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Genetic Susceptibility to Postdiarrheal Hemolytic-Uremic Syndrome After Shiga Toxin–Producing *Escherichia coli* Infection: A Centers for Disease Control and Prevention FoodNet Study

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Abstract

Background.—Postdiarrheal hemolytic-uremic syndrome (D+HUS) following Shiga toxin–producing *Escherichia coli* (STEC) infection is a serious condition lacking specific treatment. Host immune dysregulation and genetic susceptibility to complement hyperactivation are implicated in non–STEC-related HUS. However, genetic susceptibility to D+HUS remains largely uncharacterized.

Methods.—Patients with culture-confirmed STEC diarrhea, identified through the Centers for Disease Control and Prevention FoodNet surveillance system (2007–2012), were serotyped and classified by laboratory and/or clinical criteria as having suspected, probable, or confirmed D+HUS or as controls and underwent genotyping at 200 loci linked to nondiarrheal HUS or similar pathologies. Genetic associations with D+HUS were explored by multivariable regression, with adjustment for known risk factors.

Results.—Of 641 enrollees with STEC O157:H7, 80 had suspected D+HUS (41 with probable and 32 with confirmed D+HUS). Twelve genes related to cytokine signaling, complement pathways, platelet function, pathogen recognition, iron transport, and endothelial function were associated with D+HUS in multivariable-adjusted analyses ($P \leq .05$). Of 12 significant single-nucleotide polymorphisms (SNPs), 5 were associated with all levels of D+HUS (intergenic SNP rs10874639, *TFRC* rs3804141, *EDN1* rs5370, *GP1BA* rs121908064, and *B2M* rs16966334), and 7 SNPs (6 non–complement related) were associated with confirmed D+HUS (all $P < .05$).

Conclusions.—Polymorphisms in many non–complement-related genes may contribute to D+HUS susceptibility. These results require replication, but they suggest novel therapeutic targets in patients with D+HUS.

Keywords

Genetic variant; typical hemolytic-uremic syndrome; foodborne illness; Shiga toxin; verocytotoxin; STEC; diarrheal HUS

Shiga toxin–producing *Escherichia coli* (STEC) infection represents an ever-present threat to public health, with an unpredictable and potentially life-threatening disease course [1, 2]. Conservative estimates indicate that STEC causes approximately 231 000 illnesses, 3150 hospitalizations, and 30 deaths annually in the United States [3]. Most reported STEC infections in the United States are caused by *E. coli* O157:H7, but at least 150 other STEC serotypes, some equally virulent, have also been associated with outbreaks and sporadic illness [4, 5]. STEC disease ranges from uncomplicated diarrhea to hemorrhagic colitis and postdiarrheal hemolytic-uremic syndrome (the typical form of disease; hereafter, “D+HUS”), which may cause multiorgan failure as a result of diffuse microvascular thromboses (small-vessel clots) and a vascular leak syndrome [2, 6, 7]. While significantly more common among children and a leading cause of renal failure in this population, D+HUS also occurs in adults, following STEC infection acquired through consumption of contaminated food or water or contact with farm animals, with similarly serious sequelae [8]. The pathogenesis of D+HUS is incompletely understood, in part because it is vastly understudied in comparison to atypical (ie, nondiarrheal or familial) genetic or acquired forms of the disease [9]. Hyperactivation of the complement cascade and immune

dysregulation are strongly implicated in atypical HUS, which differs from D+HUS in its tendency to recur and overall worse prognosis [7, 9]. Treatment for HUS remains largely supportive, but in atypical HUS, a recently developed monoclonal antibody directed against the terminal components of complement can provide significant benefit [10].

Approximately half of individuals with atypical HUS carry mutations in complement-regulatory genes such as complement factor H (CFH), leading to altered or persistent complement activation [7, 11]. Host genetic factors predisposing to D+HUS remain uncharacterized, however, in large part because of the logistical challenges of orchestrating epidemiologic studies in the midst of infectious disease outbreaks. The outbreak of foodborne STEC O104:H4 infections in Germany in 2011, associated with contaminated sprouts, resulted in a high incidence of D+HUS among adults and children, underscoring the need to better understand D+HUS pathogenesis and identify prognostic biomarkers and therapeutic targets [6, 12, 13]. Prompt diagnosis of O157 and other highly virulent STEC infections is critical; parenteral volume expansion early in the course of infection can limit renal damage and improve clinical outcomes [14, 15]. In addition, antibiotic therapy for STEC O157 infections has been associated with more-severe disease; early and accurate diagnosis therefore facilitates appropriate treatment [16, 17]. Identification of new pathophysiological mechanisms of D+HUS and development of multipronged adjunctive therapies targeted to these mechanisms could significantly reduce morbidity and mortality.

The objective of this case-control study was to identify host genetic factors that confer susceptibility to D+HUS following STEC infection and to assess the contribution of complement-regulatory gene mutations that have previously been identified as risk factors for atypical HUS.

PATIENTS AND METHODS

Ascertainment of Study Subjects

The Foodborne Diseases Active Surveillance Network (FoodNet) is a population-based surveillance network of 10 sites, encompassing 15% of the US population [18]. FoodNet conducts active, laboratory-based surveillance for 9 types of enteric bacterial infections, including O157 and non-O157 STEC. Individuals residing within the FoodNet catchment area during 2007–2012 who developed diarrheal illness and were determined to have laboratory-confirmed STEC-related diarrhea or clinician-diagnosed D+HUS were eligible for this study. STEC infection was confirmed by culture, enzyme-linked immunoassay for Shiga toxins, and/or polymerase chain reaction for genes encoding Shiga toxins. Additionally, serological evidence of STEC O157 and O111 infections was accepted for patients with D+HUS and no STEC isolated from their stool. Demographic and clinical information, such as race, renal function, and antimicrobial use, were obtained through a concurrent but separate FoodNet cohort study of the risks of D+HUS and antimicrobial exposure, which closed in 2010; samples for DNA extraction continued to be collected from cases ascertained via routine FoodNet surveillance but with more-limited clinical data. Study participants without clinical covariate data were excluded from the present analysis.

