Genetic variation in *TERT* modifies the risk of hepatocellular carcinoma in alcohol-related cirrhosis: results from a genome-wide case-control study

Short title: *TERT* genetic variation and HCC risk

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Significance of this study

What is already known on this subject?

► Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, responsible for ~0.8M deaths per year worldwide. Most alcohol-related HCCs develop in patients with established alcohol-related cirrhosis

► Older age, male sex, obesity and type II diabetes are risk factors for the development of HCC in people with alcohol-related cirrhosis

► Only three genetic loci - *PNPLA3*, *TM6SF2*, and *WNT3A*-*WNT9A* - have been associated with the development of alcohol-related HCC, at genome-wide significance, to date. Other risk loci are likely to exist.

What are the new findings?

► We identify the rs242652 germline variant in *TERT* as a novel susceptibility locus for HCC development in alcohol-related cirrhosis (ArC)

► Specifically, the rs2242652 A allele is associated with an decreased risk of HCC development in ArC.

►Carriage of rs2242652 in *TERT* is not associated with the risk for developing alcohol-related cirrhosis

How might it impact on clinical practice in the foreseeable future?

► Exploration of the functional significance of *TERT* variants could provide important insights into the pathogenesis of HCC in people with alcohol-related cirrhosis

► Genetic profiling of patients with alcohol-related cirrhosis might inform HCC screening programs

Abstract

Objective Hepatocellular carcinoma (HCC) often develops in patients with alcohol-related cirrhosis at an annual risk of up to 2.5%. Some host genetic risk factors have been identified but do not account for the majority of the variance in occurrence. This study aimed to identify novel susceptibility loci for the development of HCC in people with alcohol related cirrhosis.

Design Patients with alcohol-related cirrhosis and HCC (cases: n=1,214) and controls without HCC (n=1,866), recruited from Germany, Austria, Switzerland, Italy and the UK, were included in a two-stage GWAS utilizing a case-control design. A validation cohort of 1,520 people misusing alcohol but with no evidence of liver disease was included to control for possible association effects with alcohol misuse. Genotyping was performed using the Infinium®Global Screening Array (version 24v2, Illumina) and the OmniExpress Array (version 24v1-0a, Illumina).

Results Associations with variants rs738409 in *PNPLA3* and rs58542926 in *TM6SF2* previously associated with an increased risk of HCC in patients with alcohol-related cirrhosis were confirmed at genome-wide significance. A novel locus rs2242652(A) in *TERT* (telomerase reverse transcriptase) was also associated with a decreased risk of HCC, in the combined meta-analysis, at genome-wide significance (p=6.41×10^{-9}, odds ratio (OR) =0.61 (95%CI, 0.52-0.70). This protective association remained significant after correction for sex, age, BMI, and type 2 diabetes (p=7.94×10^{-5}, OR =0.63 (95%CI, 0.50-0.79). Carriage of rs2242652(A) in *TERT* was associated with an increased leukocyte telomere length (p=2.12×10^{-44}).

Conclusion This study identifies rs2242652 in *TERT* as a novel protective factor for HCC in patients with alcohol-related cirrhosis.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy world-wide and is responsible for ~0.8 million deaths per annum(1). The global incidence of HCC is rising and may surpass 1 million cases annually by 2025(2). Alcohol-related liver disease (ArLD) is a leading underlying cause of HCC in Europe and Northern America(3,4). Most cases of alcohol-related HCC develop in patients with established cirrhosis. Cohort studies indicate that the cumulative incidence of HCC approaches 2.5% per annum for alcohol-related cirrhosis (ArC) patients attending specialist care centers(3,4). Clinical risk factors for the development of HCC in people with ArC include older age, male sex, type 2 diabetes and obesity(2,5) – but explain only a fraction of the total variability in HCC occurrence(6,7).

In recent years, interest has focused on dissecting the underlying host genetics of HCC through candidate gene association studies. In the studies undertaken to date, loci in the genes coding for patatin-like phospholipase domain containing 3 (*PNPLA3*; rs738409) and transmembrane 6 superfamily member 2 (*TM6SF2;* rs58542926) were robustly confirmed to increase the risk of developing HCC in ArC(8), while loci, rs72613567:TA in hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) and rs429358:C in apolipoprotein E (*APOE*), were found to attenuate risk(9–11). As the products of these genes are involved in lipid turnover and processing, it is not surprising that the same loci also modulate the risk for HCC development in people with non-alcoholic fatty liver disease (NAFLD)(12).

The variants currently identified as associated with HCC risk in ArC only account for a small proportion of the heritability risk, suggesting the existence of additional genetic modulators(7,8). Also, the genetic risk loci recognized hitherto do not relate to genes considered pivotal to hepatocarcinogenesis(13). Identifying these additional, potential

genetic modulators of hepatocarcinogenesis requires large genome-wide association studies (GWAS) in which *cases* are defined as people with alcohol-related cirrhosis with HCC and *controls* as people with alcohol-related cirrhosis who have no evidence of HCC. These definitions are critical to enable the detection of risk loci with a direct molecular link to hepatocarcinogenesis *per se*, rather than to the development of alcohol-related steatosis, inflammation or fibrosis.

A European GWAS of HCC in alcohol-related liver disease, while not conforming to this exact design, was recently undertaken by Trépo et al.(14). In their discovery analysis comparing 775 HCC cases (80% with F3/F4 fibrosis) against 1332 non-HCC controls (94% with F3/F4 fibrosis), a genome-wide significant association was identified between the rs708113:T allele locus near W*NT3A*-*WNT9A* and a reduction in the risk for developing alcohol-related HCC (14).

The aim of the present study was to undertake a GWAS in patients with HCC against a background of alcohol-related cirrhosis comprising 1066 cases and 844 controls using a case-control design.

Methods

Patient cohorts:

Germany / Switzerland / Austria Alcohol Cohort (Discovery cohort)

The diagnosis of ArC was established based on a history of long-term, sustained alcohol intake of a minimum of 40g/day in women and 60g/day in men, together with histological examination of liver tissue; or compatible historical, clinical, laboratory, radiological and endoscopic features. Patients were excluded if they had any other potential cause of liver injury, specifically if they were positive for hepatitis B surface antigen (HBsAg), anti-hepatitis C immunoglobulin G (anti-HCV IgG), antinuclear antibodies (ANA) (titre>1:80) or anti-mitochondrial antibodies (titre>1:40), had elevated serum ferritin levels with a transferrin saturation of >50%, a serum ceruloplasmin of <20mg/dl (0.2 g/dl), a serum alpha-1 antitrypsin of < 70 mg/dL (13µmol/L) or were morbidly obese. The diagnosis of HCC was made following on histological examination of tumour tissue or based on criteria applied to images obtained using multiphasic CT or dynamic contrast-enhanced MRI (15,16) (Suppl. Methods A).

