Brief Report

Absence of Relationship Between MTTP Haplotypes and Longevity

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Background. Polymorphisms for the microsomal triglyceride transfer protein (MTTP) gene have been associated with longevity and with lower risk for cardiovascular mortality. However, the association of MTTP with longevity has been contested in a large German collection of nonagenarians and centenarians.

Methods. We made a detailed characterization of MTTP haplotype carrier status in a cohort of 1398 old men and women (mean age 78 years) and a population-based cohort (n = 777) of younger controls (mean age 40 years) in Oxford, England.

Results. There were no significant differences in haplotypes for MTTP gene between the younger and older age groups.

Conclusion. This study, which adopted a more detailed genetic analysis of the MTTP gene in a large case–control study of older people provides reliable evidence against any significant association of the MTTP gene with longevity.

Most of the reported associations of genes with longevity in humans have not been replicated. The apolipoprotein E (APOE) gene, of which the ε4 variant predisposes to both Alzheimer’s disease (1,2) and cardiovascular disease (3), is one of the few genes to be consistently associated with longevity. Since the candidate gene approach is unlikely to provide novel suggestions for longevity genes, it was of great interest that a region on chromosome 4 associated with longevity was implicated in a genome-wide scan (4) of Caucasian individuals living in North America. The gene of interest on chromosome 4 was later identified as MTTP (microsomal triglyceride transfer protein gene) by fine-mapping the region of interest (5).

Like APOE, the MTTP gene codes for a gene product potentially related to cardiovascular disease by regulation of the plasma concentration of low-density lipoprotein cholesterol (6). However, although we recently observed an association between certain MTTP genetic variants and risk of cardiovascular disease in the West of Scotland Prospective Study (WOSCOPS) (7), this association appeared to be unrelated to plasma low-density lipoprotein cholesterol concentrations. Interestingly, one of the polymorphisms examined in the haplotype-based identification of the MTTP gene for longevity (5), rs2866164, is in complete linkage disequilibrium (LD) with the MTTP-493G/T variant assessed in the WOSCOPS. It, therefore, seemed plausible that the increased risk of nonfatal and fatal cardiovascular disease associated with carriers of the MTTP-493T variant might explain the association of longevity with carriers of the MTTP-493G variant.

Recently, Nebel and colleagues (8) were unable to verify any association between MTTP haplotypes and longevity in a collection of 1589 nonagenarians and centenarians with appropriately matched controls using three single-nucleotide polymorphisms (SNPs) to form haplotypes in MTTP gene (8). They argued that the initial observation indicating linkage of the MTTP gene with longevity may have been flawed by inadequate matching of the control sample. The MTTP gene is highly heterogeneous, and we have already shown that determination of haplotype structure based on either two, as in the report by Geesaman and colleagues, or three SNPs, as used by Nebel and colleagues, would provide a rather restricted view of the complex genetic variability in the MTTP gene. We have therefore determined the haplotype frequencies, based on 11 SNPs, in an Oxford-based collection of 1451 old people and compared this with 777 population-based controls in the age range 30–50 years to assess possible associations of the MTTP gene with longevity.

Methods

Populations

The DNA bank of older people derived from the Oxford Healthy Aging Project (9) comprises a random sample of 2740 people aged 65 years and older, who resided in the city of Oxford, England, when first examined between 1991 and 1994. The sample was drawn from general practice registers to provide equal numbers of participants aged 65–74 years.
and 75 years and older. Between 1994 and 1996, all surviving participants were invited to provide a blood sample. The mean age at blood collection was 77.9 ± 0.2 (standard error of the mean [SEM]) years. DNA samples were obtained from 1451 participants, and 1398 were successfully genotyped for all SNPs. All participants provided written informed consent according to procedures required by the Local Research Ethics Committee, which also approved the study protocol. Analyses of DNA and phenotypic data were carried out anonymously to the identifiers of individual participants in the study.

The younger control population was recruited from the Oxford Biobank (10), which is a population-based study of healthy 30- to 50-year-old men and women residing in Oxfordshire. The mean age at blood collection was 40.6 ± 0.2 (SEM) years. A total of 777 participants had successful genotyping at all loci tested.

