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A novel splice-affecting \textit{HNF1A} variant with large population impact on diabetes in Greenland

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Summary

Background The genetic disease architecture of Inuit includes a large number of common high-impact variants. Identification of such variants contributes to our understanding of the genetic aetiology of diseases and improves global equity in genomic personalised medicine. We aimed to identify and characterise novel variants in genes associated with Maturity Onset Diabetes of the Young (MODY) in the Greenlandic population.

Methods Using combined data from Greenlandic population cohorts of 4497 individuals, including 448 whole genome sequenced individuals, we screened 14 known MODY genes for previously identified and novel variants. We functionally characterised an identified novel variant and assessed its association with diabetes prevalence and cardiometabolic traits and population impact.

Findings We identified a novel variant in the known MODY gene \textit{HNF1A} with an allele frequency of 1.9% in the Greenlandic Inuit and absent elsewhere. Functional assays indicate that it prevents normal splicing of the gene. The variant caused lower 30-min insulin ($\beta = -232 \text{ pmol/L, } \beta_{SD} = -0.695, P = 4.43 \times 10^{-4}$) and higher 30-min glucose ($\beta = 1.20 \text{ mmol/L, } \beta_{SD} = 0.441, P = 0.0271$) during an oral glucose tolerance test. Furthermore, the variant was associated with type 2 diabetes (OR $4.35, P = 7.24 \times 10^{-6}$) and HbA1c ($\beta = 0.113 \text{ HbA1c\%}, \beta_{SD} = 0.205, P = 7.84 \times 10^{-3}$). The variant explained 2.5% of diabetes variance in Greenland.

Interpretation The reported variant has the largest population impact of any previously reported variant within a MODY gene. Together with the recessive \textit{TBC1D4} variant, we show that close to 1 in 5 cases of diabetes (18%) in Greenland are associated with high-impact genetic variants compared to 1–3% in large populations.

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These authors contributed equally.
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**Greenlandic Summary**
Qulequtaq HNF1A-mi pinngoqqaammi allannguut suussussersineqarlaaq, pinngoqqaatinik allangguinermut sunniuteqartoq. Kalaallit Nunaami diabetesimik nappaateqarnermut annertuumik sunniutilik.

**Tunuliaqutaasooq** Nagqeqatigiit Inuit akornanni nannapait pinngoqqaatikkut katitigaanerat, pinngoqqaatikkut allannguutaqagjuttunik amerlaqisunik nappaateqalersinnaanermut annertuumik sunniuteqarnermik aqoqarput. Allangguuutunik taamaatunik suussussersineq, pinngoqqaatinik nannapait patisasiutikatsisegtaaqqor, nunarsuarmlu nakorsaanaermut nappatinillu suussussersinnaanermut atallillugtu pinngoqqaatini kigqornuyattaqakani misissuinermi naliqginnerulsitsilluni. Misissuinermi matumani, pinngoqqaatini allangguuutunik, diabetesimik pinngoqqaammi akornuteqarnermik patisaeqartumi Maturity Onset Diabetes of the Young (MODY), Kalaallit Nunaami innuttasununut nalinginnaanerpaamit iliisimaneqartunik suussussersinniaaqq laaqilisinnissarlulun suniertaaqov.

**Periuseq** Innuttaasut katillugit 4497-it taakkunannga 448-it, pinngoqqaatit, pinngoqqaateqar qatqakunnaarlu ayekit, pinngoqqaatit MODY-it ilisimaneqartut 14-it, ilisimaneqartunik ilisimaneqanngitsunillu allannguuteqarnermut. Allannguutuput suussussersinneqarlaap qaqaq atituuinanik saasaavugut, diabetesillu atugaaneranun nutumalluutuqeqarnermullu inouriaatikumik patisaeqartumut ilisarnaatit innuttaasunuq diabetesimut atassutaa misissuqqaasaraarluq.