United Kingdom Alcohol Cohort (Replication cohort 1)

The United Kingdom Biobank (UKB) is a large-scale biomedical database containing in depth genetic and health information from a prospective study of approximately half a million middle-aged individuals from the UK recruited in 2006-2010(17). Participants have been deeply phenotyped and are linked to UK hospital in-patient, cancer and mortality registries. A nested case-control dataset (n=860) was created utilizing this resource. Cases were defined as participants with a hospital admission for ArC (ICD10:K70.3), and an HCC diagnosis (ICD10:C22.0 or ICD9:155.0). Controls were participants with a hospital admission for ArC but with no record of an HCC diagnosis. Analyses were restricted to participants of white British ancestry. These nested case-

control data were then pooled with 306 patients recruited from the Centre for Hepatology at the Royal Free Hospital, London who had histologically proven ArC with or without HCC, as described previously(18) (Suppl. Methods B).

Germany and Italy Alcohol Cohort (Replication cohort 2)

The replication cohort included 238 patients with ArC (42 with HCC) from the University of Bonn, and 72 patients with ArC (36 with HCC) from the University of Milan.

Validation cohorts

Patients with a history of alcohol misuse (AM) but without evidence of significant alcohol-related liver injury were recruited from psychiatric units in Germany (n=1080)(19,20) and from Hepatology Centres in Heidelberg, Germany (n=99) and London, UK (n=341)(18) (Suppl. Methods C).

Genotyping and Imputation

Discovery cohort

Genotyping was performed using genomic DNA extracted from peripheral blood samples as described previously(18). The GWAS (Stage 1) included 1910 patients with ArC genotyped on the Infinium®Global Screening Array (version 24v2, Illumina) (Table 1) (Suppl. Methods D). Genotype imputation was performed with Minimac4 to the Haplotype Reference Consortium reference panel (HRC r1.1)(21) using the Michigan Imputation Server(22) (Suppl. Methods E).

Replication and validation samples

Patients from the Royal Free Hospital, London and Germany were genotyped using the OmniExpress array (24v1-0a, Illumina)[12]. The replication (Stage 2) included 1170 patients with ArC (Table 1). Patients from Italy were genotyped on the Infinium®Global Screening Array (24v2, Illumina) (Suppl. Methods D). Genotypic data were imputed for

each cohort to the HRC reference. (Suppl. Methods E). Imputed genotypic data from 606 patients were obtained from the UKB Resource(23)

Statistical analyses:

GWAS analysis

Association analyses for 7,946,762 variants were performed using Plink 2.0(24) with allele dosages obtained after imputation (imputation info score > 0.3, minor allele frequency >1%). The lambda inflation factor λ_{GC} for the unadjusted GWAS analysis was 1.085 indicative of subtle population stratification. To account for the observed inflation, the top 20 principal components (PC) on the LD-pruned data set were calculated and the top 15 PCs of genetic ancestry included as covariates in the regression models (25). The corrected λ_{GC} was 1.03. Two discovery GWAS analyses were performed: GWAS 1 (primary GWAS analysis): included only the top 15 PCs as covariates in the regression model. The *p* value threshold for lead SNPs for replication follow-up was set to $p<5\times10^{-6}$ to allow loci with *suggestive* association to be included at the replication stage. GWAS 2 (sensitivity GWAS analysis): included sex, age and the top 15 PCs as covariates; the top 15 independent loci were follow-up at Stage 2.

Loci discovery and annotation

Independent genomic risk loci and lead variants (for $p<5\times10^{-6}$) were derived from FUMA (V.1.3.1)(26) based on GWAS summary statistics, as previously described (27). For a locus to be defined as independent it had to be separated from other loci by at least 500kb of genomic distance; the top-ranking SNPs were deemed potential lead markers.

Power analysis

The expected power to identify a true association between a SNP and HCC development in ArC was calculated using the GAS Power Calculator(28). The power for SNPs with minor allele frequencies of ≥20% was estimated to be 49% for alleles with a relative risk of 1.5, increasing to 81% for a relative risk of 1.6, for a *p* value threshold of 5×10^{-8} (Suppl. Table 1).

Replication analysis

In stage 2, the selected SNPs were validated in independent samples from the UK, Germany and Italy. Study-specific β estimates and standard errors were further analyzed using fixed-effect meta-analysis. Two criteria were required to demonstrate replication: a) $p<5.55\times10^{-3}$ (corresponding to $p<0.05$ after Bonferroni correction for nine tests in the *primary* analysis); or $p<3.33\times10^{-3}$ (corresponding to $p<0.05$ after Bonferroni correction for 15 tests in the *sensitivity* analysis) c) and consistency of allelic effect direction between discovery and replication samples (Suppl. Methods F).

Additional replication analyses

The association between novel risk loci and HCC/liver cancer were also assessed using: a) publicly available summary statistics from a recent alcohol-related HCC GWAS performed by Trépo et al.(14); b) data from two large population-based cohorts (Finngen and BioBank Japan). c) data from a UK cohort of patients with HCV-related cirrhosis (STOP-HCV) (Suppl. Methods F).

Association with other cancers (pleiotropy)

Moreover, we assessed if novel risk loci were associated with selected cancers unrelated to the liver in both the UK Biobank and FinnGen population-based cohorts. Each cancer phenotype was defined by ICD codes present in hospital admissions, death records and cancer registry records. In addition, the NHGRI-EBI Catalog of

human genome-wide_association studies was searched for association of novel risk loci with cancer phenotypes. (Suppl. Methods F).

Meta-analysis GWAS: A fixed-effect meta-analysis restricted to markers present in all data sets (n = 5,552,382) was performed using METAL(29) to: a) utilize the total study sample (n=3080) for the discovery stage and b) to determine the combined effect size of replicated loci across Stage 1 and 2 datasets.

eQTL analysis

Variants at novel loci were tested for *cis*-eQTL effect on gene expression in: a) liver tissue (n=266) using the database of the Genotype-Tissue Expression Project (GTEx) release V8(30) and b) whole-blood (n= 24,376) using the database of the eQTLGen Consortium(31).

SNP Heritability Analysis

The proportion of phenotypic variance explained by the additive genetic effect of common genome-wide significant SNPs (h^2_{SNP}: SNP heritability) was estimated using a Genomic relatedness matrix REstricted Maximum Likelihood (GREML) analysis implemented in GCTA(32) (Suppl. Methods G).

Association with HCC-related phenotypes

Replicating loci were tested in the total UKB for association with two HCC-related phenotypes: leukocyte telomere length(33) and liver fat content(34). Leukocyte telomere length was available for 474,074 participants in UKB (Field ID: 22191), whilst liver fat content was available for 8315 imaging sub-study participants (Field ID: 22436) (Suppl. Methods H).

Ethics

The study protocol was approved by the ethics committees of the participating institutions. All included individuals provided written informed consent prior to inclusion into the study.

Patient and public involvement

There was no patient and public involvement in the design and conduct of this study.

Results

Genome-wide association study and validation of the loci

After imputation a total of 7,946,762 variants with a MAF>0.01 were tested for association with HCC in 1,066 cases with ArC and HCC and 844 controls with ArC but with no evidence of HCC (Table 1).

