INTRODUCTION

The global burden of diabetes mellitus is projected to grow to 700 million people by 2045, making it one of the fastest growing diseases worldwide (1). It is currently the leading cause of micro- and macrovascular disease, leading to kidney failure, blindness, heart disease, and lower limb amputations (2). Diabetes is broadly categorized into type 1 (T1DM), type 2 (T2DM), and other rarer forms, all of which share the adverse health consequences of persistently elevated blood glucose. T1DM is caused by autoimmune destruction of insulin-producing pancreatic β cells, while T2DM is primarily mediated by peripheral insulin resistance. Both forms of diabetes eventually lead to progressive loss of pancreatic β cells and insufficient insulin secretion (3). Despite the discovery of effective medications for T2DM, such as Glucagon-like Peptide-1 (GLP1) agonists, Dipeptidyl peptidase 4 (DPP4) inhibitors, and sulfonylureas, most of these therapies rely on cells to secrete insulin. As a result, many patients with T2DM ultimately depend on daily insulin injections once endogenous insulin is no longer available, leaving a substantial unmet need for new targets for therapeutic intervention.

Understanding the genetic contributions to diabetes would help improve our understanding of underlying biological pathways, identify at-risk individuals, and guide more effective precision therapeutics. Genome-wide association studies (GWASs) have identified more than 60 loci for T1DM (4) and hundreds for T2DM (5, 6). With some exceptions, most of these variants map to noncoding regions of the genome, leaving us with few clear candidate genes (5). Without obvious leads, the consequences of these variants on glucose metabolism are challenging to explore mechanistically. GWASs are also limited in scope since they focus on common variants, which tend to have smaller effect sizes.

On the other hand, whole-exome sequencing can uncover the full spectrum of protein-coding variants, including rare and ultra-rare protein-coding variants that have demonstrably large effects on human traits. Of particular interest are loss-of-function alleles that protect against disease since inhibiting their gene products has clear, human-validated precedence for therapeutic intervention (7–9). The growing availability of whole-exome sequences in large populations with linked medical record data has ushered in a new era of gene discovery based on protein-coding variants that could constitute clinically efficacious target opportunities (10).

The largest exome sequencing study for T2DM to date included ~21,000 cases and ~24,000 controls and identified four genes that reached exome-wide significance (11). Here, we report an exome sequencing association study for diabetes in 412,394 multiancestry exomes from the UK Biobank (UKB) with linked health records. This cohort included 33,788 individuals with non–insulin-dependent T2DM, 23,880 with self-reported diabetes, and 4171 with insulin-dependent diabetes. Using our previously developed gene-level collapsing framework (12), we identified that hemizygous protein-truncating variants (PTVs) in the X chromosome gene MAP3K15 conferred 35% reduced odds of developing diabetes. This protective
effect correlated clinically with decreased circulating glucose and hemoglobin A1c (HbA1c) levels. The findings were replicated in two independent cohorts, the Mexico City Prospective Study (MCPS) and FinnGen. Within FinnGen, we identified a particular Finnish-enriched MAP3K15 PTV that is associated with decreased odds of developing both T1DM and T2DM. PTVs in MAP3K15 were not associated with any adverse phenotypes in a phenome-wide assessment of 15,719 clinical endpoints in the UKB, suggesting that this gene could be a safe and promising target for managing diabetes.

**RESULTS**

**Cohort characteristics and study design**

We processed exome sequences from 454,796 UKB participants through our previously described cloud-based pipeline (12). Through stringent quality control, we removed samples with low sequencing quality, with low depth of coverage, and from closely related individuals (Materials and Methods). For this study, we focused on five T1DM- and T2DM-related phenotypes based on self-reported and International Classification of Diseases 10th revision (ICD-10) data: unspecified diabetes mellitus (i.e., self-reported), non–insulin-dependent diabetes mellitus, insulin-dependent diabetes mellitus, “strict” insulin-dependent diabetes mellitus (excluding any individuals who were billed for both non–insulin-dependent and insulin-dependent diabetes), and use of metformin (table S1). In total, 33,788 cases mapped to at least one of the diabetes-related clinical phenotypes. The ancestral breakdown of cases included 30,359 of European ancestry, 2007 of South Asian ancestry, 1234 of African ancestry, and 188 of East Asian ancestry. We also assessed quantitative traits related to diabetes, including nonfasted blood glucose, glycosylated hemoglobin (HbA1c), and body mass index (BMI) (table S2).

We performed single-variant exome-wide association tests (ExWAS) and gene-level collapsing analyses to test for protein-coding associations with each diabetes phenotype (Materials and Methods). As previously described, our collapsing framework tests for gene-phenotype associations across 18,762 genes under 10 different non-synonymous collapsing models (including a recessive model) to evaluate a range of possible genetic architectures (Materials and Methods and table S3) (12). We performed two versions of the collapsing analysis: one restricted to individuals of European ancestry (~90% of the UKB participants here, the association between MAP3K15 and diabetes in the recessive model reached study-wide significance (unspecified/self-reported diabetes: OR = 0.70, 95% CI: [0.62, 0.79], P = 2.71 × 10⁻⁵). With the increased sample size of 394,692 European participants here, the association between MAP3K15 and diabetes in the recessive model reached study-wide significance (unspecified/self-reported diabetes: OR = 0.70, 95% CI: [0.62, 0.79], P = 5.0 × 10⁻⁶), firmly establishing a protective effect of MAP3K15 loss of function against developing diabetes.

Among the various forms of diabetes, the identified MAP3K15 variants were most significantly protective against T2DM (non–insulin dependent diabetes) (table S8). To determine whether recessive variation in MAP3K15 also protects from T1DM, we defined a T1DM-specific phenotype in the UKB (N = 881 cases) using available ICD-10 and primary care information (Materials and Methods). Under the recessive collapsing model, variation in MAP3K15 appeared to protect against T1DM, but the association did not achieve study-wide significance with the current T1DM sample size (OR = 0.52, 95% CI: [0.25, 1.09], P = 0.09) (table S8).

