*rapid communication*

# Three polymorphisms associated with low hepatic lipase activity are common in African Americans

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**Abstract We have shown previously that a hepatic lipase allele (designated −514T) is common among African Americans and contributes to low hepatic lipase activity in this population. To identify other hepatic lipase alleles associated with low hepatic lipase activity in this population, the coding region and intron–exon boundaries of the hepatic lipase gene were sequenced in 20 African American men with low hepatic lipase activity. Two polymorphisms (N193S and L334F) were associated with low post-heparin plasma hepatic lipase activity and were much more common in African Americans than in whites. This finding, together with our previous data on the −514T allele, indicates that at least three different hepatic lipase polymorphisms associated with low hepatic lipase activity are common among African Americans. Analysis of hepatic lipase haplotypes revealed that 97% of African Americans have at least one hepatic lipase allele that is associated with low hepatic lipase activity.—**Nie, L., S. Niu, G. L. Vega, L. T. Clark, A. Tang, S. M. Grundy, and J. C. Cohen. **Three polymorphisms associated with low hepatic lipase activity are common in African Americans. *J. Lipid Res.* 1998.** 39: **1900–1903.**

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Epidemiological studies have shown that plasma high density lipoprotein cholesterol (HDL-C) concentrations are higher in African American men than in white American men (1–4). This ethnic difference in plasma HDL-C concentrations cannot be accounted for by environmental factors and is, therefore, likely to be of genetic origin (2). Recently, we found that the activity of hepatic lipase, an enzyme involved in HDL catabolism, is lower in African American men than in white American men (5). In that study, plasma HDL-C concentrations were similar in African American and white American men with similar hepatic lipase activities. This observation suggests that the difference in plasma HDL-C concentrations between these two populations may be due to ethnic differences in hepatic lipase activity.

The low hepatic lipase activity observed in African Americans was due in part to the increased prevalence in that population of a hepatic lipase allele (designated −514T) that is associated with reduced hepatic lipase activity (5). Interestingly, the increased prevalence of the −514T allele did not appear to fully account for the low hepatic lipase activities observed in African Americans. When African American and white American men were grouped according to their hepatic lipase genotypes using the −514 polymorphism, hepatic lipase activities were significantly lower in African Americans in both CC and CT genotype groups (the TT genotype groups could not be compared because of the low prevalence of this genotype among whites). This observation suggests that other factors besides the −514T allele also contribute to the differences in hepatic lipase activities observed in these two populations. One possibility is that other common alleles of hepatic lipase gene (*LIPC*) that are associated with low hepatic lipase activity are more common among African Americans. To identify such polymorphisms we sequenced key regions of the hepatic lipase gene in African Americans with low hepatic lipase activity. Our data show that two previously identified polymorphisms (6, 7) that cause amino acid substitutions in hepatic lipase (N193S and L334F) are associated with low hepatic lipase activity and are much more common in African Americans than in whites.

## METHODS

The study was approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and the State University of New York Health Science Center.

### Subjects

African American and white men were recruited by advertisements posted at the University of Texas Southwestern Medical

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TABLE 1. Age, anthropometry, and plasma lipids of African American and white men

| Variable | African-American Men | White Men |
|---|---|---|
| n | 47 | 119 |
| Age | 34 ± 7 | 32 ± 11 |
| BMI (kg/m$^2$) | 26 ± 5 | 26 ± 3 |
| Plasma cholesterol (mg/dl) | 173 ± 35 | 167 ± 36 |
| Plasma triglyceride (mg/dl) | 86 ± 50 | 104 ± 56 |
| HDL-cholesterol (mg/dl) | 46 ± 11 | 40 ± 9 |

Center and at the State University of New York Health Science Center. All of the men were apparently healthy, none were diabetic, and none used lipid-lowering drugs or hormones. Age, anthropometry, and plasma lipid values of the men are given in **Table 1**.

### Assay of post-heparin plasma hepatic lipase activity

Hepatic lipase activity was measured in post-heparin plasma as described previously (8).