Associations with HCC were observed at genome-wide significance ($p<5\times10^{-8}$) for two independent genomic loci viz *PNPLA3* and *TM6SF2* (Table 2; Figure 1A, Suppl. Fig. 1). The strongest signal was at rs2294915, located in *PNPLA3* ($p = 6.21\times10^{-15}$) which encodes 1-acylglycerol-3-phosphate O-acyltransferase. This tag SNP rs2294915, located in intron 8 of *PNPLA3*, is in strong linkage disequilibrium (LD) ($r^2= 0.92$) with the functional variant rs738409 C>G p.I148M in exon 3 of *PNPLA3* that yielded a similar *p* value at the discovery stage ($p = 7.23\times10^{-15}$, OR (CI95%) = 1.71 (1.49-1.96)).

The other signal associated with HCC at genome-wide significance was rs58489806, located in intron 1 of *MAU2* ($p = 1.49\times10^{-9}$) encoding MAU2 sister chromatid cohesion factor; 49 additional genome-wide significant SNPs were mapped to this locus. The variant rs58489806 is in strong LD ($r^2= 0.80$) with the coding variant rs58542926 p.E167K at the *TM6SF2* locus (encoding transmembrane 6 superfamily member 2) that yielded ($p = 2.81\times10^{-9}$, OR (CI95%) = 1.94 (1.56-2.42)) at the discovery stage.

In stage 2, the nine lead SNPs from HCC associated loci were validated in independent cohorts from the UK, Germany and Italy in fixed-effect meta-analysis (Table 1; Suppl. Tables 2-4). In addition to rs2294915 in *PNPLA3* ($p = 6.19\times10^{-6}$) and rs58489806 in *TM6SF2/MAU2* ($p = 5.22\times10^{-4}$), disease association was replicated for the minor allele in rs2242652:A ($p = 1.07\times10^{-3}$) in *TERT* (telomerase reverse transcriptase) (Table 2). In the combined analysis of all stage 1 and stage 2 samples, the association of rs2242652:A in *TERT* with alcohol-related HCC attained genome-wide significance (*p*

= 6.41×10^{-9}, OR (CI95%) = 0.61 (0.52-0.72) (Table 2). The protective effect associated with carriage of *TERT* rs2242652:A remained significant after correction for sex, age, BMI, type 2 diabetes, and the top 15 PCs of genetic ancestry, but did not reach genome-wide significance (p = 7.94×10^{-5}; OR (95% CI) = 0.63 (0.50-0.79) (Suppl. Table 5) reflecting the loss of power associated with the high number of missing BMI and diabetes data points in the analysis (Table 1).

A sensitivity analysis in which the genome-wide analysis was additionally adjusted for sex and age also showed genome-wide significant association with HCC for two independent genomic loci *PNPLA3* and *TM6SF2* with HCC and suggestive evidence of association for *TERT* (p = 9.28×10^{-6}). (Table 2; Suppl. Figs. 2 and 3). Of the top 15 associated loci, only the variants in *PNPLA3*, *TM6SF2* and *TERT* were replicated (Table 2).

The combined GWAS meta-analyses of stage 1 and 2 data sets of the primary and the sensitivity analyses confirmed genome-wide significant association with HCC for genomic loci in rs738409 in *PNPLA3*, rs58542926 in *TM6SF2* and rs2242652 in *TERT*. No additional risk locus attained genome-wide significance $p<5.0\times10^{-8}$ (Suppl. Table 6). Forest plots showing the association between genomic loci in *PNPLA3, TM6SF2, TERT* and HCC are shown in Suppl. Figs. 4-6. Regional association plots of these three loci are shown in Figs. 1B-1D and in Suppl. Figs. 7-9.

Previously reported associations of HCC in the context of ArC with variants of *HSD17B13* rs72613567:TA (p = 8.95×10^{-3}; OR = 0.81 (0.69-0.95) and *APOE* rs429358:C (p = 5.44×10^{-3}; OR = 0.74 (0.60-0.91) were nominally significant in the present study, but did not achieve genome-wide significance in the discovery cohort (Suppl. Tables 5 and 7). In contrast, a recently reported association between rs708113:T near *WNT3A* was not confirmed (Suppl. Tables 5 and 7). Other previously

described HCC risk loci, e.g. *DEPDC5* in HCV-related HCC (35) or *STAT4* and *HLA-DQ* (36) were not significantly associated with ArC-related HCC in the present study (Suppl. Table 7).

Allelic and genotypic associations for *TERT* were highly significant, in the univariate analyses, for the comparisons HCC *vs.* ArC ($P_{allelic}$ = 2.81×10^{-11}, $P_{genotypic}$ 2.32×10^{-10}) and HCC *vs.* alcohol misuse but not for ArC *vs.* alcohol misuse using combined genotype counts from the stage 1 and 2 data sets (Suppl. Table 8; Figure 2). The protective effect for HCC was greater in homozygous carriers of *TERT* rs2242652:A (OR = 0.41 (0.25-0.67)) than in heterozygous carriers (OR = 0.61 (0.51-0.72)). In contrast, variants in *PNPLA3* and *TM6SF2* were strongly associated both with ArC and ArC-related HCC (Suppl. Tables 9-10, Figure 2).

Fine-mapping of *TERT* locus

In the primary meta-analysis of stage 1 and stage 2 samples the strongest association signal was obtained for the minor allele in rs2242652:A (p = 6.40×10^{-09}; OR = 0.61 (0.52-0.72)), although the alternative allele in rs10069690:T was similarly associated (p = 5.19×10^{-08}, OR = 0.66 (0.57-0.77)). Both variants are located in intron 4 of *TERT* and are correlated (r^2 = 0.70; Suppl. Table 11). The analysis of LD structure at the *TERT* locus showed that the association signal spans a narrow range from intron 2 to intron 6 of *TERT* – here termed LD block B-3 region (Suppl. Table 11, Suppl. Figure 7). The conditional analysis on allele dosage of rs2242652:A or rs10069690:T on each of the 20 SNPs from the B-3 region confirmed rs2242652 to be the lead locus (Suppl. Table 11 and 12). Indeed, none of the other variants within the B-3 block, including rs10069690 was associated with HCC after conditioning on rs2242652 (Suppl. Table 11).

Replication of the *TERT* variant´s association with hepatocellular carcinoma

Significant associations were observed between rs2242652:A and HCC in patients with HCV-related cirrhosis (p = 0.047; OR = 0.72 (0.53-0.99) and in the population-based FinnGen, UK Biobank, and BioBank Japan cohorts (Table 3, Suppl. Fig. 10 and Suppl. Table 13).