**Heterozygous versus hemizygous MAP3K15 PTVs**

Because the recessive collapsing model includes all nonsynonymous variants, including missense variants, we wanted to test whether the protective mechanism of MAP3K15 variation operated specifically through recessive loss of function. We thus assessed whether recessive PTVs remained associated with protection from diabetes when missense variants were excluded from the model. Because there were only 5 female homozygous carriers, we focused on hemizygous male (N = 1126) and heterozygous female carriers (N = 2604) of European ancestry to assess dose-dependent PTV effects. Heterozygous female carriers had 18% reduced odds of self-reported diabetes compared to female noncarriers (OR = 0.82; 95% CI: [0.64, 1.02], P = 0.076) (Fig. 1C and table S9). In comparison, hemizygous male carriers demonstrated a 35% decreased risk of developing diabetes compared to male noncarriers (self-reported; OR = 0.65, 95% CI: [0.48, 0.85], P = 0.001; Fig. 1C and table S9). Hemizygous male PTV carriers were also less likely to be prescribed the antidiabetic medication metformin (OR = 0.62, 95% CI: [0.40–0.92], P = 0.01) compared to heterozygous females (OR = 0.85, 95% CI: [0.60–1.17], P = 0.36) (Fig. 1C). The decrease in HbA1c levels was greater in hemizygous male carriers (β = -0.21 SD units, 95% CI: [-0.26, -0.15], P = 1.2 × 10⁻¹⁵) (table S10) than in heterozygous female carriers as well (β = -0.07 SD units, 95% CI: [-0.11, -0.04], P = 5.3 × 10⁻⁵) (Fig. 1D). While the decrease in HbA1c appears three times greater in male PTV carriers, the CIs of the point estimates are wide with the current sample size. Future studies with larger sample sizes will increase our confidence in the precise point.
estimates for hemizygous versus heterozygous PTV carriers. Nonetheless, the effect sizes for diabetes risk, HbA1c, and metformin use in hemizygous MAP3K15 PTV carriers compared to heterozygous carriers demonstrate that the protective effect of MAP3K15 loss is dose dependent.

**MAP3K15 variant-level analyses**

We find that PTVs occurred throughout the MAP3K15 sequence, with two more frequent variants accounting for 74% of the European ancestry hemizygous male carriers: Arg1122* (MAF = 0.11%) and Arg1136* (MAF = 0.35%) (Fig. 1E, fig. S1, and table S11). None of the European ancestry males carried both PTVs despite their proximity. Conditional analysis via logistic regression confirmed that both PTVs were independently associated with reduced odds of diabetes (self-reported) in hemizygous males (Arg1122*: OR = 0.30, 95% CI: [0.12, 0.72], P = 0.007; Arg1136*: OR = 0.68, 95% CI: [0.48, 0.97], P = 0.035) (table S12). Each variant was also independently associated with lower odds of hypertension and BMI compared to heterozygous carriers (Fig. 1D, table S12).

**Fig. 1. Genetic associations with diabetes and related traits among the European ancestry participants in the UKB.** (A) ORs and allele frequencies of gene-level collapsing and ExWAS associations (P < 1 × 10−8) with diabetes diagnoses. (B) Effect sizes and allele frequencies of collapsing and ExWAS associations (P < 1 × 10−8) with HbA1c. We limited associations to those also associated with changes in blood glucose levels. Both (A) and (B) include variants/genes with the largest effect sizes achieved per gene across ExWAS and collapsing models. Allele frequencies for collapsing results are defined as the QV frequency in controls. (C) ORs for diabetes and hypertension diagnoses in heterozygous female MAP3K15 PTV carriers and hemizygous male MAP3K15 PTV carriers. (D) Effect sizes of hemizygous and heterozygous PTVs in MAP3K15 for various diabetes-related traits. BP, blood pressure. P values in (A) and (C) were generated via two-tailed Fisher’s exact test, and P values in (B) and (D) were generated via a linear regression model that included age and sex (B) or age (D) as covariates. (E) Lollipop plot depicting MAP3K15 PTVs (stop, gain, and frameshift variants) observed among hemizygous males of European ancestry. Essential splice variants were not included. The y axis is capped at 40.
with lower HbA1c levels in hemizygous males (Arg1122*: β = −0.30 SD units, 95% CI: [−0.44, −0.16], \(P = 4.2 \times 10^{-5}\); Arg1136*: β = −0.19 SD units, 95% CI: [−0.27, −0.11], \(P = 2.2 \times 10^{-3}\)) when jointly tested in a linear regression model (table S12). We then performed another gene-level collapsing analysis excluding these two variants and found that the remaining 38 rarer PTVs remained significantly associated with reduced HbA1c in hemizygous males (β = −0.16 SD units, 95% CI: [−0.27, −0.04], \(P = 7.2 \times 10^{-5}\)). Hemizygous carriers of the remaining PTVs also appeared to be protected from diabetes (self-reported; \(OR = 0.83, 95\% CI: [0.52, 1.35]\)), although this association did not achieve statistical significance (\(P = 0.46\), likely due to the smaller number of carriers (\(N = 296\)) (table S12). Last, we ensured that the effect of MAP3K15 was independent of variation in the MAP3K15 gene-level collapsing analysis excluding these two variants and found a linear regression model (table S12). We then performed another recessive variants and diabetes association between MAP3K15 and diabetes in the pan-ancestry analysis (\(OR = 0.70, 95\% CI: [0.62, 0.79]\), \(P = 5.7 \times 10^{-10}\); table S16). In the pan-ancestry quantitative trait analysis, MAP3K15 was also more significantly associated with lower HbA1c (β = −0.14 SD units, 95% CI: [−0.27, −0.11], \(P = 1.1 \times 10^{-2}\)) and lower nonfasted blood glucose (β = −0.13 SD units, 95% CI: [−0.25, −0.11], \(P = 5.5 \times 10^{-10}\)) in the recessive collapsing model (table S17).

**MCPS replication**

In addition to performing pan-ancestry analysis in the UKB cohort, we evaluated the association between MAP3K15 and diabetes in 96,811 exomes from unrelated individuals of Admixed American ancestry in the MCPS. The prevalence of T2DM in Mexico is among the highest in the world, and in the MCPS, the prevalence of previously diagnosed diabetes rose from 3% at 35 to 39 years of age to greater than 20% by 60 years of age (17). We used the same recessive collapsing model applied in the UKB to test the MAP3K15 association in this cohort. Recessive nonsynonymous variants in MAP3K15 were nominally associated with reduced odds of diabetes (self-reported; \(OR = 0.81, 95\% CI: [0.66, 0.996]\), \(P = 0.046\); Fig. 2A and table S18) and were significantly associated with lower HbA1c levels (β = −0.11 SD units, 95% CI: [−0.18, −0.04], \(P = 2.2 \times 10^{-3}\); Fig. 2B and table S19).