### DNA sequencing

The exons and intron/exon boundaries of the hepatic lipase gene were sequenced in 20 African American men who had low post-heparin plasma hepatic lipase activities. Sequencing was performed using a standard protocol for cycle sequencing (9).

### Assay of hepatic lipase polymorphisms

The −514 polymorphism was assayed as described previously (10). The A to G substitution at nucleotide +651 created a Bsr I restriction site (5′ ACTGG 3′). To assay this polymorphism, a 315 bp fragment containing the restriction site was PCR amplified using the primers 5′ AGGCCCCATGAAAATGTCAGTG CTC 3′ and 5′ CCCGCGTAACCCTTACCCCTGCT 3′. The A to C polymorphism at +1075 destroyed a Mse I restriction site (5′ TTAA 3′). To assay this polymorphism, a 232 bp fragment containing the restriction site was PCR amplified using the primers 5′ TCACTGCTTAAATTATCTCTCTCTT 3′ and 5′ ACAGAGTCCA TTTATGTTCTGCAAG 3′. PCR amplified fragments were labeled by adding 0.3 pmol [$^{32}$P]dCTP to the PCR reaction mixture. The DNA fragment was digested by adding 5 U of Bsr I in 30 μl NEB buffer 3 (New England Biolabs) to the PCR reaction and electrophoresed on a 5% polyacrylamide gel.

### Statistical methods

The frequencies of hepatic lipase alleles in whites and in African Americans were compared using $\chi^2$ tests. The mean hepatic lipase activities and body mass indices were compared in African American men with the +1075AA genotype and +1075AC genotypes using *t*-tests. The mean hepatic lipase activities and body mass indices were compared in white American men with the +651AA, AG, and GG genotypes using analysis of variance.

## RESULTS

DNA sequencing revealed two polymorphisms in the coding region of hepatic lipase that were present in two or more African Americans: an A to G substitution at nucleotide +651 that changes an asparagine at codon 193 to serine (N193S), and an A to C substitution at +1075, that changes a leucine at codon 334 to phenylalanine (L334F). Both of these polymorphisms have been identified previously in whites (6, 7). The +651G allele was approximately twice as common in African American men ($P_G$ = 0.64) as in white men ($P_G$ = 0.34). The +1075C allele was approximately 20 times more common in African American men ($P_C$ = 0.20) than in white men ($P_C$ = 0.01). The observed frequencies of the +651 and +1075 hepatic lipase genotypes were consistent with Hardy-Weinberg equilibrium in both populations (**Table 2**).

TABLE 2. Hepatic lipase genotypes in African Americans and white Americans

| *LIPC* Genotype | African American (Observed) | African American (Expected) | White (Observed) | White (Expected) |
|---|---|---|---|---|
| 1) +651[a,b] | | | | |
| AA | 8 | 6 | 54 | 52 |
| AG | 18 | 22 | 49 | 53 |
| GG | 21 | 19 | 16 | 14 |
| 2) +1075[a,b] | | | | |
| CC | 1 | 2 | 0 | 0 |
| AC | 17 | 15 | 3 | 3 |
| AA | 29 | 30 | 112 | 112 |
| 3) −514[a,b] | | | | |
| CC | 10 | 10 | 67 | 67 |
| CT | 23 | 23 | 45 | 44 |
| TT | 14 | 14 | 7 | 7 |

[a] $P < 0.001$ for allele frequency in African Americans versus white Americans ($\chi^2$ test).
[b] $P > 0.7$ for observed versus expected allele frequency in African Americans and in white Americans ($\chi^2$ test).

