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ORIGINAL REPORT

Clinical and Genetic Risk Factors for Acute Pancreatitis in Patients With Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose
Acute pancreatitis is one of the common causes of asparaginase intolerance. The mechanism is unknown, and genetic predisposition to asparaginase-induced pancreatitis has not been previously identified.

Methods
To determine clinical risk factors for asparaginase-induced pancreatitis, we studied a cohort of 5,185 children and young adults with acute lymphoblastic leukemia, including 117 (2.3%) who were diagnosed with at least one episode of acute pancreatitis during therapy. A genome-wide association study was performed in the cohort and in an independent case-control group of 213 patients to identify genetic risk factors.

Results
Risk factors associated with pancreatitis included genetically defined Native American ancestry ($P < .001$), older age ($P < .001$), and higher cumulative dose of asparaginase ($P < .001$). No common variants reached genome-wide significance in the genome-wide association study, but a rare nonsense variant rs199695765 in *CPA2*, encoding carboxypeptidase A2, was highly associated with pancreatitis (hazard ratio, 587; 95% CI, 66.8 to 5166; $P = 9.0 \times 10^{-9}$). A gene-level analysis showed an excess of additional *CPA2* variants in patients who did versus those who did not develop pancreatitis ($P = .001$). Sixteen *CPA2* single-nucleotide polymorphisms were associated ($P < .05$) with pancreatitis, and 13 of 24 patients who carried at least one of these variants developed pancreatitis. Biologic functions that were overrepresented by common variants modestly associated with pancreatitis included purine metabolism and cytoskeleton regulation.

Conclusion
Older age, higher exposure to asparaginase, and higher Native American ancestry were independent risk factors for pancreatitis in patients with acute lymphoblastic leukemia. Those who inherit a nonsense rare variant in the *CPA2* gene had a markedly increased risk of asparaginase-induced pancreatitis.

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INTRODUCTION

Asparaginase is a major cause of pancreatitis that occurs in 2% to 18% of patients treated for acute lymphoblastic leukemia (ALL),[1-9] although steroids and thiopurines can also cause this complication.[10-12] Severe pancreatitis is a contraindication to further asparaginase therapy and has been associated with compromised treatment outcome.[3,7,13] Better understanding the risk factors for pancreatitis could help improve ALL therapy by identifying mechanisms of pancreatitis, which might lead to new interventions to prevent or reverse it, or by identifying patients at such high risk that consideration of non–asparaginase-containing regimens would be warranted.

The pathogenesis of asparaginase-induced pancreatitis has not been elucidated. Clinical risk factors include intensive asparaginase and older age[1-4,7,14,15]; however, most studies lack sufficient power to robustly assess risk factors.[4-8]

Studies on pancreatitis of other etiologies, such as alcoholic pancreatitis, have identified a few

genetic risk factors (eg, *PRSS1*, *PRSS2*, *SPINK1*, *CFTR*, *CTRC*, *CASR*, *CLDN2*, *CPA1*, and *HLA-DRB1**07:01), most of which relate to premature trypsin activation or sustained trypsin activity in the pancreas.[16-23] However, there has not been an agnostic genome-wide association study (GWAS) of asparaginase-induced acute pancreatitis.[24] Here we report the largest study to date to determine the clinical and genetic risk factors for acute pancreatitis in 5,398 patients treated for newly diagnosed ALL with contemporary regimens containing asparaginase.

METHODS

Patients and Treatment

We investigated patients (age 0 to 30 years) with newly diagnosed ALL treated on seven front-line protocols (Fig 1; Data Supplement Table 1): Total XIIIB (NCI-T93-0101D),[25] Total XV (NCT00137111),[26] COG P9904 (NCT00005585),[27] P9905 (NCT00005596),[27] P9906 (NCT00005603),[27] AALL0232 (NCT00075725),[28] and AALL0331 (NCT00103285) at St Jude Children's Research Hospital and in the Children's Oncology Group.

Among 5,958 patients enrolled on Total XIIIB/XV, COG P9904/P9905/P9906, and AALL0232, we studied 5,185 patients (including 117 cases) with available DNA (Data Supplement Table 2). There were 209 (4.0%) adolescents and young adults (age 18 to 30 years) in the cohort, and 3.6% (8 of 209) adolescents and young adults developed pancreatitis, making up 6.8% of the 117 cases. From approximately 5,500 patients enrolled in AALL0331, there were 103 cases of pancreatitis; of these, we included all 71 cases who had available germline DNA and successful genotyping and matched two controls to each case by age (± 2 years) and protocol-planned asparaginase doses. Patient characteristics by protocol are described in the Data Supplement Table 3. The studies were approved by the institutional review boards of all participating institutions. Informed consent was obtained in accordance with the Declaration of Helsinki.

