The FTO Gene Is Associated With an Atherogenic Lipid Profile and Myocardial Infarction in Patients With Type 2 Diabetes

A Genetics of Diabetes Audit and Research Study in Tayside Scotland (Go-DARTS) Study

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Background—Common variation in the fat mass and obesity (FTO)–related gene is associated with increased body fat and susceptibility to type 2 diabetes. We hypothesized that this would also associate with metabolic phenotypes of insulin resistance and increased risk of cardiovascular morbidity and mortality.

Methods and Results—FTO rs9939609 genotype was determined in 4897 patients with type 2 diabetes in the prospective Genetics of Diabetes Audit and Research Study in Tayside Scotland study. The A allele was associated with lower plasma high-density lipoprotein cholesterol (mean difference, 0.03 mmol/L; \( P = 0.008 \)), higher triglycerides (0.1 mmol/L, \( P = 0.007 \)), higher atherogenic index of plasma (0.03, \( P = 0.003 \)), and, as expected, increased body mass index (0.77 kg/m\(^2\), \( P = 8.8 \times 10^{-6} \)). During a mean follow-up of 3.6 years, the A allele was also associated with increased risk (hazard ratio, 2.36; CI, 1.49 to 3.74; \( P = 0.0002 \)) of fatal and nonfatal myocardial infarction (total of 324 events) in a model, including baseline age, gender, prevalent myocardial infarction, smoking status, statin, and insulin use. This association diminished but remained significant when obesity-related traits, such as body mass index, glycohemoglobin, and lipid parameters, were also included (hazard ratio, 2.01; CI, 1.18 to 3.45, \( P = 0.011 \)). There was a strong interaction of FTO genotype and statin use and cardiovascular outcome (\( P = 0.001 \)), such that cardiovascular morbidity and mortality was completely abrogated in individuals who were prescribed statins.

Conclusion—The increased fat mass in carriers of the A allele of rs9939609 of FTO is associated not only with increased risk of type 2 diabetes, but also with an increase in atherogenic lipid profile and myocardial infarction in these patients. This variant may, therefore, in the future contribute to more effective targeting of specific preventative therapy.

Key Words: genetics ■ myocardial infarction ■ diabetes mellitus ■ heart diseases ■ metabolism

The increasing global burden of obesity is associated with an increasing prevalence of type 2 diabetes (T2D) and subsequently an increase in morbidity and mortality primarily due to ischemic heart disease.\(^1\) It has been estimated that for every increase in a body mass index (BMI) measure of 1 unit there is an 8% increase in cardiovascular mortality.\(^2\)

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Recently, whole genome association studies have demonstrated that single-nucleotide polymorphism (SNP) rs9939609 in the fat mass and obesity (FTO)–associated gene region at 16q10 is strongly associated with both increased body weight and susceptibility to T2D,\(^3\) and this association has been confirmed in other studies.\(^4,5\) The original association with T2D was found to be through an association with BMI. Other SNPs in this region have also been identified with similar associations and it seems likely that their association is due to linkage disequilibrium within the region.\(^4\) Although the SNPs seem to be associated with raised BMI in multiple European populations,\(^3,4\) the association seems to be absent in some non-European populations, such as the blacks\(^4\) and the Chinese Han,\(^6\) and in oceanic populations.\(^7\) It has been demonstrated that the region is also associated with other metabolic features related to being overweight. Healthy individuals possessing the A allele were found to have not only a raised BMI but also an increased whole body resistance to insulin.\(^8\)

Given the firmly established relationship of increased BMI and T2D with cardiovascular morbidity and mortality, we...
investigated the clinical impact of genetic variation in the \textit{FTO} gene in terms of risk of myocardial infarction in a large population of patients with T2D in Scotland known as Genetics of Diabetes Audit and Research Study in Tayside Scotland (Go-DARTS).