**Paasiaq** Nagqeqatigiimmi kalaallini pinngoqqaammi allannguut ilisimaneqartumi MODY-mi HNF1A maannamut suussussersineqanngitsoq, pinngoqqaammi allannguut 1.9%-imik akulikissusilik, inuiannilu allani nassuiarneqartumit innuttaasunut annertunerusumik sunniuteqarpoq. Pinngoqqaammi allannguut qatqat qanooq ittuunanerik saasaavugut, diabetesimik allannguutip 2.5%-ia nassuiarpaa.

**Nassuiaat** Pinngoqqaammi allannguut nalunaarutigineqartuoq pinngoqqaammi MODY-mi allannguuummiit siornatigut nassuiaaerneqartumit innuttaasununut anertunerusumik sunniuteqarpoq. Pinngoqqaammi allannguut kinguaanit kigornunneqarsinniaarlu TBC1D4 ilangguullugtu, Kalaallit Nunaami diabetesimik nappaateqartut tallimaapta atatsip pallingajattup (18%)-innuttaasunut amersaluumut 1%-inut sanilliullugtu-pingqoqqaammi allannguutiut nappaateqalersinnaamut annertuumik sunniuteqarnermut atassuteqarnera takutipparput.

**Aningaasaliisut** Novo Nordisk Fonden, Danmarks Frie Forskningsfond kiisalunut Karen Elise Jensens Fond.©

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**Introduction**
Diabetes is a global health burden that disproportionately affects certain population groups.1 Identifying population-specific genetic susceptibility is necessary to recognize diabetes subtypes that may have implications for prevention, diagnosis, and clinical care, thereby improving equity in genomic medicine.

Population-specific genetic variants have been identified, both in known and novel monogenic diabetes genes. In Mexican and US Latino individuals, the E508K variant of the known Maturity Onset Diabetes of the Young (MODY) gene HNF1A is associated with an increased risk (OR = 5.5) of type 2 diabetes (T2D).2 Analysis of diverse populations revealed that this variant is very rare or absent in other populations and thus unique to individuals with Native American ancestry. In Oji-Cree individuals, the HNF1A G319S variant is associated with an increased risk of diabetes (OR 4.00 for homozygous carriers and OR 1.97 for heterozygous carriers)3 and earlier age of onset.4 Whole genome sequencing data from Pima Indians identified the R1420H variant of the known neonatal diabetes gene ABCC8, which was associated with a moderately increased risk (OR = 2.0) of diabetes, earlier age of diagnosis, and lower BMI.5 Genetic studies in diverse populations have advanced the discovery of novel disease-associated genes and loci. A novel haplotype in SLC16A11 has been reported,
which moderately increases diabetes risk and is common among the Mexican and Latin American population, but rare in Europeans. Conversely, population-specific genetic factors may also protect against disease. This is the case for two independent protein-truncating variants in SLC30A8, most common among Finnish and Icelandic individuals, and a variant near CCND2 also identified in Icelandic individuals.

We have previously shown that unique genetic variants contribute substantially to diabetes development in Greenland. A loss-of-function variant in TBC1D4 has a recessive odds ratio of ten for T2D, and leads to decreased protein expression of the GLUT4 glucose transporter and consequently postprandial muscle insulin resistance. The variant is population-specific to Inuit with an allele frequency of 17% and accounts for more than 10% of all diabetes cases in Greenland. Additionally, a low frequency splice-site variant in ADCY3 substantially increases the risk of T2D and overweight among Greenlanders, and two common variants in LARGE1 and ITGA1 are associated with T2D with moderate effect sizes. These examples show that the contribution of high-impact genetic effects related to diabetes is high in Greenland compared to larger outbred populations, where the common loci most strongly associated with diabetes, such as TCF7L2, only infer a moderately increased risk.

Motivated by the unique genetic composition of the Greenlandic population, we used whole-genome sequencing data from 448 Greenlandic individuals to screen for population-specific variants in known MODY genes, in order to investigate to which degree variation within these genes contribute to the genetic burden of diabetes in the Greenlandic population. This approach allowed us to identify a novel variant that was not captured by previous genome-wide association studies in the Greenlandic population. Here we characterise this variant in terms of its associations to cardiometabolic traits, its functional consequences and its impact on diabetes risk in the Greenlandic population.

