Familial Hypercholesterolemia and Type 2 Diabetes in the Old Order Amish

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Alleles associated with lower levels of LDL cholesterol (LDL-C) have recently been associated with an increased risk of type 2 diabetes (T2D), highlighting the complex relationship between LDL-C and diabetes. This observation begs the question of whether LDL-C–raising alleles are associated with a decreased risk of T2D. This issue was recently addressed in a large familial hypercholesterolemia (FH) screening study, which reported a lower prevalence of self-reported diabetes in FH subjects than in age-matched relatives without FH. To extend this observation, we tested the association of FH with diabetes status and glycemia in a large Amish population enriched for the FH-associated *APOB* R3527Q variant that included 640 *APOB* R3527Q carriers and 4,683 noncarriers. Each copy of the R3527Q T allele was associated with a 74.9 mg/dL increase in LDL-C. There was little difference in T2D prevalence between subjects with (5.2%) and without (4.5%) the R3527Q allele ($P = 0.23$), and there was no association between R3527Q variant and impaired fasting glucose, fasting glucose or insulin, or oral glucose tolerance test–derived measures. Our data provide no evidence supporting an association between the *APOB* R3527Q variant and T2D or glycemia and highlight the asymmetry of the LDL-C–T2D relationship and/or the gene/variant-dependent specificity of the LDL-C–T2D association.

The observation that treatment of hypercholesterolemia with statins to reduce LDL cholesterol (LDL-C) levels leads to an ~9% increased risk of type 2 diabetes (T2D) (1) has generated a large dialogue on the relation of LDL-C lowering and T2D. Studies showing that LDL-C–lowering variants in *HMGCR*, the gene encoding HMG-CoA reductase and the molecular target of statins, are also associated with increased risk of T2D (2) implicated the gene itself as the driver for the increased T2D risk rather than an off-target effect of statins. Subsequent studies have since shown LDL-C–lowering alleles at other genes also to be associated with increased risk of T2D, further suggesting that the LDL lowering itself is related somehow to increased T2D risk. An intriguing recent finding is the appearance of heterogeneity among LDL-C–lowering variants in different genes of the degree to which they increase T2D risk. For example, recent meta-analyses have revealed that LDL-C–lowering alleles at *PCSK9* and *LDLR* are associated with a small (19%, $P = 0.03$, and 13%, $P = 0.05$, respectively) increased risk of T2D per LDL-C reduction of 1 mmol/L (38.7 mg/dL), while LDL-C–lowering alleles at *NPC1L1* appear to impose a greater T2D risk (142%, $P < 0.001$) per LDL-C reduction of 1 mmol/L (3). These observations and others (4,5) suggest that mechanisms both dependent and independent of LDL-C may be associated with increased T2D risk. Uncovering these mechanisms may reveal potentially targetable pathways for diabetes prevention or treatment.

The fact that LDL-C–lowering alleles are associated with an increase in T2D risk begs the question as to whether LDL-C–raising alleles are associated with a decrease in T2D risk. This hypothesis was recently tested by Besseling et al. (6) in familial hypercholesterolemia (FH) cases identified from the national Dutch FH screening program. FH is associated with extremely high levels of LDL-C due to mutations in the LDL receptor (LDLR) pathway, with mutations occurring mainly in *APOB*, *LDLR*, and *PCSK9*. In the study

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by Besseling et al. (6), there was a 44% lower rate of (self-reported) T2D in FH cases (adjusted prevalence 1.44%) compared with unaffected relatives (adjusted prevalence 3.26%). In subanalyses, the relative protection against T2D was observed in patients with either the *LDLR* or *APOB* mutations, with the strongest effect in those with *LDLR* null mutations, which are more severe in disturbing LDL-C metabolism.

