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Fucosyltransferase 3 Polymorphism and Atherothrombotic Disease in the Framingham Offspring Study

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Abstract

Background—Previous studies have suggested a positive association between phenotypes of fucosyltransferase 3 (*FUT3)* gene (also known as Lewis gene) and coronary heart disease.

Methods—We used data on 1,735 unrelated subjects in the Framingham Offspring Study to assess whether 3 functional single nucleotide polymorphisms (SNPs) of the *FUT3* gene (T59G, T1067A, and T202C) were associated with prevalent atherothrombotic disease.

Results—Contrary to T1067A and T202C SNPs, there was evidence for an association between T59G SNP and atherothrombotic disease prevalence. In a multivariable model controlling for age, sex, alcohol intake, pack-years of smoking, ratio of total-to-HDL-cholesterol, and diabetes mellitus, odds ratios (95% CI) for prevalent atherothrombotic disease were 1.0 (reference), 0.80 (0.46-1.41), and 6.70 (1.95-23.01) for TT, TG, and GG genotypes of the T59G SNP, respectively. Minor alleles of T202C and T1067A SNPs showed a modest and non-significant association with atherothrombotic disease. Overall, FUT3 polymorphism that influences the enzyme activity (GG genotype for T59G or $\geq$ 1 minor allele of T202C or T1067A) was associated with increased atherothrombotic disease prevalence [OR: 1.57 (1.05-2.34)] and this association was stronger among abstainers (2-fold increased odds) than among current drinkers (p for interaction 0.11).

Conclusions—Our data suggest that functional mutations of the *FUT3* gene may be associated with an increased atherothrombotic disease prevalence, especially among abstainers. Additional studies are warranted to confirm these findings.

Keywords

Cardiovascular disease; *FUT3* gene; epidemiology; genetics

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Although cardiovascular disease remains the leading cause of death in the US, little is known about its genetic determinants. The α (1,3/1, 4) fucosyltransferase 3 (*FUT3*) gene (also know as Lewis gene) is located on the short arm of chromosome 19 (19p13.3)[1]and previous studies have reported that Lewis (a-b-) phenotype is associated with a 2-fold increased risk of coronary heart disease[2,3]. In addition, an earlier study has suggested that alcohol consumption may modify the Lewis gene-ischemic heart disease association[4]. The α (1, 3/1, 4) fucosyltransferase enzyme is responsible for fucosylation of proteins. Fucosylated glycoproteins are important for cell adhesion and as tumor markers[5]. While the T59G mutation reduces the availability of fucosyltransferase 3 enzyme[6,7], the T1067A and T202C mutations drastically decrease the enzyme activity[8]. We used data collected on 1,735 unrelated participants in the Framingham Offspring Study to assess whether these 3 single nucleotide polymorphisms of the *FUT3* gene[1] were associated with the prevalence of atherothrombotic disease and whether such relation was influenced by alcohol consumption.

Methods

Population

The Framingham Heart Study is a population based cohort study that began in 1948 in Framingham, MA. In 1971, 5124 offspring (and their spouses) of the original cohort (referred to as the Framingham Offspring Study) were entered into a prospective cohort study. Detailed descriptions of the Framingham Heart Study have been published previously[9,10]. DNA samples were collected during examination 6 (1993-1997) of the Offspring Study and the present study used data from 1,805 Caucasian unrelated participants. Written informed consent was obtained from study participants, and the study protocol was approved by the Institutional Review Board of Brigham and Women's Hospital.

Assessment of atherothrombotic disease in the Framingham Heart Study

Details on outcome assessment and validation in the Framingham Study have been published[11,12]. Briefly, at each examination a medical history, physical examination, a 12-lead ECG, and other tests were completed. We defined atherothrombotic disease as presence of recognized myocardial infarction (ICD-9 code 410), coronary insufficiency (ICD-9 code 411), or atherothrombotic stroke (ICD-9 code 433 and 434).

FUT3 SNP genotyping

SNPs were genotyped using the TaqMan technology[13,14] implemented on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). PCR was performed using TaqMan Universal Master Mix (Applied Biosystems), 5 ng DNA, 900 nM of each primer, and 200 nM of each probe in a 5μl reaction. SNP detection involved amplification of appropriate chromosomal regions by PCR, then a primer extension reaction using a dideoxynucleotide that is complementary to one of the SNP alleles. Genotype data were obtained from 1,735 individuals.

Other covariates

HDL was measured using a heparin-manganese chloride procedure[15,16]. Total cholesterol was measured by a manual Abell-Kendall procedure[17]. Hypertension was defined as systolic blood pressure≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or treatment for hypertension. Diabetes mellitus was defined as fasting blood glucose greater than 126 mg/dL or current use of hypoglycemic agents.

