

Short Report

GENETIC FACTORS INFLUENCING INFLAMMATION IN OBESITY

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Obesity has reached epidemic proportions and is associated with an increased risk of developing chronic diseases such as diabetes and cardiovascular diseases (CVD).¹ A significant proportion of obese individuals remains metabolically healthy while others show a moderate to severe CVD risk.² Many traditional risk factors are linked to the high morbidity related to obesity; insulin resistance, dyslipidemia and hypertension are usually grouped together and define the metabolic syndrome (MS), but other non-traditional risk factors are also emerging.² The evolution of the factors contributing to the conversion from a healthy to a risk profile remain unknown but the actual scientific knowledge support the implication of environmental and genetic factors.³ In 1993, Hotamisligil et al. realised the first association study between obesity, more specifically insulin resistance, and an inflammatory marker expressed in adipose tissue, TNF-alpha.⁴ Van Guilder et al. studied the inflammatory state and obesity, more explicitly the MS, and observed differences in levels of inflammatory biomarkers whether subjects were non-obese, obese without MS and obese with MS.⁵

Our team has previously identified, using microarrays, a list of genes related to the inflammation/immune system that are differentially expressed in visceral adipose tissue of obese individuals with versus without MS.⁶ From this list, we selected *IFI30* and *TSLP* genes since they are part of the same metabolic category: immune/inflammatory response.⁷ Our hypothesis is that plasma hs-CRP levels are influenced by SNPs in genes involved in the inflammatory/immune system that are differentially expressed in visceral adipose tissue of obese subjects with or without MS. The objective of the study was to determine whether *IFI30* or *TSLP* sequence variations are associated with levels of plasma hs-CRP and/or traditional CVD risk factors.

Materials and methods

Since June of 2000, 1311 severely obese men and women from the eastern part of the Quebec province (Canada) undergoing an anti-obesity surgery were recruited. Details of the surgical protocol have been presented elsewhere. All subjects provided written informed consent before their inclusion in the study.⁶ Of the genes found to be differentially expressed using microarrays, 2 genes have been selected for a validation of differential expression. Expression of these genes has been measured using real-time RT-PCR and the methodology TaqMan. The $\Delta\Delta C_T$ calculation method was used to assess the mean fold expression difference between the groups.⁸ For each gene, coding regions, including exon-intron junction and the promoter (~ 2000bp) were sequenced using the BigDye™ Terminator 3.1 kit, PCR forward and/or reverse primers. Samples were run on ABI® 3730/XL DNA Analyzer automated sequencers (Applied Biosystems, Foster City, CA, USA). We genotyped SNPs revealed by sequencing using custom and validated probes (Applied Biosystems, Foster City, CA, USA), as described.⁹

Statistical analyses

We used Spearman correlations to test mean fold expression difference (MFED) of microarray vs. real-time RT-PCR results. Then we selected locus tag SNPs with Haploview software (version 4.0 RC2) as well as the computation of Hardy-Weinberg equilibrium and linkage disequilibrium (LD; r^2) between these SNPs.¹² To analyze genotypic frequencies we merged homozygotes with heterozygotes for the minor allele, for genotype having a frequency $\leq 5\%$. Chi-square tested were performed in MetS+ vs.

MetS- subgroups and in low vs. high plasma CRP subgroups defined using the median CRP (8.3 mg/L) value as a cut-off point. Finally, we conducted analysis of covariance comparing mean phenotypic values across genotype groups of IFI30 and TSLP genes. All statistical analyses were performed with SAS statistical software, version 9.1 and statistical significance was defined as $p < 0.05$.

Results

Microarray results were validated for *IFI30* and *TSLP* genes. All the promoter, exon-intron junctions and coding regions were sequenced and we went through identification of SNPs. We achieved genotyping of 10 tagged SNPs (total for the 2 genes) for over 1300 obese subjects (squared in linkage disequilibrium map, see Figure 1 and 2).

Subgroups of low vs. high plasma CRP levels were significantly different for rs11554159 and rs7125 genotype frequencies in the IFI30 gene ($p < 0.05$). Also, rs2289277 in TSLP gene showed significant differences in genotype frequencies between MetS+ vs. MetS- subgroups ($p < 0.05$). Of the 10 SNPs analyzed, 2 show significant genetic association with plasma CRP-levels when adjusted for the effect of age, sex, smoking, and waist girth: rs11554159 ($p < 0.008$) and rs7125 ($p < 0.04$), both in IFI30 gene. No associations were found in TSLP gene. As for traditional risk factors, in IFI30 gene, rs7125 was associated with plasma total-cholesterol and LDL-cholesterol. In TSLP gene, rs2289277 was associated with fasting glucose.

Discussion

IFI30 and TSLP gene were shown to be differentially expressed in visceral adipose tissue of obese subjects with or without MS by 2 different methods. IFI30 and TSLP gene sequence variations show associations with hs-plasma CRP levels and traditional CVD risk factors. IFI30 sequence variations (rs7125 and rs11554159) were associated with hs-plasma CRP after adjustment for age, sex, smoking and waist girth.

Conclusion

The results obtained suggest a potential role of some sequence variations of IFI30 and TSLP genes in the inter-individual variability observed in metabolic and/or inflammatory profiles in obesity. Further studies are needed to assess the potential role of genetic variations in these genes with the chronic inflammatory state in obesity and their real impact on the metabolic profile, MS incidence and plasma hs-CRP levels.

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