Genotyping

Genotyping was performed using the Amplifluor chemistries (11) at the high-throughput facility (KBioscience, Hoddesdon, U.K.). DNA was in 96-well plates, and every 10th sample was replicated as internal control to control for genotyping error. Haplotype calculations were based on 14 SNPs in the younger cohort and 11 SNPs in the older cohort. It was clear from the younger cohort that 4 SNPs were in complete LD; therefore, only one of these was used in the older cohort. Assessment of the haplotype structure and frequency was made according to Stephens and colleagues (12) using the PhaseV2 program with a 1000 iteration setting; the assessment was repeated five times.

Participants with more than two missing SNP data points were removed before running the PhaseV2 program. This was made to ensure correct input variables as the Phase program fills in missing data. In this process the program tends to bias towards the common variant. The genotype error was well below 1% but, due to the stringent criteria on missing data, the reported numbers represent 89% (haplotype table) or 91% (Table 1) of all participants originally included. In addition, approximately 10 extremely rare (frequency less than 1%) haplotypes were removed. Although some of these haplotypes are likely to represent very uncommon variants, it is also recognized that such haplotypes may have arisen from genotyping errors, thus not representing true genetic variation of the sample.

Statistics

Differences in genotype and haplotype frequencies between the older and younger populations were assessed by means of a chi-square test estimated using SPSS (14.0). A power calculation was made using the software Quanto, version 0.5.5, available at http://hydra.usc.edu/GxE/ using an allele frequency of 0.25 in a log-additive model and a case/control ratio of 1:0.5 (this corresponds to a multiplicative, i.e., allele-wise model as actually tested). The relative risks ranged from 1.1 to 1.5, and the power from 70% to 95%, to obtain a thorough evaluation of power. With these assumptions, this study has ~70% power to detect an effect size as small as 1.2 and >95% power to detect an effect size of 1.3.

RESULTS

Successful genotyping was ascertained in 98.7 ± 0.4% of all 11 SNPs in the MTTP gene with a genotyping error of 0.3% based on the replicate samples. All SNPs were in Hardy–Weinberg equilibrium in both the younger and the older age groups. The allele frequencies of the MTTP Q95H variant and the MTTP I128T variants are shown in Table 1. There were no statistically significant differences in any of these SNPs between the younger and older populations, indicating a lack of survival benefit for any of the variants. The haplotype distribution was based on 11 SNPs in the MTTP gene and yielded eight haplotypes with a frequency greater than 1%. The LD statistics for all 14 SNPs used in the younger control population are graphically plotted in Figure 1. The r² values show that the SNPs MTTP-5038C/G, MTTP-164C/T, and MTTP-I128T are in complete LD. Of these three SNPs, only MTTP-I128T was therefore typed for the older population. Similarly, SNPs MTTP D98E and MTTP S166N were in complete LD, and MTTP D98E alone was typed in the older population. The D’ values from Figure 1 show that LD extends across the whole 52-kb region of the gene. The previously reported (7) MTTP haplotype nr 5 was split into two new haplotypes as the MTTP-410G/A (MTTP/rs6532821) has not been used before. Table 2 shows the haplotype analysis using all the available information on genetic variability in the MTTP gene and demonstrates no differences in the distribution of these haplotypes between the younger and older populations (p = .45). Table 2 also shows the minor allele frequency for each SNP. None of these frequencies showed a statistically significant difference between old people and the younger control cohort.

We also looked at the frequency distribution for each of the 11 SNPs in 5-year age bands within the older people to see if there was an underlying age-dependent trend for any particular SNP frequency. No association of any of the 11 SNPs with age was detected within the population.