Association of *TERT* variants with non-liver cancers

Associations between *TERT* rs2242652:A and the ten most frequent cancers were explored in the UKB and FinnGen (FG) cohorts (Supp. Figure 10). Significant associations were observed with bladder cancer (FG: $p = 6.10 \times 10^{-6}$, OR = 0.83 (0.67-0.90)), UKB: $p = 5.82 \times 10^{-7}$, OR = 0.84 (0.79-0.90)), and prostate cancer (FG: $p = 5.11 \times 10^{-11}$, OR = 0.87 (0.84-0.90)), UKB: $p = 6.16 \times 10^{-16}$; OR = 0.86 (0.83-0.89)) while weaker associations were observed for lung and skin cancer. The effect sizes for prostate and bladder cancer were smaller than those for HCC / primary liver cancer in these cohort (UKB: HCC: p = 0.028; OR = 0.80 (0.66-0.89), FG: primary liver cancer: p = 0.009; OR = 0.81 (0.69-0.95)). These effect sizes are broadly consistent with those reported in the NHGRI-EBI Catalog of human genome-wide association studies (Suppl. Table 14).

Additive effect of risk variants

The proportions of patients with ArC, in the discovery and validation cohorts, who developed HCC increased with cumulative carriage of the risk increasing alleles rs738409:G in *PNPLA3*, rs58542926:T in *TM6SF2* and rs2242652:G in *TERT* (Suppl. Figure 11). In the discovery cohort, the OR for alcohol-related HCC was 2.12 (1.76–2.56) in patients carrying three to four risk alleles, and 5.24 (2.82–9.77) in patients carrying five to six risk alleles (Suppl. Table 15). In the UK replication cohort, the ORs for carriage of three to four risk alleles and five to six risk alleles were even higher at

3.25 (1.84–5.73) and 17.8 (6.38–49.6), respectively. (Suppl. Figure 12, Suppl. Table 15).

Association with leukocyte telomere length and liver fat content in the UK Biobank.

The minor allele of the lead variant in *TERT* rs2242652:A ($p = 2.12\times10^{-44}$) was significantly associated with an increase in LTL, as was rs10069690:T which is in strong LD with the lead variant ($p = 4.08\times10^{-84}$) (Suppl. Table 16). Additional variants located in the tested interval, i.e. rs7726159, showed even stronger association with LTL ($p = 1.16\times10^{-219}$) despite weak LD with rs2242652 ($r^2 = 0.354$) (Suppl. Table 11). The main association signals for HCC and LTL were both located in the LD block B-3 region, but a direct correlation in the strength of association was not observed (Suppl. Figure 13, Suppl. Table 11). Lead variants in *PNPLA3* and *TM6SF2* were not significantly associated with LTL-- rs738409 ($p = 0.458$) and rs58542926 ($p = 0.475$), but showed significant associations with liver fat content *viz.* rs738409 ($p = 3.39\times10^{-61}$), rs58542926 ($p = 5.94\times10^{-45}$) respectively (Suppl. Table 16); rs2242652 in *TERT* was not significantly associated with liver fat content ($p = 0.144$).

eQTL Analysis

Carriage of rs2242652:A was associated with increased expression of *TERT* in blood leukocytes ($p= 1.39\times10^{-5}$) (Suppl. Table 11). However, no significant eQTLs were found for rs2242652 in liver using the GTEx data base or in any other tissues (30).

SNP Heritability Analysis

The percentage heritability for ArC- HCC explained by additive genome-wide SNPs expressed as h^2 was 29.6% on the observed scale (GWAS cohort) and either 20.4% or 25.7% on the liability scale assuming a disease prevalence of 1% or 2.5%, respectively (Suppl. Table 20). The proportion of phenotypic variation due to the

underlying genetic variation in the *PNPLA3* / *TM6SF2* / *TERT* LD regions, expressed as h^2, was 7.5% on the observed scale and 4.2 % or 5.3 % on the liability scale, assuming the same disease prevalence (Suppl. Table 17). The proportion of the total SNP heritability due to variance component 1 (*PNPLA3* / *TM6SF2* / *TERT* variants) was 25.5 % for model 1, adjusted for 15 PCs, and 22.2 % for model 2 adjusted for sex, age and 15 PCs. After adjustment of variance component 1 for lead variants rs738409 in *PNPLA3* / rs58542926 in *TM6SF2* / rs2242652 in *TERT h2* was reduced to 0.000001%, indicating that the genetic risk of variance component 1 was fully captured by the three identified lead variants.

Discussion

In the present study, associations at genome-wide significance were identified between HCC in ArC and previously recognized variants in *PNPLA3* and *TM6SF2,* and with a variant in *TERT* (telomerase reverse transcriptase) on chromosome 5 not previously associated with this phenotype. In combination, these three loci may explain up to 25% of the total SNP heritability in HCC in patients with ArC.

The identification of host genetic risk factors for alcohol-related HCC has been largely undertaken using a candidate gene approach. Candidate genes have invariably been selected because of their association with progression of alcohol-related liver injury and positive robust associations for variants rs738409 in *PNPLA3,* and rs58542926 in *TM6SF2* have been identified (8,9). These variants are known to modify liver fat content and signaling, but how they influence the mechanisms leading to tumor initiation or promotion is largely unknown(37,38). In the present study, the *increased* risk associations between HCC in ArC and rs738409 in *PNPLA3* and rs58542926 in *TM6SF2* were confirmed, at genome-wide significance.

Significant associations have also been identified between rs72613567 in *HSD17B13* and rs429358 in *APOE* and a *reduced* risk for developing HCC in ArC(9–11). In the present study, these protective associations were confirmed but failed to reach a detectable genome-wide significance level (Suppl. Table 5).

Further insights into the genetic landscape of HCC in the context of ArLD were recently provided by Trépo and colleagues(14) who undertook a discovery GWAS of HCC in people with a spectrum of alcohol-related liver disease in a French-Belgian collaborative effort. Similar to the present study, they confirmed genome-wide significant associations with an increased risk for developing alcohol-related HCC and variants in *PNPLA3 and TM6SF2.* In addition, they found an equally significant

association with rs708113 in the *WNT3A-WNT9A* region on chromosome 1q42, which was associated with a reduced risk for development of alcohol-related HCC. The presence of this variant was associated with increased immune cell infiltration of tumor tissues and a lower frequency of beta-catenin mutations (*CTNNB1*) which frequently precede HCC occurrence(39). This protective effect of rs708113 was not observed in people with HCC on a background of chronic HCV infection or NAFLD(14).

In the present study rs708113 in the *WNT3A-WNT9A* region was not significantly associated with the development of HCC, possibly reflecting differences in the cohort composition between the two studies although both comprised of participants of European descent. This assumption of population diversity is supported, to some extent, by the fact that in the French-Belgian cohorts the effect size of rs58542926 in *TM6SF2* surpassed that of rs738409 in *PNPLA3* which has been the strongest single genetic risk locus for ArLD in previous candidate gene association studies(40).

