Association of Genetic Variants in NUDT15 With Thiopurine-Induced Myelosuppression in Patients With Inflammatory Bowel Disease

Gareth J. Walker, MBBS; James W. Harrison, PhD; Graham A. Heap, PhD; Michiel D. Voskuil, MD; Vibeka Andersen, MD; Carl A. Anderson, PhD; Ashwin N. Ananthakrishnan, MD; Jeffrey C. Barrett, PhD; Laurent Beaugerie, PhD; Claire M. Bewshea, MSc; Andy T. Cole, DM; Fraser R. Cummings, DPhil; Mark J. Daly, PhD; Pierre Eliul, PhD; Richard N. Fedorak, MD; Eleonora A. M. Festen, MD; Timothy H. Florin, MBBS; Daniel R. Gaya, DM; Jonas Halfvarson, MD; Alisa L. Hart, PhD; Neel M. Heerasing, MBBS; Peter Hendy, MBBS; Peter M. Irving, MD; Samuel E. Jones, PhD; Jukka Koskela, MD; James O. Lindsay, PhD; John C. Mansfield, MD; Dermot McGovern, DPhil; Miles Parkes, DM; Richard C. G. Pollok, PhD; Subramaniam Ramakrishnan, MD; David S. Rampton, DPhil; Manuel A. Rivas, DPhil; Richard K. Russell, PhD; Michael Schultz, PhD; Shaji Sebastian, MD; Philippe Seksik, PhD; Abhey Singh, MBBS; Kenji So, MBBS; Harry Sokol, PhD; Kavitha Subramaniam, MBBS; Anthony Todd, MBChB; Vito Annese, MD; Rinse K. Weersma, MD; Ramnik Xavier, MD; Rebecca Ward, MSc; Michael N. Weedon, PhD; James R. Goodhand, MBBS; Nicholas A. Kennedy, MBBS; Tariq Ahmad, DPhil; for the IBD Pharmacogenetics Study Group

IMPORTANCE Use of thiopurines may be limited by myelosuppression. TPMT pharmacogenetic testing identifies only 25% of at-risk patients of European ancestry. Among patients of East Asian ancestry, NUDT15 variants are associated with thiopurine-induced myelosuppression (TIM).

OBJECTIVE To identify genetic variants associated with TIM among patients of European ancestry with inflammatory bowel disease (IBD).

DESIGN, SETTING, AND PARTICIPANTS Case-control study of 491 patients affected by TIM and 679 thiopurine-tolerant unaffected patients who were recruited from 89 international sites between March 2012 and November 2015. Genome-wide association studies (GWAS) and exome-wide association studies (EWAS) were conducted in patients of European ancestry. The replication cohort comprised 73 patients affected by TIM and 840 thiopurine-tolerant unaffected patients.

EXPOSURES Genetic variants associated with TIM.

MAIN OUTCOMES AND MEASURES Thiopurine-induced myelosuppression, defined as a decline in absolute white blood cell count to $2.5 \times 10^9/L$ or less or a decline in absolute neutrophil cell count to $1.0 \times 10^9/L$ or less leading to a dose reduction or drug withdrawal.

RESULTS Among 1077 patients (398 affected and 679 unaffected; median age at IBD diagnosis, 31.0 years [interquartile range, 21.2 to 44.1 years]; 540 [50%] women; 602 [56%] diagnosed as having Crohn disease), 919 (311 affected and 608 unaffected) were included in the GWAS analysis and 961 (328 affected and 633 unaffected) in the EWAS analysis. The GWAS analysis confirmed association of TPMT (chromosome 6, rs11969064) with TIM (30.5% [95/311] affected vs 16.4% [100/608] unaffected patients; odds ratio [OR], 2.3 [95% CI, 1.7 to 3.1], $P = 5.2 \times 10^{-9}$). The EWAS analysis demonstrated an association with an in-frame deletion in NUDT15 (chromosome 13, rs746071566) and TIM (5.8% [19/328] affected vs 0.2% [1/633] unaffected patients; OR, 38.2 [95% CI, 5.1 to 286.1], $P = 1.3 \times 10^{-8}$), which was replicated in a different cohort (2.7% [2/73] affected vs 0.2% [2/840] unaffected patients; OR, 11.8 [95% CI, 1.6 to 85.0], $P = 0.03$). Carriage of any of 3 coding NUDT15 variants was associated with an increased risk (OR, 27.3 [95% CI, 9.3 to 116.7], $P = 1.1 \times 10^{-7}$) of TIM, independent of TPMT genotype and thiopurine dose.

CONCLUSIONS AND RELEVANCE Among patients of European ancestry with IBD, variants in NUDT15 were associated with increased risk of TIM. These findings suggest that NUDT15 genotyping may be considered prior to initiation of thiopurine therapy; however, further study including additional validation in independent cohorts is required.

Corrected on April 23, 2019.

© 2019 American Medical Association. All rights reserved.
Thiopurines (mercaptopurine and its prodrug azathioprine) are commonly used in the management of inflammatory bowel disease (IBD). However, approximately 15% of patients develop adverse drug reactions that necessitate drug withdrawal.1,2 Thiopurine-induced myelosuppression (TIM) has a cumulative incidence of 7% and usually occurs within a few weeks of starting the drug.1 Most patients are asymptomatic, but serious opportunistic infections may occur and there is an estimated mortality of 1%.1

The enzyme thiopurine S-methyltransferase (TPMT) converts thiopurines to methylated metabolites, reducing the production of active 6-thioguanine nucleotides.3 Genetic variation in the TPMT gene (RefSeqGene NG_012137.2) can result in decreased TPMT enzyme activity and higher production of 6-thioguanine nucleotides, predisposing patients to bone marrow suppression.1,3,4 Pretreatment testing of TPMT is recommended by the US Food and Drug Administration to identify patients at risk of TIM.5 Among patients with reduced TPMT activity, the drug may be avoided or the dose reduced.6 However, TPMT variants are only found in 25% of patients of European ancestry affected by TIM, suggesting the presence of other genetic and environmental determinants.6,7 Studies in patients of East Asian ancestry8-9 and other populations10-14 have identified variants in nudix hydrolase 15 (NUDT15; RefSeqGene NG_047021.1) as risk factors for TIM. Although a novel NUDT15 variant (rs746071566, p.Gly17_Val18del) was described by Moriyama et al10 in a single pediatric patient of European ancestry affected by TIM, the association of the NUDT15 genetic variation with TIM in this population has not been fully evaluated.

