

Pleiotropic effects of a common *IGF2-INS-TH* haplotype on obesity, response to glucose, plasma triglycerides and smoking initiation

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See also: - HGM2006 poster 70 and HGM2006 poster 221

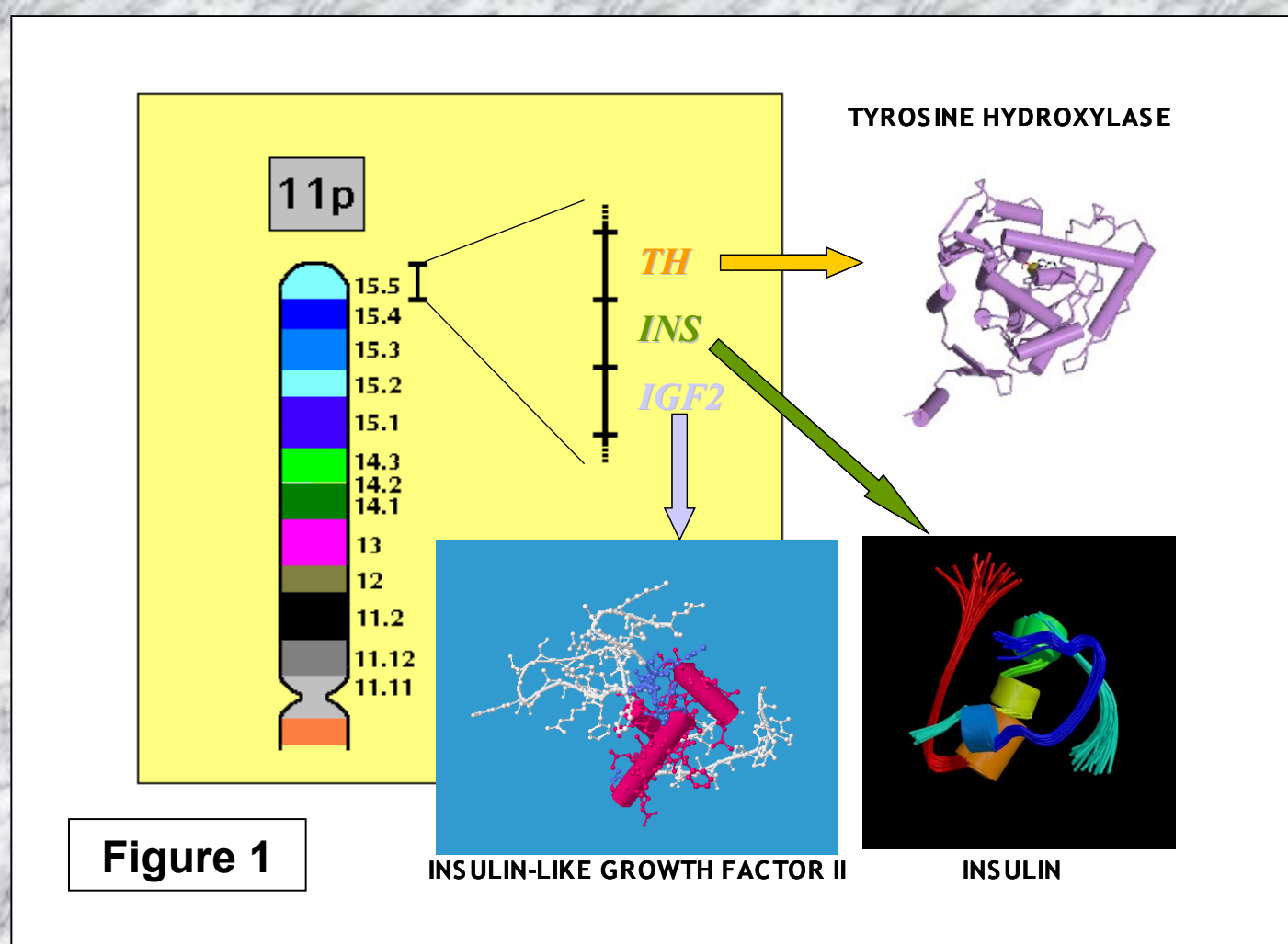


Figure 1. The human *IGF2-INS-TH* region on chromosome 11 harbours three important genes representing key elements of a growth regulatory pathway, of the insulin signalling pathway and of the catecholaminergic pathway, respectively.