The key finding in the present study was the identification of a risk locus in *TERT*, that is not related to lipid turnover, inflammation or fibrogenesis but appears to be highly influential in HCC development(41). Like any cancer, HCC arises when healthy hepatocytes acquire mutations in specific genes regulating cell division. In HCC, *TERT* is the most commonly mutated gene, with mutations (mainly in the promoter region) present in up to 60% of tumors(42). This lends clear plausibility to the association reported in this study between inherited polymorphisms in *TERT* and alcohol-related HCC. Similar relationships between germline and somatic variants have been identified for other cancers types(43). The biology of telomere regulation is still being unraveled and remains incompletely understood. *TERT* encodes the catalytic subunit (hTERT) of the enzyme telomerase, which maintains telomeres, the repeated DNA segments found at the ends of chromosomes. In most cells telomeres progressively

shorten as the cells repeatedly divide and this eventually triggers the cell to stop dividing or to undergo apoptosis. Telomerase counteracts the shortening of telomeres by adding small repetitive DNA segments to the ends of the chromosomes during each cell division cycle(44). Telomerase is also abnormally active in most cancer cells(45). *TERT* expression levels significantly affect telomerase activity in various cells and tissues(46). Previous studies show that older age, male gender and cirrhosis (all classic risk factors for HCC) are associated with shorter telomere length in liver tissue(47). Thus, the present study, showing that rs2242652:A reduces HCC risk whilst at the same time increasing telomere length, is directionally concordant with this previous work. From a mechanistic perspective, it could be that shorter telomeres leave cells more vulnerable to mutations in driver genes, thus accelerating hepatocarcinogenesis(47). It is important to point out however that the association between rs2242652 and HCC may not be entirely mediated through telomere length alone. Indeed, for variants in TERT, we found that there was not a good correlation between strength of association with telomere length and strength of association with HCC. Thus, rs2242652 is not simply acting as a surrogate for telomere length. Relevant to this point is that, as part of its non-canonical functions *TERT* also regulates the WNT/β-catenin pathway (48,49)**.** This signaling pathway is suggested to play a role in alcohol-induced fibrogenesis and hepatocarcinogenesis, too(14,50). However, regarding the risk of alcohol-induced fibrosis/cirrhosis, our data unequivocally show no association with rs2242652 in *TERT*.

There is also some support for the findings in the present study in previous publications. In the GWAS undertaken by Trépo and colleagues(14) rs2242652:A in *TERT* was associated with a reduced risk of HCC, but the odds ratio was weaker than in the present study and did not reach statistical significance (p = 0.179; OR = 0.89 (0.75-1.06)). However, carriage of rs10069690:T in *TERT* – the nearest available proxy

to rs2242652 - was associated with a significantly reduced risk of HCC development (p = 0.036; OR = 0.84 (0.71-0.99)). The significant association between rs2242652:A in *TERT* with liver and intrahepatic bile duct carcinoma in the population-based FinnGen cohort and with HCC in the BioBank Japan cohort additionally substantiate this study's findings. A case control study in Han Chinese involving 473 patients with HCC and 564 healthy volunteers, which is reported in two separate publications (Huang and Zhang (51,52)), also identified associations between variants in *TERT* and the development of HCC; carriage of rs2242652:A in *TERT* was associated with a reduced risk for HCC development (OR = 0.70, 95% CI: 0.55-0.90, p = 0.004), as was carriage of rs10069690:T (OR = 0.75, 95% CI: 0.59-0.96, p = 0.021). Patients with chronic HCV infection were excluded from this study but otherwise it is unclear whether the patients with HCC had underlying chronic liver disease and, if so, its aetiology.

A number of HCC risk loci have been identified in patients developing HCC on a background of chronic HCV(35) and chronic HBV(36), but none was significantly associated with ArC-related HCC in the present study. However, there is some evidence that variants in *TERT* may predispose to HCC in other types of chronic liver disease. Thus, a significant association between rs2242652:A and the reduced risk for developing HCC was observed in patients with HCV-related cirrhosis in the present study following reanalysis of the STOP-HCV(53) data. Also, in a small study Dong and colleagues(54) showed that carriage of the common allele T in rs10069690 is associated with an increased risk of developing HCC on a background of chronic viral hepatitis (OR = 2.78,95% CI: 1.62–4.78, p = 0.00014). Thus, the association between rs2242652 and HCC may extend beyond its relationship with ArC. Further work is warranted to assess if a similar association applies to patients with non-alcohol fatty liver disease (NAFLD). A previous study showed that rare loss of function germline mutations in *TERT* are enriched in patients with NAFLD-HCC relative to controls –

however, the specific relevance of the rs2242652 locus in this patient group is unknown(55).

TERT rs2242652 has also been implicated in the susceptibility for developing other cancers but the direction of association seems to vary between cancer types (Suppl. Table 14). In the present study rs2242652:A was significantly associated with reduced risks for developing bladder cancer and prostate cancer in the UKB and FinnGen cohorts. Kote-Jarai and colleagues(56) found that carriage of *TERT* rs2242652:A: was associated with a lower risk for developing prostate cancer and with increased *TERT* expression which has been reported to improves survival, in prostate cancer. Further large studies involving diverse populations are clearly needed.

The present study has a number of strengths including: a) use of a two stage GWAS approach; b) large, carefully selected case and control samples focusing on HCC in patients with established ArC; b) careful exclusion of confounding co-morbidities; c) uniform inclusion of Caucasians participants of European ancestry; d) the protective effect of rs2242652:A on HCC has been confirmed in the Japanese and Chinese population, suggesting that it may be applicable to East Asian population too, and e) although the study was confined, by design, to patients with HCC on a background of ArC a cohort of patients with HCV-related HCC was also included to assess the generalizability of our findings to other aetiologies. The study also has a number of limitations: a) it was performed retrospectively and hence potentially important information such as the lifetime alcohol history, information on diabetes and obesity were not generally available; b) it had comparatively low power to detect true disease associations with smaller effect sizes (odds ratio <1.4), at the levels of significance needed for GWAS analysis, and c) only a minority of the HCC cases had histological confirmation of the diagnosis so tissue specimen for molecular analyses were not available.

In conclusion, the present study identifies *TERT* rs2242652:A as a novel genetic factor for HCC development in ArC and confirmed the importance of the *PNPLA3* and *TM6SF2* as risk factors for HCC in this population. While the association between HCC and rs2242652:A in *TERT* is robust, the functional implications of carriage of this protective allele remains unclear. Carriage of rs2242652:A was significantly associated with an increase in leukocyte telomere lengths, but data on its effect on *TERT* transcription in liver tissue were not available. Thus, the functional implications of this association require further study in this specific context since the impact of *TERT* transcription, telomere length and the risk of malignancy remains controversial(57).