**UKB pan-ancestry analysis**

We next tested whether the MAP3K15 association, as well as the other gene-level diabetes associations, was shared across individuals of African (\(n = 7412\)), East Asian (\(n = 2209\)), and South Asian (\(n = 8078\)) ancestry in the UKB (tables S14 and S15). Under the recessive collapsing model, the ORs for the association between MAP3K15 and diabetes were consistently in the protective direction for each ancestry (table S15).

We then applied the Cochran-Mantel-Haenszel (CMH) test to combine the results of the full binary trait collapsing analysis results across all four ancestral groups, including Europeans (Materials and Methods). HNF4A, which did not reach study-wide significance in the European-only analysis, was significantly associated with increased odds of diabetes in the pan-ancestry analysis (\(OR = 1.60, 95\% CI: [1.37, 1.86]\), \(P = 5.3 \times 10^{-10}\)). Among the genes significantly associated in the European-only collapsing analysis, GCK and GIGYF1 became more significant, while HNF1A modestly reduced in significance in the pan-ancestry analysis (table S16). The protective association between MAP3K15 recessive variants and diabetes (unspecified/self-reported) became more significant in the pan-ancestry analysis (\(OR = 0.70, 95\% CI: [0.62, 0.79]\), \(P = 5.7 \times 10^{-10}\); table S16). Under the same recessive collapsing model, the ORs for the association between MAP3K15 and diabetes were consistently in the protective direction for each ancestry (table S15).
Notably, \( MAP3K15 \) PTV carrier frequency (0.38%; \( N = 364 \) of 96,811) was less than half of that seen in the UKB Europeans (1.1%; \( N = 4191 \) of 394,692). Consistent with this, European individuals in the Genome Aggregation Database (gnomAD) (18) have the highest frequency of \( MAP3K15 \) PTVs. In contrast, individuals of Mexican or Latin American genetic ancestry have the lowest carrier frequency among all seven represented populations (fig. S3). Thus, populations of European ancestry are most adequately powered for the detection of the protective association between \( MAP3K15 \) and diabetes. To combine evidence for the recessive collapsing model for \( MAP3K15 \) across studies, we extended our original UKB pan-ancestry analysis (comprising of four major ancestral groups) to include the MCPS cohort. In this expanded pan-ancestry analysis, the protective association between recessive nonsynonymous variants in \( MAP3K15 \) and diabetes increased in significance compared to that in the UKB pan-ancestry analysis (CMH OR = 0.73, 95% CI: [0.66, 0.80], \( P = 1.4 \times 10^{-10} \)).

The genetic architecture of T2DM is unique in individuals of Mexican descent because a well-known haplotype confers ~20% of this population’s increased risk of disease (19). This haplotype, which contains four missense variants in the gene \( SLC16A11 \), is exceptionally common in Admixed American individuals (allele frequency ~30%) and rare in Europeans (~1%). Fine-mapping studies and molecular experiments demonstrated that this haplotype results in the lower expression of \( SLC16A11 \), a transporter that influences fatty acid and lipid metabolism (20). Consistent with prior reports in Mexicans (19), carriers of the \( SLC16A11 \) haplotype in the MCPS cohort were at significantly increased odds of diabetes (OR = 1.37, 95% CI: [1.32, 1.42], \( P = 1.0 \times 10^{-65} \)) and had increased HbA1c levels (\( \beta = 0.08 \) SD units, 95% CI: [0.07, 0.09]; \( P = 1.17 \times 10^{-37} \)) (Materials and Methods and tables S20 to S22). We thus tested whether variation in \( MAP3K15 \) buffers against the increased disease risk in \( SLC16A11 \) carriers or whether these two genetic factors might operate independently (Materials and Methods). We found a strongly protective effect of recessive nonsynonymous variation in \( MAP3K15 \) in individuals who do not carry the \( SLC16A11 \) risk haplotype (OR = 0.45, 95% CI: [0.28, 0.69], \( P = 5.4 \times 10^{-5} \)), which is absent in \( SLC16A11 \) risk haplotype carriers (OR = 1.11, 95% CI: [0.86, 1.40], \( P = 0.42 \)) (table S21). This effect modification was statistically significant under a chi-squared heterogeneity test (\( \chi^2 = 11.78; 1 \) df, \( P = 6.0 \times 10^{-5} \)). Likewise, HbA1c levels were more strongly reduced in recessive \( MAP3K15 \) carriers who did not carry the \( SLC16A11 \) risk haplotype (\( \beta = 0.16 \) SD units, 95% CI: [−0.26, −0.05], \( P = 0.004 \)) than in those who carried the risk haplotype (\( \beta = 0.06 \) SD units, 95% CI: [−0.15, 0.03], \( P = 0.19 \)) (table S22). These results have important precision medicine implications, suggesting that therapeutically targeting \( MAP3K15 \) may not be as effective in individuals carrying the risk-increasing \( SLC16A11 \) haplotype.

**FinnGen replication analysis**

Among ancestral groups in gnomAD (18), PTVs in \( MAP3K15 \) were the second most common in Finnish Europeans (fig. S3). We thus sought to confirm whether the protective association between \( MAP3K15 \) and diabetes was replicated in FinnGen (release 6), which includes genotype data for 260,405 individuals of Finnish descent (21). We found that the Arg1122* PTV (rs140104197) is considerably more enriched in Finnish Europeans than non-Finnish Europeans (MAF: 0.33 versus 0.11%). This enrichment in part reflects the unique advantage of performing genetic analyses in isolated populations, such as Finland, in which alleles that are rare in other populations...
have increased in frequency due to historical bottlenecks (22). The variant had a high imputation score (INFO score 0.98), reflecting the high confidence in the genotype status of the variant in this dataset. As with the UKB population, we found that individuals carrying the Arg1122* PTV (rs140104197) were significantly protected against T2DM (OR = 0.81, 95% CI: [0.71–0.93], \( P = 2.3 \times 10^{-3} \)); additionally, this variant also protected against T1DM in FinnGen (OR = 0.60, 95% CI: [0.45–0.79], \( P = 3.7 \times 10^{-5} \) (table S23). Notably, there are nearly nine times more T1DM cases in FinnGen (\( n = 7609 \)) than in the UKB non-Finnish Europeans (\( n = 881 \)), attributable to Finland having the highest incidence of childhood T1DM globally (23). We were thus better powered to detect the association between MAP3K15 and T1DM in this population.

