specificity of the antibody to V5. (**c**,**d**) Immunofluorescent staining of ZnT8 variants in HeLa (**c**) and Ins1e (**d**) cells. ZnT8 was detected using antibodies against the C-terminal V5 tag or the N terminus of the endogenous protein (both green), as indicated. BFP-V5 and untransfected HeLa cells serve as controls. Cells were also stained with Hoechst 33342 to mark nuclei (blue). Within each row, identical exposure times were used for all images. (**e**) ZnT8 variant expression, as detected by immunostaining with antibody to V5, following 4-h treatment with inhibitors of the lysosome (chloroquine, 100 μM) or proteasome (MG132, 10 μM). Images were acquired using either a 63× (top row) or 10× (all other rows) objective and identical exposure times. Scale bars, 100 μm.

protein structure) in up to 13,884 additional individuals (6,388 cases and 7,496 controls) also yielded results consistent with the null distribution of no association with T2D (**Supplementary Fig. 3**).

Among these SNVs, we noted a nonsense variant (c.412C>T, p.Arg138*) in *SLC30A8* (transcript accession NM_173851) that trended toward protection from T2D rather than risk (7 case and 21 control observations, odds ratio (OR) = 0.38, $P = 0.05$). To propagate this variant into larger sample sizes, we added p.Arg138* to the design of the Illumina Human Exome array (along with six other variants from the original sequencing that were predicted to cause protein truncation). Using this array, we genotyped the 13,884 individuals already genotyped for p.Arg138*, as well as 7,212 additional Finnish or Swedish individuals (**Supplementary Tables 4** and **5**). In the combined analysis of 10,534 cases and 10,562 controls, the protective association for p.Arg138* remained nominally significant (OR = 0.46, $P = 0.01$; **Supplementary Table 6**). We genotyped p.Arg138* in an additional 26,566 European individuals (8,210 T2D cases and 18,356 controls; **Supplementary Table 7**), with 16 heterozygotes observed (2 cases and 14 controls). Combining all available genotype data for the variant encoding p.Arg138*, heterozygosity 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; ref. 20), which is necessary for zinc flux into β cell insulin-secretory granules[4] and subsequent insulin crystallization[10,12]. Upon co-secretion with insulin, zinc also fulfills autocrine and paracrine signaling roles[21]. A previously identified common missense variant in *SLC30A8* (rs13266634; c.973T>A, p.Trp325Arg) associates with T2D risk[7,22] and glucose[5] and proinsulin[6] levels with significance far exceeding genome-wide thresholds ($P < 5 \times 10^{-8}$).

Cellular characterization has suggested that the risk-increasing allele encoding p.Trp325Arg reduces ZnT8 zinc transport activity[8,9]. In *Slc30a8* knockout mice, observations range from no effect on insulin secretion or glucose homeostasis to modest hyperglycemia on a high-fat diet, with the phenotype varying with sex and genetic background[8–15]. Furthermore, data obtained from a recent β cell–specific *Slc30a8* knockout mouse model suggest a multi-organ effect on the phenotype, with circulating zinc also influencing hepatic insulin clearance in these mice[21]. 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.Arg138* and T2D risk was statistically modest, we sought additional evidence. Unfortunately, the near absence of the p.Arg138* variant outside of western Finland limited our ability to further characterize its effect in other populations (**Supplementary Figs. 4** and **5**). We thus sought to identify a wider spectrum of protein-truncating variants in *SLC30A8* by investigating the catalog of 35 million variants collected by deCODE Genetics through whole-genome sequencing[23]. The variant encoding p.Arg138* was not observed in this data set. However, an independent protein-truncating variant was observed at a frequency of 0.17%—a deletion (c.100_106delAAAGATC, p.Lys34Serfs*50; **Supplementary Figs. 6** and **7**) predicted to cause a frameshift and loss of all six transmembrane domains encoded by the islet-specific transcript (NM_173851) of *SLC30A8* (ref. 4).