D+HUS was defined as confirmed if all 4 of the following laboratory abnormalities were met: (1) hemoglobin level or hematocrit below age- and sex-specific thresholds, (2) platelet count of $<150 \times 10^3$ platelets/μL, (3) serum creatinine level of ≥1.0 mg/dL if <13 years old or ≥1.5 mg/dL if ≥13 years old, and (4) fragmented erythrocytes on peripheral blood smear [19]. Probable cases met the first 3 criteria but lacked evidence of erythrocyte fragmentation. Physician-diagnosed cases without sufficient data for further classification were defined as suspected D+HUS. Cases were excluded if sufficient DNA could not be extracted, if they opted out of genetic testing, or if they spoke neither English nor Spanish. Controls comprised participants with documented STEC-related diarrhea who did not develop D+HUS as defined by any of the above criteria and who were similarly ascertained via FoodNet surveillance during the same period. STEC exposures were classified as O157:H7 or non-O157:H7, and only the former group was analyzed, to ensure that microbiologic risk was similar between study participants.

Written and verbal informed consent were obtained from participants aged ≥14 years; a parent or legal guardian provided written and verbal consent for patients aged <18 years. A waiver of assent was obtained for minors aged <14 years. The study was approved by the institutional review boards of Vanderbilt University, all participating state health departments, and the Centers for Disease Control and Prevention (CDC).

Gene Selection

For candidate-gene selection, we assumed that D+HUS pathogenesis overlaps that of atypical HUS and involves vascular injury as a final common pathway. In addition to including genes and single-nucleotide polymorphisms (SNPs) that have been associated with atypical HUS, SNPs associated with vascular endothelial injury via complement- or immune-mediated mechanisms and those involved in regulating inflammation, prostaglandin synthesis, vascular integrity, and endothelial-cell activation were identified in literature searches, using the following search terms: “genetic,” “risk,” “hemolytic-uremic syndrome,” “HUS,” “polymorphism,” “typical” (or “STEC,” or “post-diarrheal”), and “thrombotic thrombocytopenic purpura.” Since iron transport is fundamentally important in immune regulation and resistance to microbial infections via the hepcidin-ferroportin pathway [20], genes encoding iron transport–related proteins were also included. The National Institutes of Health Genetic Association Database (2004–2014; available at: http://geneticassociationdb.nih.gov/) was searched for genetic variants associated with arteriovascular or renal injury, microvascular thrombosis, and renal failure [20]. Finally, candidate SNPs were searched in the SNPedia database (available at: http://www.snpedia.com/index.php/SNPedia) for additional confirmation of phenotype associations, when possible. Representative candidate genes and chromosomal loci evaluated in this study and their known disease associations are listed in Table 1. A list of the 78 genes and all SNPs evaluated is provided in Supplementary Table 1.

Laboratory and DNA Methods

Either mouthwash (oral rinse) or buccal swab kits (Oragene) were used to collect buccal cell samples. Whole-genome DNA amplification was performed on 226 samples that were collected using the mouthwash protocol to obtain adequate DNA for genetic

analysis. Genomic DNA was extracted using PureGene (Gentra Systems, Minneapolis, MN). Genotyping was performed (blinded to clinical outcome) by Sequenom assay or custom ABI TaqMan assay (ABI Prism 7900 HT Sequence Detection System, Applied Biosystems, Foster City, CA), based on the variant. Proprietary probe and primer sequences, cycling, and scanning conditions for customized TaqMan assays are available from the manufacturer upon request. Genetic data were analyzed using ABI Sequence Detection Software v2.1 software, and calls were confirmed by visual inspection of plots.

Statistical Methods

Comparison of baseline characteristics was performed using the Wilcoxon rank sum test for nonnormally distributed, continuous variables (ie, age), and either the χ^2 test or Fisher's exact test was used for categorical variables (eg, sex, race, and antibiotic use). Genetic associations were evaluated by multivariable logistic regression, with adjustment for age at diagnosis, sex, race (non-Hispanic white vs other races), and antibiotic use within 7 days of diarrhea onset and before D+HUS diagnosis (yes vs no), and the need for whole-genome amplification (yes vs no). Three D+HUS outcomes were considered: (1) individuals who fulfilled criteria for confirmed, probable, or suspected D+HUS (outcome 1); (2) probable or confirmed cases only (outcome 2); and (3) confirmed cases only (outcome 3). The referent group for analyses of all 3 D+HUS outcomes included only individuals who did not fulfill any HUS criteria. As stated above, the case-control analysis presented here was also restricted to individuals with known *E. coli* serotype O157:H7.

An unbiased, additive genetic model was used for all analyses. Bonferroni corrections for multiple statistical tests were not used, owing to the exploratory nature of the analysis and small case sample size, to limit type II error. SAS statistical software v12.0 was used for all analyses; 2-sided *P* values of $< .05$ were considered statistically significant.

RESULTS

We identified 837 individuals with diarrheal illness or clinical symptoms suggestive of D+HUS, 818 of whom were eligible for this genetic study (Figure 1). Nine subjects had insufficient DNA for genetic testing or whole-genome amplification, 8 opted out of genetic testing or did not provide written consent, and 2 provided duplicate samples. After we excluded 52 samples with call rates of <90% for ≥1 SNP and 125 individuals with either unknown or non-O157 STEC serotypes, 641 subjects were evaluable, 80 of whom were classified as D+HUS cases; 561 were controls without evidence of HUS. Since 243 of these individuals had not been enrolled in the separate STEC cohort study and therefore lacked sufficient laboratory data for further classification, 398 were available for unadjusted analyses involving more-stringent D+HUS case definitions; 12 additional individuals were excluded from multivariable analyses, because of incomplete or absent covariate data. Of 80 cases with suspected D+HUS, 41 had probable D+HUS, and 32 had confirmed D+HUS.

