Common variants in glyoxalase I do not increase chronic pancreatitis risk

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Abstract

Introduction

Chronic pancreatitis (CP) may be caused by oxidative stress. An important source of reactive oxygen species (ROS) is the methylglyoxal-derived formation of advanced glycation endproducts (AGE). Methylglyoxal is detoxified by Glyoxalase I (GLO1). A reduction in GLO1 activity results in increased ROS. Single nucleotide polymorphisms (SNPs) of GLO1 have been linked to various inflammatory diseases. Here, we analyzed whether common GLO1 variants are associated with alcoholic (ACP) and non-alcoholic CP (NACP).
Methods

Using melting curve analysis, we genotyped a screening cohort of 223 ACP, 218 NACP patients, and 328 controls for 11 tagging SNPs defined by the SNPinfo LD TAG SNP Selection tool and the functionally relevant variant rs4746. For selected variants the cohorts were extended to up to 1,441 patient samples.

Results

In the ACP cohort, comparison of genotypes for rs1937780 between patients and controls displayed an ambiguous result in the screening cohort (p = 0.08). However, in the extended cohort of 1,441 patients no statistically significant association was found for the comparison of genotypes (p = 0.11), nor in logistic regression analysis (p = 0.214, OR 1.072, 95% CI 0.961–1.196). In the NACP screening cohort SNPs rs937662, rs1699012, and rs4746 displayed an ambiguous result when patients were compared to controls in the recessive or dominant model (p = 0.08, 0.08, and 0.07, respectively). Again, these associations were not confirmed in the extended cohorts (rs937662, dominant model: p = 0.07, logistic regression: p = 0.07, OR 1.207, 95% CI 0.985–1.480) or in the replication cohorts for rs4746 (Germany, p = 0.42, OR 1.080, 95% CI 0.673–1.24; France, p = 0.19, OR 0.90, 95% CI 0.76–1.06; China, p = 0.24, OR 1.18, 95% CI 0.90–1.54) and rs1699012 (Germany, Munich; p = 0.279, OR 0.903, 95% CI 0.750–1.087).

Conclusions

Common GLO1 variants do not increase chronic pancreatitis risk.

Introduction

Chronic pancreatitis (CP) is a recurring inflammation of the pancreas with progressive fibrosis by tissue destruction that in some patients results in exocrine and endocrine pancreatic insufficiency [1]. Several studies have identified that the underlying pathomechanisms can range from premature intrapancreatic activation of proteases to local and systemic inflammatory processes, which are relevant for the initiation and progression of the disease [2]. Recently, it was demonstrated that oxidative stress (ROS) is involved in these inflammatory and fibrotic processes [3–5]. As advanced-glycation-end products (AGE) impact on ROS, they may contribute to CP development [6] as it was shown in acute pancreatitis [7].

“Dicarbonyl stress” indicates a cellular condition where α-oxoaldehyde metabolites accumulate, leading to an increased modification of protein and DNA which contribute to cellular dysfunction in ageing and disease. “Dicarbonyl stress” is mainly caused by methylglyoxal (MGO) that is formed as a by-product in glycolysis [8], ketone body metabolism and threonine catabolism [9–11]. MGO is highly reactive with nucleotides, phospholipids and proteins [12,13] with the result of a rapid formation of AGE. In addition, reducing sugars like glucose react with amino groups and trigger MGO formation and AGE generation in a non-enzymatic protein glycation within the Maillard reaction [6]. AGE themselves induce several detrimental processes on a cellular level and furthermore activate different signaling pathways via the RAGE receptor. Moreover, they induce ROS and have been associated with various disease entities [14].
For protection on the cellular level MGO is detoxified by the Glyoxalase system. Glyoxalase I (GLO1) catalyzes the conversion of α-oxo-aldehydes such as MGO and L-glutathione (GSH) to form the corresponding hemithioacetal S-D-lactoylglutathione [15]. In the next step, hydroxyacyl glutathione hydrolase (GLO2) converts S-D-Lactoylglutathione to D-lactate and GSH. Herein, GLO1 is the rate-limiting enzyme in this series of reactions [16].

Thus far, GLO1 single nucleotide polymorphisms (SNPs) were associated with distinct inflammatory diseases. The rs4746 (p.Ala111Glu) variant displayed a decrease of GLO1 enzymatic activity for the A-allele in lymphoblastoid cells of the brain [17,18]. In addition, rs4746 has been linked to diabetes [19], atherosclerosis [20], chronic renal failure [21], vascular diseases [22,23], neuropsychiatric disorders [24,25], and different cancer types [26–29]. Moreover, rs1130534 (c.372A>T, p.Gly124 = ) and rs1049346 were correlated with lower enzyme activity, but did not associate with vascular complications in diabetes mellitus [30].