Heterozygosity for the variant encoding p.Lys34Serfs*50 was associated with 80% reduced T2D risk, with 2 observations of this variant 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** and **9**). Taking into consideration the ancestral relationship between Norwegian and Icelandic populations, we genotyped the variant in 5,714 Norwegians (**Supplementary Table 8**) and observed no carriers in 1,645 cases and 3 carriers in 4,069 controls. Combining the evidence for the p.Lys34Serfs*50 and p.Arg138* variants strengthened the association

Table 1 Association of *SLC30A8* variants with T2D

Variant	Ancestry	Country	Cohort	*N*		Carriers		Allele frequency		OR (95% CI)	*P*
				Cases	Controls	Cases	Controls	Cases (%)	Controls (%)		
p.Arg138*	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		
	South Asian	Singapore	Singapore Indians	562	585	1	1	0.089	0.085		
	European	UK	UKT2D	321	319	0	1	0.0	0.16		
p.Lys34Serfs*50	European	Iceland	deCODE	2,953	67,919	2	248	0.034	0.18	0.17 (0.05–0.52)	0.0019
	European	Norway	HUNT2	1,645	4,069	0	3	0.0	0.037		
c.71+2T>A	African American	United States	WFS	501	527	1	0	0.1	0.0	0.30 (0.14–0.64)	0.0021
	African American	United States	JHS	530	533	0	1	0.0	0.094		
p.Met50Ile	European	Germany	KORA	97	91	0	1	0.0	0.55		
c.271+G>A	East Asian	Korea	KARE	520	551	0	1	0.0	0.091		
	South Asian	Singapore	Singapore Indians	562	585	0	1	0.0	0.085		
c.419–1G>C	South Asian	UK	LOLIPOP	530	537	1	0	0.094	0.0		
p.Trp152*	European	Finland	Botnia	134	180	0	1	0.0	0.28		
p.Gln174*	South Asian	UK	LOLIPOP	530	537	1	5	0.094	0.47		
c.572+1G>A	African American	United States	JHS	530	533	0	1	0.0	0.094		
p.Tyr284*	South Asian	UK	LOLIPOP	530	537	0	2	0.0	0.19		
	South Asian	Singapore	Singapore Indians	562	585	0	1	0.0	0.085		
p.Ile291Phefs*2	African American	United States	JHS	530	533	0	1	0.0	0.094		
p.Ser327Thrs*55	African American	United States	WFS	501	527	0	2	0.0	0.19		
Combined	–	–	–	30,433	118,701	19	326	–	–	0.34 (0.21–0.53)	1.7×10^{-6}

Through sequencing and genotyping of ~150,000 individuals across 5 ancestry groups, a spectrum of 12 rare predicted protein-truncating variants was identified in *SLC30A8*. Shown for each variant are ancestry group, cohort, number of genotyped cases and controls (*N*), number of cases and controls observed to carry the variant, and observed allele frequencies in cases and controls. ORs and *P* values were computed separately for three groups of variants: p.Arg138*, p.Lys34Serfs*50 and the remaining variants. For p.Arg138* and p.Lys34Serfs*50, for which more than ten carriers were observed, statistics were computed separately for each cohort (Online Methods and **Supplementary Note**) and then combined via a fixed-effects meta-analysis. For the remaining variants, an association score was computed by comparing the aggregate frequencies of variant carriers in 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 on the basis of all studied individuals, whereas ORs and *P* values were computed with correction for sample structure (population stratification and genetic relatedness; **Supplementary Note**); thus, displayed ORs differ from those computed solely from frequency estimates. CI, confidence interval.