Diagnosis of Pancreatitis and Analysis of Clinical Risk Factors

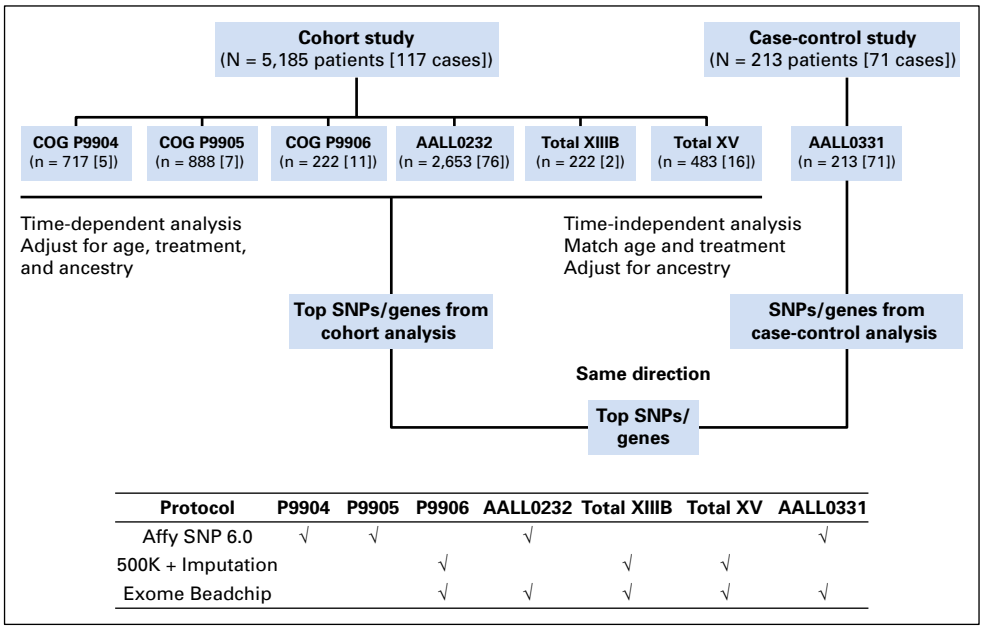
Acute pancreatitis was prospectively graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0 for AALL0232 and AALL0331, and version 2.0 for all the other protocols. The criteria were similar across all studies, including symptoms (abdominal pain, nausea, and vomiting), increased serum amylase or lipase, and radiographic or surgical findings. Only symptomatic episodes (grade 3 and 4 for v2.0 and grade 2 to 4 for v3.0) were included in the analysis regardless of severity; there were no fatal (grade 5) cases. For time-dependent analyses, time to first episode of pancreatitis was considered if the patient had more than one episode. To identify covariates to include in the GWAS, we used the Fine-Gray regression model for competing risks[29] to evaluate the association between pancreatitis and variables including age (in years) and genetic ancestry (as percentages of European, African, Asian, and Native American ancestries)[30] as continuous variables; sex, ALL immunophenotype, and treatment regimen (protocol-planned cumulative asparaginase dose $\leq$ 120,000 U/m^2 v $\geq$ 240,000 U/m^2) were also included as categorical variables. Time at risk was censored at therapy completion; failures other than pancreatitis, such as induction failure, relapse, secondary malignancy, or death were considered as competing events. For purposes of normalizing asparaginase doses across protocols, pegylated *Escherichia coli*–asparaginase (PEG-asparaginase) at 2,500 U/m^2 every other week was considered equivalent to native *Escherichia coli*–asparaginase at 25,000 U/m^2 once per week for 2 weeks.

Genotyping

Genotyping of germline DNA was performed (Fig 1; Data Supplement) for 5,398 patients with the Affymetrix Genome-Wide Human SNP 6.0 (or GeneChip Human Mapping 500K) Array (Santa Clara, CA) and 3,469 of the 5,398 with the Illumina HumanExome Beadchip Array (San Diego, CA). Genetic ancestries were determined using STRUCTURE[30] and either treated as a continuous variable or used to categorize patients as white (European ancestry > 90%), black (African ancestry > 70%), Hispanic (Native American ancestry > 10%, nonblack), Asian (Asian ancestry > 90%), and other.

Single-Nucleotide Polymorphism–Based Analysis for Pancreatitis

After excluding single-nucleotide polymorphisms (SNPs) with low call rate, 751,455 SNPs on the SNP 6.0 and 169,521 SNPs on the Exome Beadchip were interrogated in the initial GWAS. Each SNP was tested using Gray's test,[31] and those achieving $P < .05$ were subsequently analyzed.



