Table 1 Pathological features of the two study groups

Parameter	Chronic hepatitis C (group 1) (n = 124)	Non-C chronic liver disease (group 2) (n = 48)	p Value
Steatosis	50 (40.3%)	16 (33.3%)	0.550
Mild	36 (29.0%)	8 (16.7%)	
Moderate	10 (8.1%)	6 (12.5%)	
Severe	4 (3.2%)	2 (4.2%)	
Steatohepatitis	7 (5.7%)	1 (2.1%)	0.445
Modified HAI†	6.7 (2.7), 0–13	4.8 (3.4), 0–13	0.010*
Fibrosis score†	3.3 (1.9), 0–6	1.8 (1.7), 0–6	0.002*
Haemosiderosis	4 (3.2%)	0 (0.0%)	0.487

HAI, Histology Activity Index.
*Significant difference.
†Values are mean (SD), range.

increased to seven (5.6%) in group 1 and one (2.1%) in group 2 (p = 0.445) after applying the immunohistochemical staining for detection of Mallory hyaline bodies. Pericellular fibrosis was identified in all steatohepatitis sections, but neutrophil inflammatory reaction was only identified in three specimens. Necroinflammatory injury, as reflected by modified Histology Activity Index (HAI), was significantly higher in group 1 than in group 2 (6.7 (2.7) vs 4.8 (3.4), p = 0.01). The fibrosis score was significantly higher in group 1 than in group 2 (3.3 (1.9) vs 1.8 (1.7), p = 0.002). Overweight/obesity (≥25 kg/m^2) was reported in 80.6% of patients in group 1 and 62.5% in group 2 (p = 0.097), type 2 diabetes mellitus (fasting blood sugar >126 mg/dl and postprandial blood sugar 200 mg/dl on more than one occasion) was encountered in 27.4% of group 1 and 12.5% in group 2 (p = 0.038), while hypertriglyceridaemia (>200 mg/dl) was found in 6.5% of group 1 and 8.3% of group 2 (p = 0.759, table 1).

While steatosis was not associated with HCV (p = 0.555), it was significantly correlated with overweight/obesity (p = 0.001), diabetes mellitus (p = 0.001) and hypertriglyceridaemia (p = 0.019).

By multivariate analysis, only overweight/obesity and diabetes mellitus were found to be significantly associated with steatosis. Neither necroinflammation nor fibrosis was correlated with steatosis in patients with HCV genotype 4 (p = 0.953 and 0.463). This lack of correlation may be explained by the discrepancy between a high frequency of steatosis (40%) and a low frequency of steatohepatitis (5.6%) in group 1.

We conclude that hepatic steatosis is prevalent in nearly 40% of patients infected with HCV genotype 4, which is more highly attributed to associated metabolic factors. It seems that there is no correlation between necroinflammation or fibrosis and steatosis in patients with HCV genotype 4.

A El-Zayadi
Department of Tropical Medicine, Ain Shams University, Cairo and Cairo Liver Center, Giza, Egypt

M Attia
Department of Hepatology and Gastroenterology, Theodor Bilharz Research Institute, Cairo, Egypt

E M F Barakat
Department of Tropical Medicine, Ain Shams University, Cairo, Egypt

K Zalata
Department of Pathology, Mansoura University, Mansoura, Egypt

A Saeid
Cairo Liver Center, Giza, Egypt

H Hamdy
Department of Tropical Medicine, Ain Shams University, Cairo, Egypt

A El-Nakeeb
Cairo Liver Center, Giza, Egypt

Correspondence to: Professor Abdel-Rahman El-Zayadi, 5 El-Gergawy St Dokki, Giza, Egypt; clcz@tedata.net.eg

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Association of the T300A non-synonymous variant of the *ATG16L1* gene with susceptibility to paediatric Crohn's disease

The hunt for genetic variants conferring risk to inflammatory bowel disease (IBD) has been ongoing for more than 10 years, with a number of susceptibility genes implicated through various approaches. Earlier this year, through the genotyping of 16 360 non-synonymous single nucleotide polymorphisms (SNPs), Hampe *et al*[1] made an important addition to this repertoire by reporting a highly significant association between Crohn's disease and the autophagy-related 16-like 1 (*ATG16L1*) gene. Specifically, a common coding variant, rs2241880 (T300A), was shown to confer a strong risk for the disease, and this association was replicated in the same study in separate cohorts of patients with Crohn's disease but not with ulcerative colitis. Although these findings are compelling, there are continuing concerns regarding the performance of association studies in complex traits.[2] Many errors and biases can blight any individual study, whereas independent replication ensures that the original findings are indeed robust and provide a more accurate estimate of the likely effect size.[3 4]. Such independent replication efforts are now considered mandatory in the study of complex traits.[2]