The primary objective of this study was to investigate the association between genetic variants and TIM in patients of European ancestry with IBD. It was hypothesized that the frequency of these variants would be increased among patients affected by TIM and enriched in those with early-onset TIM (<8 weeks from start of maximum dose).1

Methods

Study Design and Setting

The protocol was approved by the National Research Ethics Committee (11/SW0222, Exeter pharmacogenetic PRED4 program and STBI, Exeter IBD Genetics cohort, England). All participants provided informed written consent. A retrospective case-control study of the association of genetic variants with TIM was designed as part of the Exeter pharmacogenetic PRED4 program, which aims to investigate the genetic basis of serious adverse reactions among patients prescribed commonly used drugs in gastroenterology (http://www.ibdresearch.co.uk).15,16 Platforms for both genome-wide association studies (GWAS) and exome-wide association studies (EWAS) were used to investigate common and rare genetic variation, respectively.

Study Populations and Case Definition

Thiopurine-induced myelosuppression cases (affected patients) were recruited from 82 sites within the United Kingdom and from 7 sites outside the United Kingdom between March 2012 and November 2015 and not followed up after their single study visit. Individuals affected by TIM were identified through clinical encounters, systematic searches of electronic records, recall via the Medicines and Healthcare Products Regulatory Agency Yellow Card Scheme, and by direct advertising to patients.

Inclusion criteria included all of the following: diagnosis of IBD, history of thiopurine exposure during the 7 days prior to the onset of TIM, decline in absolute white blood cell count to 2.5 × 10⁹/L or less or decline in absolute neutrophil cell count to 1.0 × 10⁹/L or less, and determination by the treating physician that use of a thiopurine was the likely cause of TIM and the dose was reduced or the drug was withdrawn.

Investigators at each site completed a custom-designed case report form (eAppendix 1 in the Supplement) that captured the following data: patient demographics (age, weight, height, ethnicity, and smoking history), adverse drug reaction data (type of thiopurine, dose, drug start and stop date, full blood cell count normal range reference values), and IBD phenotype. Each patient was diagnosed as having IBD by his or her gastroenterologist using endoscopic data, histological data, radiological data, or a combination of these, and phenotyped according to the Montreal classification of IBD.17 The Montreal system classified the extent of ulcerative colitis as limited to the rectum (E1), distal to the splenic flexure (E2), or proximal to the splenic flexure (E3). For Crohn disease, patients were categorized by age at disease onset (A1: <17 years, A2: 17-40 years, or A3: >40 years), location of disease (L1: ileal, L2: colonic, or L3: ileocolonic), and disease behavior (B1: nonstricturing and nonpenetrating, B2: stricturing, or B3: penetrating).

Consistent with our prior pharmacogenetics studies,15,16 the case report forms of all recruited patients affected by TIM were reviewed independently by at least 4 gastroenterologists and assigned an adjudication category (eAppendices 2-4, eFigure 1, and eMethods in the Supplement).18 Only patients assigned as definitely or probably affected by TIM were included in the discovery and replication analyses.

Thiopurine-exposed controls without TIM (unaffected patients) were identified from the Exeter IBD Genetics cohort recruited at the Royal Devon and Exeter Hospital (additional
Genetic Variants Associated With Thiopurine-Induced Myelosuppression in IBD

Original Investigation Research

February 26, 2019 Volume 321, Number 8 775

© 2019 American Medical Association. All rights reserved.

details appear in the eMethods in the Supplement). In the final analyses, only patients with an absolute white blood cell count of $3.0 \times 10^9/L$ or greater and an absolute neutrophil cell count of $1.5 \times 10^9/L$ or greater for the duration of their treatment with a thiopurine were included.

The replication cohort met the identical inclusion criteria and included nonoverlapping patients from the same central study site (Royal Devon and Exeter Hospital) and patients from 4 new sites (Saint-Antoine Hospital in France, University Medical Center Groningen in the Netherlands, Cedars-Sinai Medical Center in the United States, and Massachusetts General Hospital in the United States). Patients at these new sites were identified from searches of preexisting genetics cohorts in April 2017. These sites had started recruitment in 2005 (Massachusetts General Hospital and Cedars-Sinai Medical Center), 2011 (University Medical Center Groningen), and 2013 (Saint-Antoine Hospital).

Genetic Analysis
Details of genetic data generation and quality control prior to the GWAS and EWAS analyses appear in the eMethods in the Supplement. For the GWAS analysis, 245,185 variants were genotyped using the Illumina Infinium G4L GWAS array. Patients were excluded if they had variants with a call rate of less than 98%, had variants with a minor allele frequency of less than 1%, or had variants with a Hardy Weinberg equilibrium of $P < 1 \times 10^{-6}$ among unaffected patients. The principal component analysis was carried out using Genome-wide Complex Trait Analysis version 1.249 to assess data from the 1000 Genomes Project.20 Only data from patients clustering with non-Finnish European patients were included. This process minimized the potential confounding effects of population stratification, which might have resulted in an association with variants and TIM even though the association was with a specific ethnicity, which was by chance overrepresented or underrepresented among affected patients compared with unaffected patients.

We excluded patients of Finnish ancestry because their unique genetic background, which has occurred as a consequence of geographical and cultural isolation, has led to the enrichment of some disease-causing gene variants and losses of others. Other quality control measures included a sex-mismatch check (a method that used X chromosome homozygosity rates to determine sex and identify patients for whom the sex recorded in the case report form or phenotype database did not match the predicted sex based on genetic data) and relatedness checking (in which sample and pedigree integrity were both simultaneously examined by reconciling genomic data with self-reported relationships between patients).

After prephasing with the Eagle2 algorithm,21 imputation with the positional Burrows-Wheeler transform22 method was performed using phase 3 of the 1000 Genomes Project20 reference panel and the Wellcome Trust Sanger Imputation Service. Only single-nucleotide polymorphisms with a postimputation information score of less than 0.85 or a minor allele frequency of less than 0.01 were included. After all the quality control measures were implemented, 6,272,335 variants remained.

For the EWAS analysis, exonic regions were sequenced using the Illumina HiSeq platform (150 base-paired reads) and reads mapped to the Genome Reference Consortium human build 37 using the Burrows-Wheeler Alignment MEM algorithm.23 Each sample was sequenced to an average depth of 34 times with approximately 99% of the targeted regions covered by 1 time or greater, approximately 92% covered by 10 times or greater, and approximately 70% covered by 25 times or greater. Variants with a Hardy Weinberg equilibrium of $P < 1 \times 10^{-6}$ were excluded along with any variants that had a genotyping success rate of less than 0.98, a read depth of less than 10 times, or a genotype skew of $P < 5 \times 10^{-9}$ (binomial test). For quality control after the EWAS analysis, a quantile-quantile plot was used (eFigure 2 in the Supplement).