DISCUSSION

The MTTP gene associated (5) with longevity was identified after fine-mapping of a locus on chromosome 4 that had been identified by a genome-wide scan in a long-lived North American cohort (4). This finding was recently challenged in a large German case–control study that found

<p>| Table 1. Allele Frequencies for the MTTP Gene in Younger and Older Age Populations |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|</p>
<table>
<thead>
<tr>
<th>SNP</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>Chi-Square</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>128I</td>
<td>1162</td>
<td>(75%)</td>
<td>2051</td>
<td>(73%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>128T</td>
<td>392</td>
<td>(25%)</td>
<td>745</td>
<td>(27%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>95Q</td>
<td>1463</td>
<td>(94%)</td>
<td>2663</td>
<td>(93%)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>95H</td>
<td>91</td>
<td>(6%)</td>
<td>187</td>
<td>(7%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: MTTP = Microsomal triglyceride transfer protein; SNP = single-nucleotide polymorphism; NS = not significant.
no evidence for an association between MTTP gene haplotype, carrier status, and longevity. The original observation has also been challenged by Bathum and colleagues in a study of 1651 92- to 93-year-old persons (13). They studied the effect of two of the original SNPs used by Geesaman and colleagues (5) but failed to find a difference in gene frequency compared with a smaller \( n = 575 \) and younger (average age 53 years) control cohort. The present study reports no difference in haplotype frequencies for the MTTP gene between younger and older populations; this finding is consistent with the findings of the German case-control study. Nebel and colleagues pointed out that there may have been some evidence of bias in the North American younger control group, which is likely to have caused the positive identification of the MTTP gene in that study. We agree with the reasoning that the SNP allele frequency of the most crucial of the polymorphisms in the study of Geesaman and colleagues appears to be unbalanced. The MTTP-5038C/G (MTTP rs2866164), MTTP-493G/T (MTTP rs1800591), MTTP-164C/T (MTTP rs1800804), and MTTP I128T (MTTP rs3816873) are all in complete LD, are located on the same haplotype within the populations studied so far, and can therefore be compared with previously published studies. There is a surprisingly close range in distribution for the less common variant \((0.25–0.29)\) within Caucasian populations: healthy 50-year-old Swedish men \((0.25)\) (14), Irish diabetic persons \((0.25)\) (15), Norwegian persons with familial hypercholesterolemia \((0.26)\) (16), moderately hypercholesterolemic Scottish men \((0.26)\) and Scottish men with cardiovascular disease \((0.26)\) (7), and Framingham men \((0.29)\) and women \((0.26)\) (17). This is in contrast to the results in the control population reported by Geesaman and colleagues which show a frequency of 0.34 (5).

The SNPs used in this study were identified primarily from previous publications in which MTTP haplotypes have been studied, not from data within the HapMapII Project. Comparing the haplotype coverage across the MTTP region using the chosen SNPs against those within the HapMapII database, it is concluded that, of the 14 SNPs genotyped in the younger population, 7 were identifiable within HapMapII (MTTP-1428, MTTP-164, MTTP D98E, MTTP I128T, MTTP S166N, MTTP H297Q, and MTTP rs881980). Of these seven SNPs, there were two pairs in complete LD.
(MTTP-164 with MTTP I128T and MTTP S166N with MTTP D98E, respectively), and only one of each pair was typed in the older population. The remaining five HapMapII SNPs represent 51% of the variation within this region at an $r^2 > 0.5$, whereas 44% of the variation was seen at an $r^2 > 0.8$. However, the five additional SNPs we used (not included in HapMap II) (MTTP-410, MTTP-388, MTTP Q95H, MTTP Q244E, and MTTP_rs2255119) were chosen to uniquely identify four of the eight haplotypes (see Table 2). Only two of the SNPs within HapMapII uniquely define a haplotype; we therefore estimate that using the additional SNPs here is as informative in terms of haplotype coverage, or even more so, than using only the five nonredundant ones within HapMapII.

We have recently reported an association between risk of nonfatal and fatal cardiovascular disease and MTTP genotypes. The MTTP-493T variant was associated with increased risk of cardiovascular disease in the WOSCOPS study, and this may reflect a survival effect by a lower allele frequency in a long-lived cohort. We speculated that the effect was mediated through myocardial vulnerability due to the strategically important effect of MTTP for lipid transport. However, the present study provides reliable evidence against any significant association of the MTTP gene with longevity.

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