D+HUS cases were significantly younger than controls (median age, 7 vs 21 years). Participants were predominantly non-Hispanic white by self-report (Table 2). A nonsignificantly higher proportion of cases was female. The proportion of samples from individuals without HUS that required DNA amplification (44%) was higher than the

proportion of samples from suspected, probable, and confirmed D+HUS cases that required amplification (37%, 33%, and 34%, respectively; $P < .01$ for comparison across groups).

Unadjusted Genetic Analyses

To ensure as much uniformity of microbiologic risk as possible across STEC-exposed individuals, we restricted our analyses to subjects with documented STEC serotype O157:H7, comprising 80 suspected D+HUS cases and 561 controls. Results of unadjusted analyses of SNP associations with D+HUS are shown in Table 3. Significant associations with at least 1 D+HUS definition were observed for 16 evaluated SNPs, encompassing 5 gene categories: (1) complement regulation and the innate immune system (complement component 4–binding protein, α subunit [*C4BPA*]; von Willebrand factor–cleaving protease [*ADAMTS13*]; Toll-like receptor 3 [*TLR3*]; and *TLR4*), (2) the related category of vascular integrity and homeostasis (endothelin 1 [*EDN1*]; platelet glycoprotein 1b, α polypeptide [*GP1BA*]; cyclooxygenase 1 [*PTGS1*]; and *PTGS2*), (3) iron transport (divalent metal transporter 1 [*SLC11A2*/*DMT1*], transferrin receptor 1 [*TFRC*], and β−2 microglobulin [*B2M*]), and (4) inflammatory cytokine–receptor signaling (interleukin 1 receptor antagonist [*IL-1RN*], interleukin 6 receptor [*IL-6R*], and bone morphogenetic protein 2 [*BMP2*]). Two SNPs, rs20417 in *PTGS2* and rs4986791 in *TLR4*, were associated with an increased likelihood of all D+HUS outcomes, with odds ratios (ORs) of 1.8–2.7. *PTGS2* rs20417 associations with outcomes 2 and 3 ($P < .01$ for both) were also stronger than that with outcome 1 and met gene-based Bonferroni criteria for significance ($P < 6 \times 10^{-4}$). Of note, point estimates of the ORs for association were in the same direction for all D+HUS outcome definitions for all but 1 SNP.

Adjusted Genetic Analyses

Results of analyses of selected SNPs in 386 patients, adjusted for age, sex, race, antibiotic use within 1 week of symptom onset, and DNA amplification, are shown in Table 4. Manhattan plots for each D+HUS outcome definition are shown in Figure 2A–C. These analyses identified SNPs in 11 different genes and 1 intergenic SNP that were associated with ≥1 D+HUS outcome definitions.

Two variants were significantly associated only with confirmed D+HUS (outcome 3): *C4BPA* rs9943077 and *IL6R* rs8192284 (adjusted ORs, 0.47 and 1.75, respectively). These ORs indicate a decreased D+HUS risk associated with SNP rs9943077 and an increased risk associated with SNP rs8192284.

In 5 genes, SNPs were significantly associated with all 3 D+HUS outcome definitions: an intergenic SNP (rs10874639) nearest the collagen XI gene *COL11A1*, rs3804141 in *TFRC,* rs5370 in *EDN1*, rs121908064 in *GP1BA,* and rs16966334 in *B2M.* Subjects with D+HUS were more likely to have rs10874639_*G* (range of adjusted ORs, 2.2–2.6), *TFRC* rs3804141_*A* (range of adjusted ORs across case definitions, 2.4–2.9), *GP1BA* rs121908064_*A* (range of adjusted ORs, 13.3–27.4), and *B2M* rs16966334_*G* alleles (range of adjusted ORs, 4.2–5.4). In contrast, D+HUS cases were less likely to have the *EDN1* rs5370_*T* allele (range of adjusted ORs, 0.34–0.48).

Associations were observed for SNPs rs4986791 in *TLR4* and rs4792847 in *MAP3K14* (a tumor necrosis factor α receptor [*TNFR*] superfamily gene) for an increased and decreased risk of 2 D+HUS outcomes (1 and 2), respectively. Both of these SNPs also showed borderline associations with outcome 3 (confirmed HUS; $P < .075$ for both); the *TLR4* SNP, which was significant in unadjusted analyses, retained borderline significance after adjustment.

Three SNPs, *CFHR1–5* rs6677604, rs315951 in *IL1RN*, and rs2234649 in *TNFR1*, were each associated with 1 D+HUS outcome definition ($P \leq .05$ for all); one of these SNPs (rs315951_*C*) conferred a decreased risk. The *CFHR1–5* variant also showed a borderline association with outcome 1 (ie, suspected D+HUS; $P = .06$). All but 2 individuals were non-Hispanic white; hence, adjusted ORs in analyses restricted to non-Hispanic whites for all D+HUS outcomes (1, 2, and 3) yielded very similar results (data not shown).

Nine SNPs identified in unadjusted analyses, *PTGS1* rs883484, *PTGS2 rs20417, C4BPA* rs11120211, *TFRC* rs4927866 and rs480760, *TLR3* rs3775291, *SLC11A2* rs224572, *BMP2* rs1979855, and *ADAMTS13* rs652600, lost significance following adjustment, although 6 of these retained a borderline statistically significant association with at least 1 D+HUS outcome. Genetic variants associated with D+HUS with adjusted *P* values of >.05 and ≤.15 are listed in Supplementary Table 2. Borderline associations for 2 of these SNPs, rs7673587 in the fibrinogen β chain gene *FGB* and rs1801133 in *MTHFR*, were observed with all 3 D+HUS outcomes.