LEGENDS TO FIGURES

Figure 1: Genome-wide association study (Discovery GWAS) results. Principal findings of genetic analyses **Panel A)**: Manhattan plot of genome-wide association results for alcohol-related hepatocellular carcinoma (HCC) in the primary discovery cohort. P values (−log10) are shown for SNPs that passed quality control. The genome-wide significance threshold (5×10^{-8}) is shown as a black line. The threshold for replication follow-up ($P=5\times10^{-6}$) is shown as a dashed line. Gene names for replicating loci (Table 2) are shown. Variants with significance $P<5\times10^{-8}$ are highlighted in red, those with $P<5\times10^{-6}$ are highlighted in green. **Panel B)** Locus plot for HCC risk locus *PNPLA3*. The −log10 (P values, meta-analysis of discovery and replication samples) are plotted against SNP genomic position based on NCBI Build 37, with the names and location of nearest genes shown at the bottom. The variant with the lowest P value (lead variant) in the discovery analysis in the region is marked by a purple diamond. SNPs are colored to reflect correlation with the most significant SNP, with red denoting the highest LD (r2>0.8) with the lead SNP. The top association signal is located in exon 3 of *PNPLA3*. Estimated recombination rates from the 1000 Genomes Project (hg19, EUR population) are plotted in blue to reflect the local LD structure. **Panel C)** Locus plot for HCC risk locus *TM6SF2*. The top association signal is located in exon 6 of *TM6SF2*. **Panel D)** Locus plot for HCC risk locus *TERT*. Fine-mapping analysis of the *TERT* association signals. Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the Discovery GWAS cohort. The lead association signal is located in intron 4 of the *TERT* gene (annotated on the reverse strand), located in LD block B-3 spanning from intron 4 to intron 2 of *TERT*.

Figure 2: Association between novel (*TERT*) and confirmed loci (*PNPLA3*, *TM6SF2*) with HCC and cirrhosis phenotypes

Odds ratios and 95% confidence intervals for the susceptibility loci for alcohol-related hepatocellular carcinoma (HCC) and alcohol-related cirrhosis (ArC) in comparison to alcohol misusers without cirrhosis (AM). The comparison HCC versus ArC displays allelic odds ratios of combined stage 1 & 2 samples (meta-analysis), derived from allele dosage data, adjusted for age, sex, BMI, type 2 diabetes status and top 15 principal components of genetic ancestry; * The comparison HCC vs. AM and ArC vs. AM display unadjusted allelic odds ratios derived from 2×2 contingency tables of allele counts observed in the total cohort, provided in Suppl. Tables 2-4.

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Table 1: Overview of the study populations included in the discovery and replication cohorts

Variable	**Discovery (GWAS stage 1)** [a] (n= 1910)			**Replication (stage 2)** [a] (n= 1170)								**Validation** [c] Patients with alcohol misuse (n= 1520)		
	Germany-Switzerland-Austria (n= 1910)			United Kingdom (cohort 1) (n= 860)			Germany (cohort 2) (n= 238)			Italy (cohort 3) (n= 72)			Germany (n= 1179)	United Kingdom (n= 341)
	Cases (n= 1066)	Controls (n= 844)	*p* [b]	Cases (n= 70)	Controls (n= 790)	*p* [b]	Cases (n= 42)	Controls (n= 196)	*p* [b]	Cases (n= 36)	Controls (n= 36)	*p* [b]	Non-cirrhosis Controls	Non-cirrhosis Controls
Age (yr)	64.8 (8.5) (100%)	57.1 (9.7) (100%)	***	60.2 (5.9) (100%)	56.3 (8.9) (100%)	***	67.1 (9.1) (100%)	58.5 (9.7) (100%)	*	72.7 (8.0) (100%)	53.3 (8.9) (100%)	***	42.7 (10.4) (100%)	48.6 (10.5) (100%)
Proportion male (n: %)	968 (90.8)	624 (73.9)	***	67 (95.7)	577 (73.0)	***	35 (83.3)	131 (66.8)	**	32 (88.9)	31 (86.1)	*	1148 (97.4)	263 (77.1)
BMI (kg/m2) [c]	28.1 (4.8) (69%)	26.5 (5.3) (91%)	***	29.3 (4.4) (100%)	27.5 (4.8) (92%)	**	24.8 (3.5) (57%)	26.1 (5.8) (52%)	*	27.0 (3.8) (64%)	27.0 (6.8) (75%)	*	25.3 (4.5) (81%)	24.7 (2.3) (53%)
BMI kg/m2; (n: %) [c] <25	183 (24.7)	308 (40.3)	***	13 (18.6)	227 (31.3)	**	13 (54.2)	52 (51.0)	*	6 (26.1)	13 (48.1)	*	505 (52.6)	94 (52.2)
25-30	333 (45.0)	295 (38.6)		27 (38.6)	288 (39.7)		8 (33.3)	27 (26.5)		12 (52.2)	7 (25.9)		345 (35.9)	86 (47.8)
>30	224 (30.3)	162 (21.2)		30 (42.9)	211 (29.1)		3 (12.5)	23 (22.5)		5 (21.7)	7 (25.9)		110 (11.5)	0 (0)
Diabetes Type II + (n: %) [c]	337 (47.1) (67%)	136 (30.1) (54%)	***	20 (28.6) (100%)	102 (12.9) (100%)	***	14 (36.8) (90%)	36 (19.7) (93%)	**	16 (44.4) (100%)	5 (13.9) (100%)	**	58 (5.7) (86%)	8 (3.6) (66%)

[a] Cases and controls were assigned to groups as detailed in the Methods section; [b] *p* values were calculated from Student's t-test for quantitative variables and as Pearson's chi-squared test for categorical variables; *** *p* value < 0.0001; ** *p* value < 0.05; * *p* value > 0.05; [c] Validation cohorts were used in post-hoc risk assessment; [d] Data are reported as mean ± standard deviation or as number (%) ; Completeness of phenotypic information for age, BMI and type 2 diabetes status are reported as percentage of subjects with available information below the mean value.

Table 2: Association results for lead markers of regions entering the validation stage of the primary and sensitivity GWAS analysis.