We have an ongoing genome-wide association study of children with Crohn's disease using the Illumina Infinium II HumanHap500 BeadChip.[5 6] The probe for rs2241880 is present on this platform, so we were able to query our current dataset for its association with Crohn's disease as a single test. All analyses were carried out using the software package ''plink'' (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml). In order to avoid potential bias deriving from population stratification, controls were genetically matched to Caucasian patients with Crohn's disease by clustering of the pairwise identity-by-state distances. Complete linkage agglomerative clustering was used with restrictions based on a pairwise population concordance test so that one case was matched with two controls. Controls were matched with cases based on their pairwise identity-by-state as long as a pairwise population concordance test had a p value of >0.001. In some limited cases, only one genetically matched control could be found based on the restrictions. Our association analysis included 142 out of 143 unrelated Caucasian children with Crohn's disease (71 boys, age of onset 1–17 years, mean 10.9 years) who had consented to the study and 281 matched controls (140 boys) recruited from the greater Philadelphia area from 2000 to 2006 at the Children's Hospital of Philadelphia. One patient with Crohn's disease was missing genotypes and was excluded from the analysis.

The diagnosis of Crohn's disease was based on standard criteria.[7] Determination of disease site and extent was performed in each patient. The anatomical distribution of the disease was determined by involvement of regions of Crohn's disease as upper tract (oesophageal, gastric, duodenal), small bowel (jejunal, ileal), colonic and perianal. With the exception of jejunal and perianal Crohn's disease, all other areas of anatomical involvement were confirmed by microscopic evidence of chronic inflammation. Patients were categorised further into one of five groups based on the maximal extent of the disease:

- ileal, including gross involvement of the ileum without colonic disease but could include upper tract involvement (n = 12, 8.3%);
- ileocolonic, including gross involvement of the ileum and colon but could include any upper tract involvement (n = 123, 86%);
- colonic, including gross involvement of the colon without small bowel involvement (n = 8, 5.6%);
- perianal disease, including perianal fistulas, perianal abscesses, but not perianal fissures or perianal tags (this was considered a separate category and could occur with another site of involvement (n = 46, 32%); and
- upper gastrointestinal tract involvement with evidence of gross or microscopic involvement of the upper gastrointiestinal

Table 1 Genotype counts for rs2241880, rs2066843 and rs2076756 in the case-control study

SNP/gene	Genotype	Cases (n = 142)	Controls (n = 281)
rs2241880/*ATG16L1*	AA	19 (13.4%)	67 (23.8%)
	AG	65 (45.8%)	136 (48.4%)
	GG	58 (40.8%)	78 (27.8%)
rs2066843/*CARD15*	TT	15 (10.6%)	20 (7.1%)
	CT	57 (40.1%)	118 (42.0%)
	CC	70 (49.3%)	143 (50.9%)
rs2076756/*CARD15*	GG	14 (9.9%)	17 (6.0%)
	AG	58 (40.8%)	112 (39.9%)
	AA	70 (49.3%)	152 (54.1%)

SNP, single nucleotide polymorphism.

tract (n = 2, 1.4%); the latter could include any of the first four categories.

The eight subjects with colonic involvement without small bowel disease had either skip lesions in the colon or granulomas that differentiated them from ulcerative colitis. All patients with ulcerative colitis were excluded from the study.

Patients were further categorised based on the clinical history with either fistulising (n = 57, 40%), structuring (n = 26, 19%) or inflammatory (n = 60, 42%) disease behaviour using the Vienna classification guidelines criteria.[8] Fistulising or penetrating disease was defined as the presence of intra-abdominal fistulising lesions (the presence of enteroenteric fistulas) or perianal fistulising lesions (enterocutaneous). Patients with non-stricturing and non-fistulising disease at the time of presentation and throughout the follow-up period to date were classified as having inflammatory disease behaviour. Thirty subjects (21%) had a positive family history of Crohn's disease.