Statistical Analysis
Phenotype Comparisons
Continuous data were summarized using medians and interquartile ranges (IQRs) and were compared using the Mann-Whitney test. The estimate of the median difference between patients affected by TIM and unaffected patients and 95% CIs also were calculated using R version 3.5.1 (R Foundation for Statistical Computing). Categorical data were summarized as the number and percentage and compared using the Fisher exact test.

Primary Analyses
Associations for both the GWAS and EWAS analyses were determined using the Fisher exact test and PLINK version 1.9 (Cog Genomics). Manhattan plots were generated using R software to display negative log$_{10}$ $P$ values at each single-nucleotide polymorphism. A genome-wide significance threshold of $P < 5 \times 10^{-8}$ was deemed significant. Gene burden tests using PLINK sequence 0.10 and sequence kernel association tests24 were used to evaluate if an association existed between sets of rare variants across individual candidate genes and patients affected by TIM. Technical validation of the variants was carried out using Sanger sequencing (eMethods in the Supplement). For the replication cohort, case adjudication, genotype data generation, genetic quality control, and other analyses were undertaken using the same platforms and methods as the discovery cohort. Replicated variants with a Fisher exact $P < .05$ were considered significant.

Exploratory Analyses
After finding an association with a variant and TIM, the final data set was examined for any other nonmonomorphic variants within NUDT15 annotated as missense or as loss of function in the Genome Aggregation Database (gnomAD).25 Further missense variants were evaluated using in silico Protein Variation Effect Analyzer.26 Because the functional significance of this modeling is uncertain, only replicated NUDT15 variants and those previously described in other TIM cohorts8,9 were used in subsequent genotype-phenotype analyses, multivariable logistic regression analyses, and clinical usefulness analyses.

Combinations of $TPMT$ variants on the same chromosome have been reported as haplotypes; these were reconstructed using the Eagle2 algorithm21 and matched to the Clinical Pharmacogenetics Implementation Consortium definitions.27 Categorical $TPMT$ enzyme activity (ie, absent,
low, normal, or high) was measured in red blood cells using radiometric high-performance liquid chromatography as part of routine clinical practice. The relationship between TPMT haplotypes and enzyme activity was determined.

Genotype-phenotype interactions were explored using the Mann-Whitney test and the Fisher exact test. All statistical tests were 2-sided and \( P < .05 \) was considered significant. No adjustment of the \( P \) value was made for multiple comparisons of phenotype data; therefore, the results of these analyses should be considered exploratory. Weight-adjusted dose (milligrams per kilograms) was calculated using the following formulas for mercaptopurine (mercaptopurine dose in milligrams \( \times 2.08/\text{weight in kilograms} \)) and azathioprine (azathioprine dose in milligrams/weight in kilograms).

A multivariable logistic regression analysis was undertaken to assess the independent associations of NUDT15, TPMT, and weight-adjusted thiopurine dose with risk of TIM. Time to TIM (stratified by genotype) was analyzed using the Mann-Whitney test.

The potential for clinical usefulness (sensitivity, specificity, negative and positive predictive values) of genotyping for variants associated with TIM was estimated using methods adapted from Tonk et al\(^29\) and de Graaff et al\(^29\) (eMethods in the Supplement). The estimates assumed an overall risk of TIM of 7\%, either avoidance of the drug (reducing risk of TIM to 0) or target dose reduction in those patients carrying deleterious variants (reducing risk of TIM to that seen in patients with the reference haplotype or genotype), the non-Finnish European population variant carrier frequency from gnomAD,\(^25\) and the odds ratio (OR) of TIM for the variant in multivariable logistic regression analysis. The 95\% CIs for the number of patients needed to genotype were estimated using 10,000 bootstraps from the case-control cohort, from randomly generated estimates of the population with carriage of NUDT15 and TPMT variants, and from TIM rates that were based on sampling from binomial distributions. For TPMT, detailed information appears in eMethods in the Supplement.

The prevalence of NUDT15 variants in patients of other ancestry was explored using all affected patients and population data from gnomAD.\(^25\)

Results

Study Overview

Participant flow through the study appears in Figure 1. In this case-control study, 491 patients with IBD and TIM (affected patients) were recruited from 82 UK and 7 international sites between March 2012 and November 2015. One UK center recruited 843 thiopurine-exposed patients with IBD and no history of TIM (unaffected patients). Following the adjudication process, 1077 patients (398 affected and 679 unaffected patients) entered the final analysis.

After assessment using the genetic quality control measures, 70 affected patients were excluded (68 for ethnicity, 1 for relatedness, and 1 for sex mismatch) and 46 unaffected patients were excluded (31 for ethnicity, 13 for relatedness, and 2 for sex mismatch). In addition, for the GWAS analysis, 17 affected patients were excluded (10 due to failure of quality control genotyping and 7 to failure of genotyping) and 25 unaffected patients were excluded (23 due to failure of quality control genotyping and 2 to failure of genotyping). Thus, 919 patients (311 affected and 608 unaffected patients) were included in the GWAS and 961 patients (328 affected and 633 unaffected patients) were included in the EWAS analysis. Replication was conducted in 73 affected and 840 unaffected patients recruited from 5 international sites.
Phenotype Comparisons

There were no differences in sex when comparing affected and unaffected patients (female 53.0% [211/398] vs 48.5% [329/679], respectively, \( P = .17 \); Table 1). There were no differences when comparing affected and unaffected patients by type of IBD diagnosis: Crohn disease (57.8% [230/398] vs 54.8% [158/297], respectively), ulcerative colitis (39.7% [158/398] vs 44.0% [299/679], respectively), and IBD-unclassified (10.2% [230/398] vs 8.1% [89/1079], respectively, \( P = .12 \)).