ABSTRACT

Disentangling the role of genetic factors in complex traits is important to understanding pathogenesis and potentially to disease prediction of common human diseases, and pleiotropy (i.e. multiple effects of a genetic factor on different phenotypes) is one key aspect to consider. In 2004, we identified a common tagging haplotype strongly associated ($P = 0.00001$) with low weight in unrelated UK Caucasian men of the NPHSII cohort (Rodríguez et al., 2004). This extended haplotype (designated *IGF2-INS-TH**5), spans the *IGF2-INS-TH* cluster in human 11p15 (a strong candidate region influencing cardiovascular risk traits), and has been observed in more than 25% of individuals from two independent samples representative of the general UK population (NPHSII and Hertfordshire Cohort Study, HCS). In follow-up studies, we have replicated the weight-lowering effect of the *5 haplotype in HCS men, and we have found nominal associations between *5 and a wide range of phenotypes influencing metabolic syndrome risk and smoking (Rodríguez et al., 2006a; Rodríguez et al., 2006b). Multiple regression results suggest that some of the associations may be secondary to others (e.g. most, but not all, of the insulin, glucose and TG effects observed for *5 are substantially dependent on obesity indices). In contrast, other *5 effects (e.g. low weight and smoking) are independent.

INTERALLELIC LD ACROSS THE *IGF2-INS-TH* REGION AND IDENTIFICATION OF THE *IGF2-INS-TH**5 HAPLOTYPE

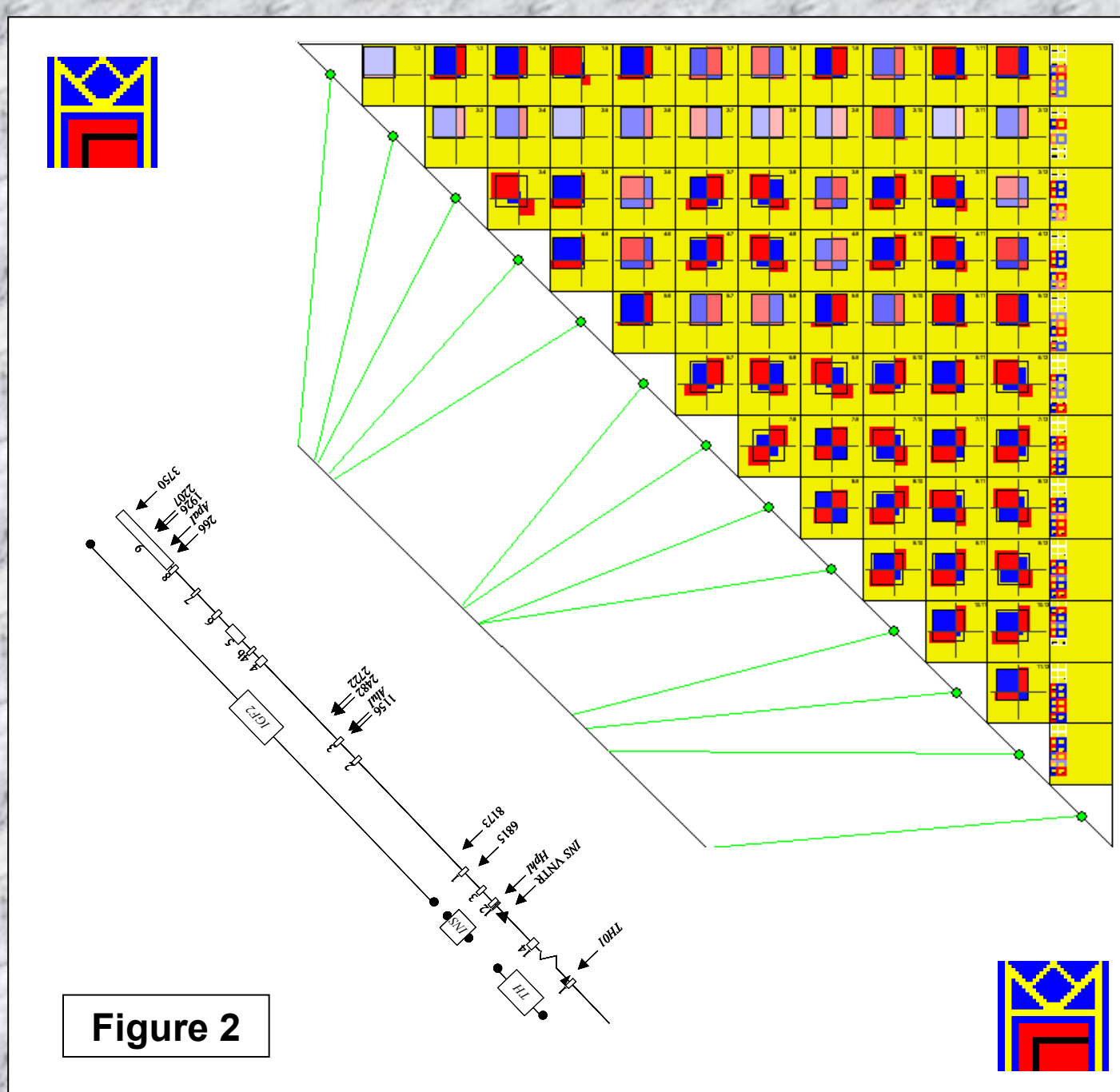


Figure 2. Interallelic linkage disequilibrium (LD) observed in NPHSII between 13 DNA markers spanning the *IGF2-INS-TH* region, using the software MIDAS (Gaunt et al., 2006). MIDAS is a new program that presents a novel approach of analysing and graphically representing the interallelic LD between multiple pairs of bi- and multiallelic markers. The graphical representation of LD incorporates information on expected haplotype frequency (under no LD), estimated haplotype frequency and D' or significance.