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, to affect activity. To test this prediction, we assessed overexpressed V5-tagged ZnT8 variants (Trp325, Arg325, *138 (as well as Arg138*) and Ser34fs*50) in HeLa cells[20] (**Fig. 1a**). Despite similar RNA transcript levels for all variants (**Supplementary Fig. 8**), only Trp325 and Arg325 ZnT8 proteins were easily detectable in cells[8], with Arg138*, *138 and Ser34fs*50 ZnT8 present at low to undetectable levels (**Fig. 1b**,**c**). Similar results were obtained using antibodies against the native protein or the V5 tag in protein blot analysis (**Fig. 1b**) and immunofluorescence (**Fig. 1c**) and in HeLa as well as in Ins1e rat insulinoma cells (**Fig. 1d**). Coexpression of *138 or Ser34fs*50 ZnT8 with Trp325 ZnT8 did not decrease expression of the full-length allele or 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. After treatment of cells with chloroquine or MG132 (lysosomal and proteasomal inhibitors, respectively[24]), higher *138 and Ser34fs*50 ZnT8 expression was detected via immunofluorescence (but remained undetectable via protein blot analysis; **Fig. 1e** and **Supplementary Fig. 10**). These results are consistent with but do not prove instability and subsequent degradation of these truncated proteins[25]. In additional experiments (data not shown), we observed zinc transport in cells expressing Arg325 and Trp325 ZnT8 but not in cells expressing *138 or Ser34fs*50 ZnT8 (an expected result given the low levels of mutant protein). Further experiments are needed to assess the *in vivo* impact of these variants, including their susceptibility to nonsense-mediated decay and potential dominant-negative effects on protein oligomerization.

These genetic and functional data suggest that *SLC30A8* haploinsufficiency reduces T2D risk. However, confidence in this conclusion would be further increased through the observation of multiple additional putative loss-of-function variants demonstrating protective effects. As part of the Genetics of Type 2 Diabetes (Go-T2D) and Type 2 Diabetes Genetic Exploration by Next-Generation Sequencing in Multi-Ethnic Samples (T2D-GENES) consortia, we sequenced *SLC30A8* exons in 12,294 individuals spanning multiple ancestry groups (**Supplementary Table 10**). Nine additional protein-truncating variants were identified—2 frameshift indels and 2 nonsense, 4 splice-site and 1 initiator codon SNV—in 23 heterozygous individuals of African-American, East Asian and South Asian ancestry (**Supplementary Data Set 1**). The variant encoding p.Arg138* was seen in three additional carriers (one case and two controls); the variant encoding p.Lys34Serfs*50 was not observed.

In aggregate, carriers of these additional variants exhibited 60% reduced T2D risk (4 observations of the variants in cases versus 18 observations in controls; OR = 0.38, $P = 0.0025$), with similar effects and statistical significance observed upon analysis of only carriers of frameshift or nonsense variants (2 observations of variants in cases versus 13 observations in controls; 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, $P = 1.7 \times 10^{-6}$), which represents a statistically significant association even after correction for the ~20,000 genes in the human genome (**Table 1**).