**Methods**

**Ethics statement**

The studies were approved by the Scientific Ethics Committee in Greenland (project 2011–13 (ref. no. 2011–056978), project 2013–13 (ref. no. 2013–090702), project 2015–22 (ref. no. 2015-16426), and project 2012–16/17 (ref. no. 2017-12997)), and conducted in accordance with the Declaration of Helsinki, second revision.

**Study participants**

Participants were pooled from two cross-sectional cohorts: B99 (n = 1788, 1998–2001) and Inuit Health in Greenland, the findings in this study underline the importance of performing genetic discovery studies in smaller populations covering all genetic ancestries, to ensure that genetic discovery is conducted equitably. Furthermore, this study challenges the notion that non-communicable diseases are primarily polygenic in aetiology, and emphasises the importance of considering ancestry in the aetiology of diabetes.
Anthropometric and biochemical measurements

Anthropometric and biochemical data collection have been described previously. HbA1c, serum total cholesterol HDL-cholesterol and triglyceride concentrations were measured at fasting. LDL cholesterol was calculated using the Friedewald formula. Plasma deoxyglucose, serum insulin, and C-peptide were measured at ∼

Participants without medication-treated diabetes 18 years of age (IHIT) or ≥35 years of age underwent an oral glucose tolerance test (OGTT) as previously described, sampled at fasting and 2 h (2-h) after glucose ingestion. In the B2018 follow-up study, the OGTT was further sampled at 30 min.

In the OGTT from the IHIT and B99 cohorts, HOMA-IR, insulin sensitivity index ISI0,120 and HOMA-B were calculated as crude measures of insulin resistance, insulin sensitivity, and beta-cell function. In the B2018 cohort, we calculated the insulinogenic index (IGI) as an expression of beta cell function, and the Matsuda insulin sensitivity index (ISI) as an expression of insulin sensitivity.

For case/control analyses, a diagnosis of T2D was defined as 1) having known T2D prior to the examination or 2) WHO 1999 criteria from the OGTT, whereas controls were normoglycemic (i.e. absence of diabetes, impaired fasting glucose, or impaired glucose tolerance).

Whole genome sequencing

DNA for whole genome sequencing (WGS) and genotyping (see below) was extracted from buffy coat stored at ~80°C.

A subset of 448 Greenlandic individuals from IHIT and B99 cohorts, selected based on sampling location independently of phenotype and disease status, underwent Illumina WGS with average sequencing depth of ~35x. Reads were cleared for adapters using bwa mem -t 24 -p -Y -K 10000000. After mapping, duplicated reads were marked. Genotype calling was done using GATK haplotype caller and variant quality score recalibration (VQSR) tools based on the GATK resource bundle. Only variants in the T98 tranche and above were used. The sites were parsed through Plink (v1.90b6), keeping the two most common alleles of multiallelic sites. The remaining variants were annotated using the Ensembl Variant Effect Predictor release 104.3.

Genetic screening

Fourteen genes in which functionally disruptive variants are known to cause MODY (OMIM entry #606391) were screened in the WGS data. Among these, we extracted variants predicted to have moderate or high impact. Variants that were already reported (found in gnomAD v3.0.0 or dbSNP build 155) and only observed in one individual were removed. The distribution of ClinVar (20210821) MODY variants in the data was analysed in order to assess the presence of known MODY variants. Given the population subset, there was >80% power to detect variants with a minor allele frequency (MAF) >0.2%.

Genotyping

A novel c.1108G>T variant was genotyped in all individuals using the KASP Genotyping Assay (LGC Genomics, Berlin, Germany). The genotyping call rate was 99.8% and there was a concordance rate of >99.9% between genotyping and imputation.

We used a previously described genome-wide genotype dataset generated from the Multi-Ethnic Global Array (MEGA chip, Illumina). After quality control, it comprised 1.6 mil. variants. We used phased 1000 Genomes and the phased Greenlandic WGS as reference for imputing common (MAF > 0.5%) variants. The imputed data was used to estimate a genetic similarity matrix (GSM) and admixture proportions. For local ancestry, we performed additional phasing and imputation which included inferred family relationships to avoid switch errors.

