# Trypsin-encoding *PRSS1-PRSS2* variations influence the risk of asparaginase-associated pancreatitis in children with acute lymphoblastic leukemia: a Ponte di Legno toxicity working group report

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# Appendix

## Supplemental table 1

**Baseline data, acute complications and risk of re-exposure in 10 participating trial groups.**

| Trial group name | Trials | N | Male sex | Age (years) | B-cell precursor ALL | WBC at ALL diagnosis ($10^9$/L) | Time from ALL diagnosis to AAP (days) | Assisted ventilation (yes [n]/available data [N]) | Acute insulin therapy (yes [n]/available data [N]) | Pseudocysts (yes [n]/available data [N]) | Second AAP if re-exposed (yes [n]/ re-exposed patients [N]) | Death due to AAP (n) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Associazione Italiana Ematologia Oncologia Pediatrica** | AIEOP-BFM ALL 2009 | 18 | 61% (11/18) | 9.8 (4.3–13.1) | 94% (16/17) | 10.7 (4.3–28) (n=17) | 117 (43–215) (n=16) | 13% (2/15) | 13% (2/15) | 21% (3/14) | 0% (0/1) | 2 |
| **Berlin-Frankfurt-Münster Austria** | AIEOP-BFM ALL 2009 | 7 | 86% (6/7) | 15.1 (10.4–16.5) | 71% (5/7) | 11.6 (6.5–15) (n=7) | 55 (45–123) (n=7) | 0% (0/6) | 0% (0/7) | 14% (1/7) | 33% (1/3) | 0 |
| **Berlin-Frankfurt-Münster** | ALL-BFM 2000 AIEOP-BFM ALL 2009 | 49 | 55% (27/49) | 9.7 (5.4–14.7) | 76% (37/49) | 12.1 (3.4–23.8) (n=49) | 37 (30–161) (n=49) | 8% (4/49) | 17% (8/48) | 20% (9/44) | 25% (2/8) | 1 |
| **The Cooperative ALL Study Group** | CoALL 97/08-09 | 4 | 50% (2/4) | 10.1 (8–12) | 100% (4/4) | 4.1 (3.1–5.7) (n=4) | 231 (159–273) (n=3) | 0% (0/1) | 0% (0/1) | 0% (0/1) | 0% (0/2) | 0 |
| **Czech Republic** | ALL-BFM 2000 ALL-IC-BFM 2002 AIEOP-BFM ALL 2009 | 4 | 0% (0/4) | 7.1 (2.5–12.3) | 100% (4/4) | 25.5 (4.8–98.8) (n=4) | 149.5 (72–228) (n=4) | 0% (0/4) | 0% (0/4) | 25% (1/4) | 0% (0/1) | 0 |
| **Dutch Childhood Oncology Group** | DCOG-ALL10/11 | 25 | 44% (11/25) | 8.2 (5.2–13.6) | 80% (20/25) | 8.1 (3.8–31.2) (n=24) | 203 (46–295) (n=25) | 12% (3/25) | 30% (7/23) | 28% (7/25) | 50% (2/4) | 1 |
| **Israel** | INS 2010 AIEOP-BFM ALL 2009 | 10 | 70% (7/10) | 11.2 (8.1–15.3) | 56% (5/9) | 41 (6.3–104) (n=9) | 38 (31–113) (n=9) | 0% (0/9) | 22% (2/9) | 33% (3/9) | 0% (0/1) | 0 |
| **Nordic Society of Pediatric Haematology and Oncology** | NOPHO ALL 2008 | 92 | 55% (51/92) | 6.3 (3.4–10.9) | 86% (65/76) | 20.6 (6.5–50) (n=76) | 110 (75–144) (n=76) | 4% (3/76) | 22% (17/76) | 28% (21/76) | 53% (10/19) | 0 |
| **Taiwan Pediatric Oncology Group** | TPOG-ALL-97/2002/2013 | 5 | 60% (3/5) | 6.9 (6.6–8.2) | 100% (5/5) | 26 (1.2–79.8) (n=5) | 34 (31–150) (n=5) | 0% (0/5) | 50% (2/4) | 40% (2/5) | 50% (1/2) | 0 |
| **United Kingdom ALL Working Party** | UKALL2003 | 30 | 50% (15/30) | 9.4 (4.7–13.3) | 83% (24/29) | 7.3 (3–41.1) (n=30) | 146 (82–225) (n=30) | 0% (0/30) | 100% (3/3) | 28% (8/29) | 20% (1/5) | 0 |
| **10 Groups** | **14 Trials** | **244** | **55% (133/244)** | **8.1 years (4.3—13.1)** | **82% (185/225)** | **15.3 (4–41) (n=225)** | **99.5 (41–182) (n=224)** | **5% (12/220)** | **22% (41/190)** | **26% (55/214)** | **37% (17/46)** | **1·8% (4/224)** |

**Legend**

Baseline data, acute complications and risk of re-exposure in 10 participating trial groups. Data are n, median (IQR), %(n/N), unless indicated otherwise.

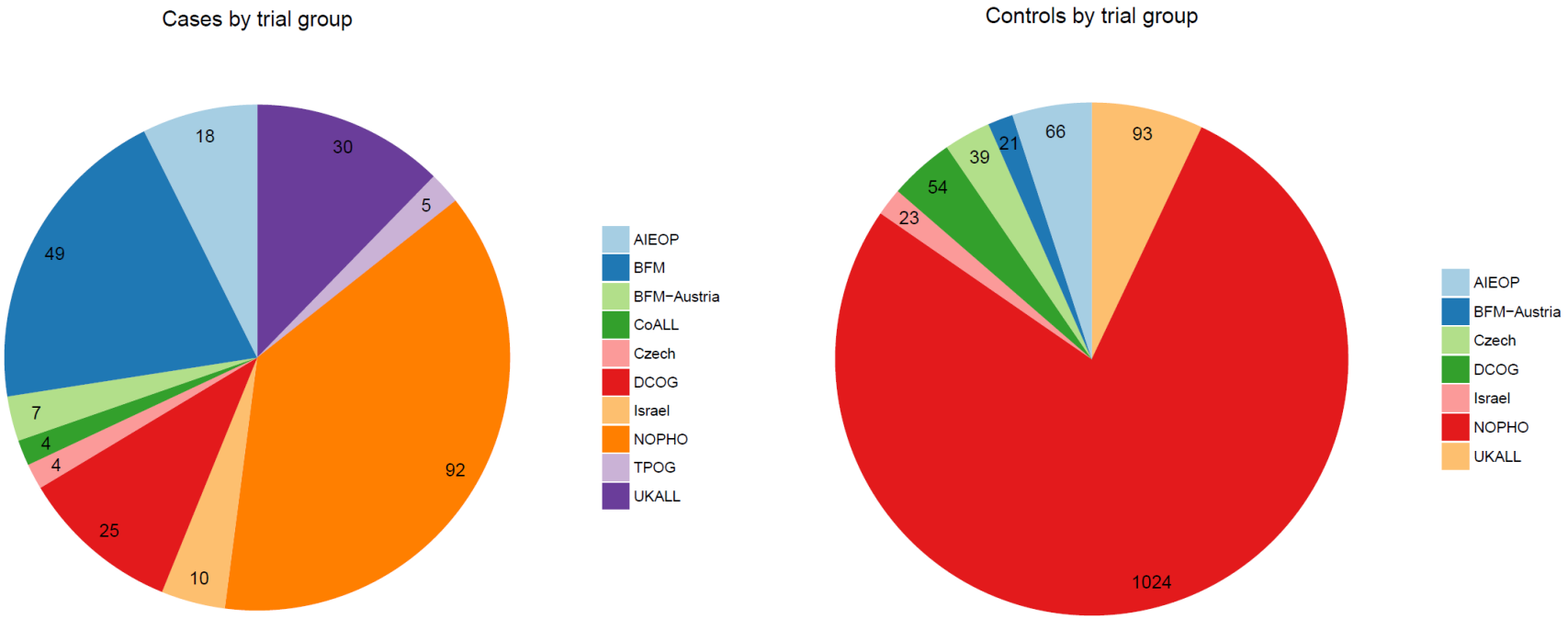
*Number of re-exposed patients with data on second AAP.

Note: BCP refers to percentage with B-cell precursor acute lymphoblastic leukemia, the remaining group had T-cell leukemia.

Abbreviations: AAP, asparaginase-associated pancreatitis; IQR, interquartile range ($25^{th}$/$75^{th}$ centiles); BCP ALL, B-cell precursor acute lymphoblastic leukemia; WBC, white blood cell count.