DISCUSSION

Ever since the 1918 influenza pandemic, public health clinicians have documented unpredictable morbidity and mortality in previously healthy individuals, triggered by an excessive inflammatory response [22–24]. Uncharacterized host factors may confer susceptibility to these life-threatening complications [17, 23, 25–27]. This exploratory study was designed to cast the net wide for host genetic factors that predispose to D+HUS following STEC exposure [6]. Furthermore, multistate FoodNet consortium resources greatly facilitated identification of carefully ascertained D+HUS cases and controls. Detailed ascertainment of likely cofactors in D+HUS development, such as antibiotic use during the first week of STEC-related diarrheal illness, was imperative to account for possible confounding in multivariable-adjusted analyses. Based on a candidate-gene list derived from published reports linking host genetic factors to renal and vascular injury and atypical HUS, this study identified many biologically plausible associations and is the first of its kind.

Several genes (*TFRC*, *EDN1*, *GP1BA*, and *B2M*), as well as an intergenic SNP nearest *COL11A1*, showed significant associations with all levels of D+HUS. Variants in *TFRC*, which encodes a critical cellular iron transporter, predispose to severe diarrhea after STEC infection in livestock, albeit by an unknown mechanism [28]. *EDN1* variants have been associated with several vascular phenotypes, including a reduced risk of diabetic retinopathy [29]. *GP1BA* encodes a platelet membrane glycoprotein, which functions as a receptor for von Willebrand factor and in platelet adhesion to the vascular endothelium after intravascular injury. GP1BA complexes with other proteins involved in platelet

signaling, enhancing platelet activation [30]. Thrombocytopenia, a well-recognized clinical feature of HUS, results from thrombotic microangiopathy and platelet consumption and is a key component of HUS pathophysiology [9]. Similarly, *B2M* encodes the serum protein β−2-microglobulin, which is required for normal cell-surface expression of other major histocompatibility complex class I proteins and is widely expressed [31]. Urinary β−2-microglobulin levels have been reported to rise precipitously preceding the onset of azotemia, thrombocytopenia, and renal failure in children with HUS [32, 33]. It is particularly noteworthy that, for many significant SNPs in this study, such as SNPs in *GP1BA*, *B2M*, *EDN1,* and *TFRC* and the intergenic SNP, the magnitude of estimated associations increased progressively with the stringency D+HUS definition or were undiminished when the most stringent case definition was used, despite smaller case numbers.

A role for complement is established in atypical HUS pathophysiology, and borderline associations identified here between suspected, probable, and/or confirmed D+HUS and SNPs in *CFHR1–5* and intronic SNP rs9943077 in *C4BPA* suggest a similar but possibly less prominent role for complement in D+HUS. *CFHR1–5* SNPs rs667604 and 16840639 have both been associated with systemic lupus erythematosus, an autoimmune disease characterized by complement hyperactivation [34]. Involvement of complement-related genes in D+HUS may explain anecdotal reports of clinical improvement in STEC-related D+HUS after treatment with eculizumab, a recombinant inhibitor of terminal complement (C5) [35]. However, these observations are based on small case series that lacked comparison groups, and reports of efficacy are inconsistent, supporting the contention that other host factors contribute to D+HUS pathogenesis [17]. If associations of *PTGS2 (COX2)* SNP rs20417, which is associated with impaired aspirin sensitivity, and SNPs in *GP1BA* and *IL1RN* are replicated and/or strengthened in larger studies, higher-dose COX-2 inhibitors or other antiplatelet agents, as well as recombinant IL-1R antagonists, may be beneficial in preventing D+HUS in individuals with these variants. *IL1RN* encodes an IL-1R ligand, which inhibits IL-1 signaling; *IL1RN* variants have been associated with microvascular complications of diabetes mellitus and with immunoglobulin A nephropathy [36–38]. Since D+HUS similarly involves microvascular injury, prior associations of *IL1RN* and *PTGS2* with other vascular pathologies argue for further exploration of these SNPs in D+HUS. Recent investigation of in vitro metabolic effects of Shiga toxins in human endothelial cells strongly implicated the eicosanoid pathway, which involves *PTGS2*-mediated prostaglandin synthesis, lending further validity to our finding of a possible association of *PTGS2* rs20147 with this phenotype [39]. It is also noteworthy that 4 genes related to iron metabolism were identified (*TFRC*, *B2M*, and more-borderline associations with *BMP2* and *SLC11A2/DMT1*). Finally, an intergenic SNP, rs10874639, which resides in a protein quantitative trait locus and has been associated with fibrinogen levels, was associated with all levels of D+HUS in our study [40]. Fibrinogen is an important component of the final common pathway of coagulation.

This study was clearly limited by the modest number of D+HUS cases (despite an intensive, multistate ascertainment effort), the lack of STEC genotype data, and the absence of associations that retained significance after correction for multiple statistical tests. To balance type I and type II error, a list of SNPs of lesser significance was also generated

for future investigation. We used both rigorous and flexible case definitions, deliberately exchanging some statistical power for diagnostic precision. The suspected, probable, or confirmed HUS outcome (outcome 1) is particularly prone to misclassification; hence, the most attention should be given to associations with confirmed D+HUS (outcome 3), followed by confirmed or probable D+HUS (outcome 2). Because the study population predominantly comprised non-Hispanic white individuals, it was unfortunately not possible to evaluate genetic variants that confer susceptibility to D+HUS in minority populations. We were, however, mindful of the possibility of selection bias and sought to minimize it by using a population-based, prospective enrollment strategy within the FoodNet catchment area. Although we cannot exclude the possibility of genetic relatedness in our sample, we believe the likelihood of this to be small. Cases were also considerably younger than controls, but age-matching was not performed, to retain as many individuals in the analysis as possible to optimize power. However, all multivariable models were age-adjusted, and none of the SNPs we evaluated is known to be associated with age. Nevertheless, we cannot completely exclude the possibility of residual confounding by age.