Protocol	P9904	P9905	P9906	AALL0232	Total XIIIB	Total XV	AALL0331
Affy SNP 6.0	√	√		√			√
500K + Imputation			√		√	√	
Exome Beadchip			√	√	√	√	√

Fig 1. Study design and genotyping platforms of the initial genome-wide association study (5,398 patients) that aimed to identify the top-ranked single-nucleotide polymorphisms (SNPs) and genes from the cohort, and the SNPs or genes significantly associated with pancreatitis ($P < .05$) in the case-control group. The number of patients and number of cases (in brackets) in each protocol are shown.

Genotype calls were coded as 0, 1, or 2 for the number of B alleles (AA, AB, or BB) assuming an additive genetic model. In the cohort (n = 5,185), further screening was performed using a Cox proportional hazards model analyzing the onset time of pancreatitis (time-dependent GWAS). Covariates included age, genetic ancestry, and treatment regimen as described above. Time to event was the length of time between the time at the first episode of pancreatitis and on-study time; for those without pancreatitis, time at risk was censored at therapy completion or at the time off treatment because of induction failure, relapse, secondary malignancy, or death. In the case-control group (n = 213), GWAS was performed using logistic regression, adjusted for genetic ancestry (time-independent GWAS).

Gene-Level Analysis of Nonsense Variants Using Sequential Kernel Association Test

SNPs were assigned to genes using Reference Sequence (Refseq) boundaries (UCSC Genome Browser [GRCh37/hg19] Assembly). For each gene, sequential kernel association test[32] was applied to test for gene-level significance for nonsense variants. For time-dependent analysis, Martingale residuals from Cox proportional hazards model with demographic and clinical covariates were used as observations of a continuous phenotype in sequential kernel association test.

Sequencing of Selected Genes

To interrogate rare and low-frequency variants in genes of interest for ALL-related phenotypes, we sequenced 283 genes in 4,217 children in the cohort and 162 in the case-control group. The 283 genes were candidate genes for a number of ALL phenotypes (eg, relapse, drug sensitivity, pharmacokinetics, leukemia risk, and several adverse effects); 42 of these were possible pancreatitis risk genes (Data Supplement Table 4). Gene-level analysis of all 283 genes and SNP-based analysis of 9,229 SNPs in the 42 pancreatitis-associated genes was performed using the same methods as in the initial GWAS (Data Supplement).

RESULTS

Pancreatitis Associated With Asparaginase

Among the 5,185 patients in the cohort, 117 were diagnosed with at least one episode of pancreatitis during therapy, with these crude rates by protocol: Total XIIIB (0.9%, two of 222), Total XV (3.3%, 16 of 483), P9904 (0.7%, five of 717), P9905 (0.8%, seven of 888), P9906 (5.0%, 11 of 222), and AALL0232 (2.9%, 76 of 2,653). The cumulative incidence of pancreatitis by protocol is shown in Figure 2A, accounting for time at risk and competing events.[29] The first episode occurred at a median of 106 days from ALL diagnosis and developed during remission induction (first 4 to 6 weeks of treatment) in 24 of 117 (20.5%) patients or during the first year of therapy (including remission induction) in 104 of 117 (88.9%) patients. The great majority of the events were clustered with asparaginase administration (Data Supplement Figure 1). Among the 71 patients developing pancreatitis on AALL0331 (case-control group), the first episode occurred at a median of 78 days and developed during remission induction in 24 of 71 (33.8%) cases and during the first year in 65 of 71 (91.5%) cases.

Considering the data herein and also prior publications of other front-line ALL protocols,[2-4,6,7,33,34] the crude rate of pancreatitis was related to cumulative asparaginase dose (R^2, 0.75; $P < .001$) and duration of asparaginase treatment (R^2, 0.83; $P < .001$; Figs 2B and 2C). Because asparaginase dose and duration were correlated (R^2, 0.64; $P = .0011$; Data Supplement Figure 2), it was not possible to determine which was more important for pancreatitis risk.

Clinical Risk Factors

A multivariate model revealed that older age (hazard ratio [HR], 1.1 per year; $P < .001$) and genetically defined Native American ancestry (HR, 1.2 for every 10% increase in Native American ancestry; $P < .001$) were associated with pancreatitis (Table 1). High-dose asparaginase regimens ($\geq$ 240,000 U/m^2) were associated with more pancreatitis (HR, 3.2; $P < .001$). Patients who received PEG-asparaginase alone did not have significantly more pancreatitis than those who received the native formulation ($P = .11$), but formulation was not the only variable that differed among protocols. Because the number of Asian patients was relatively small (n = 99), we repeated the multivariate analysis (as shown in Table 1) excluding Asian ancestry, and the prognostic importance of the other variables did not change (data not shown). To minimize the effect of dose as a confounder for evaluating formulation, we looked at P9904/P9905, the only study that included patients treated with one or the other formulation (Data Supplement Table 3), and the crude percentage with pancreatitis did not differ between those who received native asparaginase alone (0.8%, six of 723), PEG-asparaginase alone (0.9%, three of 347), or both (0.6%, three of 535). There was a positive linear relationship between the percentage of Native American ancestry and the crude rate of pancreatitis (R^2, 0.70; $P = .0024$; Data Supplement Figure 3). The univariate analysis (Data Supplement Table 5) and the classification and regression tree analysis (Data Supplement Figure 4) also depict the same clinical risk factors. Thus we used age, ancestry, and treatment regimen as covariates in the cohort and ancestry in the case-control group.