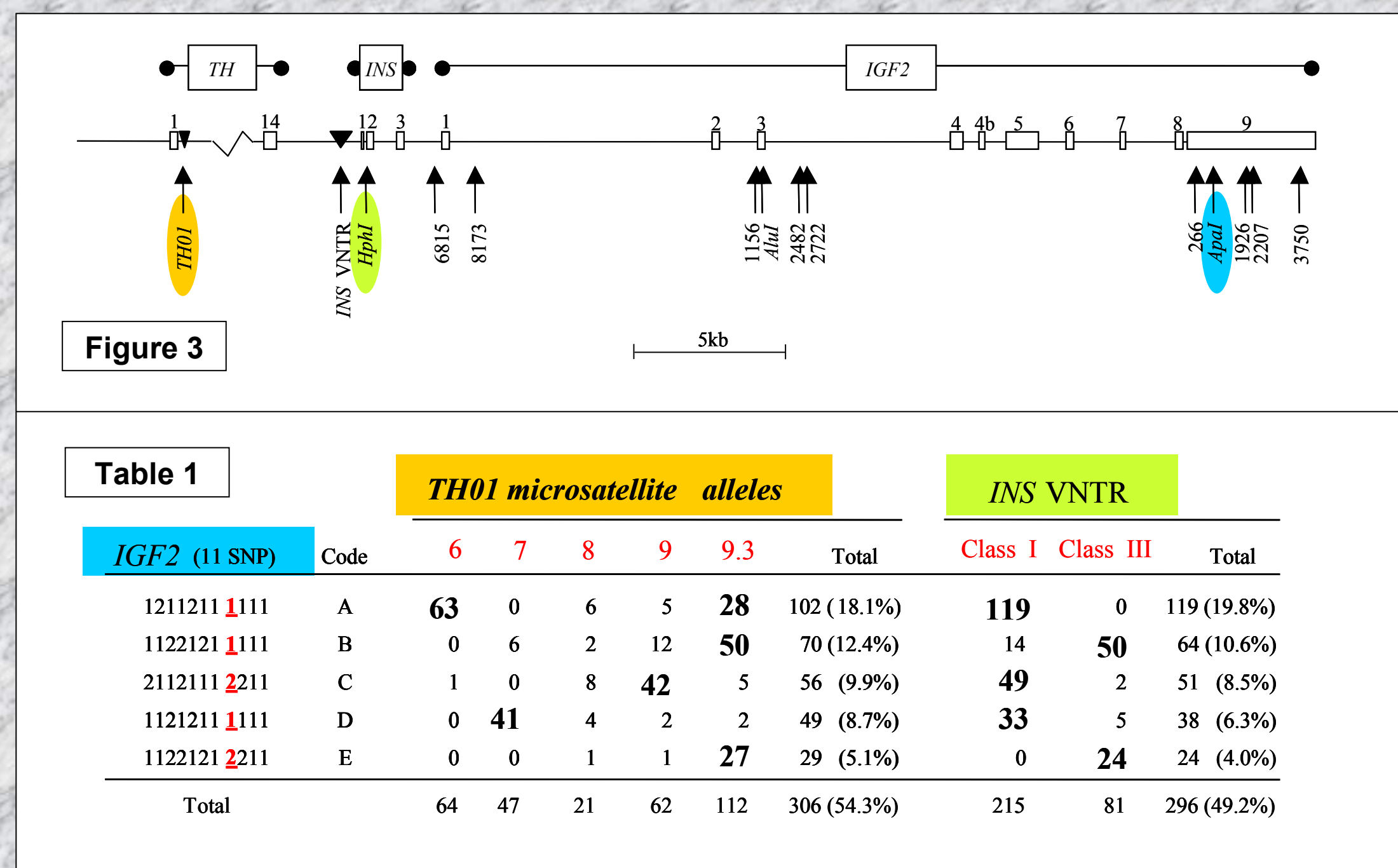


Figure 3 and Table 1. DNA markers analysed and interallelic associations found between the five commonest 11-SNP *IGF2* haplotypes and *TH01* microsatellite and *INS HphI* alleles (which mark *INS* VNTR classes I and III) in NPHSII men. The observed LD patterns allowed us to identify three tagging markers (*TH01*, *INS HphI* and *IGF2 Apal*) accounting for most of the haplotypic diversity in the region. Consideration of haplotypes defined by these markers (table 2) translated into haplotype association analyses with an increased power (due to fewer missing data) and a reduced risk of false positive findings (related to low-frequency haplotypes) which permitted the identification of genetic factors that modify the risk for cardiovascular disease (Rodríguez et al., 2004).

Haplot.	Freq.	<i>IGF2 Apal</i>	<i>INS VNTR</i>	<i>TH01</i>
*1	0.20	1 (G)	Class I	6
*2	0.17	1 (G)	Class I	7
*3	0.11	1 (G)	Class I	9.3
*4	0.10	1 (G)	Class III	9.3
*5	0.09	2 (A)	Class I	9
*6	0.08	2 (A)	Class III	9.3
*7	0.04	1 (G)	Class I	8
*8	0.04	1 (G)	Class III	8
*9	0.04	1 (G)	Class I	9
*10	0.03	2 (A)	Class I	9.3
Total	0.90			

Table 2. Designation, frequency and genetic constituents of the ten commonest *IGF2-INS-TH* extended haplotypes. The *5 haplotype is tagged by allele A of *IGF2 Apal*, allele 9 of *TH01* and allele A of *INS HphI* (marking class I alleles of the *INS VNTR*). The observed frequency of the *5 haplotype in the NPHSII cohort and in the Hertfordshire cohort is around 10%.

