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Weight loss allows the dissection of the interaction between abdominal fat and *PNPLA3* (adiponutrin) in the liver damage of obese children

To the Editor:

In the patatin-like phospholipase domain-containing 3 gene (*PNPLA3*), there is a polymorphism named rs738409, which encodes for the adiponutrin I148M variant and is a genetic determinant of liver fat and non-alcoholic fatty liver disease (NAFLD).

Many works support the correlation among the *PNPLA3* rs738409 polymorphism, ALT serum levels and hepatic steatosis. A recent study has demonstrated that this variant is associated with increased levels of liver enzymes and with liver steatosis in obese children and adolescents, suggesting that it confers genetic susceptibility to liver damage from a young age [1].

We have shown that in obese children, the magnitude of the association of *PNPLA3* with liver enzymes is driven by the size of abdominal fat expressed as waist to height ratio (W/Hr) and we stratified the individual risk of developing liver damage on the basis of the interaction between the *PNPLA3* genotypes and waist circumference [2]. Furthermore, the interplay between visceral fat and *PNPLA3* in producing liver damage has recently been confirmed in adults [3] and patients with chronic hepatitis C [4].

In addition to *PNPLA3*, a number of predisposing genetic polymorphisms have been associated with NAFLD [5]. In order to dissect the interaction between abdominal fat and *PNPLA3*, we studied 129 obese children before and after a weight loss program.

The frequency of the distribution of the different *PNPLA3* rs738409 genotypes was in Hardy-Weinberg equilibrium ($p > 0.05$).

Fifty-three patients were homozygous for the wild type allele (CC) while 51 were heterozygous (CG) and 25 were homozygous for the minor allele (GG).

The frequency of the *PNPLA3* minor allele (148M) was 0.30, which is in line with the frequencies reported in obese subjects belonging to the same geographic area [1].

By analysing the children on the basis of their *PNPLA3* genotypes, we observed that before the weight loss program, ALT and AST levels in the serum increased significantly in the children carrying the 148M allele ($p = 0.01$ and $p = 0.02$, respectively), adjusting for age, gender, pubertal stage, and BMI-SDS. Of 148M homozygotes, 24% had ALT levels above 40 IU/L compared to 16% of heterozygous and 9% of homozygous for the 148I major allele ($p = 0.02$). The prevalence of steatosis was also different according to the *PNPLA3* genotypes, with 17% of obese children 148I homozygotes showing steatosis, 33% of heterozygotes and 76% of 148M homozygotes ($p = 0.01$), adjusting for age, gender, pubertal stage, and BMI-SDS.

After weight loss, all children reduced their BMI SDS by at least 0.5. Reduction in BMI SDS and W/Hr were not statistically different among *PNPLA3* genotypes.

The group of homozygous subjects for the minor allele (148M) presented a greater reduction in serum ALT concentrations (ΔALT) and in the prevalence of steatosis compared with the group of heterozygous or homozygous subjects for the major allele ($p = 0.02$ for ALT and $p = 0.01$ for steatosis). Moreover, after weight loss, no differences among *PNPLA3* genotypes were found in ALT levels or in the prevalence of steatosis ($p = 0.1$ and $p = 0.2$, respectively).

Both the relationships between ΔBMI SDS and ΔALT and between ΔW/Hr and ΔALT were analysed, based on the different *PNPLA3* genotypes, and the slope of the relative regression lines were compared (Fig. 1).

Homozygous children for the *PNPLA3* minor allele (148M) showed a stronger correlation between ΔALT and ΔW/Hr than those carrying the other genotypes, as we observed in the comparison of regression lines ($p = 0.002$) (Fig. 1A). The same compar-