In conclusion, this study implicates variants in several non–complement-related genes (including those involved in iron transport, cytokine signaling, platelet function, pathogen recognition, and endothelial function) and, possibly, in genes encoding complement-pathway proteins, in the development of D+HUS, suggesting previously unrecognized host susceptibility factors in this serious complication of STEC infection. STEC infections and outbreaks will inevitably still occur, and this relatively unique study suggests that genetic testing might be efficiently performed on STEC-exposed individuals at risk of D+HUS using a customized SNP panel. Targeted genotyping of STEC-exposed individuals for the identified variants, reducing the multiple-testing burden, are needed to validate these findings; if the findings are replicated, therapeutic interventions focusing on these pathways may warrant concurrent investigation at the bench and at the bedside.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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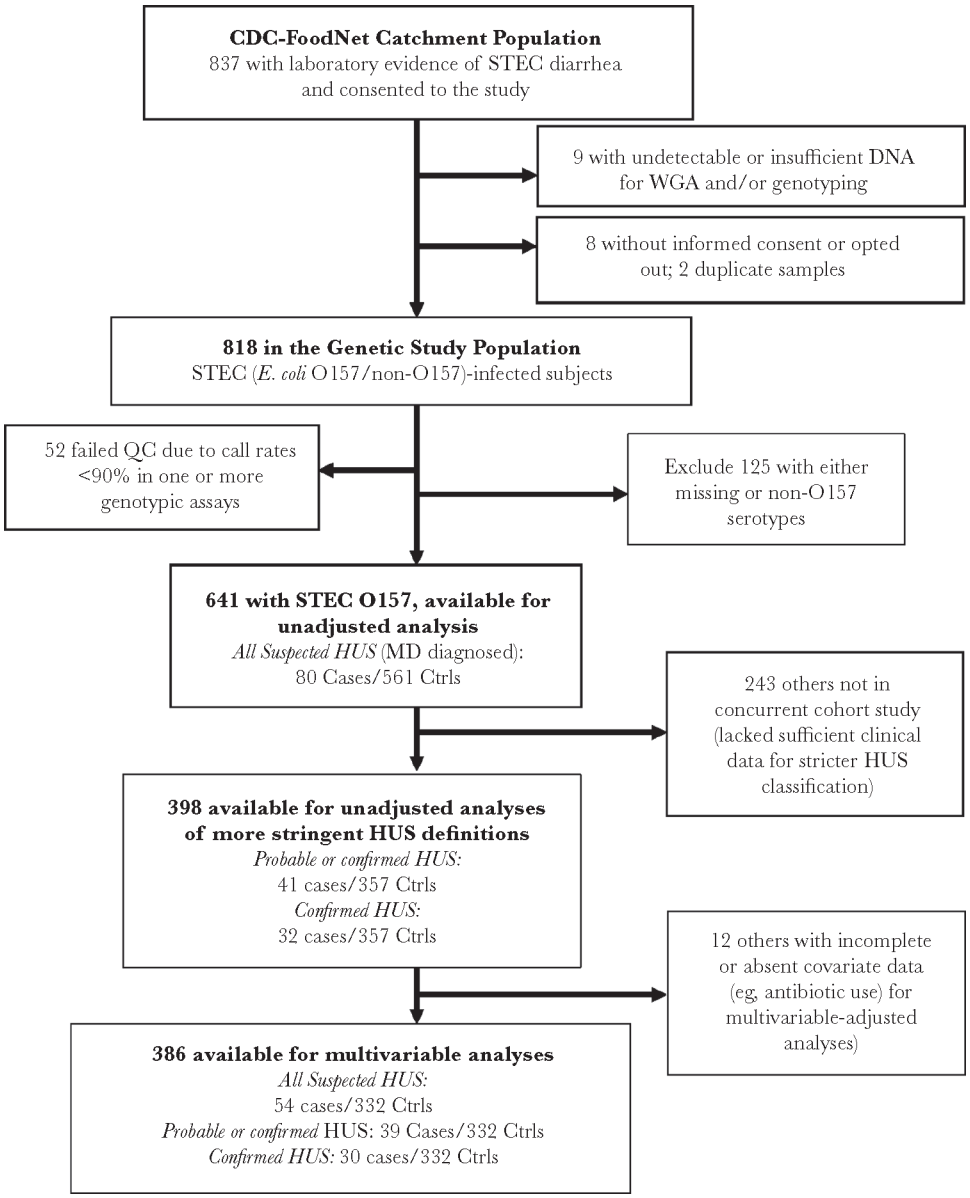

Figure 1.

Study flow diagram, showing sample sizes available for genetic analyses. Suspected hemolytic-uremic syndrome (HUS) is defined as physician-diagnosed HUS. Probable HUS is defined as HUS that met 3 of 4 laboratory criteria, including thrombocytopenia, hemolytic anemia, and/or renal failure, in the setting of Shiga toxin–producing *Escherichia coli* (STEC) exposure. Confirmed HUS is defined as HUS that met all 4 laboratory criteria. CDC, Centers for Disease Control and Prevention; Ctrls, controls; MD, physician; QC, quality control; WGA, whole-genome amplification.