Statistical analysis

We used a chi-square test to determine if the distribution of genotypes for each *FUT3* SNP was in Hardy-Weinberg equilibrium. For individual SNP analyses, wild-type genotype was used as reference. For overall analyses, we separated individuals with FUT3 genotypes that either reduce the fucosyltransferase activity (202C and 1067A) or limit is availability (GG of the T59G mutation) from the rest of the population. We used logistic regression to estimate multivariable-adjusted odds ratios for atherothrombotic disease. We assessed confounding by age, sex, hypertension, diabetes mellitus, alcohol consumption, body mass index, systolic blood pressure, smoking pack-years, HDL-cholesterol, ratio of total-to-HDL cholesterol, and education. Each variable was assessed individually and in combination with other variables. Specifically, a variable that led to a 10% or more change in odds ratio was consider as confounder and retain in the most parsimonious model. The final model included sex, age, pack-years of smoking, alcohol consumption, diabetes mellitus, and ratio of total-to-HDL cholesterol. Because most studies of FUT3 polymorphism have reported an association with coronary heart disease, we repeated these analyses excluding ischemic stroke as endpoint. All analyses were performed using PC SAS statistical analysis (SAS Inc., Cary, North Carolina).

Results

Baseline characteristics of the study participants are presented in Table 1. The genotype frequencies for T59G and T1067A SNPs but not for T202C SNP were not different from those expected under Hardy-Weinberg equilibrium (Table 2). While T1067A and T202C SNPs revealed modest and non-significant associations with atherothrombotic disease, there was evidence for an association between T59G SNP and atherothrombotic disease, with a 6.7-fold increased prevalence odds of atherothrombotic disease among subjects with GG compared with TT genotypes in a multivariable model (Table 3). This result is tempered by the fact that we only had 15 people with the GG genotype; thus, although our result is statistically significant, the estimate is imprecise. Overall, individuals with FUT3 genotypes that either reduce the fucosyltransferase activity (202C and 1067A) or limit the availability of the fucosyltransferase (GG of the T59G mutation) had a 57% increased odds of atherothrombotic disease compared with other genotypes (Table 4). Having a genotype that reduce the fucosyltransferase activity or limit its availability was associated with a 2-fold increased prevalence of atherothrombotic disease among abstainers, but not among current drinkers (p for interaction 0.11, Table 4). Exclusion of ischemic stroke cases from the outcome did not change the conclusions. For example, the GG genotype of the T59G SNP was associated with a 3.8-fold increased odds of atherothrombotic disease compared with subjects with TT genotype and having a genotype that reduce the fucosyltransferase activity or limit its availability was associated with a 2-fold increased prevalence of atherothrombotic disease among abstainers [OR=2.03 (1.11-3.72)], but not among current drinkers [OR=0.78 (0.37-1.66)] in a multivariable model.

Discussion

Individuals with FUT3 polymorphisms that influence fucosyltransferase activity or its availability an increased prevalence odds of atherothrombotic disease and this association was more evident and stronger among abstainers (borderline p value for interaction). The effect modification by alcohol consumption was restricted to SNPs known to decrease the activity of the fucosyltransferase enzyme. Previous studies have reported an increased risk of coronary artery disease among individuals with Lewis (a-b-) phenotype[2,3]. Since weak hemagglutination, inflammation, or cancer may lead to false-negative reactions[1], *FUT3* studies based on phenotype determination might misclassify *FUT3* genotype. However, earlier studies have demonstrated that individuals with Lewis negative (a-b-) phenotypes carry at least one of the 3 mutations studied in this study[1,8]. Our data are consistent with previous reports

suggesting an increased risk of coronary artery disease among Lewis negative individuals. The final product encoded by *FUT3* gene is an enzyme responsible for fucosylation of proteins. Fucosylated glycoproteins carrying α (1, 4) fucose residue s are important for cell adhesion and as tumor markers[5]. However, little is known about physiologic mechanisms by which *FUT3* gene may influence the risk of atherothrombotic disease. It has been suggested that the increased risk of coronary artery disease observed among subjects with Lewis (a-b-) may be mediated partially through elevated plasma triglycerides[2,3,18]or insulin and glucose[19]. In addition, Lewis (a-b-) has been reported as a marker of obesity[20]. Alcohol consumption and exercise are known to improve insulin sensitivity. Our findings of an increased prevalence of atherothrombotic disease among abstainers but not in current drinkers are in line with this hypothesis and consistent with another study suggesting an effect modification by alcohol of the Lewis-coronary heart disease association[4]. In addition, the fact that an increased risk of coronary death in Lewis (a-b-) subjects was observed among subjects with low but not high physical activity[18] suggests that exercise may mitigate the effects of Lewis (a-b-) on atherothrombotic disease through improvement of insulin metabolism. Although we had a relatively small sample size, the complete ascertainment for atherothrombotic disease cases and the availability of data on most potential confounders are strengths of this paper.