In conclusion, GLO1 variants with a diminished GLO1 activity cause increased MGO levels and consecutive ROS generation. Therefore, we reasoned that GLO1 SNPs contribute to the development of CP and investigated whether genetic variants in GLO1 are associated with alcoholic CP (ACP) or non-alcoholic CP (NACP).

**Material and methods**

**Patients and controls**

The study was approved by the medical ethical review committee of the Martin-Luther-University of Halle-Wittenberg (Medical ethical committee, University Halle-Wittenberg, Medical Faculty, Bearbeitungsnummer 2015–106, date: 22.01.2016, title: “Erforschung molekulargenetischer Ursachen von Pankreaserkrankungen”). All patients gave written informed consent. The diagnosis of CP was based on two or more of the following findings: history of recurrent acute pancreatitis or recurrent or persisting abdominal pain typical for CP, pancreatic calcifications and/or pancreatic ductal irregularities indicated by computed tomography imaging, magnetic resonance imaging, endoscopic retrograde pancreatography or (endo)sonography of the pancreas and/or the diagnosis of exocrine pancreatic insufficiency [31].

ACP was diagnosed in patients with a history of chronic alcohol intake (> 80 g per day for males or >60 g per day for females) for more than 2 years. NACP was diagnosed in the absence of known precipitating factors as alcohol consumption and/or smoking. Patients with a positive family history were included in the NACP group. The data on past ethanol consumption and the clinical presentation were based on research records and/or physician’s history and/or completion of a detailed questionnaire by the patient.

Patients and Controls were recruited throughout Germany and in the European centres in The Netherlands, Romania, Poland, Italy, and Hungary. Controls were blood donors and healthy volunteers as described in our former publications [32].

In the screening cohort we investigated 223 ACP, 218 NACP patients, and 328 controls for 12 common GLO1 SNPs with a minor allele frequency of a least 5%. Variants with nominal significance according to uncorrected p-values (rs1699012, rs937662, rs4746, and rs1937780) were analyzed in further subjects (extended cohorts). In addition, we screened European ACP cohorts for rs1937780 and an additional German, French, and Chinese NACP cohort for rs4746 and an independent German NACP cohort for rs1699012 (see flow chart in Fig 1). For a detailed description of the screening cohort and the extended cohorts see Table 1.

**Selection of tagging SNPs in the GLO1 locus**

We selected 11 tagging SNPs in the GLO1 locus using the SNPinfo LD TAG SNP Selection tool (LD map Figure A in S1 File). For this purpose we used an LD threshold of 0.8, a
minimum of one SNP tagged, a minimum of 5 valid genotypes to calculate LD in populations with European ancestry (CEU) and extended the region of interest by 10,000 bp in the 5’-region and the 3’-region. In addition, we analyzed SNP rs4746 that was described to be functionally relevant [17,18]. The other functionally relevant SNPs rs1049346 and rs1130534 were tagged by rs1621788 and rs13212218, respectively. Details on the selected SNPs are summarized in Table A in S1 File.

**DNA extraction and SNP genotyping**

DNA was isolated from EDTA blood using a commercial system (QIAamp Blood DNA Mini Kit; Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was conducted using One-Taq® 2X Master Mix (NEB) with 200 μM dNTPs, 1.8 mM MgCl₂ and 0.1 μM forward primer as well as 0.1 μM reverse primer (0.4 μM forward primer for rs3778443 and rs17544798; 0.2 μM reverse primer for rs4746) in a total volume of 25 μl. Cycle conditions were an initial denaturation at 95°C for 5 minutes followed by 45 cycles of 20 seconds denaturation at 95°C,

### Table A

| SNP       | Extended cohorts | Replication cohorts |
|-----------|------------------|---------------------|
| rs937662  | In total: 430 patients 621 controls | In total: # 450 patients 581 controls |
| rs1699012 | In total: 447 patients 608 controls | In total: 767 patients 1049 controls |
| rs1937780 | In total: 1441 patients* 2306 controls* | In total: 581 controls |
| rs4746    | In total: 288 patients 581 controls | In total: 928 patients 1008 controls |

Extended cohorts = screening cohort and additional patients

*European patients: see Supplementary Table 4; the German cohort includes the screening cohort

# Additional German cohort, ¤ French cohort, § Chinese cohort

Extended German cohort (n = 306) and independent replication cohort (Munich, n = 427)

**Fig 1. Flowchart of patients analyzed in this study.** Note that the extended cohorts comprise the screening cohort and novel patients for all German patients (SNPs rs937662, rs1937780, and rs4746). For rs1699012 the numbers summarize the extended with the replication cohort from Munich. The European cohorts (rs1937780) and the German, French, and Chinese replication cohorts for rs4746 are additional patients.