\textbf{Methods}

The Go-DARTS population has been described in previous studies\textsuperscript{9–11} and has been collected from the Tayside region of Scotland (population, \(\sim 400,000\)) where all healthcare activity has, for the past 2 decades, been linked to a patient-unique identifier, facilitating the creation of sophisticated regional health informatics systems. DARTS is a comprehensive, well-validated, region-wide clinical information system containing detailed data on effectively all patients with diabetes in the region.\textsuperscript{12} The data are assimilated from sources including the regional biochemistry database containing all historical serum biochemical assays and the regional dispensed prescribing database maintained by the University of Dundee’s Health Informatics Centre. This contains historical data on all medicines prescribed for all individuals in the region from 1993 to present.

Over the past 10 years, an increasing number of patients with T2D in the region have been approached to provide a sample of blood for genetic studies with consent for the genetic information to be linked anonymously to their clinical data in DARTS and associated data sets. Many of these individuals were recruited under the auspices of the Wellcome Trust UK type 2 diabetes case-control consortium and contributed significantly to the replication phase for the WTCCC GWA studies for T2D.\textsuperscript{3} The linkage of this genotypic data from this large group of patients with T2D together with the rich longitudinal clinical data sets constitutes the Go-DARTS study. We studied 4786 individuals with a diagnosis of T2D who were recruited to Go-DARTS between October 1997 and March 2006 and with an age of recruitment <85 years.

\textbf{Genotyping}

Genotyping of rs9939609 was performed as previously described using a combination of TAQMAN allelic discrimination and KASPAR genotyping (Kbiosciences).\textsuperscript{13}

\textbf{Statistics}

Each individual in Go-DARTS often has multiple measures of clinical parameters recorded over a period of time during the course of their clinical management. Therefore, we obtained a single integrated estimate as a mean value of multiple measures for each individual (intraindividual mean), obtained within a 3-year period around the date the individual was recruited into the Go-DARTS study. These clinical parameters were BMI, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, glycohemoglobin, and systolic and diastolic blood pressures. All such intraindividual mean values were adjusted for age and gender. In the case of triglycerides and triglyceride-related values, which can vary widely depending on postprandial state, intraindividual means were also adjusted for intraindividual variation as the standard deviation of the intraindividual range. Atherogenic index of plasma\textsuperscript{14} was determined by \(\log_{10}(\text{TRIG}/\text{HDL-C})\). Analysis of variance was used to determine the impact of genotype on clinical parameters.

To perform a prospective study of the impact of the \textit{FTO} variant on cardiovascular disease in T2D, we studied only individuals who had at least 1 year of follow-up data from recruitment to the genetic study with Cox’s regression being used to estimate the relative rates of cardiovascular end points. The model included exposure to both statin and insulin therapy. These were simply dichotomised as ever having at least 2 prescriptions dispensed during the study period or never having had a prescription. Other variables included in the model were smoking (recorded as ever smokers and never smokers) and a history of a previous myocardial infarction. In this study, a combined end point of fatal and nonfatal myocardial infarction was used as determined by a hospital discharge event with myocardial infarction as the diagnosis or cause of death being recorded as myocardial infarction. All data manipulation and statistical analysis were executed using STATA v9.0.

\textbf{Results}

The baseline characteristics of the 4897 Go-DARTS participants genotyped for \textit{FTO} rs9939609 who fulfilled the entry criteria for the study are shown in Table 1. The rare (A) allele had a frequency of 0.424 SE 0.005. Allele frequencies at this locus in this population did not deviate from Hardy-Weinberg equilibrium (\(P=0.96\)). Adjusted means by \textit{FTO} genotype for BMI, pulse pressure, mean arterial pressure, glycohemoglobin, HDL-C, triglycerides, total cholesterol, and atherogenic index of plasma are provided in Table 2. Inspection of the data indicated a dominant/codominant impact of the variant on all clinical parameters investigated and, therefore, the significance levels for both models are given. As expected, there was a strong association of the \textit{FTO} variant with BMI, with the adjusted mean for individuals carrying the A allele (dominant model) being 0.77 kg/m\(^2\) greater than TT homozygotes (\(P=8.8\times10^{-6}\)). Similarly, the A allele was associated with an adjusted mean HDL-C that was 0.03 mmol/L lower (\(P=0.008\)) and an adjusted mean triglyceride 0.1 mmol/L higher (\(P=0.002\)) compared with TT homozygotes. There was no association of \textit{FTO} with total cholesterol. Although individuals with the A allele were more likely to have been exposed to statin medication (\(P=0.002\)) (Table 3), accounting for this did not alter this lack of association. To further emphasize the atherogeneity of the lipid profile, we determined the atherogenic index\textsuperscript{15} and found that the A allele was associated with a significantly higher index (0.03, \(P=0.003\)). We found no significant association of genotype with glycohemoglobin parameters or treatment with insulin (data not shown).