**Supplemental figure 1**

**Pie charts showing the distribtution of cases and controls according to trialgroup**

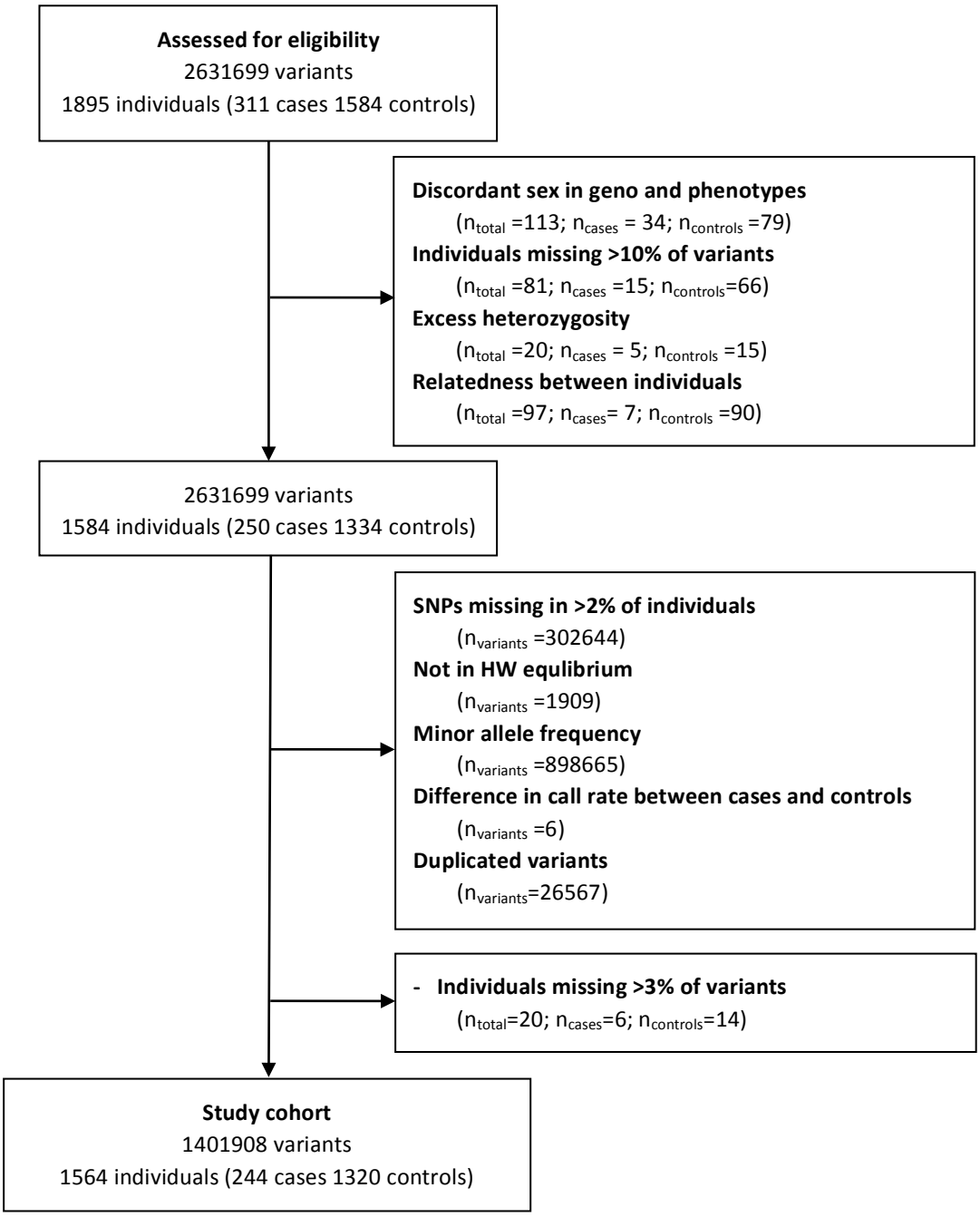



**Legend**

Pie charts showing the distribution of 244 AAP cases and 1320 controls according to childhood acute lymphoblastic leukemia trial group. Abbreviations: AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; BFM, Berlin-Frankfurt-Münster (Germany) ; BFM-Austria, Berlin-Frankfurt-Münster Austria; CoALL, The Cooperative ALL Study Group (Germany); Czech, Czech Working Group for Paediatric Haematology; DCOG, Dutch Childhood Oncology Group; DFCI, Dana Farber Cancer Institute (US); Israel, Israeli childhood cancer group; NOPHO, Nordic Society of Pediatric Haematology and Oncology (Denmark, Estonia, Finland, Iceland, Lithuania, Norway, Sweden); TOPG, Taiwan Pediatric Oncology Group; UKALL, United Kingdom ALL Working Party.

**Supplemental figure 2**

**Quality control flowchart**

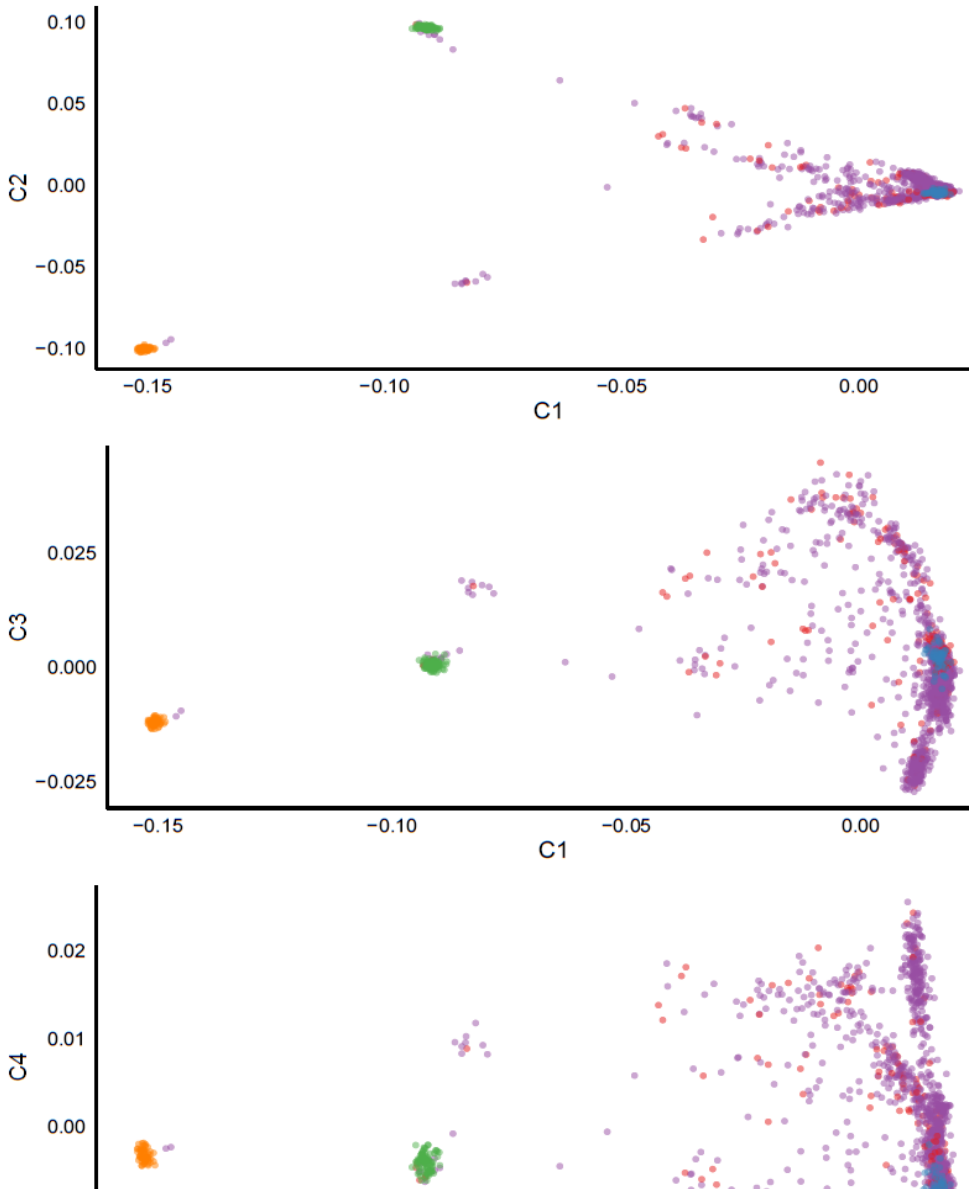



**Legend**

Flow diagram illustrating the quality control of individual and single nucleotide polymorphism data.