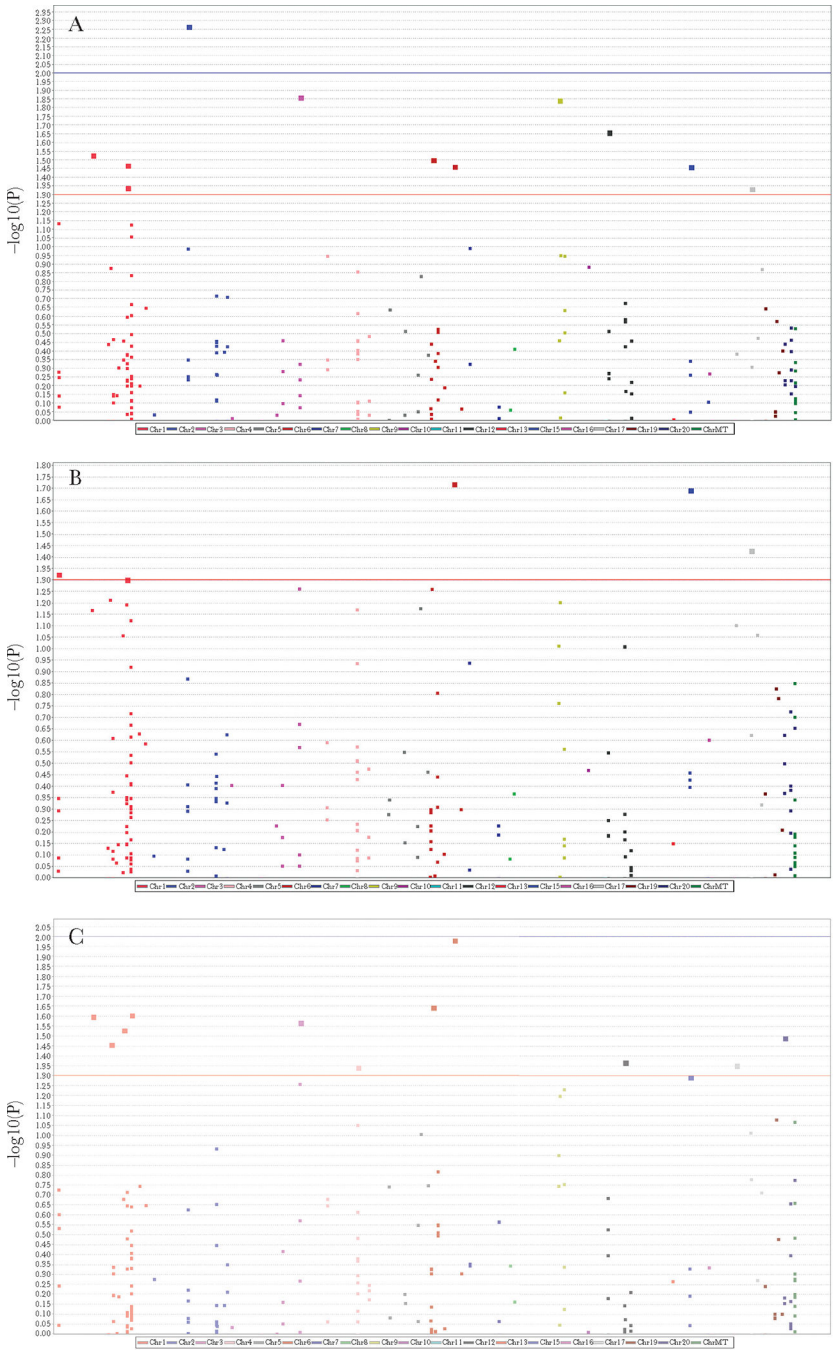

Figure 2.

Manhattan plots showing single-nucleotide polymorphism (SNP) associations with specific postdiarrheal hemolytic-uremic syndrome (D+HUS) case definitions and outcomes in the Centers for Disease Control and Prevention–FoodNet Shiga toxin–producing *Escherichia coli* study. *A*, Suspected, probable, or confirmed D+HUS (outcome 1). *B*, Probable or confirmed D+HUS (outcome 2). *C*, Confirmed D+HUS (outcome 3).

Table 1.

Representative Genes and Relevant Pathways Evaluated in the Centers for Disease Control and Prevention–FoodNet Postdiarrheal Hemolytic-Uremic Syndrome (HUS) Genetic Study

Phenotype Grouping, Candidate Gene	Locus	Relevant Pathway(s)/Gene Function	Associated Phenotype(s)
Group 1			
FGA	4q31.3	Common coagulation pathway, regulates innate immunity	Renal disease, atypical HUS, coronary artery disease
FGB	4q31.3	Controls fibrinogen protein levels	
Group 2			
ADAMTS13	9q34.2	von Willebrand factor–cleaving protease	Atypical HUS, congenital TTP impairment of renal function and thrombocytopenia in certain virus infections, ischemic stroke and cardiovascular outcomes
vWF	12p13.31	von Willebrand factor; platelet adhesion and hemostasis	
SERPINE1	7q22.1	Serine peptidase inhibitor, member 1	
GP1BA	17p13.2	Modulates platelet reactivity with von Willebrand factor and endothelium	
Group 3			
CFH	1q31.3	Regulation of complement activation in response to antigen	Age-related macular degeneration, IgA nephropathy, venous thrombosis
CFHR1-5	1q31.3	Regulation of complement activation in response to antigen	
C4BPA	1q32.2	Membrane cofactor protein	
THB	20p11.21	Thrombomodulin	
CFI	4q25	Complement receptor that inhibits complement activation	
Group 4			
TLR4	9q33.1	Recognition of bacterial lipopolysaccharide; innate immunity	Pregnancy loss, septic shock, response to gram-negative bacterial infection, age-related macular degeneration
TLR3	4q35.1	Recognition of bacterial lipopolysaccharide; innate immunity	
Group 5			
MTHFR	1p36.22	Regeneration of methionine from homocysteine	Atypical HUS, preeclampsia
Group 6			
IL1RN	2q13	Cytokine receptor signaling and downmodulation of inflammation	Chronic kidney disease, septic shock, recurrent otitis, CRP levels, autoimmune disease (SLE, rheumatoid arthritis), venous thromboembolism, diabetic nephropathy, IgA nephropathy, pregnancy loss, VTE and other vascular complications, cerebral microangiopathy, cardiovascular disease risk
TNFR1	12p13.31	Cytokine receptor–mediated signaling for TNF (proinflammatory)	
TNFR2	1p36.22	Cytokine receptor–mediated signaling for TNF (proinflammatory)	
IL6	7p15.3	Cytokine (either pro- or antiinflammatory)	