Gene-Level Analysis of Nonsense Variants Identified CPA2

Focusing on rare variants and nonsense variants, six genes were associated with pancreatitis in the cohort at $P < .05$ and in the case-control group (Table 2). In the cohort, *CPA2*, with a nonsense SNP (rs199695765 C>T) showed the strongest association ($P = 2.1 \times 10^{-9}$). This SNP was predicted to cause early termination of the CPA2 protein (Fig 3A). Two of 3,469 patients carried this variant (one in the cohort and one in the case-control group), and both developed early pancreatitis (Fig 3B); one of them had recurrent episodes after each of two asparaginase re-exposures.

GWAS of Common SNPs

Analyzing all coding and noncoding common SNPs (minor allele frequency [MAF] $\geq$ 1%) in the cohort, we identified loci in a purine metabolism gene, *FHIT* (rs9849262; $P = 8.4 \times 10^{-6}$). Among the top 20 common SNPs, 14 were assigned to seven genes, and seven of the 14 SNPs were in three genes involved in cytoskeleton regulation, including *DOCK5*, *ACTN2*, and *MICAL2* (Data Supplement Table 6). These SNPs were also associated with pancreatitis in the case-control study, although some did not reach $P < .05$ in this relatively small group. Results for top-ranked variants from single SNP analyses, including nonsense, missense, synonymous, and noncoding SNPs, are shown in the Data Supplement Tables 7, 8, and 9.