PHENOTYPIC ASSOCIATIONS OF THE *5 HAPLOTYPE

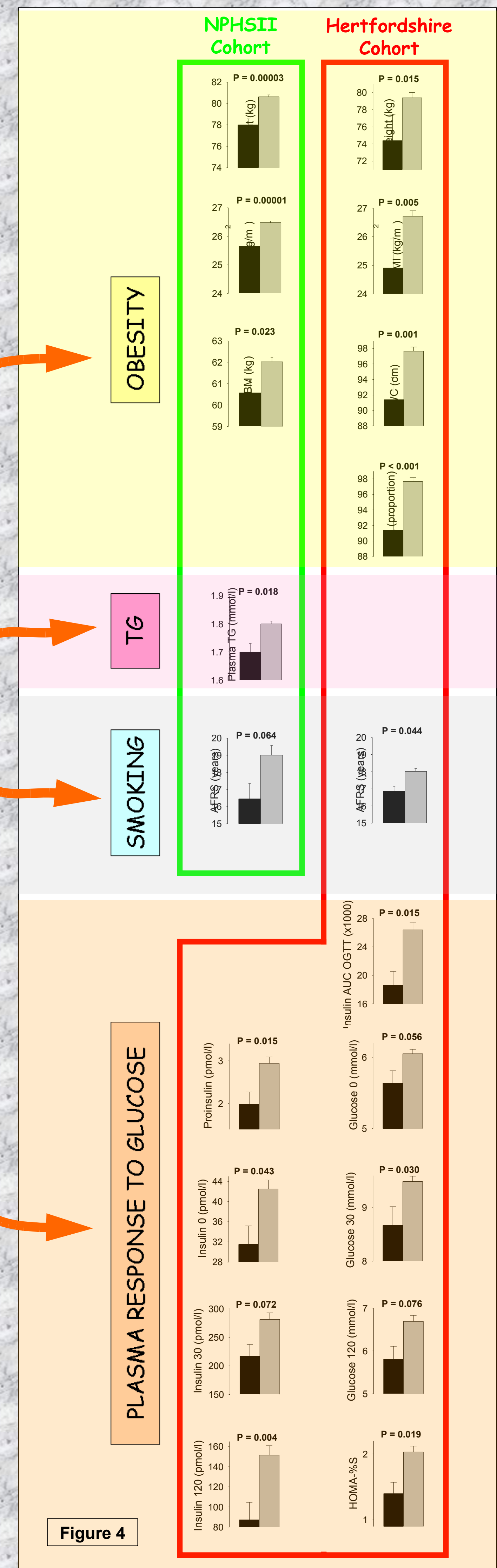


Figure 4. Graphical summary of the significant associations found for weight-related traits, plasma triglycerides, smoking initiation (as measured by age of first regular smoking, AFRS) and response to insulin traits and HOMA-%S in men. Black bars correspond to the *5 haplotype and grey bars correspond to not*5 haplotypes. The standard error of the mean is also shown for each case. P values presented are unadjusted. Results from multiple regression analyses adjusting for relevant covariates showed that associations of *5 with obesity risk traits are independent of age and smoking. The association with plasma TG was ablated by adjustment for BMI. Most of associations between plasma response to glucose traits depend also on weight, with the exception of plasma insulin levels after 120 min (Rodríguez et al., 2006a)

MATERIALS AND METHODS

Samples
NPHSII: 2743 unrelated and healthy UK men aged 51-62 years (Miller et al., 1996). Hertfordshire cohort: 1108 unrelated UK men and women aged 59-70 y (Sayer et al., 1998).

DNA markers
One microsatellite and 12 SNPs (figure 3)

Genotyping
ARMS-PCR or PCR-RFLP and Microplate Array Diagonal Gel Electrophoresis (MADGE) as previously described (Gaunt et al., 2001, Chen et al., 2002).

Phenotyping
See Rodríguez et al (2004, 2006a, 2006b) and references therein for details.

Statistical analyses
Haplotype analyses using Haplotype Trend Regression (Zaykin et al., 2002) and a combination of PHASE v2 (Stephens and Donnelly, 2003) with Student's t -test and multiple regression analyses in SPSS (v10).