					Discovery (stage 1)			Replication (stage 2)					Combined (stage 1 & 2) [c]		
Lead SNPs	Locus	Chr	SNP ID	EA, ED	*p value* [a,b]	OR [95% CI]	EAF Ca|Co|Eur	Meta *p value* [a+]	OR [95% CI]	Eff. Dir.	I^2	$p_{heterog.}$	Meta *p* [a+]	OR [95% CI]	I^2
GWAS Analysis 1 (pc adjusted) [a]															
SNP 1	*PNPLA3**	22	rs2294915	T+	**6.21×10^{-15}**	1.71 (1.50-1.96)	.49|.36|.24	**6.19×10^{-6}**	1.89 (1.44-2.50)	+++	0	0.517	**2.44×10^{-19}**	1.75 (1.55-1.97)	0
	PNPLA3	22	rs738409	G+	**7.23×10^{-15}**	1.71 (1.49-1.96)	.48|.35|.22	**9.74×10^{-6}**	1.89 (1.42-2.50)	+++	0	0.578	**4.31×10^{-19}**	1.74 (1.54-1.97)	0
SNP 2	*TM6SF2***	19	rs58489806	T+	**1.42×10^{-9}**	1.87 (1.53-2.29)	.17|.10|.08	**5.22×10^{-4}**	1.91 (1.33-2.76)	++-	54	0.110	**3.04×10^{-12}**	1.88 (1.57-2.25)	32
	TM6SF2	19	rs58542926	T+	**2.81×10^{-9}**	1.94 (1.56-2.42)	.15|.08|.07	**7.58×10^{-5}**	2.11 (1.46-3.04)	++-	61	0.076	**1.00×10^{-12}**	1.98 (1.64-2.40)	43
SNP 3	*TERT*	5	rs2242652	A-	7.87×10^{-7}	0.64 (0.53-0.76)	.13|.19|.19	**1.07×10^{-3}**	0.48 (0.31-0.74)	---	0	0.814	**6.40×10^{-9}**	0.61 (0.52-0.72)	0
SNP 4	*LINC00939*	12	rs12371263	A-	9.59×10^{-7}	0.63 (0.52-0.76)	.16|.21|.20	0.332	0.83 (0.57-1.21)	-+-	0	0.535	-	-	-
SNP 5	*DMAC2*	19	rs17318596	A-	2.49×10^{-6}	0.71 (0.61-0.82)	.33|.40|.37	0.849	1.03 (0.77-1.38)	-+-	15	0.308	-	-	-
SNP 6	*SP100*	2	rs6743289	C-	2.77×10^{-6}	0.72 (0.62-0.82)	.45|.52|.47	0.046	0.75 (0.57-1.00)	---	0	0.936	-	-	-
SNP 7	*GPIHBP1*	8	rs118088203	T-	3.60×10^{-6}	0.24 (0.13-0.44)	.01|.03|.02	0.229	1.64 (0.73-3.07)	+++	0	0.697	-	-	-
SNP 8	*CNPY1*	7	rs12698003	T+	3.65×10^{-6}	1.39 (1.21-1.60)	.46|.39|.41	0.053	0.74 (0.55-1.00)	---	41	0.179	-	-	-
SNP 9	*GLYR1*	16	rs741692	T+	4.16×10^{-6}	1.58 (1.30-1.92)	.18|.12|.15	0.783	1.05 (0.73-1.51)	++-	0	0.541	-	-	-
GWAS Analysis 2 (pc, sex, age adjusted) [b]															
SNP 1	*PNPLA3**	22	rs2294915	T+	**6.31×10^{-14}**	1.76 (1.52-2.05)	.49|.36|.24	**3.24×10^{-5}**	1.89 (1.40-2.54)	+++	0	0.428	**1.06×10^{-17}**	1.79 (1.57-2.04)	0
	PNPLA3	22	rs738409	G+	**1.67×10^{-13}**	1.75 (1.51-2.03)	.48|.35|.22	**4.17×10^{-5}**	1.85 (1.37-2.50)	+++	0	0.448	**5.35×10^{-17}**	1.77 (1.55-2.02)	0
SNP 2	*TM6SF2***	19	rs143988316	T+	**4.40×10^{-8}**	1.91 (1.51-2.41)	.16|.09|.07	5.17×10^{-2}	1.54 (1.00-2.38)	++-	0	0.621	**9.14×10^{-9}**	1.81 (1.51-2.16)	0
	TM6SF2	19	rs58542926	T+	1.21×10^{-7}	1.93 (1.51-2.45)	.15|.08|.07	**1.56×10^{-4}**	2.16 (1.45-3.22)	++-	48	0.149	**8.80×10^{-11}**	1.99 (1.61-2.44)	26
SNP 3	*SCN5A*	3	rs6599222	C+	2.86×10^{-6}	1.53 (1.28-1.84)	.25|.20|.21	0.977	1.01 (0.68-1.48)	-++	0	0.984	-	-	-
SNP 4	*intergenic*	13	rs148892410	A-	3.77×10^{-6}	0.16 (0.07-0.35)	.01|.02|.01	0.798	1.45 (0.09-24.2)	++-	17	0.299	-	-	-
SNP 5	*intergenic*	2	rs6739777	G-	5.03×10^{-6}	0.69 (0.59-0.81)	.29|.34|.30	0.388	0.86 (0.61-1.21)	-+-	40	0.193	-	-	-
SNP 6	*ENSG00000269151*	19	rs143660337	A-	5.14×10^{-6}	0.41 (0.28-0.60)	.03|.05|.04	0.151	1.68 (0.83-3.41)	++-	0	0.589	-	-	-
SNP 7	*LOC105374308*	3	rs58339845	T-	5.84×10^{-6}	0.46 (0.33-0.65)	.05|.07|.07	0.361	1.34 (0.72-2.50)	+++	0	0.919	-	-	-
SNP 8	*intergenic*	7	rs16869539	G+	5.96×10^{-6}	1.48 (1.25-1.75)	.36|.30|.37	0.537	0.90 (0.65-1.25)	---	0	0.983	-	-	-
SNP 9	*CELF2*	10	rs2277212	T+	6.84×10^{-6}	1.57 (1.29-1.91)	.75|.70|.74	0.282	1.22 (0.85-1.74)	+-+	16	0.303	-	-	-
SNP 10	*intergenic*	7	rs6462611	C+	7.82×10^{-6}	1.41 (1.21-1.64)	.49|.44|.50	0.017	0.68 (0.49-0.93)	++-	0	0.465	-	-	-
SNP 11	*ENSG00000227757*	21	rs2017196	T+	8.73×10^{-6}	1.70 (1.34-2.14)	.89|.85|.88	0.092	0.68 (0.44-1.06)	---	0	0.513	-	-	-
SNP 12	*TERT*	5	rs2242652	A-	9.28×10^{-6}	0.64 (0.52-0.78)	.13|.19|.19	**2.60×10^{-4}**	0.41 (0.25-0.66)	++-	0	0.699	**4.08×10^{-8}**	0.60 (0.50-0.72)	17
SNP 13	*RARB*	3	rs7617311	A+	9.32×10^{-6}	1.50 (1.25-1.80)	.28|.21|.26	0.454	0.87 (0.61-1.25)	-+-	0	0.751	-	-	-
SNP 14	*CIAO2A*	15	rs2922508	T+	9.53×10^{-6}	1.61 (1.30-1.99)	.17|.13|.15	0.153	1.33 (0.90-1.97)	+++	0	0.797	-	-	-
SNP 15	*intergenic*	2	rs56209271	T-	9.67×10^{-6}	0.69 (0.59-0.82)	.28|.34|.30	0.168	0.78 (0.55-1.11)	---	61	0.075	-	-	-

Abbreviations: SNP-single nucleotide polymorphism; Chr: chromosome; Ca: Cases (Cirrhosis with HCC); Co: Controls (Cirrhosis without HCC); Eur: allele frequency in north-western Europeans from gnomAD (v2.1.1). Effect Allele (EA): reference allele for odds ratio (OR); Eff.Dir (ED). Effect direction; EAF: allele frequency of the effect allele; Meta *p* value: fixed effects meta-analysis *p* value; [+] Significance derived from a fixed effect meta-analysis. Heterogeneity *p* value: Q Test for heterogeneity between cohorts (df = 2); OR: odds ratio; CI: confidence interval; $p_{heterog.}$ heterogeneity *p* value of the meta-analysis; I^2- percentage of between cohort heterogeneity; [a] Odds ratio and *p* value adjusted for top 15 PCs of genetic ancestry; [b] OR and *p* value adjusted for sex, age and top 15 PCs of genetic ancestry; [c] The results of the combined analyses are only provided for variants meeting a Bonferroni corrected p<0.05 at the replication stage (printed in bold face). * The tag SNP rs2294915 in *PNPLA3* is in LD (r2= 0.92) with the functional variant rs738409 previously reported at the *PNPLA3* locus (58,59); ** The intergenic tag SNP rs143988316 is in strong LD (r2= 0.88) with the functional variant rs58542926 previously reported at the *TM6SF2* locus (60).