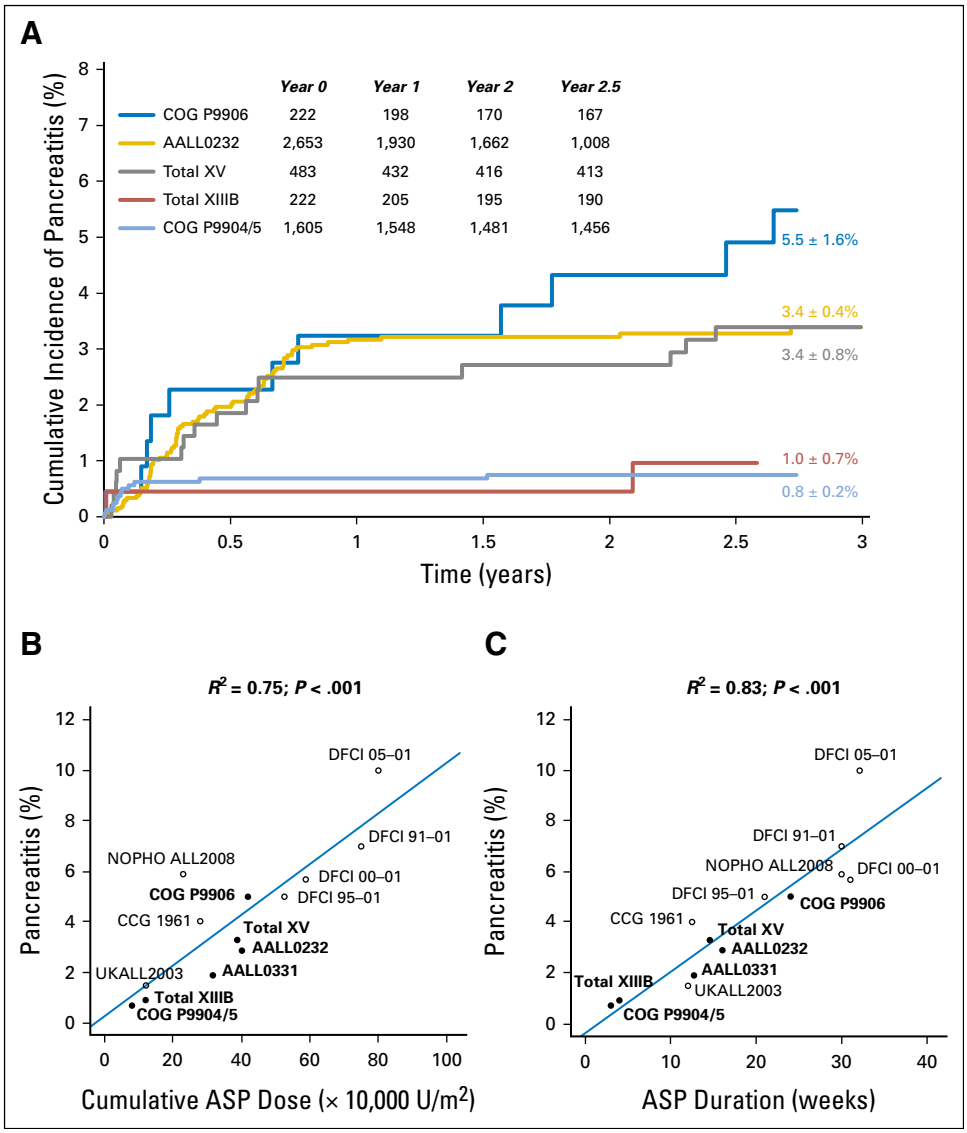


Fig 2. Incidence of pancreatitis differed by protocols. (A) Cumulative incidence estimates[29] of pancreatitis in the cohort (N = 5,185) accounting for competing risks and time at risk by different protocols. Time at risk was censored at therapy completion; failures other than pancreatitis, such as induction failure, relapse, secondary malignancy, or death, are considered as competing events. The inset table shows the number of patients at risk at 0, 1, 2, and 2.5 years into therapy. (B) The relationship between the crude rates of pancreatitis and protocol-planned dose of asparaginase (ASP), and (C) the relationship between the crude rates of pancreatitis and protocol-planned treatment duration of ASP in this study (solid circles) and in some recently reported protocols[2-4,6,7,33,34] (open circles). These rates do not account for competing events or time at risk and are not incidences. The individual points show the average protocol-planned dose or treatment duration of native *Escherichia coli*–ASP across all treatment arms for each protocol. PEG-ASP 2,500 U/m^2 every other week is considered equivalent to native *E coli*-ASP at 25,000 U/m^2 once per week for 2 weeks. The linear regression lines, R^2, and *P* values are shown. PEG-ASP, pegylated *Escherichia coli*–asparaginase.

Sequencing Detected Novel Variants in CPA2 *and Other Candidate Genes*

We identified additional variants by sequencing 283 genes, 42 of which were a priori classified as likely candidate genes for pancreatitis on the basis of our GWAS results (n = 34) or on prior links to pancreatitis in other settings (n = 8; *PRSS1*, *PRSS2*, *SPINK1*, *CFTR*, *CASR*, *CTRC*, *CPA1*, *CLDN2*; Data Supplement Table 4).

For the 4,217 children in the cohort and 162 in the case-control group with sequencing data, 380 variants were identified in *CPA2*, and 16 were associated with pancreatitis (Data Supplement Table 10). Thirteen of 24 (54%) patients who carried at least one of these variants developed pancreatitis. Interestingly, two of three patients who had three episodes carried one of these *CPA2* variants (rs199695765 and an intronic SNP chr7:129915817G>A). Patients carrying *CPA2* coding variants had more pancreatitis than those without any coding variants (HR, 2.4; 95% CI, 1.2 to 5.1; $P = .001$) after adjusting for clinical features (Fig 3B). For patients who carried the nonsense SNP rs199695765, the estimated increased risk was high (HR, 587; 95% CI, 66.8 to 5,166; $P = 9.0 \times 10^{-9}$). The positive and negative predictive values of rs199695765 were 100% (95% CI, 19.3% to 100%) and 95.6% (95% CI, 94.8% to 96.2%). Of 61,486 subjects in the Exome Aggregation Consortium (ExAC) data set,[35] 11 individuals were heterozygous for this variant, yielding an allele frequency in the general population of only 0.009% (Table 2).

Similar to the initial GWAS, we conducted a gene-level analysis of nonsense variants in the 42 putative pancreatitis genes versus the 241 nonpancreatitis genes (Data Supplement Table 11). On the basis of sequencing, a higher proportion of putative candidate pancreatitis genes had associations than the noncandidate genes at $P < .05$ (16 of 42 *v* five of 241; $P < .001$). In the gene-level analysis, *HOGA1* with a nonsense SNP (chr10:99361676 C>T, p.R255X) showed the strongest association in the cohort ($P = 6.4 \times 10^{-11}$). The only patient heterozygous for this nonsense variant developed pancreatitis early, at day 17. *CPA2* ranked second (for nonsense SNPs; $P = 6.7 \times 10^{-11}$) and remained significant when the gene-based analysis included any missense ($P = 5.5 \times 10^{-3}$) or any coding variants ($P = .045$).