### Effects of hepatic lipase polymorphisms on hepatic lipase activity

To assess the effects of the +651 and +1075 polymorphisms on hepatic lipase activity, the mean hepatic lipase activities of men with different +651 or +1075 genotypes were compared (**Table 3** and **Table 4**). Among African American men, analysis of the +651 polymorphism was confounded by the effects of the −514 and +1075 polymorphisms, both of which strongly affect hepatic lipase activity (5, 7). Of the 8 +651A homozygotes, all had at least one −514T allele, which is associated with low hepatic lipase activity, and 5 had at least one +1075C allele, which is also associated with low hepatic lipase activity. Therefore it was not possible to assess the effect of the +651 polymorphism on hepatic lipase activity independently of the −514 and +1075 polymorphisms in the Afri-

TABLE 3. Hepatic lipase +651 genotypes and hepatic lipase activity in white men

| *LIPC* Genotype | n | Hepatic Lipase Activity | Body Mass Index |
|---|---|---|---|
| +651AA | 29 | 50 ± 14 | 26.4 ± 3.3 |
| +651AG | 29 | 45 ± 16 | 26.0 ± 2.7 |
| +651GG | 8 | 38 ± 9 | 25.7 ± 2.9 |

All men were homozygotes for the −514C and +1075A alleles. $P < 0.05$ for hepatic lipase activity in AA versus AG versus GG (ANOVA). $P < 0.01$ for hepatic lipase in AA versus GG (*t*-test). $P = 0.29$ for BMI in AA versus AG versus GG (ANOVA). $P = 0.25$ for BMI in AA versus GG (*t*-test).

TABLE 4. Hepatic lipase genotypes and hepatic lipase activity in African American men

| *LIPC* Genotype | n | Hepatic Lipase Activity | Body Mass Index |
|---|---|---|---|
| 1) +651 | | | |
| AA | 8 | 29 ± 12 | 29 ± 4 |
| AG | 18 | 26 ± 10 | 25 ± 6 |
| GG | 21 | 29 ± 13 | 27 ± 5 |
| 2) +1075 | | | |
| AA | 29 | 31 ± 12[a] | 26 ± 5[b] |
| AC | 17 | 24 ± 11 | 27 ± 6 |
| CC | 1 | 16 | 29 |
| 3) −514 | | | |
| CC | 10 | 33 ± 9[c] | 26 ± 5[d] |
| CT | 23 | 28 ± 12 | 27 ± 5 |
| TT | 14 | 24 ± 12 | 27 ± 5 |

[a] $P < 0.05$ for hepatic lipase activity of AA versus AC (*t*-test).
[b] $P = 0.3$ for body mass index of AA versus AC (*t*-test).
[c] $P < 0.05$ for CC versus TT (*t*-test).
[d] $P = 0.3$ for body mass index of CC versus TT (*t*-test).

can Americans in this study. Accordingly, genotype comparisons were performed in whites, as the −514T and +1075C alleles are far less common in this population than among African Americans. In 66 white men who were homozygous for the −514C and +1075A alleles of hepatic lipase, mean hepatic lipase activity was 24% lower in +651G homozygotes and 10% lower in +651AG heterozygotes than in +651A homozygotes (Table 3). Mean body mass index was similar in all three groups (Table 3).

Mean hepatic lipase activity was 29% lower in African American men who were heterozygotes for the +1075C and +1075A alleles of hepatic lipase than in men who were homozygotes for the +1075A allele (Table 4). To adjust for possible confounding by the −514 polymorphism, the weighted average of hepatic lipase activity in the −514CC, −514CT and −514TT genotypes was calculated. After this adjustment, mean hepatic lipase activity was 23% lower in +1075CA heterozygotes than in +1075AA homozygotes.