Estimation of allele frequency

To estimate the allele frequency of the c.1108G>T variant in the Greenlandic population, we first estimated the frequency as the fraction of variant copies among the individuals genotyped for the variant. We also estimated the frequency separately for the Inuit ancestry component and for the European component of the population using ADMIXTURE v1.3.0 assuming two ancestral populations (Inuit and European).

To investigate the allele frequency of the c.1108G>T variant in other populations, we looked up frequencies in gnomAD, Human Genetic Diversity Panel (HPDP), Simons Genome Diversity Panel, and 1000 Genome Project. We used samtools, BGT and vcftools to estimate allele frequencies in each population. Finally, we used samtools to estimate allele frequencies from whole genome data from several ancient and contemporary genomes from the Americas and the Arctic.
Local ancestry
To visualise the Inuit-specific ancestry of the c.1108G>T variant, we estimated local ancestry for admixed individuals. Individuals with >99% Inuit ancestry were used as Inuit reference, and unrelated individuals from the 1000 Genome populations TSI, CEU, GBR, and IBS were used as European reference. Only sites present in both references and admixed individuals with a MAF > 5% across all three groups were used as input to RFMix (v2.03-r0).**

Association analyses
To test for associations between the c.1108G>T variant and quantitative traits, we used a linear mixed model to take relatedness and admixture into account (GEMMA**

\[ y_{rank} = \phi^{-1} \left( \frac{\text{rank}(y) - 0.5}{n} \right) \]

P-values are from analyses of transformed data and effect size estimates are reported in standard deviations as \(\beta_{SD}.\) We also estimated effect sizes from non-transformed data, reported as \(\beta.\) To test for association with T2D, we used a logistic mixed model (GMMAT**

Minigene assay
To assess the c.1108G>T variant on splicing, exon 6 of HNF1A and flanking intron sequences (86bp and 167bp) was amplified from genomic DNA of two carriers (WT and c.1108G>T) and cloned into the pCAS2 splicing minigene vector as previously described.26 As carriers were heterozygous, variant splicing effect was evaluated by comparing the effect of the corresponding WT sequence of the same individual. Total RNA was isolated from HeLa cells transiently transfected with plasmids (WT or c.1108G>T). Samples were analysed by RT-PCR followed by agarose gel electrophoresis and Sanger sequencing.

Population effect of diabetes variants
To compare population effects, we collected GWAS summary statistics of associated variants from two large studies of 898,130 individuals of European ancestry*** and 433,540 East Asian individuals.51 Summary statistics from previously reported population-specific T2D variants were collected from Amerindian,20 Icelandic,6 and Finnish populations. The effect of rare variant burden on T2D in the UK biobank (UKBB) was collected for HNF1A and GCK.33 To have comparable estimates for the explained variance, we assumed a population diabetes prevalence of 10% for all populations. For the E508K variant, the allele frequency was calculated separately for individuals with and without diabetes and weighted by diabetes prevalence of 10%. For visualisation of variants with recessive effects, the frequency of homozygous individuals was used instead of the allele frequency. The R-package Mangrove (v.1.21) was used to calculate liability-scale variance explained (LVE) for each variant separately.

Role of the funding source
None of the funding agencies had any role in the study design or collection, analysis, or interpretation of the data.

Results
Screening for known and novel functionally disruptive variants in known MODY genes
We screened fourteen MODY genes (Fig. 1A) for known and novel genetic variants using WGS data from 448
Greenlandic individuals (Supplementary Figure S1). First, we explored the presence and frequency of known, ClinVar-annotated variants. We found 91 benign variants at varying allele frequencies, nine variants with conflicting interpretations of pathogenicity at low allele frequencies (MAF<1%) and a single variant annotated as likely pathogenic (Supplementary Table S1). The likely pathogenic variant was present in only one allele in one individual who did not have diabetes. Secondly, we screened for novel functionally disruptive variants in the MODY genes. This screening led to the discovery of a variant, located in exon 6 of HNF1A, which was predicted to be a missense variant (hg19: chr 12 pos 121434344, c.1108G>T, p.Val370Phe; V370F, ENST00000257555/NM_000545.8) near a splice-site (Fig. 1A).