Table 1. Multivariate Analysis for Factors Associated With Pancreatitis in the Cohort Study (N = 5,185)

Patient Characteristic	*P*	HR (95% CI)
Native American ancestry (for every 10% interval)	< .001	1.2 (1.1 to 1.3)
Age (per year)	< .001	1.1 (1.0 to 1.2)
High-dose ASP regimen*	< .001	3.2 (1.7 to 5.9)
African ancestry (for every 10% interval)	.059	1.1 (0.997 to 1.2)
Native *Escherichia coli*–ASP only	.11	1.4 (0.9 to 2.2)
T-cell lineage ALL	.28	1.7 (0.7 to 4.5)
Asian ancestry (for every 10% interval)	.55	1.03 (0.9 to 1.2)
Male sex	.70	1.1 (0.7 to 1.6)

NOTE. Association with risk of pancreatitis was assessed using the Fine-Gray regression model for competing risks. Age and ancestry were treated as continuous variables (eg, an HR of 1.2 suggests 1.2-fold higher risk of pancreatitis for every 10% increase in Native American ancestry). Other characteristic features are dichotomized for presence *v* absence of the indicated feature.

Abbreviations: ALL, acute lymphoblastic leukemia; ASP, asparaginase; HR, hazard ratio.

*Total protocol-specified *Escherichia coli*–ASP dose ≥ 240,000 U/m^2 (ie, all patients on Total XV, COG P9906, and AALL0232) compared with ≤ 120,000 U/m^2.

Among the eight candidate genes previously associated with pancreatitis in other settings, 1,732 coding polymorphisms were identified via sequencing (Data Supplement Table 12). Eighty-one SNPs were associated at the $P < .05$ level in either the cohort or the case-control group, but none of them were significant in both groups. A variant (chr7:117119516G>A) upstream of *CFTR* showed the strongest association in the cohort ($P = 4.4 \times 10^{-9}$); this variant was carried by only one patient, who developed early pancreatitis at day 25. None of these candidate genes were associated with pancreatitis in the gene-level analysis.

DISCUSSION

Acute pancreatitis is a serious complication of asparaginase and can limit the ability to deliver curative chemotherapy. The pathogenesis and mechanisms remain unknown, and it has not been possible to identify patients at risk a priori. There is no specific treatment of acute pancreatitis; only supportive care is given.[36] Thus, the genetic and nongenetic risk factors we discovered in this largest study to date may have importance for understanding the mechanisms and developing targeted interventions for patients with predisposition to pancreatitis.

Variants in *CPA2* (carboxypeptidase A2) were associated with risk of pancreatitis in patients with ALL. This gene encodes a 417–amino acid proenzyme (proCPA2).[37] The high-risk variant we identified is a nonsense SNP (rs199695765) in exon 2, resulting in early termination in the propeptide region (Fig 3A). Among 4,379 patients, the two who carried this variant developed severe pancreatitis within weeks of their first dose of asparaginase (Fig 3B), suggesting asparaginase will not be tolerated long enough to be effective in patients who inherit this rare variant. It is unclear whether the MAF (0.023%) in this ALL cohort was comparable to or higher than the MAF in the general population (0.009%), but in any case, it is rare. In addition to rs199695765, there were 15 other variants in *CPA2* associated with pancreatitis (Data Supplement Table 10). The 16 variants were carried by 24 of 4,379 patients (collective MAF, 0.6%), and all 10 patients who carried the top 10 *CPA2* variants developed pancreatitis. These data suggest that *CPA2* variant carriers are at high risk of pancreatitis if treated with asparaginase. ALL treatment regimens vary substantially in the amount of asparaginase they include; an ALL treatment regimen that does not depend heavily on asparaginase may be preferable for these rare individuals.

The underlying mechanism by which *CPA2* variants increase risk of pancreatitis is unclear. Pancreatic carboxypeptidases are secreted from acinar cells as proenzymes, then cleaved and activated by trypsin in the duodenum. Interestingly, patients heterozygous for functionally impaired variants in a related isoform, *CPA1*, are at higher risk of chronic pancreatitis.[38]

The top-ranked gene on the basis of sequencing of 283 pharmacogenomic candidate genes was *HOGA1*, which catalyzes the breakdown of hydroxyproline.[39] Only one patient of 4,379 studied carried the *HOGA1* nonsense variant. Reduced activity of HOGA1 may cause calcium oxalate urolithiasis[40]; perhaps a similar excess of oxalate could lead to pancreatitis, because pancreas is a potential site of hydroxyproline degradation.[41]