In conclusion, the present study suggests that functional mutation of the *FUT3* gene is associated with an increased prevalence of atherothrombotic disease, particularly among abstainers and may help in risk stratification. Future studies are needed to confirm these findings and elucidate underlying biological mechanisms.

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Table 1

Characteristics of 1,735 study participants according to FUT3 genotypes in the Framingham Offspring Study

Characteristics	*FUT3* Genotype	
	FUT3 genotypes that affect enzyme activity* (n=506)	**FUT3 genotypes without effect on enzyme activity† (n=1229)**
Alcohol (g/d)	8.1±12.0	10.3±15.1
Age (y)	59.1±9.6	59.9±9.5
Body mass index (kg/m^2)	25.1±4.5	25.3±4.2
Education (y)	14.0±2.6	14.0±2.6
Systolic BP (mm Hg)	120.9±16.2	122.2±16.0
Total cholesterol (mg/dl)	196.7±39.0	193.6±36.5
HDL-cholesterol (mg/dl)	51.5±15.3	50.6±14.6
Ratio of total/HDL cholesterol	4.3±1.4	4.4±1.3
Pack-years of smoking	9.7±14.8	11.7±15.4
Sex (% male)	47.4	50.8
Hypertension (%)	17.8	17.7
Diabetes mellitus (%)	12.7	14.0

* **Defined** as presence of TA and AA genotypes of the T1067A mutation; CC and TC genotypes for T202C mutation; and GG genotype of the T59G mutation.

† **The rest of the genotypes not included above**. Data are presented as means ± standard deviation unless otherwise specified

Table 2

Frequency distribution of the 3 single nucleotide polymorphisms of *FUT3* gene among unrelated participants of the Framingham Offspring Study

SNP	Genotype	N	Observed	Expected	Chi-square	p for HWE*
T59G						
	TT	1210	83.4%	83.0%		
	TG	226	15.6%	16.2%		
	GG	15	1.0%	0.8%	1.37	0.50
T1067A						
	TT	1245	88.5%	88.8%		
	TA	162	11.5%	10.9%		
	AA	0	0.0%	0.3%	0.09	0.76
T202C						
	TT	1076	74.7%	72.0%		
	TC	303	21.0%	25.5%		
	CC	61	4.3%	2.5%	30.1	<0.0001

* HWE: Hardy-Weinberg equilibrium.

Table 3

Odds ratios (95% CI) of **atherothrombotic disease** according to *FUT3* polymorphism in the Framingham Offspring Study*

Genotypes	Cases/N	Crude	Model 2†	Model 3‡
T59G SNP				
TT	99/1210	1.0	1.0	1.0
TG	18/226	0.97 (0.58-1.64)	0.80 (0.46-1.40)	0.80 (0.46-1.41)
GG	5/15	5.62 (1.88-16.75)	6.47 (1.91-21.92)	6.70 (1.95-23.01)
T1067A				
TT	94/1245	1.0	1.0	1.0
TA	15/162	1.25 (0.71-2.21)	1.14 (0.61-2.11)	1.19 (0.64-2.24)
AA	--	--	--	--
T202C				
TT	81/1076	1.0	1.0	1.0
TC	27/303	1.20 (0.76-1.90)	1.45 (0.89-2.36)	1.46 (0.89-2.41)
CC	5/61	1.10 (0.43-2.82)	1.27 (0.47-3.45)	1.24 (0.44-3.50)

*__Atherothrombotic disease__ includes recognized myocardial infarction, coronary insufficiency, and atherothrombotic stroke; *FUT3* gene is also known as Lewis gene.

†Model 2 adjusted for age, sex, and ratio of total-to-HDL-cholesterol

‡Model 3 adjusted for variables in model 1 plus diabetes mellitus, alcohol consumption, and pack-years of smoking.

Table 4

Odds ratios (95% CI) of **atherothrombotic disease** according to *FUT3* polymorphism and alcohol consumption in the Framingham Offspring Study*

	Overall		Abstainers		Current drinkers	
***FUT3* enzyme**	**Cases/N**	**OR(95% CI)†**	**Cases/N**	**OR(95% CI)†**	**Cases/N**	**OR(95% CI)†**
Not affected	88/1229	1.0	40/531	1.0	48/698	1.0
Affected	48/506	1.57 (1.05-2.34)	31/239	2.13 (1.22-3.73)	17/267	1.16 (0.62-2.16)
P for alcohol*Lewis interaction				0.11		

*Atherothrombotic disease includes recognized myocardial infarction, coronary insufficiency, and atherothrombotic stroke; *FUT3* gene is also known as Lewis gene. **FUT3 enzyme is affected by the** presence of any allele known to reduce fucosyltransferase activity (202C and 1067A) or GG genotype for T59G mutation, known to limit availability of the fucosyltransferase enzyme; otherwise the enzyme activity is not affected.

†Model 3 adjusted for age, sex, ratio of total-to-HDL-cholesterol, diabetes mellitus, alcohol consumption, and pack-years of smoking.