Phenotype Grouping, Candidate Gene	Locus	Relevant Pathway(s)/Gene Function	Associated Phenotype(s)
IL6R	1q21.3	Interleukin 6 receptor	
IL8	4q13.3	Regulate concentrations of inflammatory cytokines	
IFNG	12q15	Immunomodulatory cytokine	
IL10	1q32.1	Antiinflammatory cytokine	
IL1B	2q14.1	Proinflammatory cytokine, mediator of acute-phase response	
CRP	1q23.2	Acute-phase protein; recognition of pathogens and cellular damage	
Group 7			
B2M	15q21.1	Intracellular and systemic iron regulation and transport; peptide antigen presentation	Resistance/response to bacterial infection in nonhuman species, resistance to tuberculosis/malaria/brucellosis, STEC susceptibility in pigs
FPN (*SLC40A1*)	2q32.2	Regulation of innate immunity and iron export via hepcidin pathway	
SLC11A2 (*DMT1*)	12q13.12	Divalent metal (eg, iron) transporter	
BMP2	20p12.3	Regulates cytokine receptor signaling	
CP	3q24-25	Plasma ferroxidase, copper transport	
TFRC	3q29	Cellular iron import, binds iron-laden transferrin	
TF	3q22.1	Iron transport in plasma	
Group 8			
HP	16q22.2	Iron binding, binds heme scavenger receptor	Cardiovascular disease, lupus nephritis
Group 9			
HFE1	6p22.2	Regulation of hepcidin, cellular iron content	Hemochromatosis (genetic iron overload)
Group 10			
PTGS2 (*COX2*)	1q31.1	Prostaglandin synthesis, vascular integrity	Atypical HUS: chronic kidney disease in Asians with hypertension; diabetic retinopathy
PTGS1 (*COX1*)	9q33.2	Prostaglandin synthesis, vascular integrity	
EDN1	6p24.1	Vasoconstriction, vascular integrity, endothelial-cell activation	

Abbreviations: *ADAMTS13*, a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13; *BMP2*, bone morphogenetic protein 2; *B2M*, β-2-microglobulin; *CD46 (MCP)*, membrane cofactor protein; *CFH*, complement factor H; *CFHR1-5*, complement factor H–related proteins 1–5; *CFI*, complement factor 1; *CP*, ceruloplasmin; *CRP*, C-reactive protein; *C4BPA*, complement component 4-binding protein, α subunit; *EDN1*, endothelin 1; *FGA*, fibrinogen α chain; *FGB*, fibrinogen β chain; *GP1BA*, platelet glycoprotein 1b, α polypeptide; *HFE*, hemochromatosis; *HP*, haptoglobin; *IFNG*, interferon γ; IgA, immunoglobulin A; *IL1RN*, interleukin 1 receptor antagonist; *IL6R*, interleukin 6 receptor; *MTHFR*, methylenetetrahydrofolate reductase; *NRAMP1*, natural resistance-associated macrophage protein 1; *PTGS1* (*COX1*), cyclooxygenase 1; *PTGS2* (*COX2*), cyclooxygenase 2; *SLC11A2* (*DMT1*), divalent metal transporter 1; SLE, systemic lupus erythematosus; STEC, Shiga toxin-producing *Escherichia coli*; *TF*, transferrin; *TFRC*, transferrin receptor 1; *THBD*, thrombomodulin; *TLR3*, Toll-like receptor 3; *TLR4*, Toll-like receptor 4; TNF, tumor necrosis factor; *TNFR1*, tumor necrosis factor receptor 1; TTP thrombotic thrombocytopenic purpura; VTE, venous thromboembolism.

Table 2.

Characteristics of the Centers for Disease Control and Prevention–FoodNet Postdiarrheal Hemolytic-Uremic Syndrome (D+HUS) Genetic Study Population

Characteristic	D+HUS Case Definition[a]			Controls[b] (n = 561)	*P* [c]
	Outcome 1 (n = 80)	Outcome 2 (n = 41)	Outcome 3 (n = 32)		
Age, y, median (IQR)	7 (3–14)	7 (3–14)	6 (3–14)	21 (8–51)	<.01
Female sex	52 (65)	29 (71)	21 (66)	328 (59)	.27
Self-reported race/ethnicity					.03
Non-Hispanic white	78 (98)	41 (100)	32 (100)	507 (90)	
Non-Hispanic black	2 (2)	0 (0)	0 (0)	11 (2)	
Hispanic	0 (0)	0 (0)	0 (0)	5 (1)	
Other or unknown	0 (0)	0 (0)	0 (0)	38 (7)	
Antibiotic use[d]	19 (24)	12 (29)	9 (28)	117 (21)	.55
Whole-genome amplification[e]	23 (26)	14 (34)	12 (38)	183 (33)	.49

Data are no. (%) of participants, unless otherwise indicated.

Abbreviation: IQR, interquartile range.

[a] Outcome 1 is defined as suspected, probably, or confirmed D+HUS; outcome 2, as probable or confirmed D+HUS; and outcome 3, as confirmed D+HUS.

[b] Subjects who did not meet any HUS case definition.

[c] For comparison of cases with outcome 1 to controls. Values of < .05 are statistically significant.

[d] During the first 7 days of the diarrheal illness; data exclude antibiotic exposure that began after development of D+HUS. A total of 255 of 641 subjects were not ascertained via the Shiga toxin-producing *Escherichia coli* cohort study and hence lacked complete clinical and/or antibiotic use data (Figure 1).

[e] Amplification of DNA in some patient samples was required for genotyping.

Table 3.

