Identifying the Genetics Underlying Nonalcoholic Fatty Liver Disease

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Medication use prior to endoscopy, in particular, acid suppression, was not reported. This makes it difficult to determine in what proportion of patients the symptoms were acid-related. It may also make interpretation of endoscopic findings, for example, the frequency of ulcers, which proton pump inhibitors (PPIs) heal, suboptimal.

Other problems that were identified in the Bonilla et al article included use of the recommended first-line therapy (PPI-CA: PPI, clarithromycin and amoxicillin) in only 69% (146/211) of patients (5). The ESPGHAN and NASPGHAN guidelines recommend the triple combination PPI-CA, given for 14 days, as the preferred regimen, if it is known that the strain is susceptible to clarithromycin and metronidazole (1). However, worldwide regional data on antibiotic susceptibilities of *H pylori* strains are scarce, despite strong recommendations that knowing pretreatment susceptibility profiles is important. Pooled US data on resistance to clarithromycin report a rate of 22% (3). In adults, but not in children, at this level of resistance 14 day twice-daily concomitant therapy (PPI, clarithromycin, amoxicillin and metronidazole) is recommended over PPI-CA (2,6,7). The problem of antibiotic resistance is important but may be different among children and adults. If antibiotic resistance data are not available, the choices of treatment need to be guided by knowledge about the actual success rates of *H pylori* therapy in the area where one practices. This is an additional argument that posttreatment testing for *H pylori* testing is done, as suggested by the guidelines.

Finally, Bonilla et al (5) report a low frequency (17%, 28/162) of obtaining culture for *H pylori* and poor culture success rate (43%, 12/28). Culturing of *H pylori*, a fastidious organism, requires dedicated laboratory technicians with special training. High-quality laboratories achieve at least 80% to 90% concordance between culture results and histology and/or UBT. Sadly, despite pleas in pediatric and adult guidelines to increase culture and antibiotic susceptibility testing of *H pylori* organisms, this has not happened in the USA. Consequently few data on antibiotic resistance are available. Susceptibility testing has become easier as, for example, for clarithromycin resistance PCR-based tests, which detect CLA-resistant mutations, are commercially available.

In conclusion, if studies on management of pediatric *H pylori* in other centers find problems similar to the Bonilla et al study, one might consider targeted interventions to improve knowledge translation in this area. Furthermore, this study may stimulate practicing pediatricians to reflect on how they manage *H pylori* infection.

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Identifying the Genetics Underlying Nonalcoholic Fatty Liver Disease: A Quest That is Far From Over

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See ''Pediatric Non-Alcoholic Fatty Liver Disease Is Affected by Genetic Variants Involved in Lifespan/Healthspan'' by Crudele et al on page 161.

It is widely accepted that the complex pathophysiology of nonalcoholic fatty liver disease (NAFLD) has important individual variability based on differences in genetic susceptibility (1). At present, the I48M variant in patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene identified in 2008 remains the major putative gene variant associated with steatosis, inflammation, and fibrosis in both children and adults. Several other putative and protective variants have been associated with NAFLD. It is, however, assumed that multiple, possibly hundreds, of other unidentified variants most of small effect size and with indirect effects play a role in the heritability of NAFLD in children and adults (1). Given the presumed importance of genetics in NAFLD, more complete understanding of the gene variants associated with advanced NAFLD holds the promise for noninvasive and early identification of those at risk of advanced NAFLD and providing new insights in the pathophysiology of NAFLD and ultimately individualizing therapy.

In this issue of the JPGN, Crudele et al study the association with NAFLD diagnosis, histological features of NAFLD, and metabolic features of 10 diverse gene variants involved in both human liver and metabolic diseases and ''healthspan,'' in a case control design involving 177 teenagers with histologically determined NAFLD and 146 controls (39 healthy children and 107 self-declared healthy adults) (2). They find that no individual variant shows a difference in frequency between cases and controls. Using multidimensional reduction (MDR) analysis, a statistical method used in genetics studies to test interaction between variants ('epistasis'), the interactions between variants in the *ANRIL* (rs1556516)

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and *IL6* (rs1800795) genes are associated with NAFLD diagnosis, however. Il-6 is a pro-inflammatory cytokine that has been associated with insulin resistance and type 2 diabetes (3). The function of ANRIL is not fully clarified, although this long noncoding RNA has been implicated in multiple diseases, such as diabetic retinopathy (4). The combined presence of ANRIL and IL-6 variants has 2.01% explained entropy, that is, positive synergistic effects of these variants on the presence of NAFLD.