**MAP3K15 protective PTV signal is not associated with changes in BMI or metabolic derangements**

Obesity is central to T2DM, both as a risk factor and as a pathologic sequela. Classically, the initial molecular triggers of insulin signaling involve activating the insulin receptor tyrosine kinase and its receptor substrates (24). In obesity, this cascade is disrupted due to the increased activity of several protein phosphatases, which dephosphorylate and terminate signaling (25, 26). As MAP3K15 encodes a member of the mitogen-activated protein kinase (MAPK) family of signal transducers, we considered whether the protective effects of MAP3K15 loss of function may be isolated from the upstream consequences of obesity on cell signaling. However, MAP3K15 appears to be conspicuously specific for glucose metabolism, with little to no effect on other aspects of metabolic syndrome such as blood pressure, BMI, total body fat mass, or body fat percentage in both the UKB and MCPS (Figs. 1, C and D, and 2, C and D, and tables S19 and S24).

To further explore whether the effect of MAP3K15 on diabetes is independent of obesity, we evaluated whether European individuals with a loss of MAP3K15 are at a lower risk of developing diabetes even after adjusting for BMI. The protective effects of hemizygous MAP3K15 PTVs toward both HbA1c (BMI unadjusted: \( \beta = -0.21 \) SD units, 95% CI: [−0.15, −0.26], \( P = 2.3 \times 10^{-3} \); additionally, this variant also protected against T1DM in FinnGen (OR = 0.60, 95% CI: [0.45–0.79], \( P = 3.7 \times 10^{-5} \) (table S23). Notably, there are nearly nine times more T1DM cases in FinnGen (\( n = 7609 \)) than in the UKB non-Finnish Europeans (\( n = 881 \)), attributable to Finland having the highest incidence of childhood T1DM globally (23). We were thus better powered to detect the association between MAP3K15 and T1DM in this population.

### Supporting evidence

Because PTVs in MAP3K15 appear to reduce the odds of T1DM and T2DM and are not associated with BMI, the protective effect is unlikely to operate through insulin sensitization. Functionally, MAP3K15 encodes an MAPK known to play major roles in regulating cell stress and apoptotic cell death (28). To gain more insight into how MAP3K15 may influence blood glucose, we examined its expression across tissues within GTEx (29). MAP3K15 is most strongly expressed in the adrenal glands, but it is also expressed in the spleen, kidney, pancreas, and pituitary glands (Fig. 3A). Single-cell expression data from human pancreatic endocrine cells indicate that MAP3K15 is most strongly expressed in islet cell subpopulations, including \( \alpha \), \( \beta \), and \( \delta \) cells (Fig. 3B) (30–34). Bulk RNA sequencing of pancreatic islet cells from 495 samples contained in the TIGER dataset (35) also revealed increased MAP3K15 expression in islet cells (fig. S4A).

MAP3K15’s elevated expression in the pituitary is also intriguing and may reflect some role in growth hormone/insulin-like growth factor 1 signaling. In examining single-cell RNA sequencing data of diabetes. However, we first sought to explore whether targeting MAP3K15 may be harmful in humans. Among European participants in the UKB, approximately 1 in every 150 (0.6%) males has a lifetime systemic and complete absence of functional MAP3K15. These individuals comprise a generally healthy cohort, suggesting that therapeutically targeting MAP3K15 function would be tolerable in humans. These patients did not exhibit any worrying changes among the 168 measured blood metabolites. We also evaluated MAP3K15’s probability of being loss-of-function intolerant (pLI) score. pLI scores reflect selective pressures against protein-truncating variants (18), with higher scores indicating greater genic intolerance. MAP3K15’s pLI score is 0, suggesting that loss of MAP3K15 is not associated with early-onset phenotypes that affect fecundity.

We also surveyed associations between nonsynonymous variants (12) in MAP3K15 and 15,719 clinical phenotypes in UKB Europeans. We did not observe any adverse phenotypic associations (\( P < 1 \times 10^{-4} \), including coronary artery or cardiovascular disease, in individuals with loss of MAP3K15 (“ptv,” “ptv5pcnt,” and “rec” collapsing models) (table S26).

Although a previous animal study reported that loss of Map3k15 in mice may raise blood pressure (27), we did not find any evidence that individuals harboring MAP3K15 PTVs were at increased risk of hypertension. In contrast, those with MAP3K15 PTVs consistently appear less likely to be hypertensive across all three studied global populations: UKB Europeans, FinnGen Finnish Europeans, and Admixed Americans in Mexico City (MCPS). Hemizygous MAP3K15 PTV status was associated with modestly lower systolic blood pressures (\( \beta = -0.07 \) SD units, 95% CI: [−0.12, −0.01], \( P = 0.01 \) in UKB Europeans (Fig. 1, C and D, and tables S9 and S10). The Finnish-enriched MAP3K15 PTV (Arg1122*; rs140104197), which is associated strongly with T1DM and T2DM, was protective against hypertension in the independent FinnGen cohort (OR = 0.85, \( P = 0.016 \); Fig. 2D and table S23). Last, in the independent MCPS cohort, the same recessive collapsing model that replicated the protective diabetes signal revealed a nonsignificant association with reduced odds of self-reported hypertension (OR = 0.87; 95% CI: [0.71, 1.06], \( P = 0.18 \); Fig. 2A). The lack of association between recessive variation in MAP3K15 and any deleterious phenotypes suggests that pharmacologically modulating MAP3K15 may be safe and worthwhile to explore in humans.

### Potential MAP3K15 safety liabilities

With evidence that loss of MAP3K15 may be protective against diabetes, targeting MAP3K15 could become an approach for managing...
the developing human adrenal gland (36), MAP3K15 expression appears to be confined to the adrenal cortex (fig. S4, B and C), suggesting a different potential role in mediating mineralocorticoid or glucocorticoid response.