**Supplemental figure 3**

**Multidimensional scaling plots of genetic ancestry in study population**

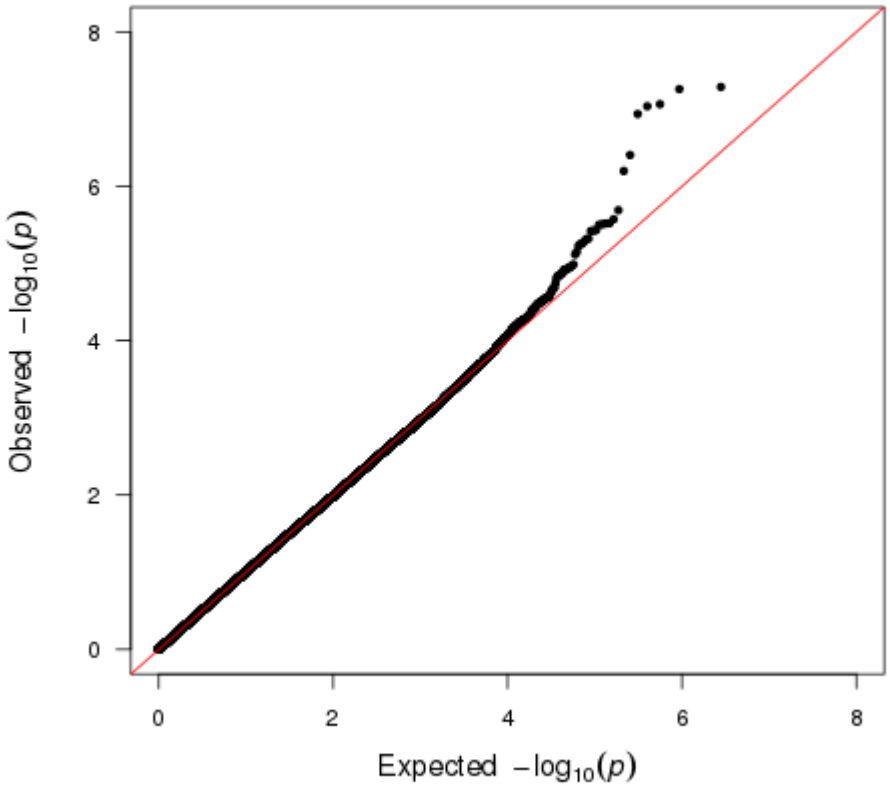



**Legend**

Multidimensional scaling plots plot of genetic ancestry of AAP cases, controls and HAPMAP controls for reference. Abbreviations: CEU, Utah residents with Northern and Western European ancestry; CHB, Han Chinese in Beijing, China; JPT, Japanese in Tokyo, Japan; YRI, Yoruba in Ibadan, Nigeria.

**Supplemental figure 4**

**Quartile-quartile plot**




**Legend**

Quartile-quartile plot showing the expected *vs* the observed p-values in the cohort of 244 cases and 1320 controls. The logistic regression analysis was adjusted for age and genetic ancestry, with a lambda of 1.02 there was no evidence of population substructure.

**Description of validation cohort**

**Replication of results in the Children's Oncology Group AALL0232 cohort**

All data is provided from Liu *et al*. *"Clinical and Genetic Risk Factors for Acute Pancreatitis in Patients With Acute Lymphoblastic Leukemia*"; Journal of Clinical Oncology 2016**.**
The AALL0232 trial was used for validation. This cohort constitutes the largest cohort, with significant duration of asparaginase treatment (~16 weeks) previously published (Liu et al. Journal of Clinical Oncology, 2016). The trial included 3058 patients, out of which genotyping data was available in 76 cases diagnosed using National Cancer Institute's Common Terminology Criteria for Adverse Events, and 2577 controls. The cohort was included, in the only other GWAS published on pancreatitis in children with ALL[1]. The AALL0232 cohort was genotyped on the Affymetrix Genome-Wide Human SNP 6.0 (or GeneChip Human Mapping 500K) Array, and linkage disequilibrium (LD) between SNPs in the investigation and validation study was assessed by the National Cancer Institute LDassoc tool.

**Diagnostic criteria for pancreatitis**

National Cancer Institute's Common Terminology Criteria for Adverse Events for Pancreatitis (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf) grade 2-4 were included as cases in the analysis:

| Adverse Event | Short Name | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 |
|---|---|---|---|---|---|---|
| Pancreatitis | Pancreatitis | Asymptomatic, enzyme elevation and/or radiographic findings | Symptomatic, medical intervention indicated | Interventional radiology or operative intervention indicated | Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis) | Death |

**Asparaginase exposure in protocol AALL0232**

| Induction | Extended Induction[a] | Consolidation | Interim Maintenance and Delayed Intensification | Total ASP dose[b] U/m$^2$ (excluding Extended induction) | Total ASP weeks[c] (excluding Extended induction) | N (%) of patients developing pancreatitis |
|---|---|---|---|---|---|---|
| PEG 2500 U/m$^2$ x1 | PEG 2500 U/m$^2$ x1 | PEG 2500 U/m$^2$ × 2 | DH/PH: PEG 2500 U/m$^2$ × 2 (single IM/DI) or PEG 2500 U/m$^2$ × 6 (double IM/DI) | 250.000–4500000 | 10–18 | 32/1359 (2.4%) |
| | | | DC/PC: PEG 2500 U/m$^2$ × 4 (single IM/DI) or × 6 (double IM/DI) | 350.000–550,000 | 14–22 | 44/1294 (3.4%) |

All asparaginase was given intramuscularly. [a]Depends on patients' bone marrow blast percent during induction or minimal residual disease (MRD) status at the end of Induction. [b]Total dose and [c]total weeks of *PEG*-asparaginase during therapy, excluded the extended Induction. Abbreviations: ASP, asparaginase; PEG, pegylated; DH/PH, dexamethasone/prednisone during induction therapy and high-dose methotrexate/leucovorin during the first interim maintenance; DC/PC, dexamethasone/prednisone during induction therapy and escalating methotrexate/leucovorin during the first interim maintenance.

**Baseline data in 2653 patients included in the AALL0232 genome-wide association study cohort**

| | ALL0232 (n=2653) |
|---|---|
| **Age** | |
| 1−10 years old | 907 (34.2%) |
| 10−30 years old | 1746 (65.8%) |
| **Gender** | |
| Male | 1468 (55.3%) |
| Female | 1185 (44.7%) |
| **Race** | |
| White | 1455 (54.8%) |
| Black | 134 (5.1%) |
| Hispanic | 710 (26.8%) |
| Asian | 60 (2.3%) |
| Other | 294 (11.1%) |
| **Immunophenotype** | |
| B-lineage | 2653 (100%) |
| T-lineage | 0 (0%) |

**Association analysis**

Time-dependent analysis (Cox proportional hazards regression) was performed adjusting for age and ancestry.

| AALL0232 (high asparaginase dose) All patients. N=2653, 76 cases | | | |
|---|---|---|---|
| | **Major>minor allele** | **HR (95% CI)** | ***P*** |
| **rs13228878** | A>G | 0.68 (0.48–0.96) | 0.03 |
| **rs10273639** | C>T | 0.69 (0.49–0.98) | 0.04 |

**Legend**

The table shows results from the replication study.

**Genotypes**

| **rs13228878** | | | |
|---|---|---|---|
| | CC | CT | TT |
| Controls | 585 | 1227 | 765 |
| Cases with pancreatitis | 11 | 35 | 30 |
| % pan | 0·02 | 0·03 | 0·04 |

**Legend**

Genotype of rs13228878. The table shows the rs13228878 genotype in cases with pancreatitis and controls without pancreatitis.

**Genotypes**

| **rs10273639** | | | |
|---|---|---|---|
| | CC | CT | TT |
| Controls | 561 | 1253 | 763 |
| Cases with pancreatitis | 11 | 35 | 30 |
| % pan | 0·02 | 0·03 | 0·04 |

**Legend**

Genotype of rs10273639. The table shows the rs10273639 genotype in cases with pancreatitis and controls without pancreatitis.