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40 seconds annealing (Table B in S1 File), 90 seconds primer extension at 72°C followed by final extension for 5 minutes at 72°C in an automated thermal cycler. Primers and probes (Table B in S1 File) were synthesized by TIB Molbiol (Berlin, Germany). Genotyping was performed using the LightCycler480® system (Roche Diagnostics).

Probe oligomers were diluted in H₂O to a concentration of 200 nM. For genotyping we used the PCR products from standard PCR (see above) with 50 nM (final) of probe oligomers followed by melting curve analysis with the following protocol: 95°C for 60 seconds, 40°C for 60 seconds, continuous increase to 70°C with various ramp rates (0.29°C/s rs12198212 and rs1621788, 0.14°C/s rs17544798, rs132212218 and rs937662, 0.19°C/s for the other seven SNPs). Call rates for all SNPs were > 95%. For quality control 2.6% of all samples were genotyped in duplicates blinded to the investigator. Resulting concordance rate was 99.7%.

**Statistical analysis**

Quality of SNP genotypes was assessed by study-wise call rate and exact test for Hardy-Weinberg disequilibrium (HWE) in patients and controls. We compared the results for genotype
frequencies of the different polymorphisms between patient cohorts and control populations with the Chi-square test and logistic binary regression. All other models (dominant, recessive, allele frequencies) were computed by using two-tailed Fisher’s Exact test. P-values were calculated using IBM SPSS Statistics 25 and GraphPad Prism 5. A p-value of less than 0.05 was considered to be significant. When a significant or nominal significant association was found, at least an extended or one replication cohort was screened.

**Results**

**No study-wide significant association of GLO1 SNPs with ACP**

We identified no significant association in logistic regression analysis and no significant difference in the genotype distribution in the ACP screening cohort compared to controls (Table 2 and Table C in S1 File). As for rs937662 the HWE was nominal significant in our controls (p = 0.047) and for both rs937662 and rs1699012 nominal significant results were observed in the NACP screening cohort in the dominant or recessive model (p = 0.08, respectively) we extended our analysis in the ACP cohort to 430 and 447 patients. Here, we found no association for both SNPs and no deviation from HWE for rs937662 in the controls (p = 0.38; controls) (Table 3). Furthermore, genotype data of rs1937780 displayed a borderline significance in the screening cohort (p = 0.08). Again, we extended our investigated groups and analysed rs1937780 in a further German ACP cohort and additional European ACP patients from The Netherlands, Hungary, Italy, Romania and Poland. In the German cohort (n = 872) compared to controls (n = 1,474) the association was statistically significant (p = 0.003) as the genotype distribution between ACP patients and controls differed (GG: 40.0% vs. 33.1%; GA 45.9% vs. 51.6%; AA 14.1% vs. 15.3%) (Table D in S1 File). Of note, there was a deviation from HWE in the controls of the extended German cohort (p = 0.01). However, we did not find a significant association in logistic regression analysis (p = 0.134, odds ratio (OR) 1.117, 95% confidence interval (95% CI) 0.963–1.290) for this cohort. Otherwise, in the overall cohort of European ACP patients and controls we found no significant association in logistic regression analysis either (p = 0.214, OR 1.075, 95% CI 0.961–1.196) (Table E in S1 File). This result was