The frequency of allele G was 63.73% in the cases and 51.96% in the controls ($p = 6.93 \times 10^{-4}$, Fisher's exact test one-sided) (see genotype counts in table 1), yielding an allelic odds ratio (OR) of 1.62 (95% CI 1.21 to 2.18) and 1.80 (95% CI 1.18 to 2.75; population attributable risk (PAR) 0.18) for homozygosity. These ORs are slightly higher than those reported previously in the German case-control cohort (allelic OR = 1.45; homozygosity OR = 1.77) and the UK case-control cohort (OR = 1.35; homozygosity OR = 1.71).[1]

Sixty-five cases derived from our patient cohort also had both parents recruited. Using a binomial exact test, we observed a significant association with rs2241880 in a transmission disequilibrium test (43:24 allele G transmitted to non-transmitted; one-sided p = 0.014); this test is more robust for population stratification and allowed us to confirm that the observations from our case-control analysis were accurate.

The *CARD15* gene has been extensively validated as a susceptibility gene for Crohn's disease.[9–11] We genotyped our patients with the HumanHap550K SNP chip from Illumina, which does not include the three main *CARD15* SNPs associated with Crohn's disease (rs2066844, rs2066845 and rs2066847) nor any SNP in significant linkage disequilibrium with them. Duerr *et al*[12] reported a significant association between Crohn's disease and two tagging SNPs on the HumanHap317 SNP chip (rs2066843 and rs2076756); however, they did not provide information on minor allele frequency (MAF) or odds ratios for these variants in their report which would be needed to determine our power to replicate their findings. We examined those two SNPs in our study cohort (table 1) and, unlike Duerr *et al*,[12] we did not detect an association between them and Crohn's disease (p = 0.45 for rs2066843 and p = 0.19 for rs2076756 for difference in allele frequencies, Fisher's exact test). With the observed MAF of 0.26 for rs2076756 and 0.28 for rs2066843 in our control group and the current sample size, we had 80% power to detect a significant association at the 5% level if the allele frequencies in the cases were 0.35 and 0.37, respectively, or higher; in contrast, the two SNPs had MAF of 0.30 and 0.31 in our cases. While these results do not support an association with the *CARD15* gene, we note that our sample size may be too small to detect an association on the basis of the *CARD15* SNPs available on the chip, and we would have to type the three classical *CARD15* SNPs directly in order to reach a definite conclusion which is beyond the scope of this paper.

Taken together, we have replicated the association of allele G of rs2241880 in the *ATG16L1* gene with Crohn's disease by demonstrating its effect in the childhood form of the disorder. Once our genome-wide association study is complete, we will have the opportunity to look for other variants in the genome that are associated with Crohn's disease as a consequence of our use of a higher resolution BeadChip. In addition, we will explore the *ATG16L1* gene further to elucidate other variants that may confer genetic susceptibility to this debilitating disorder in our cohort.

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Robert N Baldassano
Division of Gastroenterology and Nutrition, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Jonathan P Bradfield
Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Dimitri S Monos
Department of Pediatrics, University of Pennsylvania, School of Medicine and Department of Pathology and Laboratory Medicine, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Cecilia E Kim, Joseph T Glessner, Tracy Casalunovo, Edward C Frackelton, F George Otieno
Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Stathis Kanterakis
Department of Pediatrics University of Pennsylvania, School of Medicine and Department of Pathology and Laboratory Medicine, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Julie L Shaner, Ryan M Smith, Andrew W Eckert, Luke J Robinson, Chioma C Onyiah
Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Debra J Abrams
Division of Gastroenterology and Nutrition, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Rosetta M Chiavacci, Robert Skraban
Center for Applied Genomics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Marcella Devoto
Division of Human Genetics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, and CCEB, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Struan F A Grant, Hakon Hakonarson
Center for Applied Genomics, and Division of Human Genetics, Abramson Research Center, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Correspondence to: Dr Hakon Hakonarson and Dr Struan Grant, Center for Applied Genomics, Children's Hospital of Philadelphia, Abramson Research Center, 3615 Civic Center Boulevard, Philadelphia 19104, Pennsylvania, USA; hakonarson@chop.edu or grants@chop.edu

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