Because of inherent biases in using self-reported diabetes history and the potential for differential preventive care given to subjects already known to have a lipid disorder, we sought to evaluate the association of FH with T2D and glycemia in a well-phenotyped cohort. Our analyses were carried out in 5,323 Old Order Amish (OOA) individuals from Lancaster County, Pennsylvania, a population enriched for one of the known FH mutations, the R3527Q variant in the *APOB* gene (rs5742904). We have previously reported that LDL-C levels are increased by 58 mg/dL per copy of the *APOB* R3527Q allele in the OOA (7). This mutation is more common in the OOA population than in other Caucasian populations because of a founder effect. Our sample included 640 *APOB* R3527Q carriers, a number far larger than the 84 *APOB* mutation carriers reported in the Dutch study. We compared across *APOB* R3527Q genotypes T2D prevalence and mean levels of glycemia-related traits, including glucose and insulin values measured during oral glucose tolerance tests (OGTTs) in a subset of subjects. We found no evidence supporting an association between the *APOB* R3527Q variant and T2D or glycemia.

RESEARCH DESIGN AND METHODS

All 5,323 subjects included in this study were phenotyped for one or more of the study outcomes (diabetes, fasting glucose, and/or HbA_{1c}) and genotyped for *APOB* R3527Q by either the Illumina HumanExome BeadChip or by whole-exome sequencing. Paired-end 75 bp exome sequencing was performed by the Regeneron Genetics Center as previously described (8) but using xGen (Integrated DNA Technologies) capture followed by sequencing on the Illumina HiSeq 2500 System with v4 chemistry. For samples with both exome sequence and chip genotype data (n = 2,695), the concordance rate for the R3527Q variant was 99.85%. All study subjects had participated in at least one of the community-based studies our group had carried out in this population over the past 20 years as part of our Amish Complex Disease Research Program (9–13). Among these were 640 subjects heterozygous (n = 625) or homozygous (n = 15) for *APOB* R3527Q, corresponding to an allele frequency of 6% and carrier frequency of 12%, consistent with what we have previously reported (7).

For the analyses described in this report, T2D was diagnosed according to American Diabetes Association criteria of fasting glucose >7 mmol/L (126 mg/dL) or $HbA_{1c} \geq 6.5\%$ (if available). Subjects reporting current use of antidiabetes medications (n = 86) were also considered to have diabetes regardless of glucose testing. A 3-h OGTT was administered to 723 individuals who had previously participated in the Amish Family Diabetes Study (9), and subjects having a 2-h glucose level >11.1 mmol/L (200 mg/dL) were also considered to have T2D. Impaired fasting glucose was defined in individuals without diabetes as a glucose level between 5.6 and 6.9 mmol/L (100 and 125 mg/dL). Fasting glucose was measured by a Beckman glucose analyzer using the glucose oxidase method or the spectrophotometry method as implemented by Quest Diagnostics, fasting insulin by radioimmunoassay (Linco Research, Inc., St. Charles, MO), and HbA_{1c} by immunoturbidimetry (Quest Diagnostics, Horsham, PA). HOMA of insulin resistance (HOMA-IR) was defined as HOMA-IR = [fasting insulin (mU/L) × fasting glucose (mmol/L)]/22.5. Area under the curve (AUC) in OGTT was computed using the trapezoid rule. We tested the association of glucose and HbA_{1c} levels with *APOB* R3527Q genotype using a mixed model that accounts for relatedness among individuals as a random effect. Age and sex were included as covariates. Analyses were carried out under an additive genetic model using the MMAP software program (14). Logistic regression was used to test the association of T2D with genotype.

RESULTS

In the sample described in this report, each copy of the *APOB* R3527Q T allele was associated with a 74.9 mg/dL increase in LDL-C levels (95% CI 71.7–78.1), a slightly higher estimate than the 58 mg/dL per allele effect size we have reported previously in a smaller sample size (7). This effect size was present across all ages studied (i.e., ages 20 years and older). The overall prevalence of T2D in the sample was 4.6%, and there was virtually no difference by R3527Q genotype (wild type 4.5%, heterozygotes 5.1%, and homozygotes 6.7% across genotypes, P = 0.236) (Table 1). In fact, the R3527Q T allele was associated with an increased, not decreased, risk of T2D (per allele odds ratio [OR] 1.27 [95% CI 0.85–1.85] for additive model; per allele OR 1.41 [95% CI 0.07–8.08] for dominant model), although in both models the CIs were wide and included 1. Further analyses revealed no evidence for genotype * age or genotype * BMI interactions.