**Replication of results in the Dana Farber Cancer Institute consortium 87–01, 91–01, 95–01 and 00–01 ALL cohort**

A cohort of ALL children recruited from the Dana Farber Cancer Institute (DFCI) were used for the validation of the rs62228256 polymorphism on 20q13.2. Patients were treated according to DFCI ALL Consortium protocols DFCI 87-01, 91-01, 95-01, or 00-01 between January, 1987 and July, 2005 receiving variable asparaginase exposure[2–4]. This cohort is further referred to as DFCI. Genotyping was performed on a total of 318 patients, out of which 33 were cases of pancreatitis diagnosed according to the CTCAE criteria and 285 were controls. Cases were identified by retrospective evaluation of medical charts. This cohort was genotyped by allele-specific oligonucleotide hybridization as described elsewhere.[5] Briefly, while in DFCI 95-01 and DFCI 00-01, one dose of asparaginase was administered during remission induction, asparaginase was administered for 20 to 30 consecutive weeks during consolidation phase in all protocols.

**Asparaginase exposure in Dana Farber Cancer Institute protocols**

| Asparaginase according to protocol | |
|---|---|
| ***Induction (4 weeks)*** | |
| **Protocol 87-01** | *E. coli*, *Erwinia* or PEG ASP × 1 dose (randomized; investigational window; 5 days pre-day 0) |
| **Protocol 91-01** | None |
| **Protocol 95-01** | *E. coli* or *Erwinia* ASP 25 000 IU/$m^2$ × 1 dose (randomized; day 4) |
| **Protocol 00-01** | E. coli ASP 25,000 IU/m2 IM × 1 dose |
| ***Intensification (20–30 weeks) every 3-week cycle*** | |
| **Protocol 87-01** | *E. coli* ASP 25 000 IU/$m^2$ weekly |
| **Protocol 91-01** | Randomized to *E. coli* ASP 25 000 IU/$m^2$ weekly or PEG ASP 2500 IU/$m^2$ every 2 weeks |
| **Protocol 95-01** | Randomized to *E. coli* ASP 25 000 IU/$m^2$ weekly or *Erwinia* ASP 25 000 IU/$m^2$ weekly |
| **Protocol 00-01** | Randomized to fixed dosing of *E. coli* ASP (based upon BSA) and individualized dosing (based upon NSAA every 3 weeks) |

Abbreviations: ASP, asparaginase; PEG, pegylated; BSA, body surface area; NSAA, nadir serum asparaginase activity.

**Baseline data in 318 patients included in the Dana Farber Cancer Institute cohort**

| | Dana Farber Cancer Institute Cohort (n=318) |
|---|---|
| **Age** | |
| 1−10 years old | 254 (79,9%) |
| 10−18 years old | 64 (20,1%) |
| **Gender** | |
| Male | 167 (52,5%) |
| Female | 151 (47,5%) |
| **Immunophenotype** | |
| B-lineage | 294 (92.5%) |
| T-lineage | 24 (7.5%) |
| **Source of ASP** | |
| Native *E. Coli* | 289 (90,9%) |
| *Erwinia* | 29 (9,1%) |
| **DFCI protocol** | |
| 00-01 | 125 (39,3%) |
| 95-01 | 122 (38,4%) |
| 91-01 | 55 (17,3%) |
| 87-01 | 16 (5%) |

## Association analysis

Binary logistic regression analysis was performed adjusting for age.

| | DFCI ALL cohort. N=318, 33 cases | | |
|---|---|---|---|
| | **Major>minor allele** | **OR (95% CI)** | **P** |
| **rs62228256** | C>T | 1.19 (0.35–4.03) | 0.77 |

## Legend

The table shows results from the replication study of rs62228256.

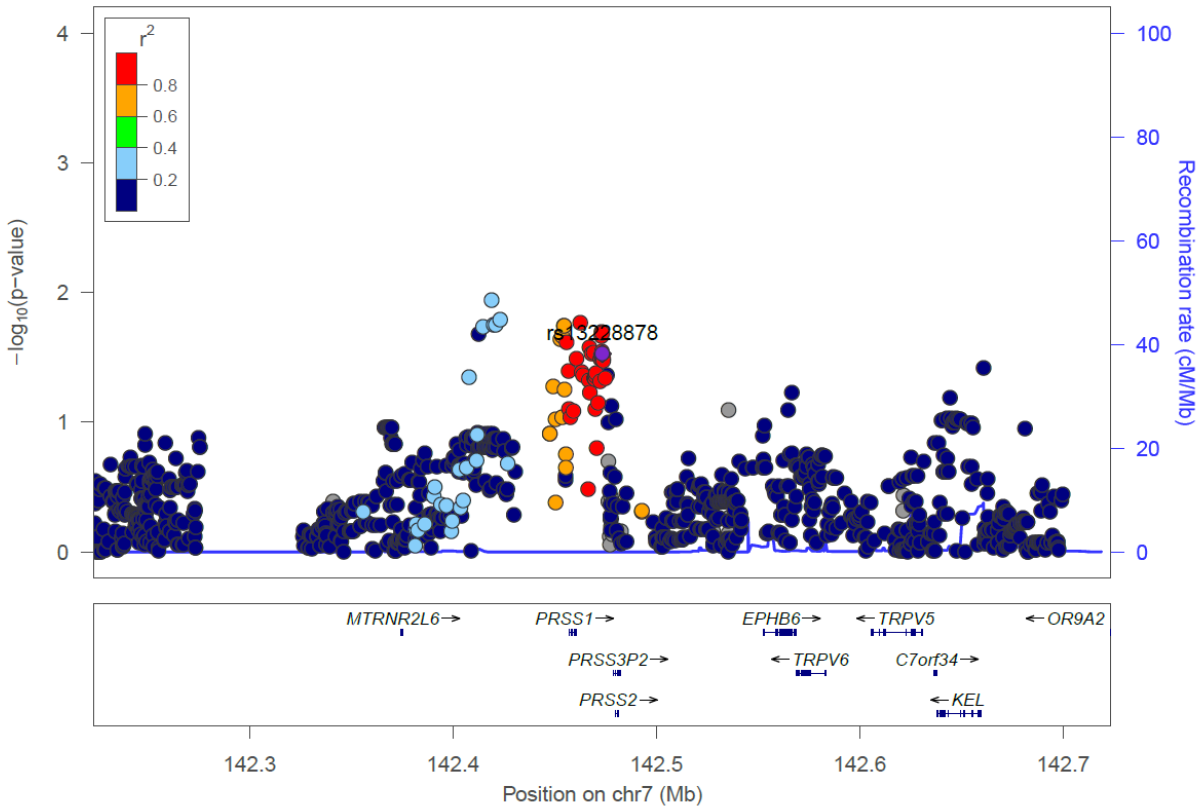
**Genotypes**

| | rs62228256 | | |
|---|---|---|---|
| | CC | CT | TT |
| Controls | 264 | 20 | 1 |
| Cases with pancreatitis | 30 | 3 | 0 |
| % pan | 0·10 | 0·13 | 0 |

**Legend**

Genotype of rs62228256. The table shows the rs62228256 genotype in cases with pancreatitis and controls without pancreatitis.