### *LIPC* haplotypes

Assuming that the −514, +651, and +1075 polymorphisms have independent effects on hepatic lipase activity, the haplotype −514C, +651A, +1075A should be associated with the highest hepatic lipase activity. The frequency of this haplotype among African Americans and white Americans was calculated using individuals who were homozygous at one or more of the polymorphic loci. Among African Americans, seven of the eight possible haplotypes were observed (data not shown). Of the 47 African American men in this study, 20 were homozygous for C at nucleotide −514, of whom 7 were homozygous for A at nucleotide +1075. Among these 7 men, none were homozygous and 6 were heterozygous for A at nucleotide +651. Therefore, the frequency of the −514C, +651A, +1075A haplotype in African Americans can be calculated from the frequency of the −514C allele in the population (0.46) multiplied by the fraction of −514C alleles that have an A at +1075 (17/20), multiplied by the fraction of −514C, +1075A alleles that have an A at +651 (6/14). Using this calculation, we estimate that the frequency of the −514C, +651A, +1075A haplotype is 0.17 in African Americans. The corresponding calculation in white Americans indicated a frequency of 0.49 for the −514C,+651A,+1075A haplotype.

## DISCUSSION

Hepatic lipase activity, an important determinant of plasma HDL-C concentrations, is lower among African American men than among white men (5). The high prevalence of a specific *LIPC* allele (designated −514T) among African Americans contributes to the low hepatic lipase activity in this population, but statistically significant ethnic differences were observed even among men with identical −514 alleles (5). In the present study, we found that two nucleotide substitutions in the coding region of *LIPC* (+651A to G and +1075A to C) that are associated with decreased hepatic lipase activity are significantly more common among African Americans than among white Americans. Analysis of the haplotypes formed by *LIPC* polymorphisms at nucleotides −514, +651, and +1075 revealed that more than 97% of African Americans have at least one hepatic lipase allele associated with decreased hepatic lipase activity.

Hegele, Tu, and Connelly (6) reported that the +651 A to G polymorphism, which changes asparagine at codon 193 to serine, was relatively common among Caucasians. The association between this polymorphism and hepatic lipase activity was not evaluated, however (6). In the present study, the effects of this polymorphism on hepatic lipase activity could not be assessed in African Americans because of confounding by the −514 and +1075 polymorphisms. To avoid the confounding influence of these polymorphisms, we studied 66 white men who were homozygotes for both −514C and +1075A. In these men, a clear gene dosage effect of the +651 polymorphism was apparent. Mean hepatic lipase activity was 10% lower in +651AG heterozygotes and 24% lower in +651G homozygotes than in +651A homozygotes. The +651 polymorphism is therefore significantly associated with hepatic lipase activity independent of sequence polymorphism at nucleotides −514 and +1075. This finding suggests that the substitution of serine for asparagine at codon 193 decreases the activity of hepatic lipase. As we have not directly compared the hepatic lipase activities of the N193 and S193 alleles in vitro, however, we cannot formally exclude the possibility that the +651 polymorphism does not affect hepatic lipase activity directly. The association observed in white men may be due to another, as yet unidentified, polymorphism that is in linkage disequilibrium with the +651 polymorphism.

The +1075 A to C substitution changes the leucine at codon 334 to phenylalanine and was originally identified in two Finnish families with hepatic lipase deficiency (7, 11). In vitro expression studies indicate that the substitution causes a 70% decrease in hepatic lipase activity. In the present study, heterozygosity for phenylalanine at codon

334 was associated with a 29% decrease in hepatic lipase activity, an effect consistent with the in vitro findings.

For each of the three *LIPC* polymorphisms identified, the common allele in whites is associated with higher hepatic lipase activity. In contrast, for two of the three polymorphisms the allele associated with lower activity is the common allele among African Americans. Larger association studies will be required to determine whether these three polymorphisms fully account for the differences in hepatic lipase activity between African American and white men. However, analysis of the *LIPC* haplotypes created by the −514, +651, and +1075 polymorphisms indicated that 83% of the *LIPC* alleles in the African American population have at least one polymorphism associated with decreased hepatic lipase activity. Therefore more than 97% of African Americans have at least one *LIPC* allele that is associated with decreased hepatic lipase activity. Consequently, high hepatic lipase activity is rare, and low hepatic lipase activity is common among African Americans.