Results of the 3-h OGTTs are shown in Fig. 1 for the subset of 723 subjects who underwent this procedure, and as indicated, the response profiles, measured as AUC for glucose and insulin changes following glucose challenge, were virtually indistinguishable across genotypes (P = 0.73 and 0.42, respectively). Table 2 compares prevalence of impaired fasting glucose and mean values of fasting glucose, fasting insulin, and HbA_{1c} as well as HOMA-IR in individuals without diabetes according to genotype. These results also reveal little differences between genotypes (P = 0.42–0.73).

DISCUSSION

Our study is unique in its large sample size of FH subjects (n = 640) carrying a single *APOB* mutation who were

Table 1—Levels of metabolic variables according to *APOB* R3527Q genotype

Trait	All subjects (n = 5,323)	CC (n = 4,683)	TC (n = 625)	TT (n = 15)	β	P
Age (years)	43.0 ± 16.9	43.2 ± 16.9	41.0 ± 16.3	50.8 ± 18.8	−1.790	0.02
Male sex (%)	44 (2,365/5,323)	45 (2,088/4,683)	43 (271/625)	40 (6/15)		0.80
BMI (kg/m^2)	26.6 ± 0.07 (n = 5,289)	26.6 ± 0.07 (n = 4,652)	26.5 ± 0.20 (n = 622)	27.2 ± 1.35 (n = 15)	0.091	0.68
LDL (mg/dL)	134.0 ± 0.6 (n = 5,248)	124.8 ± 0.5 (n = 4,615)	198.9 ± 1.9 (n = 618)	294.1 ± 16.6 (n = 15)	74.894	<0.00001
Waist circumference (cm)	89.2 ± 0.2 (n = 5,164)	89.3 ± 0.2 (n = 4,548)	88.5 ± 0.5 (n = 601)	91.2 ± 3.1 (n = 15)	−0.48	0.38
Diabetes prevalence (%)	4.6 (245/5,323)	4.5 (212/4,683)	5.1 (32/625)	6.7 (1/15)	0.240†	0.23
Taking diabetes medication (%)	1.6 (86/5,298)	1.6 (75/4,661)	1.8 (11/622)	0 (0/15)	0.111	0.74

Data are mean ± SD unless otherwise indicated. †OR 1.27 (95% CI 0.85–1.85).

recruited as part of a community-based population survey and tested for diabetes. Our data provide no evidence supporting an association (protective or otherwise) of the *APOB* R3527Q variant with either T2D or any other glycemia-related trait. Notably, the 95% CI surrounding our estimate of the T2D–*APOB* R3527Q association (0.85–1.85) is not consistent with a 35% reduced odds of having T2D as reported by Besseling et al. (6) but could be associated with as much as a 15% decreased odds of T2D or as much as a 85% increased odds. Our analysis is also consistent with the lack of association reported for this variant with T2D from the GoT2D Consortium (OR 1.69, P = 0.17) based on 76 allele carriers and >75,000 noncarriers (http://www.type2diabetesgenetics.org). The absence of association observed in our study (and GoT2D) contrasts with the results obtained by Besseling at al. (6) that were based on self-reported diabetes history in the Netherlands FH registry. The protective association of FH with T2D reported in the Dutch study was present for both *LDLR* and *APOB* mutations; notably, the R3527Q variant present in the Amish also appears to be the predominate *APOB* variant observed in the Netherlands study (15). A possible explanation for this discrepancy is that heightened surveillance of FH cases and carriers in the Netherlands FH registry may have altered behaviors related to T2D risk, while this was unlikely to be the case for Amish *APOB* R3527Q carriers as subjects were unaware of their genotypes at the time of subject enrollment and few Amish seek primary care; in fact, only 3.1% of our Amish FH subjects reported taking lipid-lowering medications.