The impact of \textit{FTO} genotype on cardiovascular morbidity and mortality was modeled by determining the relative hazard of possession of the A allele of rs9939609 for developing a fatal or nonfatal myocardial infarction after recruitment in the 4101 individuals with complete data. Age, gender, smoking

\begin{table}
\centering
\caption{Population Characteristics}
\begin{tabular}{ll}
\hline
\textbf{Parameter} & \textbf{Mean} & \textbf{SD} \\
\hline
Age, y & 63.7 & 11.7 \\
Female, % & 55.3 & \ldots \\
Prevalent MI, % & 10.9 & \ldots \\
Statin prescription, % & 69.8 & \ldots \\
Insulin prescription, % & 32.4 & \ldots \\
BMI, kg/m\(^2\) & 30.7 & 5.9 \\
Systolic BP, mm Hg & 140.9 & 7.8 \\
Diastolic BP, mm Hg & 77.7 & 7.7 \\
Glycated hemoglobin, % & 7.7 & 1.3 \\
Triglycerides, mmol/L & 2.4 & 1.6 \\
Total cholesterol, mmol/L & 4.8 & 0.9 \\
HDL cholesterol, mmol/L & 1.3 & 0.4 \\
\hline
\end{tabular}
\end{table}

\textit{MI} indicates myocardial infarction.
status, prevalent myocardial infarction, and having a history of statin and insulin use were included in the initial model. The model included an interaction term of genotype with statin use. During a mean follow-up of 3.6 years, there were a total of 324 events with 94 (6.82%) occurring in the 1377 TT homozygotes and 230 (8.44%) in the 2724 individuals with the A allele. The hazard ratio (HR) for possession of the A allele compared with TT homozygotes in this model was 2.36 (CI, 1.49 to 3.74; \( P = 0.0002 \)). With the subsequent inclusion of obesity-related parameters, ie, BMI, glycohemoglobin, mean arterial pressure, HDL-C, triglycerides, and total cholesterol in the model, the impact of FTO genotype was reduced but remained significant (HR, 2.01; CI, 1.18 to 3.45, \( P = 0.011 \)). As a result of the finding of a significant interaction term (\( P = 0.001 \)) for statin use by FTO genotype, we stratified the data according to statin use. The Figure shows the Kaplan-Meier hazard functions for possession of the A allele compared with TT homozygotes in these 2 groups, showing that the increased risk is seen in the 1266 nonstatin users (HR, 2.3; CI, 1.4 to 3.7, \( P = 0.0005 \)), but is abolished by statin use (HR, 0.9; CI 0.7 to 1.3, \( P = 0.64 \)).

### Discussion

We have demonstrated in this large prospective longitudinal study of patients with T2D that variation in the FTO gene is not only associated with an increased BMI, but also with a specific dyslipidemic phenotype that is characteristic of insulin resistance. This, in turn, is associated with an increase in risk of myocardial infarction and cardiovascular death. These observations come from a population with established T2D, and the genotypic effect of the FTO variant on lipids is, therefore, over and above the dyslipidemic profile and cardiovascular risk we would expect in this population. This suggests that within the spectrum of phenotypic subtypes of T2D the FTO variant is associated with one that is marked by greater insulin resistance and at greater cardiovascular risk.