To further explore how MAP3K15 may contribute to the pathophysiology of diabetes in pancreatic tissue, we examined its expression in transcriptomic data collected from pancreatic cell lines harboring mutations in Nkx6-1, a gene tightly associated with maturity-onset diabetes of the young (MODY) (37). MODY is an early-onset, autosomal dominant presentation of diabetes with a clear heritable component and is thus ripe for studying the genetics of insulin dysregulation and hyperglycemia. One cell line included a mutation known to impair Nkx6-1 function and served as a positive control, whereas the other two carried MODY-associated variants. Across all three pathologic lines, MAP3K15 was the most significantly up-regulated gene (Fig. 3C), strongly implicating increased MAP3K15 activity in the pathophysiology of diabetes. Understanding how MAP3K15 contributes to MODY will be an important avenue for future work.

Evidence derived from two in silico tools further supports the role of MAP3K15 in diabetes. We first explored Gene-SCOUT, which uses gene-level collapsing analysis statistics for 1419 UKB quantitative traits to identify genes that result in similar biomarker profiles when mutated (38). Using Gene-SCOUT, we found that variation in the zinc transporter gene SLC30A8 is associated with the most similar human biomarker profile to those with variation in MAP3K15 (Fig. 4, A and B, and fig. S5). SLC30A8 is expressed in pancreatic islet α and β cells, with specific variants exerting a protective effect against T2DM, similar to our findings with MAP3K15 (39, 40).

We also tested whether MAP3K15 was predicted to be associated with diabetes or diabetes-related phenotypes using Mantis-ML (41). This automated machine learning framework predicts potential gene-phenotype relationships using several features, such as tissue expression, genic intolerance, and preclinical models. Among the top 1% of predicted gene-phenotype relationships for MAP3K15 were "diazoxide-resistant diffuse hyperinsulinism" and "hyperinsulinemic hypoglycemia" (Fig. 4C and table S11). While Mantis-ML does not distinguish between disease-causing and disease-protective effects, these results provide strong evidence that MAP3K15 is associated with diabetes-related biology.

Fig. 3. Tissue expression profile of MAP3K15. (A) Expression of MAP3K15 in human tissues contained in the GTEx database. TPM, transcripts per million. We only included tissues with a median TPM > 0.1. (B) MAP3K15 expression in major subpopulations of human pancreatic cells derived from a previously published single-cell RNA sequencing dataset (30–34). UMI, unique molecular identifier. (C) Volcano plot depicting differential gene expression in mouse insulinoma cell lines stably expressing three variants in Nkx6-1: two MODY-associated variants (P329L and S317L) and a control mutation known to functionally impair Nkx6-1 (EEDD321RPPR) (37). FDR, false discovery rate.
MAP3K15-associated diseases from Open Targets

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Fig. 4. MAP3K15 quantitative trait and disease signatures. (A) Genes with the most similar quantitative trait profiles to MAP3K15 in the UKB, derived from Gene-SCOUT (38). (B) Linear regression coefficients for HbA1c and glucose from collapsing analysis models for genes in (A) (genes are sorted from top to bottom in decreasing order of similarity to MAP3K15). (C) Mantis ML (41) predictions of MAP3K15 disease associations.

DISCUSSION

This exome sequencing study of 456,796 UKB participants increases our understanding of high–effect size genetic factors involved in both propensity for and protection from diabetes in humans. We found that recessive PTVs in MAP3K15 reduce the odds of developing diabetes by 35% and significantly decrease HbA1c and blood glucose. Although the protective signal was strongest for T2DM, MAP3K15 PTVs were also protective against T1DM in both the UKB and FinnGen cohorts. Despite being distinct in their etiologies, T1DM and T2DM ultimately share some common pathophysiological pathways such as β cell dysfunction and persistent hyperglycemia (3–42). Our findings here supply a genetic link between T1DM and T2DM that ties together their shared clinical presentation of hyperglycemia and its many adverse health consequences.

Genes with loss-of-function mutations that protect against human disease present opportune therapeutic targets. As PTVs in MAP3K15 are strongly associated with lower odds of developing T1DM and T2DM, targeting it may have therapeutic value across the spectrum of diabetes. In our previously published work, MAP3K15 was 1 of 15 genes strongly associated with glucose and/or HbA1c (12). Another recent independent study on the UKB exomes (43) also suggested a relationship between MAP3K15 and T2DM among a list of gene-trait associations; however, this observation did not achieve study-wide significance (OR = 0.85, P = 2.8 × 10^{-6}). In a prior transethnic GWAS, a MAP3K15 intronic variant was among 318 significant common variant loci reported for T2DM (6). This common variant is not associated with any other complex trait besides T2DM in Open Targets, consistent with our MAP3K15 PTV–based phenome-wide results (44). With the addition of 150,000 more exomes in the present study, we now observe that loss of MAP3K15 is associated with a statistically significant reduced risk of diabetes diagnosis in addition to reduced HbA1c. Loss of MAP3K15 correlates consistently with lower blood glucose and HbA1c levels, which are predictive measures of microvascular sequelae such as peripheral neuropathy, nephropathy, and retinopathy. These convergent associations have important implications for the interpretation of genetic biomarker associations, as genetic associations with clinically relevant biomarkers are not always related to the pathophysiology of a disease. Here, we anchor genetic signals with both biomarkers of diabetes and its clinical diagnosis. Therapeutically, this suggests that targeting MAP3K15 may influence the pathophysiology underlying diabetes rather than only reducing blood glucose.

While PTVs in MAP3K15 seem to associate with protection from diabetes broadly, a notable exception was in Admixed American individuals in MCPS who carried the well-known SLC16A11 risk haplotype (20). Curiously, SLC16A11 and MAP3K15 appear to influence different arms of carbohydrate metabolism, and there is no evidence in Search Tool for Retrieval of Interacting Genes/Proteins (STRING) suggesting that these proteins physically interact (45). SLC16A11 seems particularly important in regulating lipid metabolism by modulating the rates of fatty acid β-oxidation, with knockdown of SLC16A11 leading to elevated levels of intracellular acylcarnitines and triacylglycerols (45). In contrast, individuals with MAP3K15 PTVs do not differ much in the serum lipid profile compared to non-PTV carriers but vary significantly in their serum glucose levels. For individuals harboring pathogenic variants in SLC16A11, the resulting consequences...
in lipid metabolism may drive their likelihood of developing diabetes much more so than any effect MAP3K15 may have on glucose uptake or gluconeogenesis. Regardless, future experimental work would help disentangle these two effects. Because therapeutically targeting MAP3K15 is likely to be more efficacious in individuals who do not carry the SLC16A11 risk haplotype, this has potentially important implications regarding precision medicine and clinical trial design.