| SNP         | ACP p-value | OR   | 95% CI       | NACP p-value | OR   | 95% CI       |
|-------------|-------------|------|--------------|--------------|------|--------------|
| rs2736655   | 0.261       | 0.806| 0.554–1.174  | 0.292        | 0.838| 0.603–1.164  |
| rs9380765   | 0.345       | 1.143| 0.866–1.507  | 0.077        | 1.374| 0.966–1.953  |
| rs13212218  | 0.936       | 0.983| 0.642–1.505  | 0.578        | 0.898| 0.614–1.313  |
| rs937662    | 0.621       | 0.952| 0.783–1.157  | 0.070        | 1.207| 0.985–1.480  |
| rs1621788   | 0.548       | 1.085| 0.831–1.417  | 0.526        | 1.083| 0.847–1.384  |
| rs12198212  | 0.483       | 1.116| 0.822–1.514  | 0.216        | 1.196| 0.901–1.589  |
| rs1699012   | 0.508       | 0.935| 0.767–1.140  | 0.009        | 0.763| 0.623–0.933  |
| rs1616723   | 0.639       | 1.127| 0.684–1.855  | 0.962        | 1.012| 0.630–1.623  |
| rs1937780   | 0.134       | 1.117| 0.966–1.290  | 0.112        | 1.176| 0.963–1.436  |
| rs3778443   | 0.992       | 1.003| 0.564–1.783  | 0.323        | 1.348| 0.746–2.438  |
| rs4746      | 0.918       | 0.987| 0.770–1.264  | 0.008        | 1.342| 1.080–1.669  |
| rs17544798  | 0.133       | 0.752| 0.518–1.091  | 0.786        | 1.053| 0.727–1.525  |

Abbreviations: SNP, single-nucleotide polymorphism; OR, Odds ratio; CI, confidence interval; ACP, alcoholic chronic pancreatitis; NACP, non-alcoholic chronic pancreatitis.

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confirmed for the corresponding genotype data and none of the five replication cohorts demonstrated a statistically significant association in the different genetic models used for computations (Table 4 and Tables D and E in S1 File). Except for the named SNPs and SNP rs2736655 (ACP patients p = 0.026), all SNPs corresponded to the HWE.

Table 3. Data of the analysed GLO1 SNPs in patients with alcoholic chronic pancreatitis (ACP) and controls. For the calculations different genetic models were used.

| SNP/Genetic model for calculation | p-value | OR   | 95% CI     |
|----------------------------------|---------|------|------------|
| rs2736655 G/A                   | 0.36    | 0.849| 0.613–1.176|
| rs2736655 GG + GA/AA             | 0.19    | 0.778| 0.538–1.242|
| rs2736655 GG/GA + AA             | 0.48    | 2.034| 0.407–10.18|
| rs9380765 A/G                   | 0.61    | 1.074| 0.838–1.377|
| rs9380765 AA + AG/GG             | 0.32    | 1.218| 0.828–1.794|
| rs9380765 AA/AG + GG             | 0.91    | 0.971| 0.627–1.505|
| rs13212218 G/A                  | 1.00    | 0.991| 0.675–1.543|
| rs13212218 GG + GA/AA            | 0.83    | 0.944| 0.623–1.430|
| rs13212218 GG/GA + AA           | 0.65    | 2.777| 0.308–25.03|
| rs937662 C/T                    | 0.99    | 0.995| 0.834–1.187|
| rs937662 CC + CT/TT             | 0.80    | 0.950| 0.683–1.321|
| rs937662 CC/CT + TT             | 0.89    | 1.021| 0.787–1.324|
| rs1621788 A/G                   | 0.54    | 1.087| 0.853–1.385|
| rs1621788 AA + AG/GG            | 0.76    | 1.089| 0.724–1.639|
| rs1621788 AA/AG + GG            | 0.49    | 1.143| 0.778–1.679|
| rs12198212 T/A                  | 0.37    | 1.136| 0.864–1.494|
| rs12198212 TT + TA/AA           | 0.87    | 1.108| 0.567–2.164|
| rs12198212 TT/TA + AA           | 0.33    | 1.192| 0.845–1.682|
| rs1699012 A/G                   | 0.32    | 0.909| 0.759–1.089|
| rs1699012 AA + AG/GG            | 0.33    | 0.878| 0.680–1.133|
| rs1699012 AA/AG + GG            | 0.65    | 0.942| 0.730–1.215|
| rs1616723 T/C                   | 0.81    | 0.942| 0.590–1.503|
| rs1616723 TT + CT/CC            | 1.00    | 1.390| 0.125–15.44|
| rs1616723 TT/CT + CC            | 0.79    | 0.921| 0.561–1.512|
| rs1937780 G/A                   | 0.61    | 1.073| 0.834–1.381|
| rs1937780 GG + GA/AA            | 0.30    | 0.763| 0.466–1.251|
| rs1937780 GG/GA + AA            | 0.15    | 1.324| 0.927–1.890|
| rs3778443 G/A                   | 0.49    | 0.813| 0.479–1.381|
| rs3778443 GG + GA/AA            | 1.00    | 1.952| 0.079–48.18|
| rs3778443 GG/GA + AA            | 0.39    | 0.776| 0.447–1.344|
| rs4746 T/G                      | 0.54    | 1.072| 0.862–1.333|
| rs4746 TT + TG/GG               | 0.92    | 1.045| 0.705–1.550|
| rs4746 TT/TG + GG               | 0.45    | 1.137| 0.818–1.581|
| rs17544798 A/T                  | 0.20    | 0.794| 0.569–1.107|
| rs17544798 AA + AT/TT           | 0.12    | 0.380| 0.110–1.314|
| rs17544798 AA/AT + TT           | 0.33    | 0.819| 0.561–1.194|