There were no differences in behavior of IBD when comparing affected and unaffected patients using the Montreal classification of IBD: B1 (nonstricturing and nonpenetrating, 57.7% [230/398] vs 58.9% [175/297], respectively), B2 (stricturing, 29.1% [62/213] vs 27.6% [82/297]), and B3 (penetrating, 13.1% [28/213] vs 13.5% [40/297], \( P = .94 \)). There were no differences in the extent of ulcerative colitis and IBD-unclassified when comparing affected and unaffected patients using the Montreal Classification system: E1 (limited to the rectum, 9.4% [15/160] vs 6.0% [14/234], respectively), E2 (distal to the splenic flexure, 45.6% [73/160] vs 47.9% [112/234]), and E3 (proximal to the splenic flexure, 45.0% [72/160] vs 46.2% [108/234], \( P = .46 \)).

In contrast, affected patients were younger at the time of IBD diagnosis (median, 30.1 years [IQR, 19.3-43.1 years]) compared with unaffected patients (median, 31.6 years [IQR, 22.2-44.7 years], \( P = .02 \)) and received a higher weight-adjusted thiopurine dose (median, 2.07 mg/kg [IQR, 1.69-2.45 mg/kg] vs 1.84 mg/kg [IQR, 1.48-2.19 mg/kg], respectively, \( P < .001 \)). In addition, affected patients with Crohn disease were more likely to have colonic or ileo-colonic disease than unaffected patients (L1 [ileal]: 24.9% [57/229] vs 44.0% [132/300], respectively; L2 [colonic]: 32.3% [74/229] vs 26.3% [79/300], respectively; and L3 [ileo-colonic]: 42.8% [98/229] vs 29.7% [89/300], \( P < .001 \)).

Among the 398 affected patients, 143 (36%) episodes of TIM occurred within 8 weeks of therapy with the maximum dose of thiopurine (eTable 1 in the Supplement). The median time from commencement of thiopurine to TIM was 28.3 weeks (IQR, 9.0-81.1 weeks) and the median time from maximum dose of thiopurine to TIM was 14.7 weeks (IQR, 5.9-37.9 weeks). Phenotype data for the replication cohort appear in eTable 2 in the Supplement.

Primary Analyses

GWAS Analysis

Data from 311 affected and 608 unaffected patients (eTable 3 in the Supplement) were included in the GWAS discovery cohort. The association of TIM with TPMT rs11969064 was confirmed in 30.5% (95/311) of affected patients compared with 16.4% (100/608) of unaffected patients (OR, 2.3 [95% CI, 1.7-2.9]).
778 JAMA February 26, 2019 Volume 321, Number 8

Research Original Investigation

A genetic variant within NUDT15 was confirmed in the replication cohort analysis among patients affected with late-onset TIM. The patients affected with early-onset TIM were significantly enriched with the variant (OR, 3.3 [95% CI, 1.6 to 6.9], P = 1.5 × 10−12). The association with these NUDT15 variants was enriched in patients affected with early-onset vs late-onset TIM (OR, 3.3 [95% CI, 1.6 to 6.9], P < .001).

Of the included patients in the EWAS analysis (discovery cohort), 75% (717/961) had TPMT activity levels available for analysis. All 10 patients with absent TPMT activity and 73% of patients (80/109) with low TPMT activity carried variant TPMT haplotypes (eFigure 6 and eTables 7-9 in the Supplement).

Figure 2. Manhattan Plot for the Discovery Exome-Wide Association Studies Analysis Among 328 Individuals Affected by Thiopurine-Induced Myelosuppression and 633 Thiopurine-Tolerant Unaffected Individuals

Each colored dot represents a single variant within each respective chromosome. The negative log10 P value represents a Fisher exact test analysis between affected and unaffected patients. The orange dotted horizontal line indicates genome-wide significance at Fisher exact P = 5.0 × 10−8. Gene names correspond to the gene in closest proximity to the variant with the lowest P value at each locus if within 50 kilobase pairs.

EWAS Analysis

Data from 328 affected and 633 unaffected patients were included in the EWAS discovery cohort (eTable 4 in the Supplement). The EWAS analysis, which was performed to investigate the role of rare coding variants, revealed a TIM association with a 6–base pair in-frame deletion at position 48611918 of chromosome 13 in exon 1 of NUDT15 (rs746071566, p.Gly17_Val18dup [also annotated as p.Val18_Val19insGlyVal]) was noted; however, the duplication did not meet genome-wide significance (1.5% [95% CI, 0.8 to 3.1], P = 5.2 × 10−9; eFigure 3 in the Supplement). This association was enriched in patients affected early (≤8 weeks of starting maximum thiopurine dose; OR, 4.0 [95% CI, 2.8 to 5.8], P = 1.8 × 10−15) compared with those affected later (OR, 1.6 [95% CI, 1.1 to 2.2], P = .01; eFigure 4 in the Supplement). No other genetic associations with TIM exceeded the a priori threshold for statistical significance.

To 3.1], P = 5.2 × 10−9; eFigure 3 in the Supplement). This association was enriched in patients affected early (≤8 weeks of starting maximum thiopurine dose; OR, 4.0 [95% CI, 2.8 to 5.8], P = 1.8 × 10−15) compared with those affected later (OR, 1.6 [95% CI, 1.1 to 2.2], P = .01; eFigure 4 in the Supplement). No other genetic associations with TIM exceeded the a priori threshold for statistical significance.

Exploratory Analyses

Sequence data for NUDT15 were examined for the presence of all coding variants either previously associated with TIM8–10 or identified in gnomAD25 and predicted as deleterious using the Protein Variation Effect Analyzer26 (Table 2 and eFigure 5 in the Supplement). However, 4 (p.Lys33Glu, p.Val75Gly, p.Cys28GlyfsTer28, and p.Met1?) of the 7 NUDT15 variants were each only found in a single individual. Therefore, only variants either meeting genome-wide association in this analysis (p.Gly17_Val18del) or previously associated with TIM in other analyses (p.Arg139Cys and p.Gly17_Val18dup) were included for subsequent exploratory analyses.

Overall, 9.5% (31/328) of the non-Finnish European TIM discovery cohort carry any of the 3 NUDT15 coding variants compared with 0.5% (3/633) of unaffected patients (OR, 20.9 [95% CI, 6.4 to 68.6], P = 1.5 × 10−12). The association with these NUDT15 variants was enriched in patients affected with early-onset vs late-onset TIM (OR, 3.3 [95% CI, 1.6 to 6.9], P < .001).