Through additional phenome-wide association studies in the 454,796 human participants, we find that loss of MAP3K15 is not associated with any critically adverse phenotypes that would otherwise preclude attempts to target it pharmacologically. Prior work observed that knocking out Map3k15 in mice led to increased blood pressure (27), but our extensive human study found that MAP3K15 PTVs appear to provide a protective effect against hypertension.

Although PTVs most often lead to complete loss of protein function, they can also confer partial loss-of-function or, on rarer occasions, gain-of-function effects. PTVs conferring partial loss- and gain-of-function effects tend to preferentially occur at the 3′ end of a gene and escape nonsense-mediated decay (46). Here, we find that the MAP3K15 PTV signal is distributed throughout the entire gene body, strongly suggesting a loss-of-function mechanism. Moreover, a prior study found that deletions downstream of amino acid 1179 reduce MAP3K15’s basal kinase activity and render it unable to form molecular condensates in response to osmotic stress (47). Coincidentally, the two more common PTVs that we identified (Arg1122* and Arg1136*) occur upstream of these previously characterized variants. Together, our results suggest that loss of function of MAP3K15 protects against diabetes, but future functional studies will help fully dissect the mechanism of these PTVs.

Exactly how the loss of MAP3K15 may influence insulin signaling and hyperglycemia is still unclear. The tissue expression profile of MAP3K15 demonstrates strong expression in several islet cell subpopulations and adrenal glands, suggesting that MAP3K15 might be involved in pancreatic islet cell functional maintenance and/or stress response pathways. Consistent with this, the ASK (MAP kinase) family of genes is known to influence stress response in diabetes (48, 49) (e.g., apoptosis and inflammation) with external stimuli (28). These provide important clues regarding the otherwise unknown pathways that mediate the protective effect between MAP3K15 and diabetes. Given the notable up-regulation of MAP3K15 in cellular models of MODY, these models could offer valuable insight into MAP3K15’s role in diabetes.

Although obesity is generally a central driver of type 2 diabetes, we find that the protective effects of MAP3K15 loss are notably independent of BMI. While not currently available for UKB participants, other quantitative measures of insulin resistance in MAP3K15 PTV carriers such as fasting glucose, glucose tolerance tests, and α-hydroxybutyrate levels would further illuminate how MAP3K15 modulates the insulin/glucaagon signaling balance and influences hyperglycemia. Nonetheless, our results suggest that pharmacologically targeting MAP3K15 could be an orthogonal approach to managing diabetes outside the traditional arsenal.

**MATERIALS AND METHODS**

**Discovery cohort**

Discovery genetic association studies were performed using the 454,796 exomes available in the UKB cohort (50). The UKB is a prospective study of approximately 500,000 participants aged 40 to 69 years at the time of recruitment. Participants were recruited in the United Kingdom between 2006 and 2010 and are continuously followed. Participant data include health records that are periodically updated by the UKB, self-reported survey information, linkage to death and cancer registries, collection of urine and blood biomarkers, imaging data, accelerometer data, and various other phenotypic endpoints. All study participants provided informed consent, and the UKB has approval from the North-West Multi-centre Research Ethics Committee (11/NW/0382).

**Replication cohorts**

**Mexico City Prospective Study**

The MCPS cohort consists of ~150,000 Mexican adults of Admixed American ancestry. Participants were aged at least 35 years and were recruited between 1998 and 2004. Phenotypic data were recorded during household visits. Available phenotypes include age, sex, socioeconomic status, lifestyle factors (e.g., alcohol intake, smoking status, and physical activity), current medications, and medical history (including previously diagnosed diabetes). Height, weight, waist and hip circumferences, and measured blood pressure were measured while the patient was sitting. The full characteristics of this cohort have been described in detail previously (17, 51). The MCPS study was approved by the Mexican Ministry of Health, the Mexican National Council for Science and Technology, and the University of Oxford.

**FinnGen**

The FinnGen cohort (release 6) includes 260,405 individuals from Finland with genotype and health registry data. Phenotypes have been derived from nationwide health registries (21). Patients and control subjects in FinnGen provided informed consent for biobank research, based on the Finnish Biobank Act. Alternatively, older research cohorts, collected before the start of FinnGen (in August 2017), were collected on the basis of study-specific consents and later transferred to the Finnish biobanks after approval by Fimea, the National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Fimea. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) approved the FinnGen study protocol no. HUS/990/2017. The FinnGen study is approved by the Finnish Institute for Health and Welfare.

**Phenotypes**

**UK Biobank**

We harmonized the UKB phenotype data as previously described (12). Briefly, we studied two main phenotypic categories: binary and quantitative traits taken from the February 2020 data release that was subsequently refreshed with updated Hospital Episode Statistic and death registry data as released ad hoc by the UKB on July 2020 (UKB application 26041). We parsed phenotypic data using our previously described R package, PEACOCK (https://github.com/astrezanca-cgr-publications/PEACOK) (12). In addition, as previously described (12), we grouped relevant ICD-10 codes into clinically meaningful “Union” phenotypes. For all binary phenotypes, we matched controls by sex when the percentage of female cases was significantly different (Fisher’s exact two-sided P < 0.05) from the percentage of available female controls.

To discover genes associated with the risk of diabetes, we considered five binary diabetes-related phenotypes (table S1): Union#E11#E11 Non-insulin-dependent diabetes mellitus, Union#E14#E14 Unspecified...
diabetes mellitus, Union#E10#E10 Insulin-dependent diabetes mellitus, Union#E10#E10 Insulin-dependent diabetes mellitus strict (defined as individuals who were never also billed for non-insulin-dependent diabetes mellitus), and 20003#1140884600#metformin. We also included two related quantitative phenotypes: blood glucose and HbA1c (table S2). When considering HbA1c associations, we specifically focused on genes also associated with changes in blood glucose, as HbA1c can be confounded by any traits that affect red blood cell morphology.