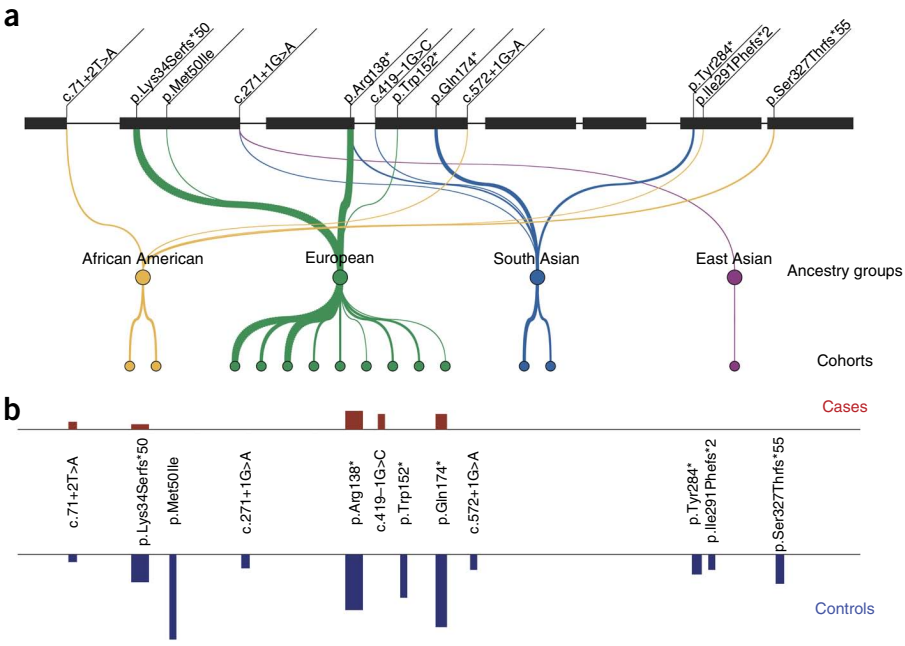

Figure 2 Protein-truncating variants identified in *SLC30A8*. Through sequencing and genotyping of nearly 150,000 individuals across 5 ancestry groups, we identified 12 *SLC30A8* variants—each rare and predicted to cause premature protein truncation. (**a**) Shown is the position of each variant on the islet-specific *SLC30A8* transcript (NM_173851; ref. 20). p.Met50Ile is predicted to alter the initiator codon in other transcripts of *SLC30A8*. Lines are drawn from each variant to the ancestry groups in which carriers were observed, with greater width corresponding to more observations. Lines are further drawn from each ancestry group to the populations (cohorts) in which carriers were identified. From left to right, the cohorts are JHS, WFS, Botnia, Danish, deCODE, Finnish, HUNT2, KORA, Malmö, PIVUS/ULSAM, WTCCC, LOLIPOP, Singapore Indians and KARE (cohort information appears in the **Supplementary Note**). Ancestry groups or cohorts with no observations are not shown. (**b**) Graphical representation of the case (red) and control (blue) frequencies for each observed variant. Wider bars correspond to more observations. A quantitative and complete representation of these data is given in **Table 1**.

We investigated potential confounding factors for the observed protective association. First, we assessed whether the haplotypic background encoding the p.Trp325Arg variant might influence results. Whereas the variants encoding p.Lys34Serfs*50 and p.Met50Ile were observed on the protective common variant haplotype, the remaining variants (including the one encoding p.Arg138*) were observed on the risk-associated common variant haplotype. Thus, independent protective protein-truncating variants were observed on opposite haplotypic backgrounds encoding p.Trp325Arg. Second, we tested for a survivor effect, where carriers of rare variants with diabetes would die at a younger age than non-carriers. However, (i) carrier ages did not differ significantly from non-carrier ages for either p.Arg138* (69.6 ± 8.4 years versus 65.5 ± 11.0 years for cases ($P > 0.1$), 46.4 ± 15.7 years versus 50.3 ± 15.5 years for controls ($P > 0.1$)) or p.Lys34Serfs*50 (70.5 ± 4.5 years versus 65.6 ± 13.8 years for cases ($P > 0.1$), 48.5 ± 20.1 years versus 50.0 ± 23.2 years for controls ($P > 0.1$)), and (ii) p.Lys34Serfs*50 association attained equivalent significance even when the analysis was restricted to age-matched controls. Finally, although we employed well-established methods using linear mixed models or principal components to control for population structure[26], we acknowledge the noted challenges to address this potential confounder in rare variant association studies[27]. We had insufficient data to control for stratification via a family-based transmission disequilibrium test (pedigree information was only available for Icelanders, with three carrier parents all transmitting the risk-associated allele to affected children). However, the consistent association of multiple independent protein-truncating variants across multiple cohorts and ancestry groups argues against population stratification being responsible for 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 encoding p.Trp325Arg is associated with proinsulin and fasting plasma glucose levels at genome-wide significance[5,6], as well as with 2-h glucose levels after an oral glucose tolerance test (OGTT) at nominal significance (**Supplementary Table 11**)[27]. 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 the traits analyzed (**Supplementary Table 12**), the strongest statistical evidence for association was observed in Icelanders between p.Lys34Serfs*50 and random (non-fasting) glucose levels: non-diabetic carriers of the protective allele had lower glucose levels ($\beta = -0.17$ 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 levels were lower at 1 h in the small number of p.Lys34Serfs*50 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 levels, although the directions of effect were consistent with those described above—these levels were 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 parallel directionality of glucose levels and T2D risk is similar to the pattern observed for p.Trp325Arg, where the T2D-protective allele also associates with lower glucose levels (**Supplementary Table 9**), providing further evidence against a survivor effect or population stratification 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 the ~20,000 genes in the human genome ($P = 1.7 \times 10^{-6}$). Non-diabetic Icelandic carriers of p.Lys34Serfs*50 also demonstrated lower glucose levels ($\beta = -0.17$ s.d., $P = 4.6 \times 10^{-4}$). Notably, initial sequencing of 115 genes in 758 individuals with extreme T2D phenotypes resulted in only 2 observations of the variant encoding p.Arg138*, 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 (with correction for 20,000 genes) required genotyping of ~150,000 individuals spanning multiple ancestry groups. Detecting similar effects in genes without previous evidence of association may require analysis on a similar or larger scale, for not only T2D but also for other complex traits.