© 2019 American Medical Association. All rights reserved.
Table 2. Association of Genetic Variants in NUDT15 With Thiopurine-Induced Myelosuppression in Patients With Inflammatory Bowel Disease Using Data From the Exome-Wide Association Studies Analysis

<table>
<thead>
<tr>
<th>Individuals Affected by Thiopurine-Induced Myelosuppression (n = 328)</th>
<th>Minor Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Heterozygotes</td>
<td>Variant Heterozygotes</td>
</tr>
<tr>
<td>NUDT15 Reference Allele</td>
<td>No. of Minor Alleles</td>
</tr>
<tr>
<td>rs746071566</td>
<td>p.Gly17_Val18del</td>
</tr>
<tr>
<td>rs116855232</td>
<td>p.Arg139Cys</td>
</tr>
<tr>
<td>rs777311140</td>
<td>p.Cys28GlyfsTer28</td>
</tr>
<tr>
<td>rs686715121</td>
<td>p.Val18_Val19insGlyVal</td>
</tr>
<tr>
<td>rs1377711140</td>
<td>p.Met18Cys</td>
</tr>
<tr>
<td>rs86111833</td>
<td>p.Met18Ile</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; SNP, single-nucleotide polymorphism.

*Calculated as (No. of variant alleles in population)/(2 × No. of participants). No affected or unaffected patients were homozygous for p.Gly17_Val18del, 5 affected patients were heterozygous for p.Gly17_Val18dup, and 304 affected patients were considered reference homozygous (328 patients in total).

**Previously annotated as p.Val18_Val19insGlyVal.

b Calculated using the Fisher exact test. A genome-wide threshold of <5 × 10^{-8} was considered significant.

c This site is multiallelic and both of these variants occur at the same chromosome position (48611918). Nineteen patients without risk variants (7.7 weeks [IQR, 5.7-20.0 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively; P = .009) and in those who carried double TPMT variants (6.1 weeks [IQR, 4.2-7.6 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively; P = .002).

d Among all affected patients in the EWAS analysis, the median time to TIM was 15 weeks (IQR, 6-41 weeks) and 34% (111/328) experienced early-onset TIM. Of note, 18% (59/328) presented with an opportunistic infection, 23% (77/328) were admitted to a hospital with a median length of stay of 6 days (IQR, 2-9 days), and 9% (31/328) required granulocyte colony-stimulating factor rescue therapy.
was seen in patients carrying 1 variant TPMT haplotype compared with affected patients without risk variants (13.9 weeks [IQR, 5.9-40.4 weeks] vs 20.0 weeks [IQR, 7.6-48.3 weeks], respectively, \( P = .14 \)).

Patients carrying either NUDT15 or TPMT and those with variants for both genes developed lower neutrophil counts than affected patients without these variants (median, 0.8 × 10⁹/L [IQR, 0.4-1.1 × 10⁹/L] vs median, 1.0 × 10⁹/L [IQR, 0.7-1.2 × 10⁹/L], respectively, \( P < .001 \)), were more likely to be admitted to the hospital (40% [39/97] vs 17% [38/231], \( P < .001 \)), and were more likely to receive granulocyte colony-stimulating factor rescue therapy (20% [19/97] vs 5.2% [12/231], \( P < .001 \)); eTables 10-11 in the Supplement).

The success of another challenge with thiopurine according to genotype also was explored. Among the 51% (167/328) of affected patients rechallenged, 57% (95/167) were able to tolerate a lower dose (median successful rechallenge dose, 1.2 mg/kg [IQR, 0.9-1.5 mg/kg]). Neither weight-adjusted dose, type of thiopurine, patient age, TPMT genotype, nor NUDT15 genotype were associated with subsequent tolerance after rechallenge (eTable 12 in the Supplement).

Multivariable Logistic Regression Analysis

In a multivariable logistic regression model analysis, the odds of TIM among patients with any of 3 coding variants in NUDT15 (OR, 27.3 [95% CI, 9.3 to 116.7]; \( P = 1.1 \times 10^{-7} \)) and in TPMT (in heterozygotes: OR, 2.2 [95% CI, 1.4 to 3.3]; \( P = 3.5 \times 10^{-3} \); in homozygotes: OR, 53.4 [95% CI, 10.4 to 980.1]; \( P = 1.5 \times 10^{-4} \)) were independent of thiopurine-weight adjusted dose (OR, 2.2 [95% CI, 1.8 to 2.8]; \( P = 5.3 \times 10^{-11} \); Table 3).

Estimated Potential Clinical Effectiveness

For NUDT15, the estimated number of patients needed to genotype to prevent 1 patient from developing TIM was 95 patients (95% CI, 62-143 patients). For every 10 000 patients genotyped, 164 would test positive for a NUDT15 variant, and of these patients, 105 would have developed TIM if they had not received an alternative treatment (positive predictive value, 64% [95% CI, 43%-100%]; eMethods in the Supplement). Genotyping 10 000 patients for NUDT15 would prevent 105 cases of TIM, which is 95 patients genotyped for every case prevented. The number needed to genotype assumed a cumulative incidence for TIM of 7% [95% CI, 6%-8%] based on a meta-analysis of 8302 patients, a drug avoidance strategy in NUDT15 variant carriers, a population carriage frequency of 1.6% [95% CI, 1.5%-1.8%], and ORs derived from bootstrapping the affected and unaffected population (sampling with replacement to estimate the variability of the OR). If a dose reduction strategy was used in NUDT15 variant carriers instead, thus reducing risk of TIM to that of patients with the reference genotype (absolute risk, 6% [95% CI, 5%-7%]), the number needed to genotype would be 105 patients (95% CI, 65-168 patients).

For TPMT, the estimated number needed to genotype was 123 patients (95% CI, 75-235 patients). For every 10 000 patients genotyped, 996 would test positive for a TPMT variant and need to receive an alternative therapy to prevent TIM in 81 patients (95% CI, 43-133 patients). Genotyping 10 000 patients for TPMT would prevent 81 cases of TIM, which is 123 genotyped for every case prevented. This assumed the following for patients carrying 2 TPMT variant haplotypes: drug avoidance, a population carrier frequency of 0.26% (95% CI, 0.19%-0.34%), and an OR of 53.4 [95% CI, 10.4-980.1]. For patients carrying 1 TPMT haplotype, this assumed the following: a thiopurine dose reduction, a population carrier frequency of 9.7% [95% CI, 8.4%-11.0%], and an OR of 2.2 [95% CI, 1.4-3.3].

In the wider cohort of 398 affected patients who were adjudicated and when including patients of non-European ancestry (who had been excluded from the GWAS and EWAS analyses), carriage of NUDT15 variants was more frequent than in patients of non-Finnish European ancestry (100% [44/44] for South Asian patients vs 9% [31/328] for non-Finnish European patients, \( P = 1.1 \times 10^{-4} \); and 56% [23/41] for East Asian patients vs 9% [31/328] non-Finnish European patients, \( P = 2.0 \times 10^{-11} \); eTable 13 in the Supplement).