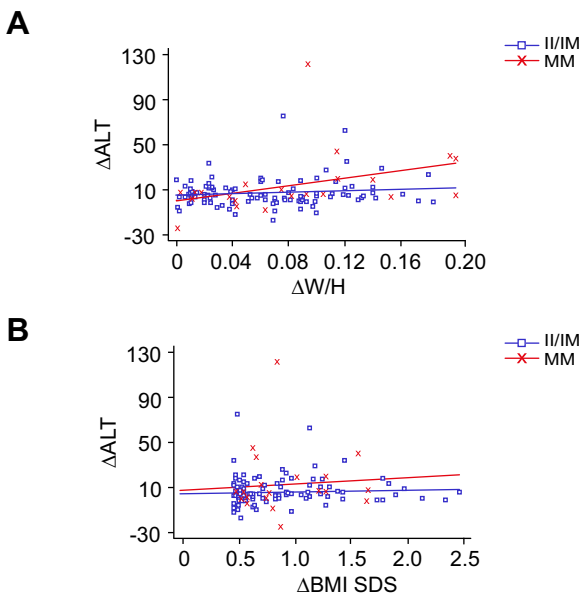

Fig. 1. Correlations between ALT levels reduction (ΔALT) and waist circumference to height ratio, W/Hr (ΔW/Hr) or BMI SDS (ΔBMI SDS) decrease according to *PNPLA3* genotypes in a group of 129 obese children subjected to a weight loss program. (A) Regression analysis describing the relationship between ALT levels reduction (ΔLT) and W/Hr reduction (ΔW/Hr) in patients homozygous for *PNPLA3* 148M variant and in patients carrying the *PNPLA3* 148I variant. The regression between ΔALT and ΔW/Hr in the group of patients homozygous for *PNPLA3* 148M variant is described by the equation $y = 0.29 + 169.4 * x$ ($r = 0.40$; $p = 0.004$). The equation for *PNPLA3* I/M and I/I was $y = 5.0 + 32.6 * x$ ($r = 0.12$; $p = 0.2$). The comparison between the slope of the two regression lines is significant ($p = 0.002$). (B) Regression analysis describing the relationship between ALT levels redution (ΔALT) and BMI SDS decrease (ΔBMI SDS) in patients homozygous for *PNPLA3* 148M variant and in patients carrying the *PNPLA3* 184I variant. The regression between ΔALT and ΔBMI SDS in the group of patients homozygous for *PNPLA3* 148M variant is described by the equation $y = 8.706 + 5.16 * x$ ($r = 0.07$; $p = 0.73$). The equation for *PNPLA3* I/M and I/I was $y = 5.86 + 1.54 * x$ ($r = 0.05$; $p = 0.57$). The comparison between the slope of the two regression lines is not significant ($p = 0.38$). (This figure appears in colour on the web.)

ison performed for ΔBMI SDS was not significant ($p = 0.38$) (Fig. 1B). These results support the idea that it is the abdominal fat (as expressed by W/Hr) more than the overall adiposity (expressed by BMI), the external (not genetic) factor which modulates the effect of *PNPLA3* 148M allele on liver damage.

Exploring whether the *PNPLA3* 148M allele influences the ability of weight loss to decrease liver fat, Sevastianova *et al.* [6] investigated 18 subjects placed on a hypocaloric low-carbohydrate diet for 6 days and demonstrated that weight loss is an effective way to decrease liver fat, irrespective of the *PNPLA3* genotype. Indeed, short term weight loss decreases liver fat content more in homozygous carriers of *PNPLA3* 148M allele than in those carrying the 148I allele. These results are in agreement with our data.

The mutant *PNPLA3* 148M allele is partially unable to hydrolyse intra-hepatocytic triglycerides [7,8], which increases the risk of liver steatosis. Studies have also shown that there is about a three-fold increase of *PNPLA3* expression in obese human subjects after the completion of a weight loss program [9]. It is possible, therefore, that the interaction between adiposity and *PNPLA3* may well be modulated by the effect of adipose tissue changes on *PNPLA3* expression.

An important clinical implication of the present study is that obese children with fat liver carrying the *PNPLA3* 148M allele are the patients who would most benefit from weight loss.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Uncovering the molecular events associated with increased intestinal permeability in liver cirrhosis: The pivotal role of enterocyte tight junctions and future perspectives

To the Editor:

We read with great interest the recently published research study by Dr. Du Plessis and colleagues on the role of intestinal macrophages in intestinal epithelial barrier dysfunction and hyperpermeability in patients with cirrhosis [1]. The authors demonstrated that decompensated liver cirrhosis was associated with the presence of significantly higher numbers of activated intestinal macrophages ($CD33^+/CD14^+/Trem\text{-}1^+$) expressing iNOS and secreting NO and IL-6, in conjunction with increased duodenal paracellular permeability and increased expression of the tight junction (TJ) protein Claudin-2. They speculated that activation of intestinal macrophages might represent an important molecular event implicated in the disruption of the intestinal epithelial barrier in cirrhosis through secretion of TJ-regulating factors such as NO and IL-6.

Despite the indisputable important pathophysiological role of gut permeability alterations in the development of complications of cirrhosis from diverse organs, little is known of its underlying molecular and/or cellular mechanisms and relevant evidence comes from extrapolation of data from animal studies. However, animal models can never reproduce human diseases to the desired level. Therefore, it is very positive that recent efforts focus on clinical studies dealing with this emerging concept; such studies might endow us with a better understanding of the pathogenetic mechanisms in humans. Our research group has recently shown, for the first time in humans, that altered expression of key structural