The absence of an association observed in our study between the R3527Q variant and T2D contrasts with the increased risk of T2D that has been reported in Mendelian randomization studies of *HMGCR*, *PCSK9*, *NPC1L1*, and *LDLR*. One speculation is that the critical determinant of lipid-associated T2D risk is more closely tied to an LDL receptor–regulated mechanism than to an upstream defect affecting the quality of the APOB ligand. As elucidated in the pioneering research of Brown and Goldstein (16), a dimeric complex between SREBPF chaperone (SCAP) and insulin-induced genes (INSIG1 and INSIG2) functions as an intracellular receptor for cholesterol. Together, these proteins mediate effects of cholesterol on SREBP pathway–mediated regulation of gene expression. It is plausible that this SREBP pathway might mediate some of the observed associations between LDL-C and the risk of diabetes. Because the INSIG/SCAP/SREBP pathway is regulated by intracellular levels of cholesterol, this raises the question of whether the R3527Q alters the rate at which LDL-C enters cells. Boren et al. (17) reported that the R3527Q substitution decreases the affinity of APOB for the LDL receptor by ~6.4-fold, suggesting that a ~6.4-fold higher concentration of Q3527-APOB would compensate for the lower binding affinity as compared with R3527-APOB. In this study, we report an ~2.4-fold increase in LDL-C level, which is less than the theoretical prediction of a 6.4-fold increase in the concentration of APOB. At least two biological factors may

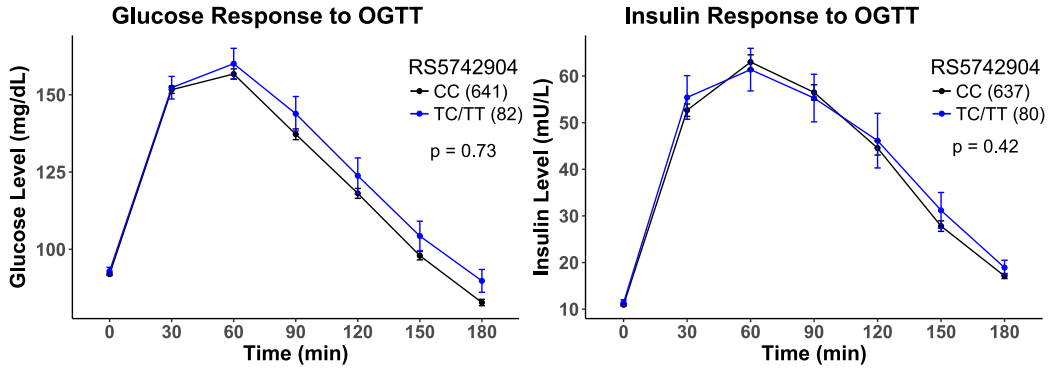

Figure 1—Glucose and insulin response to a 3-h OGTT by *APOB* R3527Q (rs5742904) genotype.

contribute to the apparent discrepancy. First, it is possible that the ratio of APOB:LDL-C may be higher in individuals who are homozygous for Q3527-*APOB*. Second, a decrease in LDL receptor occupancy would be predicted to induce upregulation of the number of LDL receptors on the cell surface by decreasing the rate of ligand-induced receptor degradation (18). Taken together, an increase in the number of LDL receptors and an increase in the concentration of APOB would be predicted to preserve relatively normal levels of receptor-mediated endocytosis.