Estimates of the rate of carrying 1 or more NUDT15 risk alleles in the general population using the gnomAD reference database ranged from 0.7% in patients of African ancestry to 29.2% in patients of East Asian ancestry (eTable 14 in the Supplement).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-adjusted thiopurine dose</td>
<td>2.2 (1.8 to 2.8)</td>
<td>( 5.3 \times 10^{-11} )</td>
</tr>
<tr>
<td>NUDT15 genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference genotype/reference genotype</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Variant genotype/variant genotype</td>
<td>27.3 (9.3 to 116.7)</td>
<td>( 1.1 \times 10^{-7} )</td>
</tr>
<tr>
<td>TPMT haplotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference haplotype/reference haplotype</td>
<td>1 [Reference]</td>
<td></td>
</tr>
<tr>
<td>Reference haplotype/variant haplotype</td>
<td>2.2 (1.4 to 3.3)</td>
<td>( 3.5 \times 10^{-3} )</td>
</tr>
<tr>
<td>Variant haplotype/variant haplotype</td>
<td>53.4 (10.4 to 980.1)</td>
<td>( 1.5 \times 10^{-4} )</td>
</tr>
</tbody>
</table>

a There were 42 observations missing.
b From logistic regression with all 3 variables included.
c For every 1 mg/kg increase in azathioprine equivalent dose. Represents the maximum azathioprine equivalent dose prior to thiopurine-induced myelosuppression adjusted for weight (milligrams per kilograms).

d Carriage of 1 or more of 3 NUDT15 variants: rs746071566 [p.Gly17_Val18del], rs746071566 [p.Gly17_Val18dup], and rs116855232 [p.Arg193Cys]. One patient with TIM possessed 2 NUDT15 variants (rs746071566 [p.Gly17_Val18dup] and rs116855232 [p.Arg193Cys]); however, it was not possible to ascertain if this represented a compound heterozygote or 2 variants on the same strand (*2 NUDT15 haplotype). For the purpose of the analysis, this case was considered as a single NUDT15 variant carrier (*NUDT15variant*).
Discussion

In this case-control study involving both GWAS and EWAS analyses, an association between an NUDT15 variant (p.Gly17_Val18del) and TIM has been identified and replicated in independent cohorts of patients of non-Finnish European ancestry. In total, 3 NUDT15 coding variants, including p.Gly17_Val18del, were identified and collectively associated with TIM independent of TPMT genotype and thiopurine dose. Patients with variants of either NUDT15 or TPMT, or among those with variants of both genes, had a faster onset of TIM, more severe TIM, and had a greater need for granulocyte colony-stimulating factor therapy.

To our knowledge, this is the first study to describe the association of an NUDT15 variant with TIM in patients of European ancestry at genome-wide significance. This extends previous work by Moriyama et al10 that first described this p.Gly17_Val18del variant in 2 pediatric patients with acute lymphoblastic leukemia and TIM; one of whom was of European, and the second, of African ancestry.

The p.Arg139Cys variant has previously been associated with TIM in a North American IBD cohort study for which the minor allele frequency reported was 2.7% in affected patients and 0.3% in unaffected patients (OR, 9.50; 95% CI, 1.05-86.5; P = 4.6 × 10^-4).9 In contrast, to our knowledge, the p.Gly17_Val18dup variant had only been reported in cohorts of East Asian ancestry.8

NUDT15 is hypothesized to hydrolyze nucleoside triphosphate active metabolites (6-thio-dGTP, 6-thio-GTP, and dGTP) thus preventing their incorporation into DNA where they would otherwise lead to futile mismatch repair and apoptosis.8,9,31 Functional experiments confirm that NUDT15 variants result in lower enzymatic activity, leading to higher levels of thiopurine active metabolites and a greater risk of TIM.8,10,31 The p.Gly17_Val18dup variant reduces NUDT15 activity to approximately 15% of normal activity, whereas p.Gly17_Val18del and p.Arg139Cys are nearly void of enzyme activity, suggesting that patients with these variants may be particularly sensitive to thiopurines.8,10

Given the widespread use of the thiopurines, these findings may have ramifications beyond the management of IBD in patients of European ancestry. Although NUDT15 variants were first associated with TIM in East Asian patients with IBD,9 this phenomenon has now been demonstrated in oncology and other immune-mediated diseases32,33 as well as in other populations.9,11-14 For population stratification reasons, patients of non-European ancestry were excluded from the genetic analyses of this study. However, it is interesting to note in the absence of TPMT variants, the frequency of variant NUDT15 haplotypes is 29.2% in populations of East Asian ancestry compared with 20.7% in Latin American populations, 13.4% in South Asian populations, and 1.6% in non-Finnish European populations.25

As expected, in the wider cohort of affected patients who were adjudicated, patients of non-European descent demonstrated a higher carriage frequency of NUDT15 variants and a lower carriage frequency of TPMT variants. If replicated in additional studies, these findings suggest that NUDT15 testing may be considered prior to thiopurine therapy irrespective of the ethnic background of the patient.

The positive predictive value of NUDT15 genotyping estimated in this study together with the recent development of alternative but more expensive therapies suggests potential clinical utility of pretreatment testing and drug avoidance in genetically at-risk patients. Recommendations regarding pretreatment NUDT15 genotyping based on data from East Asian populations are under review by the Clinical Pharmacogenetics Implementation Consortium.27 Our data suggest that pretreatment sequencing of the NUDT15 gene, including the p.Gly17_Val18del deletion, may also be considered in patients of European ancestry. However, this will not obviate the requirement for regular monitoring with blood tests for the duration of treatment among patients deemed at low risk of TIM.

The estimated number of patients needed to genotype for NUDT15 is 95, similar to the number needed to genotype reported herein and by others14 for TPMT (123 and 100, respectively). However, further validation studies including a cost-effectiveness analysis should be conducted prior to implementation of pretreatment NUDT15 genotyping.

Limitations

This study has several limitations. First, inclusion was restricted to patients with IBD of non-Finnish European ancestry. Further research is required to evaluate the association of these variants with TIM in other ancestries and disease groups. Second, the replication cohort was not exclusively recruited from independent sites. The central site recruited affected and unaffected patients to the discovery cohort and then additional patients to the replication cohorts.