**Regional association plot of the *PRSS1* and *PRSS2* loci on chromosome 7 in AALL0232**

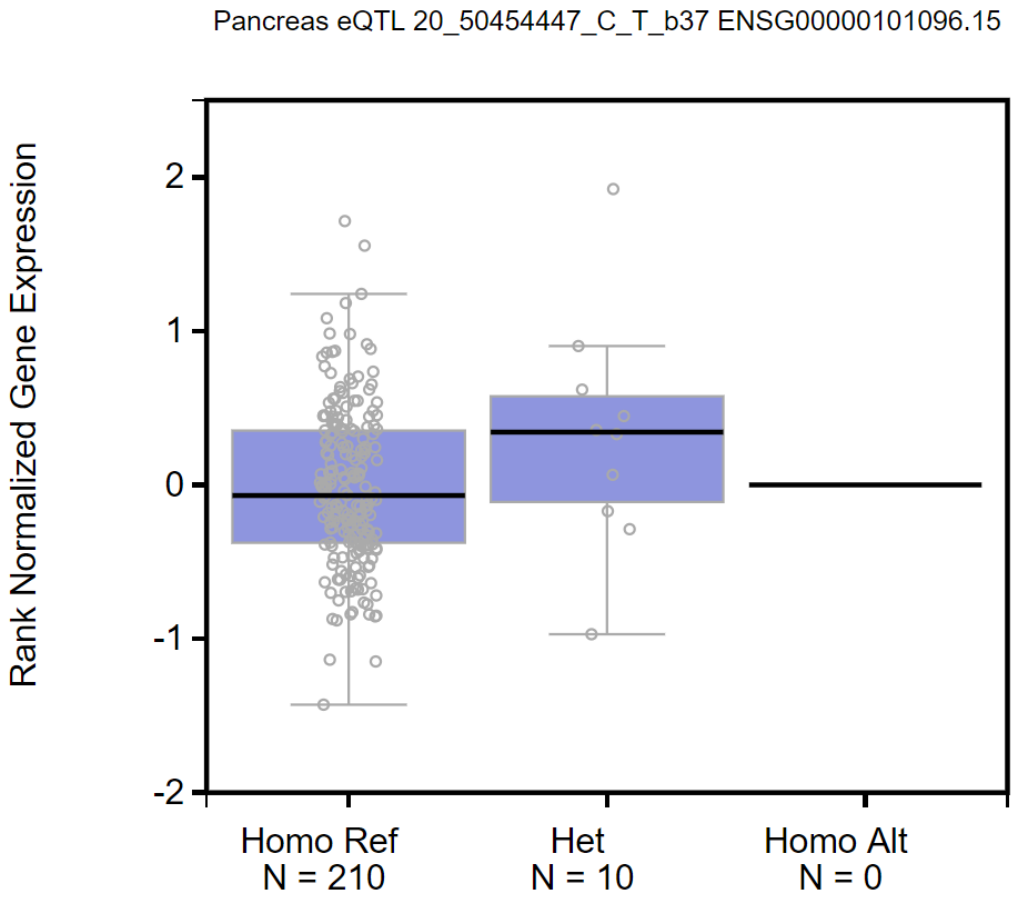



**Legend**

Regional asssociation plot showing SNPs associated with asparaginase-associated pancreatitis in 76 cases and 2570 controls. The *x* axis represents genomic location, and the *y* axis represents the *P* value for the SNP's association calculated using logistic regression adjusting for age and ancestry. The color of the dots reflects the linkage disequilibrium of the genotyped SNPs and rs13228878. LD is based on 1000 genomes European CEU samples, November 2014. The human assembly *GRCh37* was used for reference.

**Supplemental figure 5**

**Rs62228256 and Nuclear factor of activated T cells (*NFATC2)***

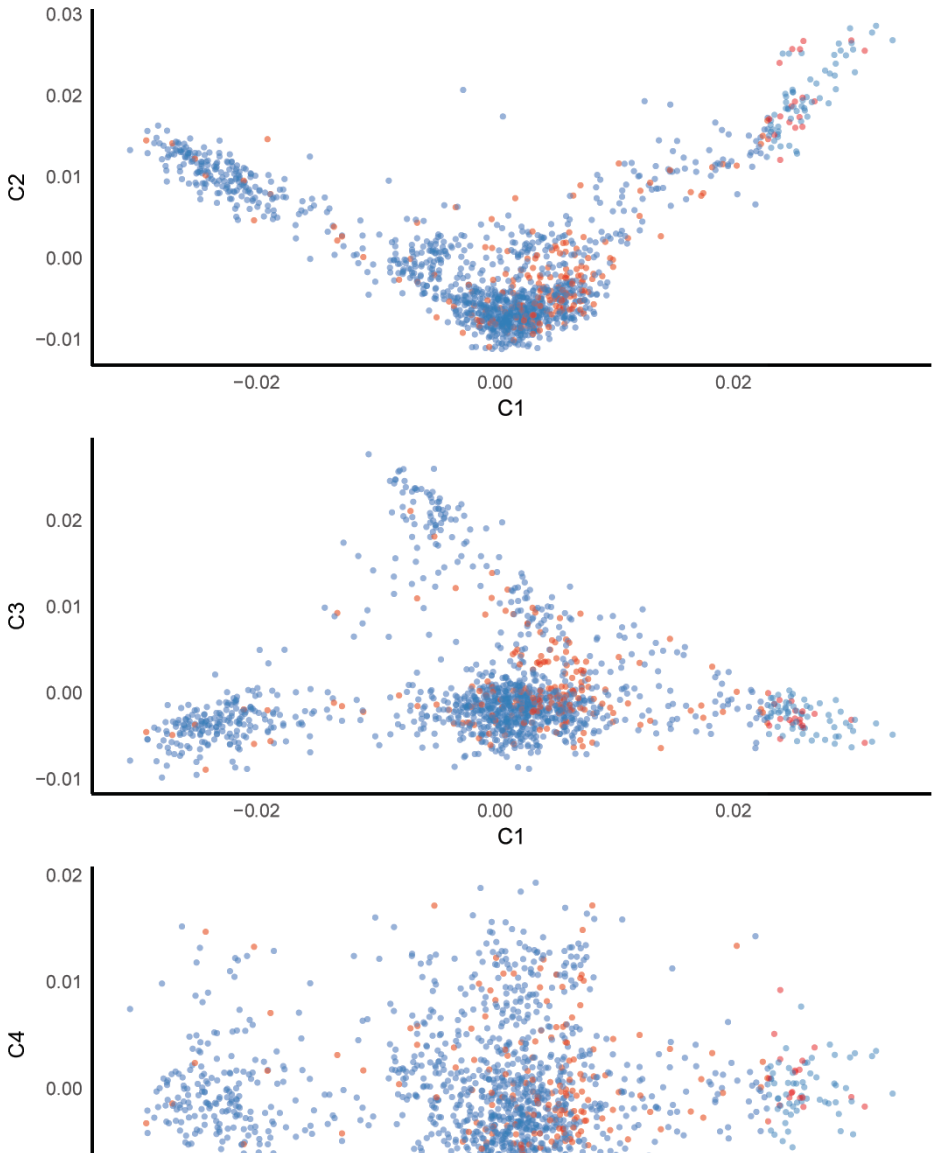



**Legend**

*NFATC2* gene expression according to rs62228256 genotype. Homo Ref refers to the major C allele, Homo Alt refers to the minor T allele. The difference between groups is statistically significant, *P*=0.045. Plot is downloaded from www.gtexportal.org/home/.

**Supplemental figure 6**

**Multidimensional scaling plots of genetic ancestry from CEU cohort**

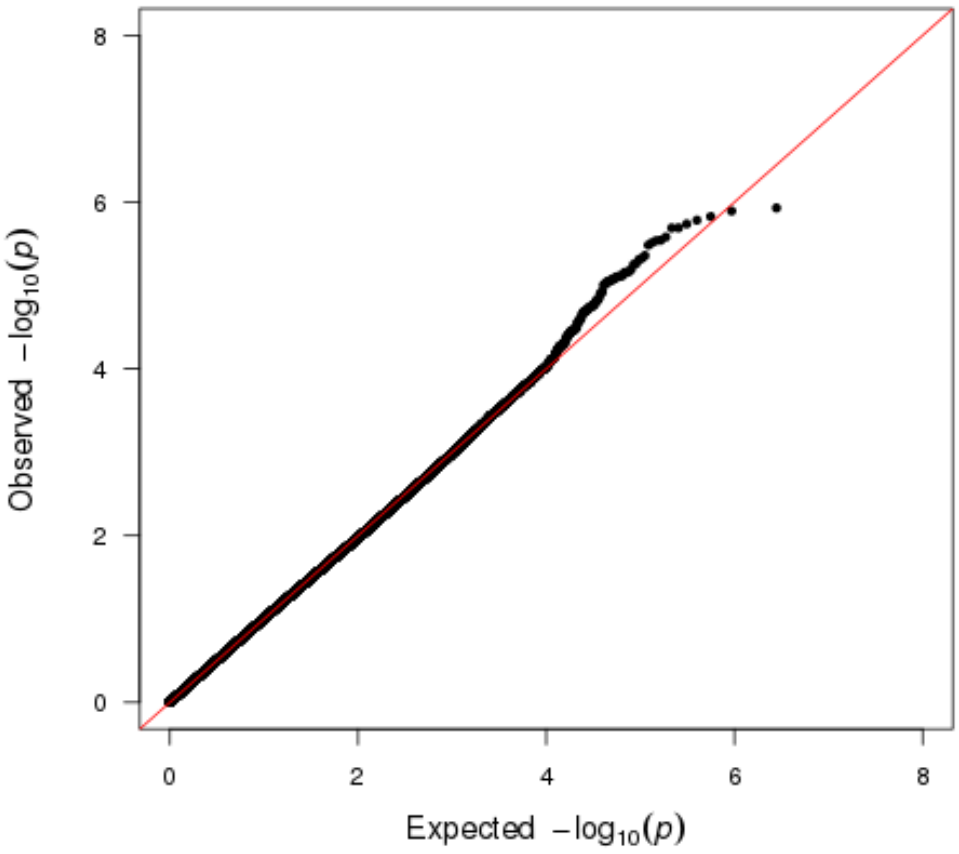



**Legend**

Multidimensional scaling plots plot of genetic ancestry in AAP cases and controls from the CEU population. Defined as individuals >16 standard deviations away from the HapMap-defined CEU (Northern and Western European) centroid mean.

