211; Gastroenterology 2007;132:1665–1671). Indeed, this is also described in the Plantinga et al study, where similar increases in IL-1β and IL-6 are observed in both healthy individuals as well as Crohn's patients carrying the ATG16L1 Thr300Ala risk variant.

This study serves to highlight the complex interactions and factors that are required for initiation and progression of Crohn's disease, including key genetic differences. Indeed, an elegant study, continuing on from the initial observations relating to deficiency in Paneth cells, has shown that ATG16L1-deficient mice, when rederived in specific pathogen-free conditions, had Paneth cells that were indistinguishable from litter mate controls (Cell 2010;141:1135–1145). Studies have identified persistent murine norovirus as the contributing factor, along with the loss of the ATG16L1 autophagy protein, which gives rise to this deficient Paneth cell phenotype. Furthermore, after administration of the colitis- and injury-inducing agent, dextran sodium sulphate, only those mice deficient for ATG16L1, in tandem with persistent norovirus infection, experienced extensive pathologic changes reminiscent of human Crohn's disease (Cell 2010;141:1135–1145).

The study by Plantinga et al emphasizes the importance of studying and validating specific genetic polymorphisms associated with chronic inflammatory diseases, such as Crohn's disease, in the human population. Specifically, the study provides further evidence that deficiencies in autophagy pathways, because of specific polymorphisms, can lead to potentially damaging clinical outcomes. Overproduction of cytokines by immune cells coding for the 300Ala risk allele could play an important contributory role in the excessive inflammation and subsequent pathogenesis of this disease. Notably, the study also shows a direct interaction between 2 Crohn's disease risk alleles, ATG16L1 and NOD2, and further adds to the findings linking susceptibility genes in a single, but functionally different pathway to that described previously. Nonetheless, we must remember that, although autophagy does seem to contribute to Crohn's disease pathogenesis, many other cell-specific mechanisms must also occur for progression to chronic inflammation.

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PROTEOMICS TO PREDICT HEPATITIS C THERAPY OUTCOME: WHERE DO WE STAND?

Patel K, Lucas JE, Thompson JW, et al. **High predictive accuracy of an unbiased proteomic profile for sustained virological response in chronic hepatitis C patients. Hepatology 2011;53:1809–1818.**

Regimens based on pegylated interferon (IFN) and ribavirin (RBV) are the standard of care for patients chronically infected with the hepatitis C virus (HCV), leading to a permanent virus eradication (sustained virologic response [SVR]) in approximately 50% of all treated patients. The enthusiasm for anti-HCV therapy, however, is mitigated by the chasm between the efficacy demonstrated by registration trials and the effectiveness of pegylated IFN observed in the clinical practice, where comorbidities and adverse events may adversely affect patient compliance, and cause dose adjustments or treatment discontinuation, which shrinks the likelihood of an SVR (Ann Intern Med 2002;136:288–292). Not surprisingly, therefore, many studies have analyzed the cost–utility ratio of anti-HCV treatment, with the primary aim to spare morbidities from unnecessary exposure to IFN and RBV. These studies have had the added benefit of identifying both pretreatment as well as early on-treatment predictors of treatment outcome, providing patients and clinicians with rough estimates of the individual chances of an SVR. Pretreatment estimation of the attainable SVR rates remains fundamental in the era of directly antiviral agents (DAAs), such as telaprevir and bocepravir. However, as the use of DAAs leads to increasing SVR rates, this will come at the expenses of more treatment-related adverse events and higher costs. Several pretreatment factors have been identified as positive predictors of treatment outcome like female sex, mild histologic hepatitis, low viral load, and HCV-2 and HCV-3 genotypes. However, on-treatment predictors, such as serum HCV-RNA clearance at week 4 (rapid virologic response [RVR]) and week 12 of therapy (early virologic response), are the most powerful predictors of SVR and are used to individualize treatment duration (J Hepatol 2011; 55:245–264). RVR is such a strong positive predictive of SVR that if this virologic milestone is reached, then the predictive power of any pretreatment factor is overshadowed. This holds true even for the recently discovered genetic predictors of treatment outcome, such as single nucleotide polymorphisms near the interleukin (IL)-28B gene, which have been shown to predict treatment outcome in the difficult-to-cure genotypes 1 and 4 of HCV (Nature 2009;461:399–401; Gastroenterology 2010;139:120–129). The discovery of such polymorphisms is the direct consequence of improvements of the so-called -omics platforms (genomics, transcriptomics, proteomics, metabolomics, etc) that are now available as potentially powerful tools to unravel the pathogenesis and predict the clinical course of a variety of diseases. Indeed, functional genomics arises from the wealth of human genome sequence information now available, coupled with the recent technological advances in robotics, nanotechnology, mass spectrometry, and bioinformatics systems. Among these, serum proteomics is a most promising tool, because it potentially yields clinically sound information starting from an easy-to-obtain biologic sample like serum.