The different models comprise (order from top to bottom), allele frequencies, the dominant and the recessive model for computations. The number of patients and the genotype distribution of each variant are summarized in Table C in S1 File. Note: For rs937662 and rs1699012 the extended German cohorts have been used for computations. Calculations were performed using the Fisher’s exact test. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.
No study-wide significant association of GLO1 SNPs with NACP

In the screening cohort the SNPs rs937662, rs1699012, and rs4746 displayed a nominal significant difference in the recessive or dominant model (p = 0.08, 0.08, and 0.07, respectively) (Table 5). Otherwise, no differences in the genotype distributions were observed (Table F in S1 File). Furthermore, we detected a significant association for SNP rs1699012 (p = 0.009, OR 0.763, 95% CI 0.623–0.933) and rs4746 (p = 0.008, OR 1.342, 95% CI 1.080–1.669) in logistic regression analysis (Table 2). To elucidate a potential association, we extended the German NACP cohort and found no statistically significant association of the genotype distribution for rs937662 (p = 0.15), whereas rs1699012 and rs4746 still displayed a significant association compared to controls (p = 0.02 and p = 0.008, respectively) (Table G in S1 File). Therefore, we investigated rs4746 in an independent German (n = 450, patients; n = 581, controls), French (n = 767, patients; n = 1,049, controls), and Chinese (n = 928, patients; n = 1,008, controls) NACP cohort. Here, no association was found in all three replication cohorts (Table 6; for genotype distribution see Table G in S1 File). Finally, for SNP rs1699012 we observed no significant association (p = 0.279, OR 0.903, 95% CI 0.750–1.087) in an independent German NACP cohort of 427 patients in logistic regression analysis (for different genetic models see Table 6). For the rare SNP rs3778443 we observed no significant association (p = 0.279, OR 0.903, 95% CI 0.750–1.087) in an independent German NACP cohort of 427 patients in logistic regression analysis (for different genetic models see Table 6).

Discussion

There is a biological plausibility that GLO1 is relevant in inflammatory processes and as such for the development of CP, although conflicting results have been reported in other diseases

Table 4. Data of the European alcoholic chronic pancreatitis (ACP) replication cohorts for rs1937780 in comparison to controls. Calculations were performed with different genetic models.

| SNP/Genetic model for calculations | p-value | OR  | 95% CI |
|-----------------------------------|---------|-----|--------|
| rs1937780 (Germany)               |         |     |        |
| G/A                              | 0.006   | 1.187 | 1.051–1.340 |
| GG + GA/AA                       | 0.46    | 1.103 | 0.870–1.399 |
| GG/GA + AA                       | 0.0009  | 1.348 | 1.133–1.604 |
| rs1937780 (Hungary)              |         |     |        |
| G/A                              | 0.82    | 0.939 | 0.601–1.465 |
| GG + GA/AA                       | 1.00    | 1.141 | 0.463–2.811 |
| GG/GA + AA                       | 0.63    | 0.815 | 0.423–1.571 |
| rs1937780 (The Netherlands)      |         |     |        |
| G/A                              | 0.81    | 0.968 | 0.764–1.226 |
| GG + GA/AA                       | 0.73    | 1.112 | 0.700–1.766 |
| GG/GA + AA                       | 0.5     | 0.887 | 0.636–1.238 |
| rs1937780 (Romania)              |         |     |        |
| G/A                              | 0.45    | 1.293 | 0.717–2.333 |
| GG + GA/AA                       | 0.34    | 2.121 | 0.513–8.767 |
| GG/GA + AA                       | 0.66    | 1.286 | 0.539–3.068 |
| rs1937780 (Poland)               |         |     |        |
| G/A                              | 0.78    | 1.095 | 0.622–1.927 |
| GG + GA/AA                       | 0.42    | 1.882 | 0.582–6.088 |
| GG/GA + AA                       | 0.84    | 0.855 | 0.376–1.946 |
| rs1937780 (Italy)                |         |     |        |
| G/A                              | 0.49    | 1.141 | 0.815–1.599 |
| GG + GA/AA                       | 0.43    | 1.298 | 0.713–2.364 |
| GG/GA + AA                       | 0.70    | 1.126 | 0.673–1.883 |
| rs1937780 (all)                  |         |     |        |
| G/A                              | 0.05    | 1.103 | 1.003–1.214 |
| GG + GA/AA                       | 0.05    | 1.151 | 1.004–1.319 |
| GG/GA + AA                       | 0.24    | 1.122 | 0.931–1.351 |