In a previous study we noted that African American and white men with similar hepatic lipase activities had similar plasma HDL-C concentrations (5). This finding suggests that the well-known difference in plasma HDL-C between African American and white men may be due to ethnic differences in hepatic lipase activity. However, it is also possible that the ethnic differences in hepatic lipase activity and plasma HDL-C concentrations are not causally related. By matching men for hepatic lipase activity, we may also have inadvertently matched them for factors that affect plasma HDL-C, such as adiposity, alcohol use, and plasma triglyceride concentrations. The results of the present study suggest a possible genetic approach that could be applied in future studies of this question. If ethnic differences in the frequency of *LIPC* polymorphisms are a major cause of ethnic differences in hepatic lipase activity, then African American and white men with the same *LIPC* alleles should have similar hepatic lipase activities. In this case, matching African American and white men for the same *LIPC* alleles would abolish the ethnic difference in hepatic lipase activity while avoiding confounding by secondary factors. Comparison of plasma HDL-C concentrations in these men would indicate whether genetic differences in hepatic lipase activity account for the differences in plasma HDL-C between African American and white men.

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## REFERENCES

1. Tyroler, H. A., C. J. Glueck, B. Christensen, and P. O. Kwiterovich, Jr. 1980. Plasma high-density lipoprotein cholesterol comparisons in black and white populations. The Lipid Research Clinics Program Prevalence Study. *Circulation*. **62:** IV99–107.
2. Sprafka, J. M., S. W. Norsted, A. R. Folsom, G. L. Burke, and R. V. Luepker. 1992. Life-style factors do not explain racial differences in high-density lipoprotein cholesterol: the Minnesota Heart Survey. *Epidemiology.* **3:** 156–163.
3. Webber, L. S., S. R. Srinivasan, W. A. Wattigney, and G. S. Berenson. 1991. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. *Am. J. Epidemiol.* **133:** 884–899.
4. Brown, S. A., R. Hutchinson, J. Morrisett, E. Boerwinkle, C. E. Davis, A. M. Gotto, Jr., and W. Patsch. 1993. Plasma lipid, lipoprotein cholesterol, and apoprotein distributions in selected US communities. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler. Thromb.* **13:** 1139–1158.
5. Vega, G. L., L. T. Clark, A. Tang, S. Marcovina, S. M. Grundy, and J. C. Cohen. 1998. Hepatic lipase activity is lower in African American than in white American men: Effects of 5′ flanking polymorphism in the hepatic lipase gene. *J. Lipid Res.* **39:** 228–232.
6. Hegele, R. A., L. Tu, and P. W. Connelly. 1992. Human hepatic lipase mutations and polymorphisms. *Hum. Mutat.* **1:** 320–324.
7. Knudsen, P., M. Antikainen, S. Ehnholm, M. Uusi-Oukari, H. Tenkanen, S. Lahdenpera, J. Kahri, M. Tilly-Kiesi, A. Bensadoun, M. R. Taskinen, and C. Ehnholm. 1996. A compound heterozygote for hepatic lipase gene mutations Leu334→Phe and Thr383→Met: correlation between hepatic lipase activity and phenotypic expression. *J. Lipid Res.* **37:** 825–834.
8. Blades, B., G. L. Vega, and S. M. Grundy. 1993. Activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma of patients with low concentrations of HDL cholesterol. *Arterioscler. Thromb.* **13:** 1227–1235.
9. ABI PRISM™. 1997. Dye Terminator Cycle sequencing ready reaction kit Protocol. Protocol # 402078.
10. Guerra, R., J. P. Wang, S. M. Grundy, and J. C. Cohen. 1997. A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. *Proc. Natl. Acad. Sci. USA.* **94:** 4532–4537.
11. Knudsen, P., M. Antikainen, M. Uusi-Oukari, S. Ehnholm, S. Lahdenpera, A. Bensadoun, H. Funke, H. Wiebusch, G. Assmann, M. R. Taskinen, and C. Ehnholm. 1997. Heterozygous hepatic lipase deficiency, due to two missense mutations R186H and L334F, in the HL gene. *Atherosclerosis.* **128:** 165–174.