Ancestry component analysis and population screening
To investigate the frequency of the c.1108G>T variant, the variant was genotyped in 4497 Greenlanders from the population-based cohorts (Supplementary Figure S1). We identified 119 heterozygous, and no homozygous carriers, leading to an estimated allele frequency in the Greenlandic population of 1.3%. The Greenlandic population is admixed and we estimated the allele frequency to be 1.9% in the Inuit component and 0.0% in the European. We did not find the variant in sequencing data from more than 200,000 ancient and contemporary individuals from across the world (Supplementary Table S2). Local ancestry of all admixed and c.1108G>T-heterozygous Greenlandic individuals (n = 87) showed that the variant was exclusively found on a background of Inuit ancestry, whereas the reference allele (HNF1A WT) was found on both European and Inuit ancestry (Fig. 1B). Collectively, this suggests that the variant is common in the Greenlandic population and absent, or extremely rare, in non-Inuit populations.

Associations with T2D and related phenotypes
We tested the association of the variant with T2D and related phenotypes (Table 1). The variant showed strong association with T2D (OR = 4.35, P = 7.24 × 10−6) and...
higher HbA1c levels ($\beta = 0.11$ HbA1c%, $\beta_{SD} = 0.20$, $P = 7.84 \times 10^{-3}$) (Fig. 1C and D). Among the T2D cases, 6.8% were heterozygous for the c.1108G>T variant whereas 2.2% of glucose-tolerant individuals were heterozygous (phenotypes stratified by diabetes status are presented in Supplementary Table S3). Combined with the previously described recessive $TBC1D4$ variant, 18% of diabetes cases in the cohort were either heterozygous for the c.1108G>T variant or homozygous for the $TBC1D4$ variant, compared to 4% of the glucose-tolerant individuals. The median age of NGT carriers was lower than that of the T2D carriers (NGT median age 38.5 versus T2D median age 55.5).

In the B2018 follow-up study, we further assessed indices of insulin sensitivity and beta cell function (Table 1). Interestingly, c.1108G>T carriers had a much lower 30-min insulin ($\beta = -232$ pmol/L, $\beta_{SD} = -0.70$, $P = 4.43 \times 10^{-4}$) and a higher 30-min glucose ($\beta = 1.20$ mmol/L, $\beta_{SD} = 0.44$, $P = 0.027$), while the fasting and 2-h timepoints showed no significant differences (Fig. 1E and F). We found strong association with estimated beta cell function IGI ($\beta = -4.19$, $\beta_{SD} = -0.860$, $P = 1.15 \times 10^{-5}$) but no association with estimated insulin sensitivity ISI ($\beta = 0.99$, $\beta_{SD} = 0.296$, $P = 0.1359$). The lack of association to fasting and 2-h measures was further supported in the OGTT data from the B99 and IHIT cohorts (Supplementary Table S4).

### Functional analyses

The c.1108G>T variant is located within the transactivation domain of $HNF1A$ in the first nucleotide of exon 6 (Fig. 2A) and could have functional effects either through the amino acid substitution and/or by affecting splicing. We analysed the potential effects of the amino acid substitution on transcriptional activity and protein abundance, and the effect on splicing in a minigene splice assay.

The c.1108G>T variant showed near-normal levels of activity compared to WT $HNF1A$ protein (mean normalized luciferase activity 105.5% ± 4.5) (Fig. 2B). We compared this to the Mexican/US Latino T2D-risk variant E508K, and two known MODY variants (P112L and P447L). The E508K variant exhibited an almost 50% reduction in activity (53.5% ± 7.9) whereas the P112L and P447L variants, showed a severe effect on $HNF1A$ activity (16.3% ± 1.8 and 19.6% ± 2.8), similar to what has been described earlier.