In determining metabolic phenotype, we have been able to exploit the availability of multiple measures over time in Go-DARTS to determine a mean value for an individual and in this way accommodate the temporal fluctuation in single values that may obscure genuine associations. The risk allele for increased weight and T2D is associated with a lower plasma HDL-C level, raised plasma triglycerides and therefore a raised atherogenic index, and as expected this is largely driven by the effects of FTO on body weight. Interestingly, the FTO variant seems to be associated with overall fat mass in terms of both central abdominal obesity and subcutaneous fat and, therefore, not associated with a body fat distribution typically associated with the insulin-resistant state. On the other hand, the clear association with the risk of development of T2D implicates insulin resistance. Furthermore, in a study of 1286 healthy individuals in whom diabetes had been excluded, the FTO A allele was found to be associated with peripheral insulin resistance as measured by hyperinsulinemic euglycemic clamp. The insulin resistance, however, was again entirely dependent on body weight.

Carriers of the FTO A allele were over twice as likely to have a myocardial infarction or cardiovascular death than the TT homozygotes. The strength of this association seems to be reduced, although remains significant by including obesity-related parameters in the model. This observation seems initially to be divergent from the association of FTO genotype with T2D, which is completely explained by the effect of the FTO genotype on BMI. In this case, it is likely to be due to the correction of the obesity-driven dyslipidemia in the individuals who receive statins, thus, disconnecting obesity risk from cardiovascular risk. This is consistent with previous observations that, for a similar cholesterol reduction, statins are more beneficial in patients who have a low HDL-C and high triglyceride.

In this observational study, the strong modulating influence of statin therapy with FTO genotype–associated outcome raises several important questions. We chose the simple metric of having had 2 prescriptions for a stain dispensed for pragmatic reasons, given the known significant impact statins

### Table 2. Influence of FTO Genotype on Clinical Parameters

<table>
<thead>
<tr>
<th>Genotype Count, %</th>
<th>TT 1625 (33.2)</th>
<th>TA 2391 (48.8)</th>
<th>AA 881 (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
</tr>
<tr>
<td></td>
<td>30.16 0.14</td>
<td>30.80 0.12</td>
<td>31.29 0.19</td>
</tr>
<tr>
<td><strong>Pulse pressure, mm Hg</strong></td>
<td>62.77 0.25</td>
<td>63.31 0.17</td>
<td>63.84 0.31</td>
</tr>
<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
<td>98.5 0.2</td>
<td>98.8 0.1</td>
<td>99.2 0.2</td>
</tr>
<tr>
<td><strong>Glycated hemoglobin, %</strong></td>
<td>7.7 0.03</td>
<td>7.7 0.02</td>
<td>7.8 0.04</td>
</tr>
<tr>
<td><strong>HDL, mmol/L</strong></td>
<td>1.32 0.008</td>
<td>1.30 0.005</td>
<td>1.29 0.01</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>2.36 0.03</td>
<td>2.44 0.02</td>
<td>2.52 0.03</td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/L</strong></td>
<td>4.83 0.02</td>
<td>4.84 0.01</td>
<td>4.85 0.03</td>
</tr>
<tr>
<td><strong>AIP</strong></td>
<td>0.20 0.006</td>
<td>0.22 0.004</td>
<td>0.24 0.08</td>
</tr>
</tbody>
</table>

All values adjusted for age and gender (measures including triglycerides also adjusted for intrinsic intraindividual variation). AIP indicates atherogenic index of plasma.

### Table 3. Individuals Receiving a Prescription for a Statin During the Course of the Study in Entire Cohort

<table>
<thead>
<tr>
<th>Statin Prescription</th>
<th>TT 478 (30)</th>
<th>TA/AA 823 (25)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yes</strong></td>
<td>1140 (71)</td>
<td>2433 (75)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1618</td>
<td>3256</td>
</tr>
</tbody>
</table>