The different models comprise (order from top to bottom), allele frequencies, the dominant and the recessive model for computations. The number of patients and the genotype distribution of each variant are summarized in Table D in S1 File. Note, the German cohort used here comprises the screening cohort and further samples. Calculations were performed using the Fisher’s exact test. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.

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In this work, we investigated a potential genetic association of GLO1 variants with ACP and NACP. We failed to identify an association between CP and one functionally relevant variant and 11 tagging SNPs covering the GLO1 locus. In our German ACP patients (total cohort) rs1937780 genotypes differed significantly between patients and controls (p = 0.003) and significance was also observed in the dominant model. Contrary, we found neither an association nor a comparable trend in the distinct

### Table 5. Data of the analysed GLO1 SNPs in patients with non-alcoholic chronic pancreatitis (NACP) and controls.

For the calculations different genetic models were used.

| SNP/Genetic model for calculations | p-value | OR     | 95% CI      |
|-----------------------------------|---------|--------|-------------|
| rs2736655                         | G/A     | 0.35   | 0.853       | 0.614–1.184 |
|                                   | GG + GA/AA | 0.11 | 0.423       | 0.150–1.218 |
|                                   | GG/GA + AA | 0.63 | 0.909       | 0.624–1.324 |
| rs9380765                         | A/G     | 0.31   | 0.874       | 0.682–1.119 |
|                                   | AA + AG/GG | 0.44 | 0.844       | 0.551–1.294 |
|                                   | AA/AG + GG | 0.35 | 0.807       | 0.536–1.217 |
| rs13212218                        | G/A     | 0.56   | 0.889       | 0.610–1.295 |
|                                   | GG + GA/AA | 1.00 | 0.900       | 0.199–4.065 |
|                                   | GG/GA + AA | 0.53 | 0.875       | 0.579–1.322 |
| rs937662                          | C/T     | 0.16   | 1.201       | 0.935–1.542 |
|                                   | CC + CT/TT | 0.71 | 1.115       | 0.679–1.834 |
|                                   | CC/ CT + TT | 0.08 | 1.388       | 0.967–1.993 |
| rs1621788                         | A/G     | 0.62   | 1.070       | 0.838–1.366 |
|                                   | AA + AG/GG | 0.84 | 1.057       | 0.702–1.592 |
|                                   | AA/AG + GG | 0.55 | 1.128       | 0.766–1.663 |
| rs12198212                        | T/A     | 0.24   | 1.182       | 0.898–1.563 |
|                                   | TT + TA/AA | 0.48 | 1.385       | 0.677–2.833 |
|                                   | TT/TA + AA | 0.29 | 1.207       | 0.854–1.705 |
| rs1699012                         | A/G     | 0.12   | 0.813       | 0.631–1.048 |
|                                   | AA + AG/GG | 0.08 | 0.650       | 0.402–1.051 |
|                                   | AA/AG + GG | 0.37 | 0.842       | 0.589–1.204 |
| rs1616723                         | T/C     | 0.91   | 0.974       | 0.608–1.560 |
|                                   | TT + CT/CC | 1.00 | 1.390       | 0.125–15.44 |
|                                   | TT/CT + CC | 0.90 | 0.956       | 0.581–1.575 |
| rs1937780                         | G/A     | 0.32   | 1.145       | 0.882–1.486 |
|                                   | GG + GA/AA | 0.39 | 1.336       | 0.749–2.380 |
|                                   | GG/GA + AA | 0.45 | 1.161       | 0.803–1.680 |
| rs3778443                         | G/A     | 0.37   | 1.341       | 0.729–2.470 |
|                                   | GG + GA/AA | 0.56 | 0.319       | 0.029–3.541 |
|                                   | GG/GA + AA | 0.27 | 1.519       | 0.790–2.920 |
| rs4746                            | T/G     | 0.17   | 1.194       | 0.931–1.531 |
|                                   | TT + TG/GG | 0.82 | 1.076       | 0.680–1.702 |
|                                   | TT/TG + GG | 0.07 | 1.415       | 0.978–2.047 |
| rs17544798                        | A/T     | 0.17   | 1.194       | 0.931–1.531 |
|                                   | AA + AT/TT | 1.00 | 1.329       | 0.241–7.324 |
|                                   | AA/AT + TT | 0.77 | 1.066       | 0.719–1.579 |