We considered several additional phenotypes in follow-up analyses of MAP3K15. In terms of binary phenotypes, we tested for associations with hypertension [Union#E10#E10 Essential (primary) hypertension] and a custom-defined T1DM phenotype that was based on ICD-9 and ICD-10 codes, primary care data, and medication prescriptions. We also analyzed two quantitative traits related to hypertension (systolic blood pressure and diastolic blood pressure) (table S2). In analyzing systolic and diastolic blood pressure, we adjusted for commonly prescribed blood pressure medications in our linear regression collapsing model (described in the “Collapsing analysis” section below) (table S27). Last, we included quantitative traits related to adiposity, including BMI (UKB Field 23104), whole body fat mass (Field 23100), and body fat percentage (Field 23099). All quantitative phenotypes were normalized using rank-based inverse-normal transformation. Effect sizes for these traits are reported as SD units.

**Mexico City Prospective Study**

We assessed three self-reported binary phenotypes in MCPS: recall of a previous diagnosis of diabetes, recall of a previous diagnosis of hypertension, and recall of use of an antidiabetic drug. We also assessed six quantitative traits: baseline HbA1c, diastolic blood pressure adjusted for antihypertensive drug use (plus 10 mmHg), systolic blood pressure adjusted for antihypertensive drug use (plus 15 mmHg), hip circumference, waist circumference, and waist-hip ratio. Collapsing analyses for quantitative traits included BMI as a covariate. Sex matching for each phenotype was performed as described above for the UKB cohort.

**FinnGen**

We extracted all phenotypic associations for one PTV of interest (rs140104197). We focused on four diagnoses: “diabetes (varying definitions),” “type 1 diabetes,” “type 2 diabetes,” and “hypertension, essential.”

**Genetic sequencing**

Exome sequencing data for 454,988 UKB participants and 143,440 MCPS participants were generated at the Regeneron Genetics Center. Genomic DNA underwent paired-end 75–base pair whole-exome sequencing at Regeneron Pharmaceuticals using the IDT xGen v1 capture kit on the NovaSeq6000 platform. Conversion of sequencing data in BCL format to FASTQ format and the assignments of paired-end sequence reads to samples were based on 10-base barcodes, using bcl2fastq v2.19.0. Initial quality control was performed by Regeneron and included sex discordance, contamination, unresolved duplicate sequences, and discordance with microarray genotyping data checks. A total of 454,796 UKB exomes and 141,046 MCPS exomes passed these quality control measures.

In FinnGen, genotyping was performed using a Thermo Fisher Scientific Axiom custom array. In addition to the core GWAS markers (about 500,000), it contains 116,402 coding variants enriched in Finland, 10,800 specific markers for the human leukocyte antigen (HLA) and killer cell immunoglobulin-like receptors (KIR) genes, 14,900 ClinVar variants, 4600 pharmacogenomic variants, and 57,000 selected markers.

**AstraZeneca Centre for Genomics Research**

**bioinformatics pipeline**

The 454,796 UKB and 141,046 MCPS exome sequences were reprocessed at AstraZeneca from their unaligned FASTQ state. A custom-built Amazon Web Services cloud computing platform running Illumina DRAGEN Bio-IT Platform Germline Pipeline v3.0.7 was used to align the reads to the GRCh38 genome reference and perform single-nucleotide variant (SNV) and insertion and deletion (indel) calling. SNVs and indels were annotated using SnpEff v4.3 (52) against Ensembl Build 38.92. We further annotated all variants with their gnomAD MAFs (gnomAD v2.1.1 mapped to GRCh38) (18). We also annotated variants using missense tolerance ratio (MTR) scores (53) to identify whether they mapped to genic regions under constraint for missense variants and rare exome variant ensemble learner (REVEL) scores (54) for their predicted deleteriousness.

**Additional quality control**

To complement the quality control performed by Regeneron Genomics Centre, we passed the UKB and MCPS exome sequences through our internal bioinformatics pipeline as previously described (12). Briefly, we excluded sequences that achieved a VerifyBAMID freemix (a measure of DNA contamination) of more than 4% and samples where less than 94.5% of the consensus coding sequence (CCDS release 22) achieved a minimum of 10-fold read depth. The cohorts were also screened to remove related participants, as determined using KING v2.2.3 (55): In the UKB, we excluded participants that were second-degree relatives or closer as estimated using the --ibdseg function (equivalent to kinship coefficient > 0.0884), and in the MCPS, we excluded participants that were first-degree relatives or closer as estimated using the --ibdseg function (equivalent to kinship coefficient > 0.1769). Given the large proportion of related individuals in the MCPS, we followed the following order of prioritizing individuals when doing the relatedness pruning to maximize statistical power for our replication analysis: individuals with a higher number of death records, the presence of a diagnosis of diabetes, a higher number of binary self-reported phenotypes, male predicted sex, and available HbA1c data. After the above quality control steps, there remained 412,394 unrelated UKB and 98,922 MCPS exomes of any genetic ancestry.

**Genetic ancestry**

The primary discovery analysis was performed in UKB participants of European ancestry. We predicted the genetic ancestry of UKB participants using PEDDY v0.4.2 (56) with sequences from the 1000 Genomes Project as population references (57). To define the European UKB cohort, we selected individuals with >0.99 Pr(European) ancestry who were within 4 SD of the means for the top four principal components. In total, 394,692 of the 422,488 unrelated UKB exomes (93%) were used for the European ancestry case-control analyses. We also used PEDDY-derived ancestry predictions to identify case-control cohorts from three other major ancestral groups (N > 1000) represented in the UKB: African, East Asian, and South Asian. Using a PEDDY cutoff of >0.95 for each of these ancestral groups, we identified 7412 African, 2209 East Asian, and 8078 South Asian UKB participants for case-control analyses.
In MCPS, we retained individuals with PEDDY-derived Pr(Admixed American ancestry) ≥ 0.95. As above, we only retained individuals within 4 SD of the mean for principal components 1 to 4. In total, 96,811 of the 98,922 unrelated MCPS exomes (98%) were of Admixed American ancestry.