Unadjusted Odds Ratios (ORs) for Associations of Specific Genetic Polymorphisms With ≥1 Case Definition of Postdiarrheal Hemolytic-Uremic Syndrome (D+HUS) Following Shiga Toxin–Producing *Escherichia coli* (STEC) Infection

		D+HUS Case Definition, All Subjects[a]					
SNP (_Allele)	Gene	Outcome 1		Outcome 2		Outcome 3	
		Unadjusted OR	*P* [b]	Unadjusted OR	*P* [b]	Unadjusted OR	*P* [b]
rs883484_A	*PTGS1* (*COX1*)	0.84	.39	0.50	.04	0.44	<.05
rs20417_C[c]	*PTGS2* (*COX2*)	1.84	.03	2.32	<.01	2.69	<.01
rs11120211_A	*C4BPA*	1.71	.04	0.84	.72	0.65	.46
rs9943077_T	*C4BPA*	0.80	.21	0.54	.03	0.42	.01
rs315951_C	*IL-1RN*	0.66	.03	0.64	.10	0.65	.16
rs4927866_T	*TFRC*	0.61	.03	0.71	.25	0.71	.32
rs480760_T	*TFRC*	1.09	.83	1.86	.18	2.44	<.05
rs3775291_A	*TLR3*	1.44	.03	1.61	<.05	1.54	.11
rs4986791_C[c]	*TLR4*	1.79	.04	2.44	.01	2.54	.02
rs652600_C	*ADAMTS13*	0.59	.01	0.65	.12	0.46	.03
rs8192284_C	*IL6R*	1.31	.10	1.65	.02	1.85	.01
rs16966334_G	*B2M*	1.73	.22	3.89	.01	4.00	.02
rs5370_T	*EDN1*	0.66	.08	0.50	.05	0.35	.02
rs121908064_A	*GP1BA*	1.79	.47	4.51	.09	5.91	.04
rs224572_A	*SLC11A2* (*DMT1*)	1.34	.09	1.48	.10	1.72	<.05
rs1979855_C	*BMP2*	1.04	.87	1.42	.18	1.78	.04

Data are for individuals with a known *Escherichia coli* O157 serotype.

Abbreviations: *ADAMTS13*, a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13; *BMP2*, bone morphogenetic protein 2; *B2M*, β-2-microglobulin; *C4BPA*, complement component 4–binding protein, α subunit; *EDN1*, endothelin 1; *GP1BA*, platelet glycoprotein 1b, α polypeptide; *IL1RN*, interleukin 1 receptor antagonist; *IL6R*, interleukin 6 receptor; *PTGS1* (*COX1*), cyclooxygenase 1; *PTGS2* (*COX2*), cyclooxygenase 2; *SLC11A2* (*DMT1*), divalent metal transporter 1; *TFRC*, transferrin receptor 1; *TLR3*, Toll-like receptor 3; *TLR4*, Toll-like receptor 4.

[a] Outcome 1 is defined as suspected, probably, or confirmed D+HUS (80 cases and 561 controls); outcome 2, as probable or confirmed D+HUS (41 cases and 357 controls); and outcome 3, as confirmed D+HUS (32 cases and 357 controls).

[b] For comparisons of subjects meeting a D+HUS case definition to all subjects who did not meet any criteria for HUS. Values are not corrected for multiple testing, and those < .05 are statistically significant.

[c] Significantly associated with all 3 D+HUS outcomes.

Table 4.

Multivariable-Adjusted Odds Ratios (ORs) for Associations of Genetic Polymorphisms With Case Definitions of Postdiarrheal Hemolytic-Uremic Syndrome (D+HUS)

		D+HUS Case Definition, All Subjects[a]					
		Outcome 1 (n = 54)		Outcome 2 (n = 39)		Outcome 3 (n = 30)	
SNP (_Allele)	Gene or (Nearest Gene)	Adjusted OR[b]	*P* [c]	Adjusted OR[b]	*P* [c]	Adjusted OR[b]	*P* [c]
rs10874639_G [d]	Intergenic (*COL11A1*)	2.27	.02	2.15	<.05	2.58	.02
rs315951_C	*IL1RN*	0.48	<.01	0.61	.10	0.60	.13
rs6677604_A	*CFHR1-5*	1.57	.07	1.72	<.05	1.54	.16
rs3804141_A [d]	*TFRC*	2.66	<.01	2.36	.04	2.87	.02
rs5370_T [d]	*EDN1*	0.47	.03	0.48	<.05	0.34	.03
rs121908064_A [d]	*GP1BA*	13.3	.04	15.5	.02	27.4	.01
rs4986791_T	*TLR4*	2.31	.01	2.14	.05	2.21	.06
rs2234649_C	*TNFR1*	7.34	.02	3.42	.32	4.19	.25
rs16966334_G [d]	*B2M*	4.21	.03	4.99	.03	5.40	.03
rs4792847_A	*MAP3K14* [e]	0.60	.04	0.54	.03	0.57	.07
rs9943077_T	*C4BPA*	0.65	.10	0.59	.08	0.47	.04
rs8192284_C	*IL6R*	1.46	.09	1.58	.07	1.75	.04

Abbreviations: *B2M*, β-2-microglobulin; *CFHR1-5*, complement factor H–related proteins 1–5; *C4BPA*, complement component 4–binding protein, a subunit; *COL11A1*, collagen type XI, EDN1, endothelin 1; *GP1BA*, platelet glycoprotein 1b, α polypeptide; *IL1RN*, interleukin 1 receptor antagonist; *IL6R*, interleukin 6 receptor; *MAP3K1*, mitogen-activated protein kinase kinase kinase 14; *TFRC*, transferrin receptor 1; *THBD*, thrombomodulin; *TLR4*, Toll-like receptor 4; *TNFR1*, tumor necrosis factor receptor 1.

[a] Outcome 1 is defined as suspected, probably, or confirmed D+HUS; outcome 2, as probable or confirmed D+HUS; and outcome 3, as confirmed D+HUS. A total of 26 cases were not included in adjusted analyses owing to insufficient laboratory, clinical, or covariate information (Figure 1). The total number of controls in each analysis was 332.

[b] Adjusted for age, sex, race, antibiotic use within 1 week of symptom onset, and requirement for whole-genome amplification (yes or no).

[c] Values are not corrected for multiple testing, and those < .05 are statistically significant.

[d] Significantly associated with all 3 D+HUS outcomes.

[e] Involved in the tumor necrosis factor superfamily member 1β signaling pathway.