Although variants in *CPA2* and *HOGA1* were rare, the GWAS of common variants (Data Supplement Table 6) provided insights into other possible mechanisms. The lead gene *FHIT* is involved in purine metabolism.[39] Because thiopurines are essential to ALL therapy and can also induce acute pancreatitis,[11,12] this finding may link thiopurines to some cases of pancreatitis in our study. Other top genes (*DOCK5*, *ACTN2*, and *MICAL2*) clustered in cytoskeleton function, supporting the hypothesis that cytoskeleton disruption may be involved in pancreatitis.[42,43] Indeed cerulein, causing cytoskeleton disorganization, is used to initiate pancreatitis in animal models.[44]

In this study, SNPs in the genes associated with non–drug-induced pancreatitis were not as strongly associated as the variants discovered using a GWAS agnostic approach (Data Supplement Table 12). Using gene-based approaches, none of these eight genes were among those with $P < .05$ (Data Supplement Table 11). *HLA-DRB1*07:01* (tagged by rs17885382) has been associated with thiopurine-induced pancreatitis,[22] but it was not associated with pancreatitis in our study ($P = .1$). These findings suggest different mechanisms of asparaginase-induced versus other forms of pancreatitis. That asparaginase-induced pancreatitis seems dose dependent (Fig 2), whereas thiopurine-induced pancreatitis does not,[22] also suggests different pathogenesis caused by these two antileukemic drugs.

Consistent with previous reports,[1-3,15,45] older age was associated with pancreatitis in our study. Although steroids and thiopurines can cause pancreatitis, asparaginase is most likely the cause in the majority of our patients, because of its early onset (coinciding with asparaginase; Data Supplement Figure 1) and its relationship to asparaginase dose and duration[2-4,6,7,34,43] (Figs 2B and 2C). The association with genetically defined ancestry is consistent with an important contribution of genetics in the development of pancreatitis (Table 1; Data Supplement Figures 3 and 4); for example, risk alleles for 16 of the top 20 common variants (Data Supplement Table 6) were present at a higher frequency (at $P < .05$) in the Hispanic- and African-ancestry groups than in others.

In summary, we showed older age, higher exposure to asparaginase, and higher Native American ancestry were independent risk factors for pancreatitis during ALL treatment. Rare nonsynonymous variants in *CPA2* were highly penetrant, and more

Table 2. Six Genes Associated With Pancreatitis From the Gene-Level Analysis of Nonsense SNPs

Gene	Chr	Nonsense SNPs	Major Allele/Risk Allele	Population RAF% of Nonsense SNP (No.)*	Cohort (n = 3,256)				Case-Control (n = 213)				PPV/NPV§ (N = 3,469)
					RAF % in Patients With Pancreatitis (n = 95)	RAF % in Patients Without Pancreatitis (n = 3,161)	Coefficient† (SE)	*SKAT P*‡	RAF % in Cases (n = 71)	RAF % in Controls (n = 142)	Coefficient‡ (SE)	*SKAT P*‡	
CPA2	7	rs199695765	C/T	0.009 (11)	0.5	0	6.5 (1.1)	2.1×10^{-9}	0.8	0	15.8 (7.4)	.03	100/95.6
SERPINA9	14	rs78283108	G/A	0.6 (732)	2.7	0.7	1.2 (0.4)	.006	0.8	0	16.2 (6.7)	.02	11.5/95.6
HSD17B2	16	rs8191246	A/G	1.4 (1,557)	3.3	0.7	1.5 (0.5)	.006	3.4	0	17.1 (5.9)	.004	17.2/95.7
		rs200540657	T/G	0.001 (1)	0.1	0			0	0			0/94.8
GCKR	2	rs146053779	C/T	0.09 (111)	1.1	0.3	1.7 (0.7)	.015	0.8	0	15.4 (8.5)	.07	16.7/95.6
ZNF233	19	rs144014948	G/A	0.09 (112)	0.5	0.1	1.9 (0.8)	.019	0.8	0	15.5 (8.3)	.06	25.0/95.6
		rs148348903	T/A	0.09 (114)	1.1	0.1				0			20.0/95.6
SLC6A18	5	rs143864547	C/A	0.003 (4)	0.5	0.02	5.0 (2.3)	.028	0	0	16.0 (7.4)	.03	50.0/95.6
		rs200802505	C/T	0.03 (41)	0	0.08			0.8	0			20.0/95.5

NOTE. The genes were rank ordered by SKAT *P* value in the cohort study, and then filtered by the direction in the case-control study. Only patients with exome array data are included in this analysis of nonsense SNPs.
Abbreviations: chr, chromosome; NPV, negative predictive value; PPV, positive predictive value; RAF, risk allele frequency; SKAT, sequential kernel association test; SNP, single-nucleotide polymorphism.
*Population risk allele frequency and number of carriers of the nonsense SNP on the basis of the ExAC data set[35] of 61,486 unrelated individuals of European, African, Latino, and Asian origins.
†Coefficient, SE, and *P* value were estimated using Cox proportional hazard model after collapsing all nonsense SNPs.
‡Coefficient, SE, and *P* value were estimated using logistic regression model after collapsing all nonsense SNPs.
§PPV = (number of patients who carried the nonsense SNP and developed pancreatitis)/(number of patients who carried the nonsense SNP). NPV = (number of patients who did not carry the nonsense SNP or develop pancreatitis)/(number of patients who did not carry the nonsense SNP). The number of patients from the cohort and the case-control group were combined.