Values in parenthesis are percentages. OR, 1.24; CI, 1.09 to 1.42; \( P = 0.002 \).
have on cardiovascular event rates, however, there are many potential biases that could influence this metric. For example, we have demonstrated that the risk allele of FTO was associated with a significantly higher level of prescribing of statins, despite no differences in cholesterol levels by genotype. However, no association was found between cholesterol levels and FTO genotype in either individuals who were prescribed statins or those not prescribed statins (data not shown) and, thus, the increased tendency to receive a statin seems unlikely due to this indication in those carrying the risk allele. This suggests that the increased statin prescribing may reflect other clinical aspects or risks associated with the obese phenotype, which is supported by the finding that this allele was associated with significantly increased tendency to be prescribed other medications, such as the fibrates and thiazolidinedines (data not shown). This observation may, therefore, further support the major finding of this study that this allele is associated with a significantly higher level of prescribing of protective medications being prescribed. In such an observational study, such potential biases are challenging to tease out, and as such this finding would require to be replicated in a randomized prospective study of statin use. It would also be appropriate to consider replication of this finding in a nondiabetic population; however, given the assumed biological association of this FTO variant with outcome is through an engendered metabolic phenotype, the event rate in the nondiabetic population is likely to be very low, requiring very large numbers of individuals. This is a likely reason that FTO genotype is associated with a metabolic phenotype, which, in turn, is associated with greater cardiovascular risk. Thus, the observed impact of statins may be partly due to the fact that the individuals at increased risk have a greater number of protective medications being prescribed. In such an observational study, such potential biases are challenging to tease out, and as such this finding would require to be replicated in a randomized prospective study of statin use. It would also be appropriate to consider replication of this finding in a nondiabetic population; however, given the assumed biological association of this FTO variant with outcome is through an engendered metabolic phenotype, the event rate in the nondiabetic population is likely to be very low, requiring very large numbers of individuals. This is a likely reason that FTO has not been a striking feature from genome-wide association studies of early-onset cardiovascular disease.

At present, the mechanism of action of the FTO variant on increasing weight gain and increased insulin resistance is uncertain. There are no features suggesting that rs9939609 is the causal variant and, indeed, there are many other variants that are in complete linkage disequilibrium with this variant and are, therefore, linked to obesity and diabetes. However, based on the rapid fall off of linkage disequilibrium with other SNPs beyond 47 kb, it has been concluded that the functional variant is likely to lie within this area of the FTO gene. Indeed, rs8050136 has recently been implicated in modulating the binding of a transcription factor CUTL1, and CUTL1 knockdown by small interfering RNA was shown to repress both FTO expression and expression of the neighboring gene FTM. This SNP lies 4 kb from rs9939609 and these variants are in complete linkage disequilibrium in the white population (D' of 1.00 and R² of 0.996). Through the use of bioinformatics and recombinant functional studies, it has recently been demonstrated that the FTO gene encodes a nucleic acid demethylase. Although the physiologically relevant substrate for this enzyme has yet to be determined, expression seems to be highest in the brain and, particularly, in hypothalamic nuclei involved in energy balance. Furthermore, expression was found to be modulated by feeding and fasting, and correlated with the expression of various peptide hormones known to modulate eating behavior, including neuropeptide Y. The neighboring gene FTM, which encodes a ciliary protein, seems to be coregulated with FTO in the arcuate nucleus and may also play a role in the observed phenotypes. This molecular data support direct observations from our own group that the eating behavior of young children is modulated by FTO genotype. Indeed, the FTO genotype has now been associated with weight gain from the age of 2 weeks postbirth. This, together with this study, clearly indicates that this genotype is having a significant role in human health from the cradle to the grave, and highlights recent concerns regarding the increased levels of childhood obesity and subsequent cardiovascular morbidity and mortality.

Conclusion

We have demonstrated in a large group of patients with T2D that the A allele of rs9939609 in the FTO gene is associated not only with a raised BMI but also with a tendency to a dyslipidemic phenotype seen typically in the insulin resistance syndrome. This translates into an increased risk of myocardial infarction. Prescription with statins seemed to ameliorate this association, indicating that it is possible for intervention to prevent the serious life-threatening consequences of this genotype.

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Disclosures

None.
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