**Discovery analyses**

**Collapsing analysis**

We performed our previously described gene-level collapsing analysis framework (12) for both binary and quantitative traits. We focused on the European-only analysis as the discovery cohort, given the much larger sample size. We included 10 nonsynonymous collapsing models, including 9 dominant models and 1 recessive model, plus an additional synonymous variant model as an empirical negative control (table S2). For the dominant collapsing models, the carriers of at least one QV in a gene were compared to the noncarriers. In the recessive model, individuals with two copies of QVs in either homozygous or putatively compound heterozygous form were compared to the noncarriers. Hemizygous genotypes for X chromosome genes also qualified for the recessive model.

Using SnpEff annotations, we defined synonymous variants as those annotated as "synonymous_variant." We defined PTVs as variants annotated as exon_loss_variant, frameshift_variant, start_lost, stop_gained, stop_lost, splice_acceptor_variant, splice_donor_variant, gene_fusion, bidirectional_gene_fusion, rare_amino_acid_variant, and transcript_ablation. We defined missense as missense_variant_splice_region_variant and missense_variant. Nonsynonymous variants included exon_loss_variant, frameshift_variant, start_lost, stop_gained, stop_lost, splice_acceptor_variant, splice_donor_variant, gene_fusion, bidirectional_gene_fusion, rare_amino_acid_variant, transcript_ablation, conservative_inframe_deletion, conservative_inframe_insertion, disruptive_inframe_deletion, missense_variant_splice_region_variant, missense_variant, and protein_altering_variant.

For binary traits, the difference in the proportion of cases and controls carrying QVs in a gene was tested using Fisher’s exact two-sided test. For quantitative traits, the difference in mean between the carriers and noncarriers of QVs was determined by fitting a linear regression model, correcting for age and sex. For analysis of systolic and diastolic blood pressure measurements, we included an indicator variable in the linear regression as a covariate to denote whether individuals were on commonly prescribed antihypertensives (table S27).

For all models, we applied the following quality control filters: minimum coverage $10x$; annotation in CCDS transcripts (release 22; approximately 34 Mb); at most, 80% alternate reads in homozygous genotypes; percent of alternate reads in heterozygous variants ≥ 0.25 and ≤ 0.8; binomial test of alternate allele proportion departure from 50% in heterozygous state $P > 1 \times 10^{-6}$; genotype quality score (GQ) ≥ 20; Fisher’s strand bias score (FS) ≤ 200 (indels) ≤ 60 (SNVs); mapping quality score (MQ) ≥ 40; quality score (QUAL) ≥ 30; read position rank sum score (RPRS) ≥ −2; mapping quality rank sum score (MQRS) ≥ −8; DRAGEN variant status = PASS; the variant site is not missing (that is, less than $10x$ coverage) in 10% or more of sequences; the variant did not fail any of the aforementioned quality control in 5% or more of sequences; the variant site achieved 10-fold coverage in 30% or more of gnomAD exomes; and if the variant was observed in gnomAD exomes, 50% or more of the time, those variant calls passed the gnomAD quality control filters (gnomAD exome AC/AC_raw ≥ 50%). $P$ values were generated by adopting Fisher’s exact two-sided test. Three distinct genetic models were studied for binary traits: allelic (A versus B allele), dominant (AA + AB versus BB), and recessive (AA versus AB + BB), where A denotes the alternative allele and B denotes the reference allele. For quantitative traits, we adopted a linear regression (correcting for age and sex) and replaced the allelic model with a genotypic (AA versus AB versus BB) test.

**Phenome-wide analysis for MAP3K15**

We performed phenome-wide associations between MAP3K15 and 15,710 binary phenotypes and 1419 quantitative phenotypes in the 394,692 European UKB individuals using the identical parameters published in our prior PheWAS publication (12). We have made all statistics publicly available through our PheWAS portal (https://azphewas.com/geneView/7e2a7f9b-97f0-45f7-9297-f976f7e667c8/MAP3K15/grl/binary).

**P value threshold**

We defined the study-wide significance threshold as $P < 1 \times 10^{-8}$. We have previously shown, using an $n$-of-1 permutation approach and the empirical null synonymous model, that this threshold corresponds to a false-positive rate of 9 (of a total of 3.6 billion tests) and 2 (of a total of 346.5 million tests), respectively, for binary traits in the setting of collapsing analysis PheWAS (12).

**Secondary association analyses**

A total of 40 unique PTVs in MAP3K15 were observed among the hemizygous male carriers. Two of these PTVs (Arg1122* and Arg1136*) were relatively more frequent. We excluded carriers of these two alleles and reperformed the collapsing analyses for the remaining MAP3K15 PTVs: Fisher’s exact test for diabetes (“Unspecified#E14#E14 Unspecified diabetes mellitus”) and linear regression for HbA1c.
To determine whether the protective effect of MAP3K15 on diabetes is independent of BMI, we performed additional analyses in which we regressed HbA1c and the self-reported diabetes phenotype (Union#E14#E14 Unspecified diabetes mellitus) on MAP3K15 PTV carrier status in males with BMI (UKB Field ID: 23104) as a covariate. To investigate the joint effects of MAP3K15 and a nearby significantly associated indel in PDHA1 (X-19360844-AAC-A), a gene that overlaps the 3′ untranslated region of MAP3K15, we regressed HbA1c and the diabetes phenotype (Union#E14#E14 Unspecified diabetes mellitus) on the carrier status for the two frequent MAP3K15 PTVs (Arg1122* and Arg1136*) and the PDHA1 indel in hemizygous males.

**MCPS SLC16A11 analysis**

A common haplotype spanning the SLC16A11 gene that harbors four missense variants (17-7041768-G-T, 17-7042164-C-T, and 17-7043011-C-T) and an indel in hemizygous males. We performed association analyses on the subset of patients with diabetes-related complications. We used this tool to identify genes that were most similar to the "seed gene" MAP3K15.

**Mantis-ML**

Mantis-ML (41) is a gene prioritization machine learning framework that integrates a diverse set of annotations, including intolerance to variation, tissue expression, and animal models. We used this tool to obtain the top disease predictions for MAP3K15 across 2536 diseases parsed from Open Targets.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abbd5340

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