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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, Sebastianova 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-hepatic 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.

References


Pierluigi Marzuillo*
Anna Grandone
Laura Perrone
Emanuele Miraglia del Giudice
Department of women and children and general and specialized surgery, Seconda Università degli studi di Napoli, Napoli, Italy
*Corresponding author.
E-mail address: pierluigi.marzuillo@gmail.com

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-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
elements of enterocyte TJ components might represent an important molecular basis of increased intestinal paracellular permeability in cirrhosis [2]. We have demonstrated in our study that the expression of the TJ-related proteins occludin and claudin-1 was significantly decreased leading to increased gut permeability and endotoxemia. The findings presented by Dr. Du Plessis et al. provide additional evidence on the importance of enterocyte TJ alterations as a molecular mechanism underlying the increase of intestinal permeability demonstrating increased expression of Claudin-2. The difference in the implicated TJ-related molecules between the two studies is difficult to be fully explained presently. There is increasing awareness that the building blocks of tight junctions, namely occludin and numerous forms of claudins, serve tissue-specific and cell type-specific needs, combining strict structural requirements and the need for quick and versatile adaptive requirements [3]. Interactions between enterocyte TJ macromolecules are certainly complex, depending, apart from local cell-type specific factors, on concentration and post-translational modifications [4]. Our knowledge on the role of such factors is still rudimentary and, therefore, in most cases it is difficult to predict from mere expression levels the final physiological outcome. However, increased expression of Claudin-2, as shown by the authors, as well as decreased expression of claudin-1 or occludin, as shown in our study, all have been associated with a reduced transepithelial resistance and increased paracellular permeability in intestinal epithelial cells [5,6].

Of note, in the authors’ study, intestinal epithelial TJ alterations were found only in patients with decompensated cirrhosis, all of which had evidence of clinically significant portal hypertension (ascites). Portal hypertension constitutes the pathophysiological basis of most complications of cirrhosis and has been previously shown to be strictly correlated with intestinal hyperpermeability [7]. Therefore, a question raised is whether portal hypertension is implicated as an early pathophysiological event that might start the process of enterocyte TJ alterations in cirrhosis. As opposed to the authors’ study, we have previously shown that altered expression of TJ proteins occurs not only in advanced cirrhosis but in compensated cirrhosis as well, where clinically significant portal hypertension (esophageal varices) was detected only in half of the patients [2]. Despite the fact that we also demonstrated an inverse correlation of esophageal varices grade (an index of portal hypertension severity) with intestinal TJ expression in cirrhotic patients, our findings in early cirrhosis raise doubts concerning the potential central role of portal hypertension in intestinal TJ alterations. Clearly, future studies need to focus on these questions, e.g., by investigating intestinal TJs in pre-hepatic portal hypertensive animal models produced by partial portal vein ligation.

Up to now, much attention has been paid to the role of luminal factors (e.g., bacterial overgrowth) in enterocyte TJ regulation and gut permeability alterations [8]. The role of the microbiome should be considered of major importance. In a recent review by Wells et al. [9], the epithelial crosstalk with the “inner” and “outer” environment has been discussed and in this context the role of Toll-like receptors (TLR) has been emphasized. Further support for this notion has been provided by in vivo studies showing that TLR-2 signaling in mucosal monocytes and TLR-4 in enterocytes are implicated in intestinal epithelial barrier dysfunction [10,11]. The recently published study by Du Plessis and colleagues, investigating further the molecular events implicated in intestinal hyperpermeability in patients with liver cirrhosis, adds the underlying lamina propria as an important compartment, pointing to macro-phages as critical players in the process. We can anticipate that in the future another compartment may be added: that of epithelial cells in the vicinity, which by laterally releasing critical mediators in an autocrine fashion (either directly or via exosomes) may play a role in spreading alterations in intestinal permeability.