The different models comprise (order from top to bottom), allele frequencies, the dominant and the recessive model for computations. The number of patients and the genotype distribution of each variant are summarized in Table F in S1 File. Calculations were performed using the Fisher’s exact test. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.

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European cohorts. The same variant has recently been investigated in pancreatic cancer patients and an association was absent [33]. As functional consequences of this variant are unknown and overall genetic data are statistically not significant, it is unlikely that rs1937780 plays a prominent role in CP development.

In the NACP cohort the three variants rs937662, rs1699012, and rs4746 showed borderline significant results in distinct analysis models that, however, were not confirmed in the extended or replication cohorts. For the variants rs937662 and rs1699012 no prior clinical relevance has been reported and therefore an association with CP is again unlikely. Contrary, rs4746 was associated with a wide spectrum of disorders ranging from diabetes [19], atherosclerosis [20], chronic renal failure [21], vascular diseases [22,34], neuropsychiatric disorders [24,25], and even to cancer [26–29]. In our work the borderline significance of the screening cohort was disproved in three large NACP cohorts from Germany, France, and China. As such, although, a functional relevance for this variant has been reported, our data show no association with CP.

We investigated cohorts with a reasonable number of patients and extended these cohorts whenever statistically or nominal significant results were obtained. Therefore, a prominent disease association of GLO1 variants with CP can be ruled out with high certainty. Nevertheless, our approach is not capable of identifying rare associating variants. We therefore analyzed

Table 6. Data of the replication cohorts of GLO1 SNPs rs937662, rs1699012, rs4746 in patients with non-alcoholic chronic pancreatitis (NACP) and controls. For computations different genetic models were used.

| SNP/Geneic model for calculations | p-value | OR     | 95% CI        |
|----------------------------------|---------|--------|---------------|
| rs937662                         | C/T     | 0.06   | 1.212         | 0.994–1.477     |
|                                  | CC + CT/TT | 0.25   | 1.279         | 0.865–1.892     |
|                                  | CC/CT + TT | 0.07   | 1.299         | 0.980–1.722     |
| rs1699012                        | A/G     | 0.01   | 0.766         | 0.627–0.936     |
|                                  | AA + AG/GG | 0.01   | 0.607         | 0.413–0.893     |
|                                  | AA/AG + GG | 0.08   | 0.768         | 0.579–1.018     |
| rs1699012 (Germany replication)  | A/G     | 0.31   | 0.907         | 0.755–1.089     |
|                                  | AA + AG/GG | 0.77   | 0.938         | 0.640–1.375     |
|                                  | AA/AG + GG | 0.22   | 0.854         | 0.664–1.099     |
| rs4746 (Germany)                 | T/G     | **0.003** | 1.360 | 1.108–1.669     |
|                                  | TT + TG/GG | 0.09   | 1.407         | 0.952–2.079     |
|                                  | TT/TG + GG | **0.004** | 1.571 | 1.168–2.112     |
| rs4746 (Germany replication)     | T/G     | 0.34   | 1.093         | 0.917–1.303     |
|                                  | TT + TG/GG | 0.63   | 1.097         | 0.797–1.510     |
|                                  | TT/TG + GG | 0.31   | 1.152         | 0.883–1.503     |
| rs4746 (France)                  | T/G     | 0.11   | 1.117         | 0.979–1.276     |
|                                  | TT + TG/GG | 0.06   | 1.262         | 0.998–1.596     |
|                                  | TT/TG + GG | 0.44   | 1.087         | 0.888–1.331     |
| rs4746 (China)                   | T/G     | 0.27   | 1.175         | 0.897–1.539     |
|                                  | TT + TG/GG | 0.81   | 0.815         | 0.312–2.132     |
|                                  | TT/TG + GG | 0.25   | 0.837         | 0.621–1.129     |

The different models comprise (order from top to bottom), allele frequencies, the dominant and the recessive model for computations. The number of patients and the genotype distribution of each variant are summarized in Table G in S1 File. Note, for rs937662, rs1699012, and rs4746 the extended German cohorts comprise the screening cohort and further German samples. For rs1699012 the replication cohort from Munich is displayed. Calculations were performed using the Fisher’s exact test. Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval.