The observed interaction was not unequivocal as variants did not consistently show a putative or protective effect in the different genotype combinations. Only genotype GG at IL-6 rs1800795 was consistently found to be a risk genotype. In a subsequent analysis, the ANRIL (rs1556516) and IL6 (rs1800795) variants also showed a significant interaction associated with fasting plasma blood glucose levels, in line with the prominent role of glucose metabolism pathogenesis of NAFLD. This effect did partially overlap with the risk for NAFLD; however, 2 genotypes associated with higher NAFLD risk and 1 associated with lower risk were not associated with a higher and lower blood glucose level, respectively. As the authors righteously point out, other genetic variant combinations potentially modified by nongenetic factors may explain the nonlinear relation of IL-6/ANRIL genotypic combinations for both NAFLD and glucose levels. Including established variants associated with NAFLD, particularly in the *PNPLA3* (rs738409) and *GCKR* (rs1260326) genes, the latter also being involved in glucose metabolism, could possibly have provided more insight. It is, however, uncertain whether the observed interaction would retain significance in a study of this size, if these variants were included. The authors conclude that larger studies are required to confirm their findings and to elucidate mechanisms by which these 2 genes interact.

This candidate gene study adds 2 more variants to the body of over 20 variants previously associated with pediatric NAFLD mostly through candidate gene studies. Like in this study, most of the identified variants are involved in lipid biosynthesis and metabolism or glucose metabolism. The study by Crudele et al distinguishes itself as it is one of the largest genetic studies in histology-proven NAFLD allowing to associate the variants to inflammation grade or fibrosis stage. Contrary to most candidate gene studies in pediatric NAFLD that selected variants based on previous adult studies, the authors took an original approach by selecting all but one of the included variants based on their association with longevity in longitudinal cohort studies.

Candidate gene studies have helped to shape our understanding of the genetics involved in the susceptibility to NAFLD and functional studies of these variants have helped advance our understanding of the pathogenesis of pediatric NAFLD (5). This understanding, however, remains fragmented as most variants are evaluated alone or in a conjunction with a limited set of other variants and validation is lacking for many variants. Remarkable in that respect is the absence of an association of the *KLB* gene (β-Klotho) rs17618244 variant with histological features of NAFLD in this study. This variant was included in this study as it was recently associated with ballooning and lobular inflammation in larger cohort of biopsy-proven pediatric NAFLD patients from the same centre (6).

For a more complete understanding of the relevance of the variants identified in candidate gene studies, their evaluation in conjunction within a genome-wide association (GWAS) approach is needed. Such untargeted approach is also the most effective and unbiased way to identify novel variants relevant for pediatric NAFLD. At present, 1 pediatric GWAS discovery study was performed in a selected cohort of 208 Hispanic men with histologically confirmed NAFLD, which identified two novel variants associated with histologic traits of NAFLD distinct from those recognized by adult GWAS studies (7). Another, analysing a mixed adult and pediatric population from North America (107 cases of biopsy-proven pediatric NAFLD) identified 2 novel variants (8). These results remain to be validated. The large population GWAS studies needed in studies to identify novel small effect size genes underlying the genetic variability in NAFLD is illustrated by a large GWAS study in adults by the EPoS Consortium involving almost 1500 histologically determined NAFLD cases and over 17,000 controls, which identified only 1 novel variant associated with steatosis (PYGO1) and 3 other possibly relevant novel variants (LEPR, C2ORF16, IDO2/TC1[C8orf4]) for which larger (meta) analyses are needed to assess their relevance (9).

Understanding the genetic susceptibility to NAFLD and its progression in children and adults is a quest that is far from over. Only collaboration and perseverance can lead to pediatric cohorts that are sufficient in size to detect more than the usual suspects through GWAS analysis. Collaborative data and sample collection within the national NASH Clinical Research Network has been ongoing for many years in North America. In the EU-pediatric NAFLD registry it is at its inception (10). Hopefully in the future, through these collaboration, large GWAS studies will be performed. It remains to be seen whether in such studies, the genes identified by Crudele et al will stand out.

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