**Supplemental figure 7**

**Quartile-quartile plot of CEU cohort**

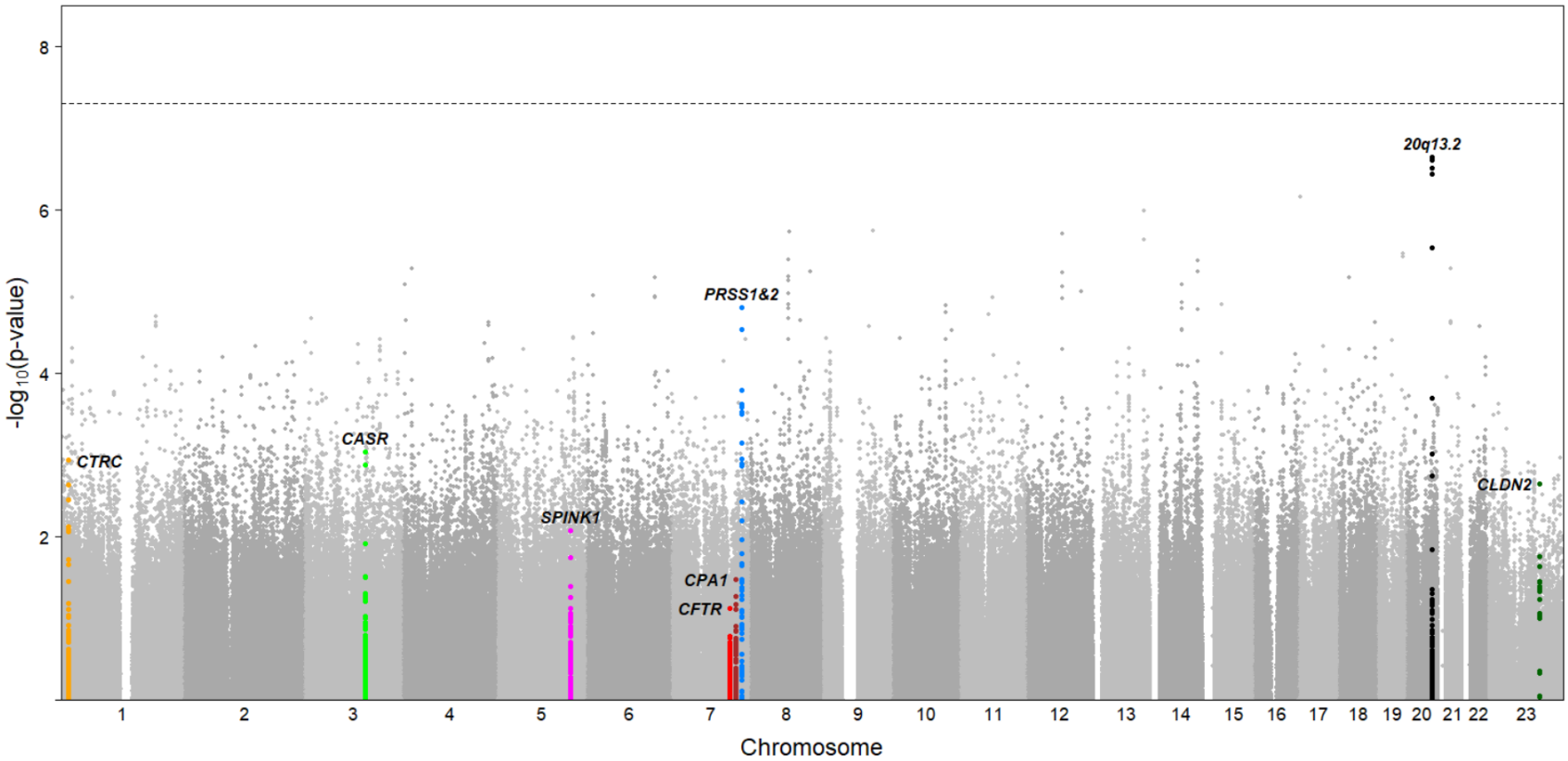



**Legend**

Quartile-quartile plot showing the expected *vs* the observed p-values in the CEU cohort of 205 cases and 1185 controls. The logistic regression analysis was adjusted for age and genetic ancestry, with a lambda of 1.02 there was no evidence of population substructure.

**Supplemental figure 8**

**Manhattanplot of CEU cohort**




**Legend**

Manhattanplot showing SNPs associated with asparaginase-associated pancreatitis in 205 cases and 1185 controls in the CEU cohort. The *x* axis represents genomic location, and the *y* axis represents the *P* value for the SNP's association calculated using logistic regression adjusting for age and ancestry. Genes previously associated to pancreatitis are marked in color. SNPs are annotated to genes based on genomic location (10 kb

**Supplemental table 2**

**Top SNPs in genes previously associated with risk of pancreatitis in CEU cohort**

| Gene | CHR | BP | SNP | Major>minor allele | MAF Cases | MAF Controls | OR (95%CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| ***CASR*** | 3 | 121913370 | rs937627 | G>T | 0.17 | 0.23 | 0.73 (0.55–0.97) | 0.03 |
| *CASR* | 3 | 121908434 | rs4678029 | T>C | 0.17 | 0.23 | 0.73 (0.55–0.97) | 0.03 |
| *CASR* | 3 | 121919740 | rs16832787 | G>A | 0.17 | 0.23 | 0.73 (0.55–0.97) | 0.03 |
| *CASR* | 3 | 121936323 | rs13320637 | G>A | 0.22 | 0.27 | 0.76 (0.59–0.98) | 0.04 |
| *CASR* | 3 | 121936370 | rs13327652 | A>G | 0.22 | 0.27 | 0.76 (0.59–0.99) | 0.04 |
| ***CFTR*** | 7 | 117145102 | rs56296320 | T>C | 0.007 | 0.02 | 0.34 (0.11–1.11) | 0.07 |
| *CFTR* | 7 | 117178754 | rs17449197 | A>G | 0.11 | 0.14 | 0.79 (0.57–1.09) | 0.15 |
| *CFTR* | 7 | 117119183 | rs4148682 | T>G | 0.1 | 0.08 | 1.26 (0.88–1.82) | 0.21 |
| *CFTR* | 7 | 117181509 | rs4148703 | G>A | 0.1 | 0.09 | 1.26 (0.87–1.81) | 0.22 |
| *CFTR* | 7 | 117219835 | rs1469486 | C>T | 0.13 | 0.12 | 1.22 (0.88–1.68) | 0.23 |
| ***CLDN2*** | 23 | 106134938 | rs12853674 | C>T | 0.12 | 0.08 | 1.67 (1.11–2.53) | 0.01 |
| *CLDN2* | 23 | 106140325 | rs4409525 | G>A | 0.34 | 0.28 | 1.4 (1.06–1.83) | 0.02 |
| *CLDN2* | 23 | 106160702 | rs12008279 | A>G | 0.51 | 0.44 | 1.31 (1.02–1.69) | 0.03 |
| *CLDN2* | 23 | 106183670 | rs12014762 | C>T | 0.22 | 0.17 | 1.32 (0.97–1.8) | 0.07 |
| *CLDN2* | 23 | 106136910 | rs5962770 | T>C | 0.28 | 0.3 | 0.98 (0.74–1.3) | 0.87 |
| ***CPA1*** | 7 | 130037805 | rs73152870 | A>G | 0.039 | 0.02 | 1.67 (0.92–3.01) | 0.09 |
| *CPA1* | 7 | 130018863 | rs10954269 | C>T | 0.09 | 0.1 | 0.77 (0.53–1.12) | 0.18 |
| *CPA1* | 7 | 130033556 | rs17389898 | T>C | 0.07 | 0.05 | 1.36 (0.87–2.12) | 0.18 |
| *CPA1* | 7 | 130019491 | rs13226219 | T>C | 0.09 | 0.1 | 0.79 (0.54–1.15) | 0.21 |
| *CPA1* | 7 | 130028089 | rs17330508 | C>T | 0.037 | 0.03 | 1.3 (0.71–2.36) | 0.39 |
| ***CTRB1-2*** | 16 | 75254970 | rs57833904 | C>T | 0.015 | 0.005 | 2.56 (0.92–7.16) | 0.07 |
| *CTRB1-2* | 16 | 75230638 | rs1019537 | C>T | 0.13 | 0.15 | 0.74 (0.54–1.03) | 0.07 |
| *CTRB1-2* | 16 | 75230739 | rs1019539 | C>T | 0.12 | 0.15 | 0.74 (0.54–1.03) | 0.08 |
| *CTRB1-2* | 16 | 75230230 | rs1559362 | T>C | 0.32 | 0.34 | 0.85 (0.67–1.07) | 0.16 |
| *CTRB1-2* | 16 | 75263661 | rs7190458 | G>A | 0.06 | 0.05 | 1.39 (0.87–2.24) | 0.17 |
| ***CTRC*** | 1 | 15758963 | rs35994710 | C>T | 0.16 | 0.23 | 0.63 (0.47–0.84) | 0.002 |
| *CTRC* | 1 | 15768304 | rs10436957 | G>A | 0.17 | 0.24 | 0.65 (0.49–0.86) | 0.003 |
| *CTRC* | 1 | 15757666 | rs10754889 | G>A | 0.30 | 0.36 | 0.71 (0.56–0.9) | 0.004 |
| *CTRC* | 1 | 15760092 | rs7541863 | C>T | 0.31 | 0.37 | 0.71 (0.56–0.9) | 0.005 |
| *CTRC* | 1 | 15763340 | rs6693417 | A>G | 0.33 | 0.4 | 0.72 (0.57–0.91) | 0.005 |
| ***PRSS1-2*** | 7 | 142473466 | rs13228878 | A>G | 0.31 | 0.42 | 0.6 (0.48–0.76) | $2.1 \times 10^{-5}$ |
| *PRSS1-2* | 7 | 142456928 | rs10273639 | C>T | 0.32 | 0.43 | 0.62 (0.49–0.78) | $3.8 \times 10^{-5}$ |
| *PRSS1-2* | 7 | 142487836 | rs2734222 | C>T | 0.38 | 0.47 | 0.66 (0.53–0.82) | 0.0002 |
| *PRSS1-2* | 7 | 142455538 | rs3757377 | G>A | 0.3 | 0.39 | 0.65(0.52–0.83) | 0.0004 |
| *PRSS1-2* | 7 | 142488688 | rs2734224 | G>A | 0.39 | 0.47 | 0.7(0.56–0.87) | 0.001 |
| ***SPINK1*** | 5 | 147207678 | rs17107315 | A>G | 0.02 | 0.01 | 2.78(1.29–5.97) | 0.009 |
| *SPINK1* | 5 | 147220041 | rs4705045 | T>G | 0.11 | 0.14 | 0.73(0.52–1.02) | 0.07 |
| *SPINK1* | 5 | 147211393 | rs4705203 | A>G | 0.12 | 0.14 | 0.78(0.56–1.08) | 0.13 |
| *SPINK1* | 5 | 147204192 | rs11319 | C>T | 0.05 | 0.05 | 1.35(0.82–2.2) | 0.23 |
| *SPINK1* | 5 | 147205839 | rs17703305 | G>T | 0.46 | 0.43 | 1.13(0.91–1.41) | 0.27 |