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whole exome sequencing data from an ongoing project but did not identify rare variants enriched in our NACP patients (unpublished data). Nonetheless, our study is limited in its restriction to a Caucasian (German) cohort as only one SNP was analyzed in an Asian replication cohort and therefore we may have missed specific associations in other ethnicities.

In summary, we performed a comprehensive investigation of GLO1 variants and did not demonstrate a prominent role for CP development in alcoholic and the non-alcoholic etiologies of the disease.

Supporting information

S1 File. Figure A. Linkage disequilibrium figure of the GLO1 locus generated by the SNPinfo LD TAG SNP Selection tool.

To generate the linkage disequilibrium (LD) figure, the SNPinfo LD TAG SNP Selection tool (https://snpinfo.niehs.nih.gov/snpinfo/snptag.html) with the following parameters was applied: LD threshold of 0.8; a minimum of one SNP tagged; a minimum of 5 valid genotypes to calculate LD in populations with European ancestry (CEU); integrated region with 10.000 bp in the 5’-region and the 3’-region of GLO1. Abbreviations: SNP, single nucleotide polymorphism; LD, linkage disequilibrium; CEU, Northern Europeans from Utah. For the following SNPs we used tagging SNPs in our study: rs10484854 was tagged by rs12198212; rs1781735 by rs1621788; rs6458064 by rs937662; and rs9394523 by rs13212218. As demonstrated in the figure the tagging SNPs represented the depicted haplotypes. In Table A in S1 File the information on the SNPs selected according to the published literature and by SNPinfo is summarized.

Table A. Overview of screened GLO1 SNPs that were identified by SNPinfo or by a literature research.

We used the SNPinfo LD TAG SNP Selection tool to identify SNPs in the GLO1 locus that cover the haplotypes of the gene. As several variants have been reported in the literature, we included these using tagging SNPs, where possible. The corresponding literature for the screened SNPs is indicated in brackets. For rs4746 several studies reported associations and functional data are available in addition. These SNPs have been tagged by the screened SNP rs1616723, rs9380765, rs13212218, and rs1621788 respectively.

Table B. Polymerase chain reaction (PCR) primers and probes for melting curve analysis of all GLO1 SNPs. Abbreviations: fw, forward; rv, reverse; XI, internal dye modified base; LC610, 5’-LightCycler Red 610; LC640 (sensor probe), LightCycler Red 640 (sensor probe); FL, 3’-Fluorescein labelling (anchor probe); PH, 3’-phosphate.

Table C. Genotype data of the analysed GLO1 SNPs in patients with alcoholic chronic pancreatitis (ACP) and controls.

Note: For rs937662 and rs1699012 the extended German cohorts are shown. Calculations were performed using the Chi-square test (two-sided). Abbreviations: Contr. = controls, Pat. = patients.

Table D. Genotype data of the GLO1 SNP rs1937780 in patients with alcoholic chronic pancreatitis in European cohorts including Germany.

Note: The German cohort comprises the screening cohort and additional samples. Calculations were performed using the Chi-square test (two-sided). Abbreviations: Contr. = controls, Pat. = patients.

Table E. Results of logistic regression with covariate gender for the GLO1 SNP rs1937780 in patients with alcoholic chronic pancreatitis in European cohorts including Germany.

Note: The German cohort comprises the screening cohort and additional samples. Calculations were performed using logistic regression. Abbreviations: OR, Odds ratio; CI, confidence interval; ACP, alcoholic chronic pancreatitis.

Table F. Genotype data of the analysed GLO1 SNPs in German patients with non-alcoholic
chronic pancreatitis (NACP) and controls. Calculations were performed using the Chi-square test (two-sided). Abbreviations: Contr. = controls, Pat. = patients.

Table G. Genotype data of the analysed GLO1 SNPs rs937662, rs1699012, rs4746 in the extended NACP cohorts. Note: For rs937662, rs1699012, and rs4746 the extended German cohorts comprise the screening cohort and additional German samples. Calculations were performed using the Chi-square test (two-sided). Abbreviations: Contr. = controls, Pat. = patients. $ NACP replication cohort and controls from Germany. $ Independent NACP replication cohort from Munich. $ NACP cohort and controls from France. € NACP cohort and controls from China.

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