When assessing the effect on protein expression, levels were unchanged for the c.1108G>T/V370F variant compared to WT $HNF1A$ levels (mean relative protein

### Table 1: Associations between the c.1108G>T and measures of glucose metabolism, lipid metabolism, and body composition.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>$\beta_{SD}$ (SE)</th>
<th>$\beta$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>4443</td>
<td>0.0850 (0.0957)</td>
<td>0.2926</td>
<td>0.3741</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>4412</td>
<td>0.0715 (0.0874)</td>
<td>0.0051</td>
<td>0.4130</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4443</td>
<td>0.2047 (0.0770)</td>
<td>0.1127</td>
<td>7.84 $\times 10^{-3}$</td>
</tr>
<tr>
<td>Fasting serum total cholesterol (mmol/L)</td>
<td>4227</td>
<td>-0.0443 (0.0908)</td>
<td>-0.0662</td>
<td>0.6522</td>
</tr>
<tr>
<td>Fasting serum HDL-cholesterol (mmol/L)</td>
<td>4468</td>
<td>-0.0002 (0.0916)</td>
<td>0.0011</td>
<td>0.9979</td>
</tr>
<tr>
<td>Fasting serum LDL-cholesterol (mmol/L)</td>
<td>3818</td>
<td>-0.0470 (0.0987)</td>
<td>-0.0660</td>
<td>0.6337</td>
</tr>
<tr>
<td>Fasting serum triglyceride (mmol/L)</td>
<td>3979</td>
<td>0.0485 (0.1011)</td>
<td>0.0016</td>
<td>0.6314</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>3981</td>
<td>0.0879 (0.0968)</td>
<td>1.4425</td>
<td>0.3628</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>3981</td>
<td>-0.0098 (0.0928)</td>
<td>0.5572</td>
<td>0.9162</td>
</tr>
<tr>
<td>Cases/control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2D</td>
<td>311/2565</td>
<td>1.47 (0.33)</td>
<td>4.35</td>
<td>7.24 $\times 10^{-5}$</td>
</tr>
<tr>
<td>T2D (no adj. for age &amp; sex)</td>
<td>311/2565</td>
<td>1.15 (0.28)</td>
<td>3.18</td>
<td>5.61 $\times 10^{-5}$</td>
</tr>
</tbody>
</table>

SE = standard error, n = number of individuals available for the association analysis. The effect size is estimated without transformation ($\beta$) or after rank-based inverse normal transformation ($\beta_{SD}$).

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The c.1108G>T variant is located within the transactivation domain of $HNF1A$ in the first nucleotide of exon 6 (Fig. 2A) and could have functional effects either through the amino acid substitution and/or by affecting splicing. We analysed the potential effects of the amino acid substitution on transcriptional activity and protein abundance, and the effect on splicing in a minigene splice assay.

The c.1108G>T variant showed near-normal levels of activity compared to WT $HNF1A$ protein (mean normalized luciferase activity 105.5% ± 4.5) (Fig. 2B). We compared this to the Mexican/US Latino T2D-risk variant E508K, and two known MODY variants (P112L and P447L). The E508K variant exhibited an almost 50% reduction in activity (53.5% ± 7.9) whereas the P112L and P447L variants, showed a severe effect on $HNF1A$ activity (16.3% ± 1.8 and 19.6% ± 2.8), similar to what has been described earlier.

When assessing the effect on protein expression, levels were unchanged for the c.1108G>T/V370F variant compared to WT $HNF1A$ levels (mean relative protein...
expression 93.7% ± 14.9) (Fig. 2C). In comparison, the P447L control variant showed lower protein expression levels (55.8% ± 14.0) while a slight reduction was observed for the E508K and P112L variants.

To address the variant’s effect on potential splice events, we performed a minigene splice assay (Fig. 2E). This assay revealed that the c.1108G>T variant allele yielded a transcript around 200bp shorter than the WT allele suggesting that the variant caused skipping of the entire 202bp length of exon 6 within the context of the minigene assay (Fig. 2D). This is suggestive of a splice-affecting variant although the assay cannot determine the exact nature of the full mutant HNF1A transcript.