**Legend**

Top-five SNPs associated with asparaginase-associated pancreatitis in the CEU cohort of 205 cases and 1185 controls. SNPs were annotated to genes if ≤10kb upstream or downstream from transcription start site or transcription terminator, respectively. Genefunctions below are defined by Genecards (www.genecards.org) and UniPort (www.uniprot.org).

Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; *CASR*, calcium-sensing receptor (G protein-coupled receptor, small changes in circulating calcium concentration are monitored affecting intracellular signaling pathways); CFTR, cystic fibrosis transmembrane conductance regulator (cAMP-regulated cell channel, conducting chloride and thiocyanate ions across epithelial membranes); CLDN2, claudin-2 (Major integral membrane protein localized exclusively at tight junctions); CPA1: Carboxypeptidase A1 (Member of the serine protease family, A1 form of the pancreatic procarboxypeptidase produced in pancreatic acinar cells, preferentially cleaves C-terminal branched-chain and aromatic amino acids from dietary proteins); CTRB1-2m, chymotrypsin B1-B2 (member of the serine protease family of enzymes, regulating activation and degradation of trypsinogens and procarboxypeptidases by targeting specific cleavage sites within their zymogen precursors); CTRC: chymotrypsin C (serum calcium-decreasing factor with chymotrypsin-like protease activity, regulating activation and degradation of trypsinogens and procarboxypeptidases by targeting specific cleavage sites within their zymogen precursors); PRSS1-2, protease, serine, 2 (encoding for cationic and anionic trypsinogen); SPINK1, serine peptidase inhibitor, kazal type 1 (Prevention of trypsin-catalyzed premature activation of zymogens within the pancreas and the pancreatic duct).

**Supplemental table 3**

**Top SNPs in genes previously associated with risk of pancreatitis in the total cohort**

| Gene | CHR | BP | SNP | Major>minor allele | MAF Cases | MAF Controls | OR (95% CI) | *P* |
|---|---|---|---|---|---|---|---|---|
| ***CASR*** | 3 | 121965199 | rs9859381 | G>T | 0.18 | 0.13 | 1.3 (0.99-1.71) | 0.06 |
| *CASR* | 3 | 121913370 | rs937627 | G>T | 0.19 | 0.23 | 0.78 (0.61-1.01) | 0.063 |
| *CASR* | 3 | 121919740 | rs16832787 | G>A | 0.19 | 0.23 | 0.78 (0.61-1.01) | 0.064 |
| *CASR* | 3 | 121908434 | rs4678029 | T>C | 0.19 | 0.23 | 0.79 (0.61-1.02) | 0.067 |
| *CASR* | 3 | 121971512 | rs937625 | T>G | 0.11 | 0.08 | 1.37 (0.98-1.92) | 0.068 |
| ***CFTR*** | 7 | 117145102 | rs56296320 | T>C | 0.01 | 0.02 | 0.3 (0.09-0.97) | 0.044 |
| *CFTR* | 7 | 117178754 | rs17449197 | A>G | 0.11 | 0.14 | 0.77 (0.56-1.04) | 0.09 |
| *CFTR* | 7 | 117181509 | rs4148703 | G>A | 0.12 | 0.09 | 1.3 (0.94-1.79) | 0.11 |
| *CFTR* | 7 | 117119183 | rs4148682 | T>G | 0.12 | 0.09 | 1.28 (0.92-1.76) | 0.14 |
| *CFTR* | 7 | 117279147 | rs6466615 | G>A | 0.01 | 0.01 | 0.4 (0.12-1.39) | 0.15 |
| ***CLDN2*** | X | 106134938 | rs12853674 | C>T | 0.11 | 0.08 | 1.71 (1.16-2.52) | 0.007 |
| *CLDN2* | X | 106140325 | rs4409525 | G>A | 0.34 | 0.28 | 1.35 (1.05-1.73) | 0.02 |
| *CLDN2* | X | 106160702 | rs12008279 | A>G | 0.53 | 0.45 | 1.28 (1.01-1.62) | 0.04 |
| *CLDN2* | X | 106183670 | rs12014762 | C>T | 0.21 | 0.17 | 1.3 (0.97-1.74) | 0.08 |
| *CLDN2* | X | 106136910 | rs5962770 | T>C | 0.26 | 0.29 | 0.99 (0.76-1.3) | 0.1 |
| ***CPA1*** | 7 | 130037805 | rs73152870 | A>G | 0.04 | 0.02 | 1.88 (1.08-3.27) | 0.03 |
| *CPA1* | 7 | 130028089 | rs17330508 | C>T | 0.04 | 0.03 | 1.59 (0.92-2.74) | 0.1 |
| *CPA1* | 7 | 130018863 | rs10954269 | C>T | 0.09 | 0.10 | 0.75 (0.53-1.06) | 0.1 |
| *CPA1* | 7 | 130019491 | rs13226219 | T>C | 0.10 | 0.11 | 0.77 (0.55-1.09) | 0.14 |
| *CPA1* | 7 | 130033556 | rs17389898 | T>C | 0.07 | 0.05 | 1.36 (0.89-2.06) | 0.16 |
| ***CTRB1-2*** | 16 | 75252306 | rs8056797 | G>T | 0.03 | 0.01 | 2.63 (1.22-5.69) | 0.01 |
| *CTRB1-2* | 16 | 75254970 | rs57833904 | C>T | 0.03 | 0.01 | 2.46 (1.17-5.16) | 0.02 |
| *CTRB1-2* | 16 | 75230638 | rs1019537 | C>T | 0.12 | 0.14 | 0.74 (0.54-1) | 0.05 |
| *CTRB1-2* | 16 | 75230739 | rs1019539 | C>T | 0.12 | 0.14 | 0.75 (0.55-1.01) | 0.06 |
| *CTRB1-2* | 16 | 75263661 | rs7190458 | G>A | 0.06 | 0.04 | 1.45 (0.93-2.26) | 0.1 |
| ***CTRC*** | 1 | 15768304 | rs10436957 | G>A | 0.17 | 0.23 | 0.69 (0.53-0.89) | 0.005 |
| *CTRC* | 1 | 15758963 | rs35994710 | C>T | 0.16 | 0.22 | 0.68 (0.52-0.89) | 0.005 |
| *CTRC* | 1 | 15760092 | rs7541863 | C>T | 0.33 | 0.38 | 0.76 (0.61-0.94) | 0.01 |
| *CTRC* | 1 | 15763340 | rs6693417 | A>G | 0.35 | 0.41 | 0.77 (0.62-0.95) | 0.01 |
| *CTRC* | 1 | 15757666 | rs10754889 | G>A | 0.32 | 0.37 | 0.77 (0.62-0.96) | 0.02 |
| ***PRSS1-2*** | 7 | 142473466 | rs13228878 | A>G | 0.35 | 0.44 | 0.61 (0.5-0.76) | $7.1 \times 10^{-6}$ |
| *PRSS1-2* | 7 | 142456928 | rs10273639 | C>T | 0.35 | 0.44 | 0.62 (0.5-0.77) | $1.1 \times 10^{-5}$ |
| *PRSS1-2* | 7 | 142487836 | rs2734222 | C>T | 0.40 | 0.48 | 0.65 (0.53-0.8) | $4.8 \times 10^{-5}$ |
| *PRSS1-2* | 7 | 142488688 | rs2734224 | G>A | 0.41 | 0.49 | 0.68 (0.55-0.83) | 0.0002 |
| *PRSS1-2* | 7 | 142455538 | rs3757377 | G>A | 0.33 | 0.40 | 0.67 (0.54-0.83) | 0.0002 |
| ***SPINK1*** | 5 | 147207678 | rs17107315 | A>G | 0.02 | 0.01 | 2.86 (1.4-5.85) | 0.004 |
| *SPINK1* | 5 | 147220041 | rs4705045 | T>G | 0.12 | 0.14 | 0.73 (0.54-1) | 0.05 |
| *SPINK1* | 5 | 147215120 | rs4705204 | T>G | 0.23 | 0.18 | 1.23 (0.96-1.57) | 0.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *SPINK1* | 5 | 147211393 | rs4705203 | A>G | 0.13 | 0.15 | 0.78 (0.58-1.06) | 0.11 |
| *SPINK1* | 5 | 147205839 | rs17703305 | G>T | 0.46 | 0.42 | 1.18 (0.96-1.44) | 0.12 |