Regarding the potential mechanism(s) of regulation of enterocyte TJ formation and function, the authors propose that IL-6 and NO released by activated intestinal macrophages might exert an important role. It has been shown by the important study of Hartmann and colleagues that inflammatory mediators released locally from intestinal monocytes might disrupt enterocyte TJs through activation of the Rho GTPase pathway which leads to myosin light chain phosphorylation [10]. This pathway has been implicated in displacement and reorganization of TJ macromolecules [12]. However, beyond the implication of altered signaling, the regulation of expression of enterocyte TJ components and the resulting formation and functionality of the TJ can be anticipated to happen at several levels. First at the transcriptional level, where a combination of transcription factors and regulatory elements (close or at a distance from the promoter) can influence the initiation and efficiency of the transcriptional machinery. Second, following transcription, at the stability of the generated message, where a constellation of RNA molecules (mainly microRNAs) can influence the produced protein. Finally, at the level of post-translational modifications, where several additions (especially of phosphate groups but not restricted to them) can affect protein trafficking and TJ formation and degradation. We propose that in future studies special emphasis should be put on the role of microRNAs, as initial studies indicate an important role [13]. Although this field is at a very early stage, the promise for specific pharmaceutical interventions for enterocyte TJs should be underlined.

In conclusion, we think that the alterations of enterocytes’ TJs is the critical anatomical change which, by a combination of molecular mechanisms that we just start to uncover, is leading to intestinal hyperpermeability in patients with cirrhosis. In our view, we have reached a point where there is an emerging need to generate more data on human material by adopting two methodological approaches: first, larger numbers of human samples should be used, taken from certified biobanks with detailed case history; second, this precious material should be subjected to study with Systems Biology approaches, namely using genomics, transcriptomics and, more importantly, proteomics for the analysis of samples. Such global biology approaches will certainly allow us to discover novel macromolecules and regulatory pathways for enterocyte TJ formation, function and turnover. The fact that these methodologies can be applied to formalin fixed paraffin embedded material existing in Pathology Departments should generate more incentive to pathologists to contribute to this field in the near future [14,15]. However, the –omics approaches can give us a wealth of information regarding macromolecules and their involvement in discrete pathways but will not allow us to reach an in depth understanding without the performance of parallel physiological studies assessing intestinal permeability and barrier function. Intestinal permeability assessment in humans could be performed with the use of differentially sized orally administered non-metabolizable probes and measuring their urinary excretion or by ex vivo set ups like Ussing chambers, whilst gut barrier integrity could be assessed using markers of pathological bacterial translocation e.g., serum levels of endotoxin, anti-endotoxin antibodies, lipopolysaccharide binding protein or...
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detection of bacterial DNA by polymerase chain reaction technique in blood. In addition, more sophisticated techniques using fluorescent markers to monitor trans-epithelial permeability and providing us with real-time information with in vivo imaging could be developed. The combination of the approaches suggested above will lead to a more global understanding of the pathophysiology of enterocyte TJs in human liver cirrhosis and to the discovery of more specific targets (disrupting a signaling pathway, interfering with a regulatory RNA or protein molecule, others) and will likely lead to more effective and potentially individualized pharmacological control of intestinal hyperpermeability, thus preventing the endotoxin-associated systemic complications and improving clinical outcome.

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Conflict of interest

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References


Reply to: “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 thank Assimakopoulos and colleagues for their interest in our recent article [1]. As suggested, important efforts are being made to understand why the intestinal barrier fails in cirrhosis and the mechanism by which viable bacteria translocate from the gastrointestinal tract.
In our study, we assessed the following:
Firstly, we studied the morphology and function of the intestinal barrier in decompensated and compensated ASH/NASH cirrhotic patients. We could show that the morphology of the intestinal barrier was intact at the ultrastructural level, suggesting that permeability was functionally altered and not just secondarily increased due to widened intracellular junctions induced by portal hypertension [2,3]. At a functional level, we could indeed confirm that transepithelial resistance was decreased and permeability increased in patients with decompensated cirrhosis. We fully agree with the comments by Assimakopoulos et al. that emphasize the important role of TJ proteins in intestinal barrier function in cirrhosis as demonstrated in their recent study [4]. The difference in the implicated TJ proteins between our study [1] and that of Assimak-