In their recent paper, Patel et al aimed at assessing whether pretreatment serum proteomic analysis could lead to the identification of peptides and/or proteins

differently expressed among patients with an SVR and those with a treatment failure (nonresponders [NRs]). This could effectively define a signature to confidently predict pegylated IFN plus RBV treatment outcome in HCV patients. They analyzed 2 cohorts (training and validation) of patients, carefully selected from the Duke Hepatology Clinical Research database. SVR and NR patients in each cohort were carefully matched with respect to their clinical and demographic data, including age, gender, race, and HCV load. Sera from these patients were tested using a label-free liquid chromatography mass spectrometry (LC-MS)-based proteomics discovery platform, and peptides were identified by Proteinlynx Global Server v.2.4 database search engines. MS/MS identifications were curated using a forward/reverse database search, at a false discovery rate of 1%. In all patients the IL-28 genotype polymorphism rs12979860 was tested with the Illumina Human610-quad BeadChip (Illumina, San Diego, CA). The training cohort consisted of 55 HCV patients, 18 NR (HCV-1) and 37 with an SVR (17 HCV-1, 20 HCV-2/3) to IFN-based regimens. By using a not-standard serum proteomics approach, they identified a 3-protein signature able to accurately predict SVR in these HCV patients. This signature included vitamin D binding protein (VTDB), alpha 2 HS glycoprotein (FETUA), and complement C5 (CO5). This signature was subsequently validated in a cohort of 41 patients infected with HCV-1 genotype (26 SVR and 15 NR). In fact, by using a "metaprotein" classification approach, 105 proteins for a total of 3768 peptides were found to be differently expressed at baseline among SVR and NR patients in the training cohort, thus providing the basis for a predictive model of response to therapy, with decreased levels of VTDB and FETUA and increased levels of CO5 emerging as the single best algorithm to predict an SVR. In the validation cohort, 112 proteins for a total of 3211 peptides were identified that significantly differed between SVR and NR patients, including VTDB, FETUA, and CO5. When combined in a regression model, VTDB, FETUA, and CO5 were able to exactly predict an SVR in >90% of the patients (88% in the validation set), providing an area under the receiver operating characteristic curve (AUROC) for SVR of 0.90 (0.86 in the validation cohort). In the training set, the inclusion in the algorithm of important demographic variables like gender, ethnicity, and serum HCV-RNA levels inflated the AUROC to 0.94, which was not significant ($P = .98$), probably as a consequence of the matching of SVR and NR patients with respect to their clinical and demographic variables known to affect treatment response. In the validation cohort, when comparing the predictive value for an SVR of the IL28 rs12979860 CC-genotype and the 3-metaprotein signature, the latter showed a better sensitivity (0.77 vs 0.73), but a lower specificity (0.80 vs 0.93). When applied to non-CC patients, the 3-metaprotein signature was able to accurately identify SVR patients with an overall accuracy of 71%, and sensitivity and specificity of 0.57 and 0.78, respectively. The authors found most of the proteins differently regulated in SVR and non-SVR patients to share a common pathway related to IFN response, thus corroborating the association between the IL-28B signaling pathway and response signature. Finally, based on these interesting results, Patel et al conclude that a serum-based protein signature can accurately predict treatment response in most HCV patients.

Comment. The efficacy of interferon-based treatment of hepatitis C is challenged by the occurrence of potentially serious adverse events that interfere with adherence to therapy (Gastroenterology 2002;123:1061–1069). Not surprisingly, all scientific societies recommend against universal treatment of HCV-infected patients. They advocate pretreatment patient selection to avoid unnecessary costs and treatment-related morbidity. This may be even more important with the advent of DAAs that are expected to significantly improve the rates of SVR at the expense of more adverse events and higher costs. Despite the fact that many pretreatment predictors of anti-HCV treatment outcome have been indentified, virus genotype and, to some extent, IL-28B polymorphisms are the only ones that have effectively entered the therapeutic algorithm (Am J Gastroenterol 2011;106:38–45). Although the discovery of IL-28B–associated single nucleotide polymorphisms and their association with antiviral treatment outcome reflect the advance of the genomics platforms, RVR and early virologic response still hold the greater potential for response prediction in individual patients, de facto representing an effective and pragmatic rate-limiting approach to optimizing hepatitis C therapy.

The conceptual background for serum proteomics to act as disease or treatment predictors is the proteome being a dynamic protein mirror of genome with a complementary role with respect to the information provided by genomic and transcriptomic approaches. Notably, the concordance between the relative expression abundance of a gene and its biological active protein product is <40%, telling us that measurement of serum mRNA levels cannot predict phenotypic variations of the proteins resulting from downstream regulatory events that confer or modify protein function (Hepatology 2006;44:299–308). In contrast with either genomics or transcriptomics, proteomics has the potential to explore differences of several orders of magnitude in protein levels while detecting minute posttranscriptional and posttranslational modifications that are often just as important for protein activity as protein expression levels. Moreover, although the amount of mRNA in organic fluids is limited, peptides and proteins circulate in abundance, thus theoretically easing the proteome analysis. As proteomics relies on a combination of sophisticated techniques, most frequently including 2-dimensional gel electrophoresis, image analysis, mass spectrometry, amino acid sequencing and bioinformatics, the recently improved proteomic technologies, such as proteomic profiling technology, enable global visualization of the proteome by a high-throughput method, allowing for the identification of either isolated