Third, in keeping with all case-control studies, the data are likely to be susceptible to recall bias and there was greater recruitment of more severely affected patients. We estimate that our affected patients represent 5% of the total eligible patients with IBD and an episode of TIM. This is based on a UK IBD prevalence of 388 patients per 100 000 population,35 a thiopurine exposure rate of 31%, using the recommendations of the European Society for Medical Oncology36 and a rate of TIM of 7%.1 This recall bias might explain the IBD phenotype differences observed between cases and controls and an overestimate of the risk associated with NUDT15 variants and TIM.

Fourth, 4.9% (16/328) of affected and 0.2% (1/633) of unaffected patients had 2 of the known TIM-associated TPMT variant haplotypes despite the recommended practice of pretreatment measurement of TPMT enzyme activity and thiopurine availability in patients deficient of TPMT enzyme activity. These patients arguably should not have received treatment with a thiopurine, regardless of the presence of NUDT15 variants.

Fifth, the proposed mitigation strategy of thiopurine avoidance rather than dose reduction in patients with NUDT15 coding variants may be overly cautious. Previous studies in patients of East Asian ancestry have shown that even patients with 2 low-functioning NUDT15 alleles may successfully tolerate a dose reduction of thiopurine by 90%,8,31,33 Likewise, in NUDT15 knockout mice models, accumulation of thiopurine metabolites was noted to be in a mercaptopurine dose-related fashion, suggesting that dose reduction might be an effective strategy.34 However, as discussed above, not all variants
effect NUDT15 enzymatic function to the same extent and the magnitude of the deleterious effect of individual variants may differ across ethnic groups.37 Furthermore, it is unknown whether such a marked dose reduction would compromise the therapeutic effect of thiopurines in patients with IBD. In our study of patients of non-Finnish European ancestry, almost 50% of patients with a single variant did not tolerate a rechallenge with thiopurine at a lower dose. These arguments may justify the use of alternative, more expensive therapies in this small group of patients at high risk of TIM. However, further data are needed to explore whether thiopurine dose reduction with enhanced monitoring or drug avoidance is the safer, less expensive, and more clinically effective strategy.

Conclusions
Among patients of European ancestry with IBD, variants in NUDT15 were associated with increased risk of TIM. These findings suggest that NUDT15 genotyping may be considered prior to initiation of thiopurine therapy; however, further study including additional validation in independent cohorts is required.

ARTICLE INFORMATION
Accepted for Publication: January 23, 2019.
Correction: This article was corrected on April 23, 2019, to fix 3 numerical errors in 1 sentence in the Results section and to add a sentence after the one with the corrected numbers.

Author Affiliations: Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, England (Walker, Heap, Heerasing, Hendy, Singh, So, Goodhand, Kennedy, Ahmad); IBD Pharmacogenetics Group, University of Exeter, Exeter (Walker, Heap, Bewesha, Heerasing, Hendy, Goodhand, Kennedy, Ahmad); University of Exeter Medical School, Exeter, England (Harrison, Jones, Ward, Weedor); Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, the Netherlands (Voskull, Festen, Weersma); Medical Department, Regional Hospital Viborg, Viborg, Denmark (Andersen); Wellcome Trust Sanger Institute, Hinxton, England (Anderson, Barrett); Department of Gastroenterology, Massachusetts General Hospital, Boston (Ananthakrishnan); Department of Gastroenterology, Saint-Antoine Hospital and Sorbonne Universite, Paris, France (Beaugerie, Seksik, Sokol); Derby Digestive Diseases Centre, Royal Derby Hospital, Derby Teaching Hospitals NHS Foundation Trust, Derby, England (Cole); Department of Gastroenterology, Southampton General Hospital, University Hospital Southampton NHS Foundation Trust, Southampton, England (Cummings); Broad Institute, Harvard University, Cambridge, Massachusetts (Daly, Koskela, Risling, Xavier); Department of Gastroenterology, Mater Dei Hospital, Msida, Malta (Ellul); Division of Gastroenterology, University of Alberta, Edmonton, Canada (Fedorko); Mater Research Institute, University of Queensland, South Brisbane, Australia (Florn); Department of Gastroenterology, Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde, Glasgow, Scotland (Gaya); Division of Gastroenterology, Örebro University, Örebro, Sweden (Halfvarson); Department of Gastroenterology, St Mark’s Hospital, London North West Healthcare NHS Trust, Harrow, England (Hart); Department of Gastroenterology, Guy’s and St Thomas’ NHS Foundation Trust, London, England (Irving); Centre for Immunobiology, Blizard Institute, Barts and the London School of Medicine, Queen Mary University of London, London, England (Lindsay); Department of Gastroenterology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, England (Mansfield); F. Widjaja Foundation Inflammatory Bowel Disease and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, California (McGovern); Department of Gastroenterology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, England (Parkes); Department of Gastroenterology, St George’s Healthcare NHS Trust, Tooting, England (Pollok); Gastrointestinal and Liver Services, Warrington and Halton Hospitals NHS Foundation Trust, Warrington, England (Ramakrishnan); Department of Gastroenterology, Royal London Hospital, Barts Health NHS Trust, London, England (Rampton); Department of Paediatric Gastroenterology, Royal Hospital for Children, NHS Greater Glasgow and Clyde, Glasgow, Scotland (Russell); Dunedin Hospital, Dunedin, New Zealand (Schultz); Gastroenterology and Hepatology, Hull and East Yorkshire Hospitals NHS Trust, Hull, England (Sebastian); Canberra Hospital, Canberra, Australia (Subramaniam); Department of Haematology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, England (Todd); Division of Gastroenterology, Azienda Ospedaliero Universitaria Careggi, Florence, Italy (Annesse).