Previous modeling of the relationship between ZnT8 activity and T2D risk centered on p.Trp325Arg, which results in mildly attenuated zinc transport that is concomitant with increased T2D risk[8], and *Slc30a8* knockout mice, where phenotypic heterogeneity is observed[13,15]. We find a clear and consistent association between putative *SLC30A8* loss-of-function variants and T2D risk across multiple ancestry groups, demonstrating convincingly that a 50% reduction in gene dosage protects against T2D in humans. These data reject the model in which loss of ZnT8 function is associated with as little as a 1.2-fold increase in T2D risk (similar to the increase in risk observed for the common p.Trp325Arg variant) at significance of $P \approx 1 \times 10^{-9}$. Phenotypic interrogation of human mutation carriers is needed to determine the physiological mechanism behind this protective association and to establish the effects of *SLC30A8* haploinsufficiency in the pancreas and other tissues[21].

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 ZnT8 activity and T2D risk in humans. The expanded *SLC30A8* allelic series offers a more functionally informative catalog of variation compared to the p.Trp325Arg variant alone, enabling future experiments investigating potential mechanisms. Although substantial work is required to understand how reduced ZnT8 activity lowers T2D risk, the current observations motivate experiments to test ZnT8 inhibition in T2D treatment in human populations.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. *SLC30A8* transcript, NM_173851 (ref. 20); ZnT8 protein, NP_776250 (ref. 20).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

This manuscript describes an analysis spanning four initially distinct sequencing studies—a collaborative project between Pfizer, Massachusetts General Hospital, the Broad Institute and Lund University 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[23], 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 J.F. and D.A., with final analysis combining data from all variants collected by J.F. The manuscript was written by J.F., D.A. and K.S., and all authors reviewed, edited and approved the manuscript. Author contributions specific to the sequencing or genotyping studies are as follows.

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE 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 from other cohorts drawn from different European populations, were genotyped for the *SLC30A8* nonsense SNV encoding p.Arg138* using the Illumina HumanExome v1.1 array. All sequenced individuals were also genotyped, with data showing 100% concordance.

Icelandic individuals were genotyped for the frameshift variant encoding p.Lys34Serfs*50 using a combination of whole-genome sequencing and imputation (either direct imputation based on chip genotyping or family-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 additional carriers of rare variants) 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.Arg138*, p.Lys34Serfs*50 and the remaining variants. For p.Arg138*, association analysis was separate for each analyzed cohort and used a linear mixed model to account for sample structure, including population stratification and genetic relatedness. Results were combined via a fixed-effects meta-analysis. For p.Lys34Serfs*50, association analysis was performed in Iceland using logistic regression, with controls matched to cases on the basis of 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, 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.

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