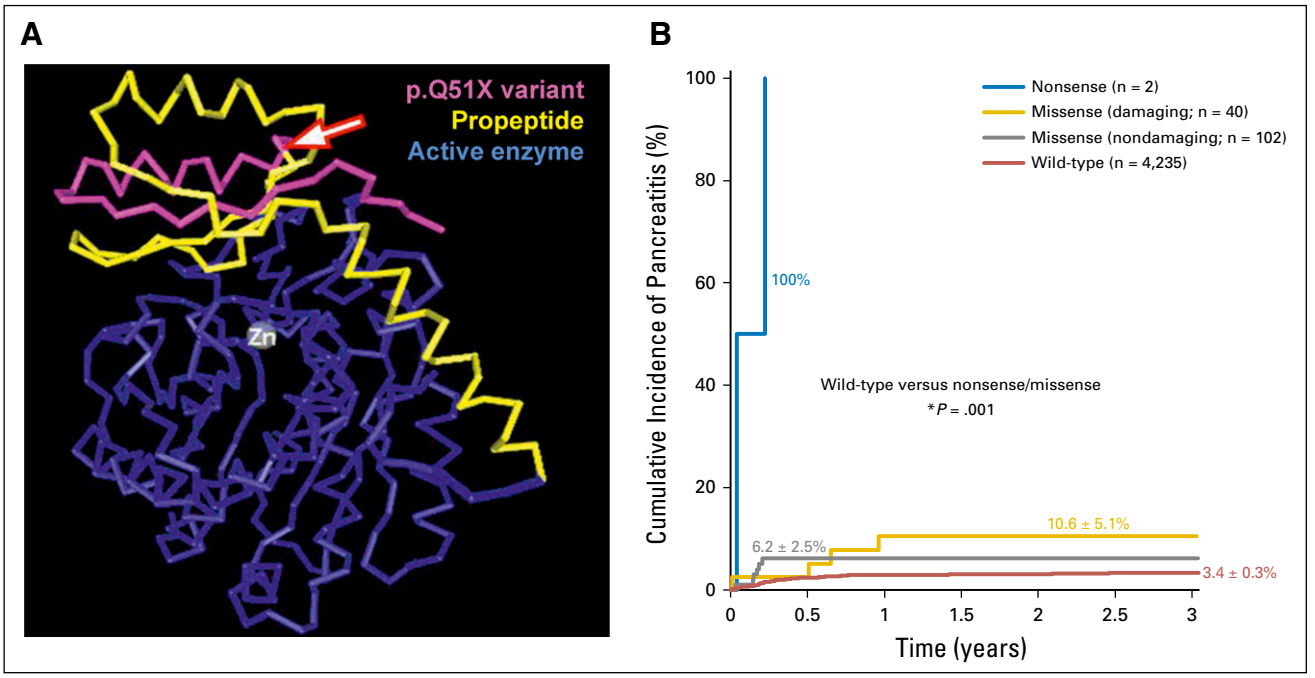


Fig 3. Nonsense single-nucleotide polymorphism (SNP) rs199695765 in *CPA2* associated with pancreatitis. (A) Protein structure of proCPA2 (without the 16–amino acid signal peptide) with the nonsense SNP rs199695765 (arrow) located in the propeptide domain. The unaffected region of p.Q51X variant (magenta), the affected propeptide (yellow), and the active enzyme moiety (blue) are shown. (B) Cumulative incidence of pancreatitis in patients in the cohort or in the case-control group who carried any *CPA2* nonsense or missense SNPs (variant) was higher than that in patients who are classified as wild type (ie, they carried no or only synonymous or noncoding SNPs). *P = .001 using the Fine-Gray competing risks regression after adjusting for clinical characteristics.

common variants in genes critical to purine metabolism and cytoskeleton function were also associated with development of pancreatitis. Our findings are consistent with a mixed genetic architecture underlying serious adverse drug effects, wherein a combination of rare but highly penetrant and common but weakly penetrant genetic risk factors contribute to genetic predisposition. For the small number of patients carrying the highly penetrant *CPA2* variants, a precision medicine approach using ALL regimens containing less asparaginase should be considered.

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Disclosures provided by the authors are available with this article at www.jco.org

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Clinical and Genetic Risk Factors for Acute Pancreatitis in Patients With Acute Lymphoblastic Leukemia

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