Conflict of Interest Disclosures: Dr Walker reported serving as a consultant for AbbVie UK; receiving honoraria from Falk and AbbVie UK; receiving grants from Crohn’s & Colitis UK and Tillot’s Pharmaceuticals; having a fellowship from the UK National Institute for Health Research; and receiving travel reimbursement from Merck Sharp & Dohme and Norgine. Dr Heap reported receiving travel reimbursement from AbbVie and being a current employee of AbbVie and owning stock in the company. Dr Andersen reported receiving personal fees from Merck Sharp & Dohme and Janssen. Dr Ananthakrishnan reported receiving a grant from Pfizer; and receiving personal fees from Takeda. Dr Beaugerie reported receiving advisory board fees from Allergan, Janssen, and Pfizer; receiving a grant from Hospira; and receiving grants and honoraria from AbbVie, Merck Sharp & Dohme, Ferring, Takeda, and Tillot’s Pharmaceuticals. Dr Cummings reported receiving personal fees from AbbVie, Takeda, Biogen, Janssen, Merck Sharp & Dohme, Amgen, Hakim Pharmaceuticals, and Pfizer/Hospira; and receiving grants from Takeda, Biogen, AstraZeneca, and Pfizer/Hospira. Dr Halfvarson reported receiving personal fees from AbbVie, Hospira, Janssen, Medivir, Merck Sharp & Dohme, Pfizer, Renapharma/AbbV, Takeda, Tillot’s Pharmaceuticals, Celtgene, Sandzoz, and Shire; and receiving grants from Janssen, Merck Sharp & Dohme, and Takeda. Dr Hart reported serving as a consultant for AbbVie; receiving personal fees from AbbVie, Takeda, Ferring, Pfizer, Lilly, Merck Sharp & Dohme, Samsung, and Sandoz; and receiving travel grants from Takeda and Merck Sharp & Dohme. Dr Lindsay reported receiving advisory board fees from Atlantic Health, AbbVie UK/global, Merck Sharp & Dohme, Shire UK, Vifor Pharma, Ferring International, Celltrion, Takeda, Napp, Pfizer, and...
University Hospitals NHS Foundation Trust, Basildon, UK; Jane F. McGovern, MBBS (F. Widjaja Foundation), MRC Path (Inflammatory Bowel Disease and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, California); Alistair McNair, PhD (Queen Elizabeth Hospital, London, UK); Anita Modi, MD (Luton and Dunstable University Hospital, Luton, UK); Kevin Monahan, PhD (West Middlesex University Hospital, Middlesex, UK); Alex Moran, MD (North Devon Healthcare Trust, Barnstaple, UK); Mary-Anne Morris, MD (Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK); Marianne Mortimore, MBBS (Mater Research Institute, University of Queensland, South Brisbane, Australia); Craig Federal, MD (Ninewells Hospital, NHS Tayside, Dundee, UK); Raheeq Muhammed, MD (Birmingham Children’s Hospital, Birmingham, UK); Charles D. R. Murray, PhD (Royal Free Hospital, Royal Free London NHS Foundation Trust, London, UK); Hanlie Olivier (IBD Pharmacogenetics Group, University of Cape Town, South Africa); Tim Francis, MD (Imperial College Healthcare NHS Trust, London, UK); Simon Panter, MD (Ashton-under-Lyne, UK); Rosemary Phillips, MD (Integrated Care NHS Foundation Trust, Tyneside District Hospital, South Tyneside, UK); Vinod Patel, MBBS (Tameside and Glossop Integrated Care NHS Foundation Trust, Ashton-under-Lyne, UK); Rebecca Saich, PhD (Basingstoke and North Hampshire Hospital, Basingstoke, UK); Jack Satsangi, PhD (Western General Hospital, NHS Lothian, Edinburgh, UK); Stefan Schreiber, PhD (Kiel University, Kiel, Germany); Sandip Sen, MD (Royal St George’s Hospital, Stoke-on-Trent, UK); Neil Shah, MD (Great Ormond Street Hospital, London, UK); Richard Shenderay, MBBS (Airedale NHS Foundation Trust, Keighley, UK); Achant Sneyd, MD (Colchester University Hospital NHS Foundation Trust, Colchester, UK); James Shutt, DM (Dorset County Hospital NHS Foundation Trust, Dorchester, UK); Mark Silverberg, PhD (Mount Sinai Hospital, Toronto, Ontario, Canada); Alison Simmons, PhD (Oxford University Hospitals, Oxford, UK); Jonathan Simmons, DM (Royal Berkshire Hospital, Royal Berkshire NHS Foundation Trust, Reading, UK); Sall Singh, PhD (Bolton NHS Foundation Trust, Bolton, UK); Malcolm Smith, MBChB (Aberdeen Royal Infirmary, Aberdeen, UK); Mark Smith, MD (Southend University Hospital NHS Trust, Southend, UK); Susan Solowardi, MD (Calderdale Royal Hospital, Halifax, UK); Christine R. Stevens, PhD (Broad Institute, Harvard University, Cambridge, Massachusetts); Giacomo Sturmiolo, PhD (Università di Padova, Padova, Italy); Sreesh Narasimhan, MD (Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK); Amanda Thomas, MBBS (Department of Gastroenterology, Royal Devon and Exeter Hospital NHS Foundation Trust, Exeter, UK); Mark Tighe, BM (Poole Hospital NHS Foundation Trust, Poole, UK); Franco Torreto, MD (Department of Gastroenterology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK); Mark Tremelling, MD (Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK); Epameinondas Tsiason, PhD (University Hospital of Ioannina, Ioannina, Greece); Deven Vani, MD (Mid Yorkshire Hospitals NHS Trust, Wakefield, UK); Alissa Walsh, MBBS (St Vincent’s Hospital, Sydney, Australia); Gillian Watermeyer, MBChB (Grote Schuur Hospital, Cape Town, South Africa); David Watts, MBChB (Forth Valley Royal Hospital, Larbert, UK); Gill Watts, MD (Wynheshawe Hospital, South Manchester, UK); Sean Weaver, PhD (Royal Bournemouth General Hospital, Bournemouth, UK); Emma Wesley, MBBS (Musgrove Park Hospital, Taunton and Somerset NHS Hospitals, Taunton, UK); Anne Willmott, MBChB (Leicester Royal Infirmary-Paediatric, Leicester, UK); Karen Yearsley, BM (Nevill Hall Hospital, Abergavenny, UK); Veena Zambra, MBBS (Leeds General Infirmary, Leeds, UK); and Sebastian Zeissig, MD (University Medical Center Schleswig-Hostein, Kiel, Germany). These individuals identified and recruited patient(s) to the study and provided comments on a draft of the manuscript.

Additional Contributions: We thank all the participants of this study. We also thank Matthew R. Nelson, PhD (director of statistical genetics at GlaxoSmithKline in North Carolina), for comments on a draft of the manuscript. We also thank the study group coordinators: Hanlie Olivier, Marian Parkinson, BSc, and Helen Gardner-Thorpe, BA (all 3 with the IBD Pharmacogenetics Group, University of Exeter, Exeter, England), for their administrative support. Only the study coordinators were remunerated for their roles.

REFERENCES


© 2019 American Medical Association. All rights reserved.