**Population impact**

In order to contextualise the population impact of c.1108G>T compared to T2D variants identified in other large and small populations, we plotted the odds ratio as a function of the minor allele frequency (Fig. 3A). For the majority of variants with an allele frequency above 1% we observed moderate odds ratios in the range of 0.8 to 1.4. In the European and Asian populations, larger effects were only found for variants with allele frequencies below 1% or in the burden of rare variants in HNF1A and GCK from UKBB. We also included other population-specific variants (Mexican/US Latino HNF1A, SLC16A11 haplotype, Pima ABCC8, Finnish and Icelandic SLC30A8, Icelandic CCND2, and previously identified diabetes-associated Greenlandic variants). For these population-specific variants, the observed effects were higher compared to other variants with similar allele frequencies. However, the Greenlandic-specific novel HNF1A c.1108G>T and the recessive TBC1D4 variant had strongly increased odds ratios relative to the allele frequencies. This difference is further accentuated when comparing the population T2D variance explained by each variant (Fig. 3B). Here, TBC1D4 and HNF1A c.1108G>T variants explained 7.3% and 2.5% of the T2D variance in Greenlanders, respectively, whereas the variance of T2D explained by the European TCF7L2 in Europeans was less than half that (1.1%) and all other variants explained less than 1% of the T2D variance. The G319S Oji-Cree variant was identified in 55 unrelated diabetic and 148 unrelated controls. The study did not take population structure into account and we were unable to obtain corrected effect size estimates and allele frequencies. For this reason the variant was not included in Fig. 3. However, assuming the reported MAF of 9.85% and OR of 1.97 are not biased by population structure, we estimate the liability-scale variance explained to 2.2%.
In this study, we analysed the genomes of Greenlandic individuals for the presence of variants in known MODY genes in order to address whether common variants in these genes, which have previously been shown to harbour population-specific variants that increase the risk of diabetes, contribute to the burden of T2D in Greenland. We identified a previously unknown variant, c.1108G>T in the known MODY gene HNF1A, which was associated with a highly increased risk (OR = 4.4) of developing T2D and decreased beta-cell function, and which was revealed in ancestry analysis to be unique to the Greenlandic population. We showed that the variant is likely to affect splicing and that it had the largest population-impact of any previously identified variant within a MODY gene and that almost 1 in 5 diabetes cases in this representative population cohort are associated with high-impact genetic variants, compared to 1–3% in European populations.

In the present study, the c.1108G>T variant was associated with significantly lower 30-min insulin levels and higher 30-min glucose levels but not with fasting or 2-h measures of glucose, insulin or C-peptide. The 30-min results from an OGTT are closely associated with first-phase insulin secretion derived from an intravenous glucose load, unlike the 2-h measures which are affected by both the insulin secretory capacity and peripheral insulin resistance. Accordingly, we observed strong associations between the variant and the beta cell function index IGI, indicative of a primary beta-cell defect, which is consistent with the known effects of HNF1A variants. Patients with HNF1A-MODY have lower insulin levels compared to non-diabetic controls, as do non-diabetic carriers of pathogenic MODY variants compared to WT individuals, and carriers of less disruptive variants in HNF1A, such as the common A98V have lower C-peptide at 30-min in an OGTT, similar to the lower 30-min insulin levels found for the identified c.1108G>T variant.

MODY is characterised by a lack of features associated with T2D, such as insulin resistance, higher body weight or abdominal fat deposition, and further by an altered blood lipid composition. In this study, we did not see any association between the c.1108G>T variant and any measure of lipid metabolism, insulin sensitivity, or body composition. However, screenings of MODY genes in an unselected diabetic population indicate that common clinical features are inconsistent in discriminating between clinical MODY and a T2D-like risk phenotype.

Similarly, the population-specific HNF1A E508K variant was not associated with any examined clinical characteristic between carriers and non-carriers, including age of diagnosis, BMI, or fasting glucose levels despite the substantial impact on diabetes risk.

Our functional studies indicate that the molecular effects of the c.1108G>T variant are caused by defective splicing, rather than by the isolated effect of the amino acid substitution.
acid substitution. While splice variants are well-established in \textit{HNF1A-MODY}, there are limited \textit{in vitro} functional studies reported on these variants.\textsuperscript{11} The most likely molecular mechanism of the identified c.1108G>T variant is haploinsufficiency, consistent with the known pathogenesis of \textit{HNF1A} variants.\textsuperscript{3,4} The variant effect indicated by the functional assays and the variant effect size may seem incongruent. However, the effect of a genetic variant may be modified by other genetic or environmental effects,\textsuperscript{65–69} and, as mentioned above, it is challenging to compare effect sizes, penetrance, and defining clinical characteristics between case–control and population studies. The functional assays used in this study are standard in the functional characterization of \textit{HNF1A}, which is only expressed in tissues that are difficult to extract. However, an ideal functional characterization would be based on beta cells cultured from Greenlandic carriers, in order to ensure that the variant is characterised in the relevant tissue and in the unique biological context of Greenlanders.