POSSIBLE MECHANISMS OF ACTION OF THE *5 HAPLOTYPE ON BODY WEIGHT

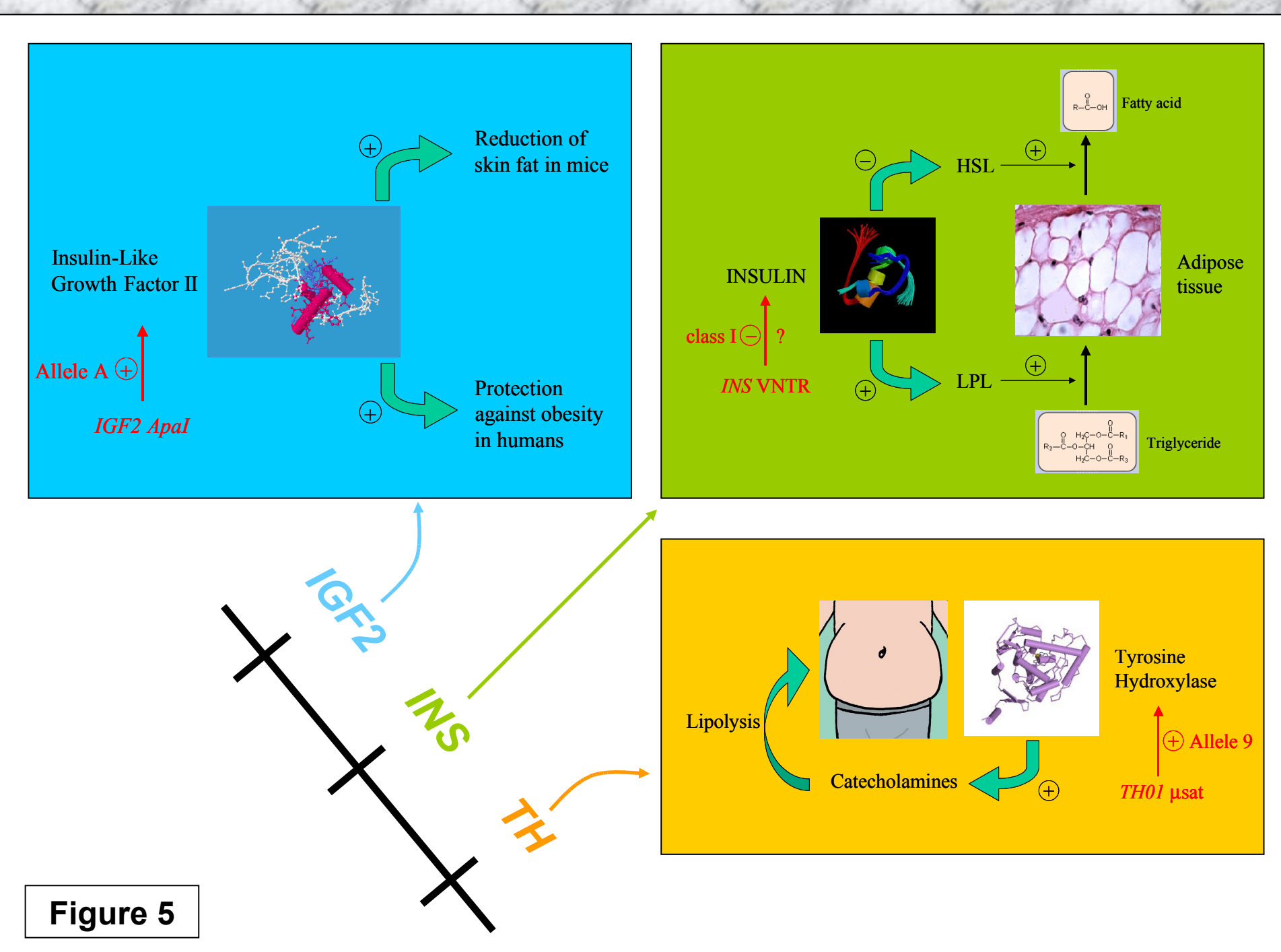


Figure 5. The functional element(s) of *5 determining the weight effect could reside in any of the three genes. This schematic representation shows the possible effects mediated by each of the constituents of the *5 haplotype (see details in Rodríguez et al., 2006a).

CONCLUSION

Taken together, our results suggest that the *5 haplotype may have a pleiotropic effect on different cardiovascular risk traits in men, by influencing both independent chain of events and interrelated intermediary phenotypes.

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