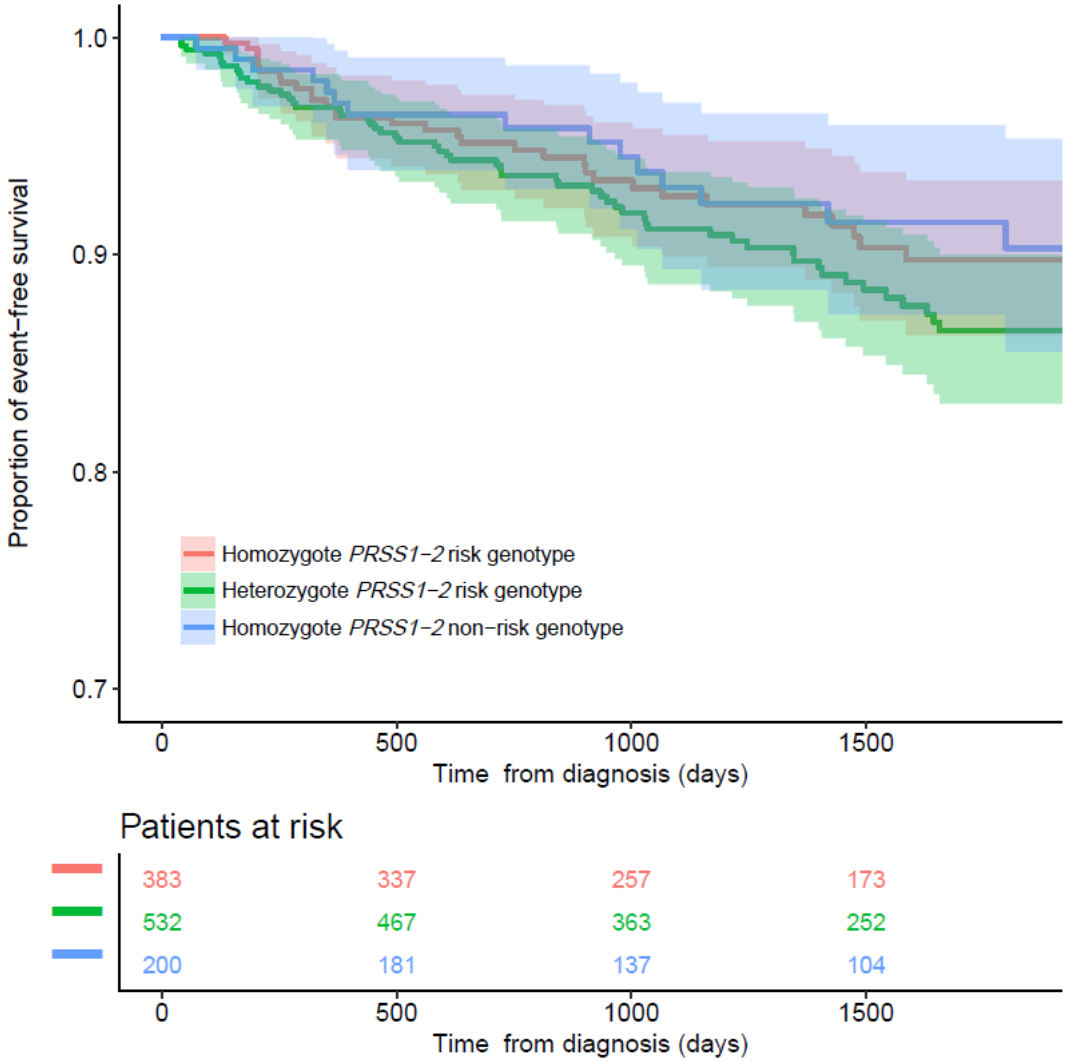
**Legend**

Top-five SNPs associated with asparaginase-associated pancreatitis in 244 cases and 1320 controls. SNPs were annotated to genes if ≤10kb upstream or downstream from transcription start site or transcription terminator, respectively. SNPs and genes were identified from litterature search (see methods section), genefunctions are defined by Genecards (www.genecards.org) and UniPort (www.uniprot.org).

Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; *CASR*, calcium-sensing receptor (G protein-coupled receptor, small changes in circulating calcium concentration are monitored affecting intracellular signaling pathways); *CFTR*, cystic fibrosis transmembrane conductance regulator (cAMP-regulated cell channel, conducting chloride and thiocyanate ions across epithelial membranes); *CLDN2*, claudin-2 (Major integral membrane protein localized exclusively at tight junctions); *CPA1*: Carboxypeptidase A1 (Member of the serine protease family, A1 form of the pancreatic procarboxypeptidase produced in pancreatic acinar cells, preferentially cleaves C-terminal branched-chain and aromatic amino acids from dietary proteins); *CTRB1-2*, chymotrypsin B1-B2 (member of the serine protease family of enzymes, regulating activation and degradation of trypsinogens and procarboxypeptidases by targeting specific cleavage sites within their zymogen precursors); *CTRC*: chymotrypsin C (serum calcium-decreasing factor with chymotrypsin-like protease activity, regulating activation and degradation of trypsinogens and procarboxypeptidases by targeting specific cleavage sites within their zymogen precursors); *PRSS1-2*, protease, serine, 2 (encoding for cationic and anionic trypsinogen); *SPINK1*, serine peptidase inhibitor, kazal type 1 (Prevention of trypsin-catalyzed premature activation of zymogens within the pancreas and the pancreatic duct).

**Supplemental figure 9**

**5-year event free survival according to *PRSS1-PRSS2* genotype**




**Legend**

Five-year event free survival in the Nordic subset of cases (n=92) and controls (n=1024) grouped according to PRSS1-2 genotype (rs13228878). Event was defined as death, relapse or second malignant neoplasm. No difference in five-years event-free survival was found using the 2-sided log-rank test (*P*=0.4). See through colors denote 95% confidence intervals.