Future studies are necessary to address the exact composition of the mutant transcript, and the mechanism of disease for example through gene-editing or studies of induced pluripotent stem cells. Importantly, we show that the combination of the large effect size and high frequency of the \textit{HNF1A} c.1108G>T variant constitutes the largest population impact of any variant within a known MODY gene reported to date. Collectively, the previously reported recessive \textit{TBC1D4} variant and the c.1108G>T variant explain the largest amount of variance for single variants by a large margin compared to all other replicable previously reported T2D-associated variants. A possible exception is the G319S variant from the small Sandy Lake Oji-Cree community with an estimated LVE% only slightly lower than c.1108G>T.\textsuperscript{3,4} However, a direct comparison is difficult because estimates of allele frequency and effect size for the G319S variant do not take population structure into account. The ancestral Inuit population to the present day Greenlanders is known to have been isolated and extremely small for thousands of years and was therefore strongly affected by genetic drift.\textsuperscript{71–73} As a consequence, high impact disease-causing genetic variants segregate at higher frequencies in the Greenlandic population than in larger populations. These results underline that while the genetic component of T2D disease burden within Europe and East Asia is highly polygenic, the Greenlandic population harbours common high penetrance variants, and serves as a reminder that this may be the case for other small populations. In fact, close to 1 in 5 of diabetes cases in Greenland carry either the \textit{HNF1A} c.1108G>T variant or are homozygous for the previously identified Inuit-specific \textit{TBC1D4} stop-gain variant. Therefore the combination of these two common high impact variants alone contribute to a large fraction of the heritability of T2D in Greenland. It also serves as a reminder of the strengths of studies based in small populations, where the number of carriers of the same deleterious variants is higher and the genetic causes of disease are more homogenous.

While individuals with \textit{HNF1A-MODY} generally respond well to treatment with sulphonylureas,\textsuperscript{74–76} it is not always the case that variants in \textit{HNF1A} result in an increased insulin secretory response to sulphonylurea. Notably, a study on the short-term response to the sulphonylurea glipizide showed that neither non-diabetic nor diabetic carriers of the Mexican/US Latino \textit{HNF1A} E508K variant were sensitive to sulphonylurea stimulation.\textsuperscript{75} Genome-wide meta-analysis also does not implicate any common \textit{HNF1A} variants in the glycemic response to sulphonylurea treatment.\textsuperscript{7} Whether carriers of the c.1108G>T variant could benefit from treatment with sulphonylurea should be pursued within the context of a randomised clinical trial establishing both short and long-term efficacy of sulphonylurea in these patients. Beyond potential pharmacogenetic benefits lie further personalised medicine potentials for diagnosis and management that take into account the individual’s ancestral, genetic, and environmental background, and underlines the importance of a genetically-driven approach to diabetes management in Greenland. This includes tailored early detection approaches in family members, and a more precise stratification of diabetes within the population that provides an avenue for directed health care efforts in the future. These potentials are in line with the multifaceted areas defined within precision medicine, including precision diagnosis, prognostics, as well as treatment.\textsuperscript{77}

In conclusion, the novel \textit{HNF1A} c.1108G>T is unique to Greenlandic Inuit and has the largest population T2D impact of previously reported variants in known MODY genes to date. This finding emphasises that the Greenlandic population is uniquely impacted by common high-penetrance variants, as the previously identified \textit{TBC1D4} variant and the newly identified c.1108G>T variant affect 18% of Greenlanders with diabetes, and underlines the importance of population-specific genetic discovery as a driver of genomic-based personalised medicine.

\textbf{Contributors}

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Data sharing statement
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Appendix A. Supplementary data
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