Several limitations of our study should be acknowledged. First, the Amish subjects included in our study were relatively young (mean age 43 years), and some subjects destined for future T2D may not have developed it yet. Second, our sample included only 245 subjects with T2D, and the minimal detectable OR at 80% power in our sample is only 0.61. However, this estimate is approximately identical to the OR of 0.62 reported by Besseling et al. (6). Moreover, our sample provides >90% power to detect a significant association of the R3527Q variant with fasting glucose if this variant accounted for as little as 0.4% of the variation in fasting glucose levels, corresponding to a change of <1 mg/dL in glucose levels. Thus, our sample size is well powered for detecting associations between the R3527Q variant and glycemic traits.

The lack of association of this FH mutation with T2D adds to the complexity of the LDL-C–T2D relationship. While LDL-C–lowering alleles appear to be associated with a modest increase in T2D risk, recent studies also indicate that the increased T2D risk is not necessarily correlated with the magnitude of the LDL-C–lowering effect. Our study adds to this story by considering a single LDL-C–raising variant, albeit one leading to a very large increase in LDL-C. Notably, we find no association with either T2D or any other glucose parameter. In contrast, other FH-associated variants, including some in *APOB*, have been associated with self-reported diabetes. These discrepancies could reflect an asymmetry in the LDL-C–T2D relationship, with T2D risk increasing with lower LDL-C levels but unchanged with markedly higher LDL-C levels, or these discrepancies could highlight a gene (or variant)-dependent specificity of the LDL-C–T2D association similar to the heterogeneous effect shown by Lotta et al. (3). Given these possibilities, Mendelian randomization studies that have shown genetic risk scores for high LDL-C to be inversely correlated with T2D risk (2,19,20) should be interpreted cautiously, as the risk score–driving risk alleles included in these analyses typically fall in a range of different genes and their effects on T2D risk may represent widely varying mechanisms rather than a single unified one. Sorting out

Table 2—Fasting and OGTT-derived measurements in individuals without diabetes

Trait	All subjects	CC	TC	TT	β	*P*
Impaired fasting glucose (%)	7.1 (359/5,046)	7.1 (316/4,442)	6.9 (41/590)	14.2 (2/14)	0.120†	0.47
Glucose (mg/dL)	85.6 ± 0.1 (*n* = 5,046)	85.6 ± 0.1 (*n* = 4,442)	85.9 ± 0.4 (*n* = 590)	86.4 ± 3.3 (*n* = 14)	0.256	0.52
Insulin (mU/L, ln)	2.22 [1.95, 2.49] (*n* = 2,468)	2.22 [1.96, 2.50] (*n* = 2,171)	2.18 [1.92, 2.48] (*n* = 291)	2.41 [2.25, 2.50] (*n* = 6)	0.016	0.61
HOMA-IR	0.69 [0.38, 1.01] (*n* = 2,274)	0.68 [0.40, 1.01] (*n* = 2,001)	0.66 [0.33, 1.02] (*n* = 267)	0.95 [0.80, 1.16] (*n* = 6)	0.018	0.55
HbA_{1c} (%, ln)	1.69 [1.63, 1.74] (*n* = 2,743)	1.69 [1.63, 1.74] (*n* = 2,417)	1.71 [1.63, 1.74] (*n* = 313)	1.69 [1.65, 1.74] (*n* = 13)	0.003	0.49
OGTT-derived measures						
Glucose AUC	389.3 ± 4.7 (*n* = 723)	374.5 ± 3.4 (*n* = 641)	387.5 ± 12.3 (*n* = 80)	403.8 (*n* = 2)	3.250	0.73
Insulin AUC	129.6 ± 2.4 (*n* = 717)	130.3 ± 3.1 (*n* = 637)	133.6 ± 10.7 (*n* = 78)	85.8 (*n* = 2)	6.930	0.42

Data are mean ± SD except for insulin, HOMA-IR, and HbA_{1c} (median [Q1, Q3]) unless otherwise indicated. SD not presented for glucose and insulin AUC in the TT genotype group because of the small number of subjects. ln, natural logarithm. †OR 1.17 (95% CI 0.80–1.56).

the mechanisms and pathways involved may reveal important insights for diabetes prevention and treatment.

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