HAPLOTYPIC ANALYSES OF THE IGF2-INS-TH GENE CLUSTER IN RELATION TO CARDIOVASCULAR RISK TRAITS

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ABSTRACT

Three important genes are clustered on human chromosome 11p15: insulin-like growth factor 2 (IGF2), insulin (INS) and tyrosine hydroxylase (TH). We undertook haplotypic studies of the whole cluster with regard to nine traits related to body composition, plasma triglyceride (TG) levels and blood pressure (BP) in 2743 middle aged males. Six out of the ten common haplotypes found showed significant association with at least one trait. Two specific haplotypes protect against obesity. Raised plasma TG levels and elevated BP and fat mass are associated with two different haplotypes containing the same specific alleles for TH and INS genes. A retrospective power analysis based on the observed haplotype frequencies indicates that our study has a 90% power to detect small differences between the mean values for each haplotype and those observed for all individuals, for the cardiovascular traits analysed. Our haplotype studies also integrate a complex literature mostly based on only one of the genes. The simplest hypothesis is that haplotypes containing a long repeat insertion in the INS gene promoter (INS "class III") alleles may be predisposing for metabolic syndrome traits (including hypertriglyceridaemia, high BP, type II diabetes and PCOS), whereas low body mass, and type I diabetes reflect the effects of at least one different haplotype, which is marked by the shortest alleles of the "class I" group in the INS promoter.

Figure 1. Genetic map of the IGF2-INS-TH region, spanning around 40 kb of 11p15. Exons of TH, INS and IGF2 are numbered and represented by boxes. A red asterisk indicates that the information given by the 12 markers analysed (see table 1).

Figure 2. Significant associations (in bold and underlined) between IGF2-INS-TH haplotypes and cardiovascular risk traits. The bars represent the percentage difference observed between the mean value of a given haplotype and the overall sample mean for each trait.

Figure 3. Percentage of detectable differences between the mean values for given haplotypes and the mean values for all individuals for each trait. There is a 90% power to detect very small differences for weight, systolic BP, diastolic BP, BMI and body fat (less than 4% for the rarest haplotype and around or less than 1.5% for the commonest one) (Figure 3). Detectable differences for fat mass, percentage fat and LBM were around 10% for the rarest haplotype, and approximately 20% for plasma TG (Figure 4) whereas considerably smaller differences could be detected with a 90% power for the more frequent haplotypes.

Figure 4. Distribution of detected haplotypes in the cluster.

Figure 5. Cladogram relating the 10 haplotypes considered at the IGF2-INS-TH region. The commonest haplotypes, which are likely the oldest (3), marked by the presence of the common alleles at IGF2 Apal and INS HphI, do not display trait association. From this clad, two new clades with effect on cardiovascular traits emerged and these are tagged by alleles either of IGF2 Apal or INS HphI. Mutation at IGF2 Apal marks weight-lowering haplotypes, whereas mutation at INS HphI (equivalent to class III alleles at the INS VNTR), marks haplotypes with increased risk for metabolic syndrome traits. Finally, a more recent clade marked by the presence of the rarer alleles at both SNPs has effects on plasma TG variation.

Table 1. Interallelic associations found between the five component IGF2 haplotypes and TH01 and INS VNTR alleles in NPHSII men. The information given by TH01 and the 11 IGF2 SNPs can be reduced to a combination of the microsatellite and a single IGF2 SNP (Apal in red). Addition of the INS VNTR alleles permits distinction between class/class II alleles at the INS VNTR locus (1).

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>IGF2 Apal</th>
<th>INS VNTR</th>
<th>TH01</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Class III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td>119</td>
<td>119 (19.8%)</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td>50</td>
<td>60 (10.6%)</td>
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<tr>
<td>C</td>
<td></td>
<td></td>
<td>42</td>
<td>42 (7.1%)</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td>5</td>
<td>8 (1.4%)</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td>1</td>
<td>1 (0.2%)</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the IGF2-INS-TH haplotypes considered for association analysis with cardiovascular traits. Consistent with approaches to reduce risk of false positive findings related to low-frequency haplotypes (2), we considered only haplotypes with a frequency higher than 3%, representing 89% of all haplotypes.

Table 3. Summary of published apparent traits or disease effects associated with the particular alleles and haplotypes at the IGF2-INS-TH region.

Conclusions

The analysis of the haplotype effects of the IGF2-INS-TH region on a spectrum of cardiovascular risk traits revealed two weight-lowering haplotypes (*5 and *10) and two haplotypes conferring increased metabolic syndrome risk (*4 and *6). The weight-lowering effect of *10 (BMI unit) represents about a 10% increase in the rate of coronary events (6). The potential significance of haplotype *4 to cardiovascular risk is not trivial. A 1.22WgMg higher diastolic BP equates with about a 6-8% increase in stroke risk and a 4-5% increment in CHD risk (6). Comparable estimations can be made for the 10% fat mass increment and for the 10% higher plasma TG of haplotype *5 in relation to the metabolic syndrome. The systematic description identifies tagging markers and specific haplotypes (particularly *4 for risk and *6 for protection), permitting deeper epidemiological and functional investigation of IGF2-INS-TH as a cardiovascular risk polygene.

Material and methods

Experimental conditions for the genotyping of polymorphisms are as previously described (7). In brief, the eleven SNPs at IGF2 and HphI were typed by PCR adopting the Amplification Refractory Mutation System (ARMs) (8) by digesting each amplification product by the corresponding restriction enzyme. The TH microsatellite TH01 and the INS microsatellites were amplified with primers and PCR conditions previously described (9,11). The amplification products were separated in a 2% agarose gel. The bands were visualized by ethidium bromide staining. The relative positions of the bands were the same obtained from the Southern blot analysis. The haplotypes were obtained by the combination of the bands. The haplotypes were confirmed by direct sequencing of the amplified DNA fragments.

References

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