Table 3 Replication of *TERT* variants in patients with alcohol-related and chronic HCV-related cirrhosis and in population-based cohorts

Cohort	Controls	Cases phenotype (ICD-10)	N cases | controls	*TERT* Variant	EA	*P*	OR (CI95%)
Current study[a]	ALD cirrhosis[a]	C22.0 Liver cell carcinoma (HCC) in alcohol-related cirrhosis	1214 | 1866	rs2242652	A	6.40×10^{-9}	0.61 (0.52-0.72)
Replication cohorts							
Trépo *et al.*[c]	ALD F0-F4 fibrosis	C22.0 HCC in alcohol-related liver disease (F0-F4 fibrosis)	775 | 1332	rs2242652	A	0.179	0.89 (0.75-1.06
Stop-HCV [b]	HCV cirrhosis	C22.0 Liver cell carcinoma (HCC) in HCV-related cirrhosis	169 | 890	rs2242652	A	0.047 *	0.72 (0.53-0.99)
Zhang *et al*.[d]	Healthy volunteers	C22.0 Liver cell carcinoma (HCC)	473 | 564	rs2242652	A	0.004	0.70 (0.55-0.90)
Dong *et al*.[e]	Healthy volunteers	C22.0 Liver cell carcinoma (HCC) (hepatitis-induced)	162 | 106	rs10069690	T	0.00014*	0.36 (0.21-0.63)
FinnGen [f]	General population	C22 malignant neoplasm of liver and intrahepatic bile duct	442 | 204,070	rs2242652	A	0.007	0.80 (0.68-0.94)
UKBB [g]	General population	C22 malignant neoplasm of liver and intrahepatic bile duct	874 | 348,465	rs2242652	A	0.027	0.87 (0.78-0.97)
UKBB [g]	General population	C22.0 Liver cell carcinoma (HCC)	383 | 348,956	rs2242652	A	0.028	0.80 (0.66-0.98)
BBJ Japan [h]	BBJ population **	C22.0 Liver cell carcinoma (HCC)	1866 | 195,745	rs72709458	T	0.00031	0.84 (0.76-0.92)

HCV, hepatitis C virus; EA: effect allele; *p: p* value and odds ratios (OR) derived from logistic regression analysis*;* ICD: International Classification of Diseases; C22.0, ICD-10 code for liver cell carcinoma; C22, ICD-10 code for Malignant neoplasm of liver and intrahepatic bile ducts; UKBB United Kingdom Biobank; FinnGen: FinnGen Biobank; BBJ: BioBank Japan;

[a] Combined effect estimates of stage 1 and 2 samples of current study as shown in Table 1 (for comparison).

[b] Cases: patients with HCV related cirrhosis and HCC, controls: patients with HCV related cirrhosis without HCC (Suppl. Methods F).

[c] Cases: patients with ALD (80% with F3-4 fibrosis; 20% F0-2 fibrosis) and HCC, controls: patients with ALD (90% with F3-4 fibrosis, 10% F0-2 fibrosis) from Trépo *et al*.(14).

[d] Zhang *et al*.(52), Huang et al.(51) Han Chinese patients with HCC (individuals were excluded from the study if they had hepatitis C virus).

[e] Dong *et al*.(61,62) male Han Chinese patients with viral hepatitis-induced primary hepatocellular carcinoma (r2= 0.85 between rs10069690:T and rs2242652:A, both variants are in high linkage disequilibrium).

[f] General population controls (excluding all cancers).

[g] As UKB data were incorporated into our discovery analysis, further interrogation of liver cancer phenotypes from UKB does not constitute independent validation.

[h] Variants rs2242652 and rs10069690 were not available in the summary GWAS data from Ishigaki et al.(63) (PMID: 32514122, publicly available from http://jenger.riken.jp/en/result) (rs72709458 is the closest proxy to rs2242652 (r2=0.973)).

** Removed diseases from control samples (biliary tract cancer, esophageal cancer, gastric cancer, colorectal cancer and pancreatic cancer)

*Allelic odds ratios were calculated from 2 × 2 tables on allele counts. Significance was calculated as one degree of freedom Chi-squared test.

LEGENDS TO FIGURES

Figure 1: Genome-wide association study (Discovery GWAS) results. Principal findings of genetic analyses **Panel A)**: Manhattan plot of genome-wide association results for alcohol-related hepatocellular carcinoma (HCC) in the primary discovery cohort. P values (−log10) are shown for SNPs that passed quality control. The genome-wide significance threshold (5×10^{-8}) is shown as a black line. The threshold for replication follow-up ($P=5\times10^{-6}$) is shown as a dashed line. Gene names for replicating loci (Table 2) are shown. Variants with significance $P<5\times10^{-8}$ are highlighted in red, those with $P<5\times10^{-6}$ are highlighted in green. **Panel B)** Locus plot for HCC risk locus *PNPLA3*. The −log10 (P values, meta-analysis of discovery and replication samples) are plotted against SNP genomic position based on NCBI Build 37, with the names and location of nearest genes shown at the bottom. The variant with the lowest P value (lead variant) in the discovery analysis in the region is marked by a purple diamond. SNPs are colored to reflect correlation with the most significant SNP, with red denoting the highest LD (r2>0.8) with the lead SNP. The top association signal is located in exon 3 of *PNPLA3*. Estimated recombination rates from the 1000 Genomes Project (hg19, EUR population) are plotted in blue to reflect the local LD structure. **Panel C)** Locus plot for HCC risk locus *TM6SF2*. The top association signal is located in exon 6 of *TM6SF2*. **Panel D)** Locus plot for HCC risk locus *TERT*. Fine-mapping analysis of the *TERT* association signals. Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the Discovery GWAS cohort. The lead association signal is located in intron 4 of the *TERT* gene (annotated on the reverse strand), located in LD block B-3 spanning from intron 4 to intron 2 of *TERT*.

Figure 2: Association between novel (*TERT*) and confirmed loci (*PNPLA3*, *TM6SF2*) with HCC and cirrhosis phenotypes

Odds ratios and 95% confidence intervals for the susceptibility loci for alcohol-related hepatocellular carcinoma (HCC) and alcohol-related cirrhosis (ArC) in comparison to alcohol misusers without cirrhosis (AM). The comparison HCC versus ArC displays allelic odds ratios of combined stage 1 & 2 samples (meta-analysis), derived from allele dosage data, adjusted for age, sex, BMI, type 2 diabetes status and top 15 principal components of genetic ancestry; * The comparison HCC vs. AM and ArC vs. AM display unadjusted allelic odds ratios derived from 2×2 contingency tables of allele counts observed in the total cohort, provided in Suppl. Tables 2-4.

Figure 1: Genome-wide association study (GWAS) results.

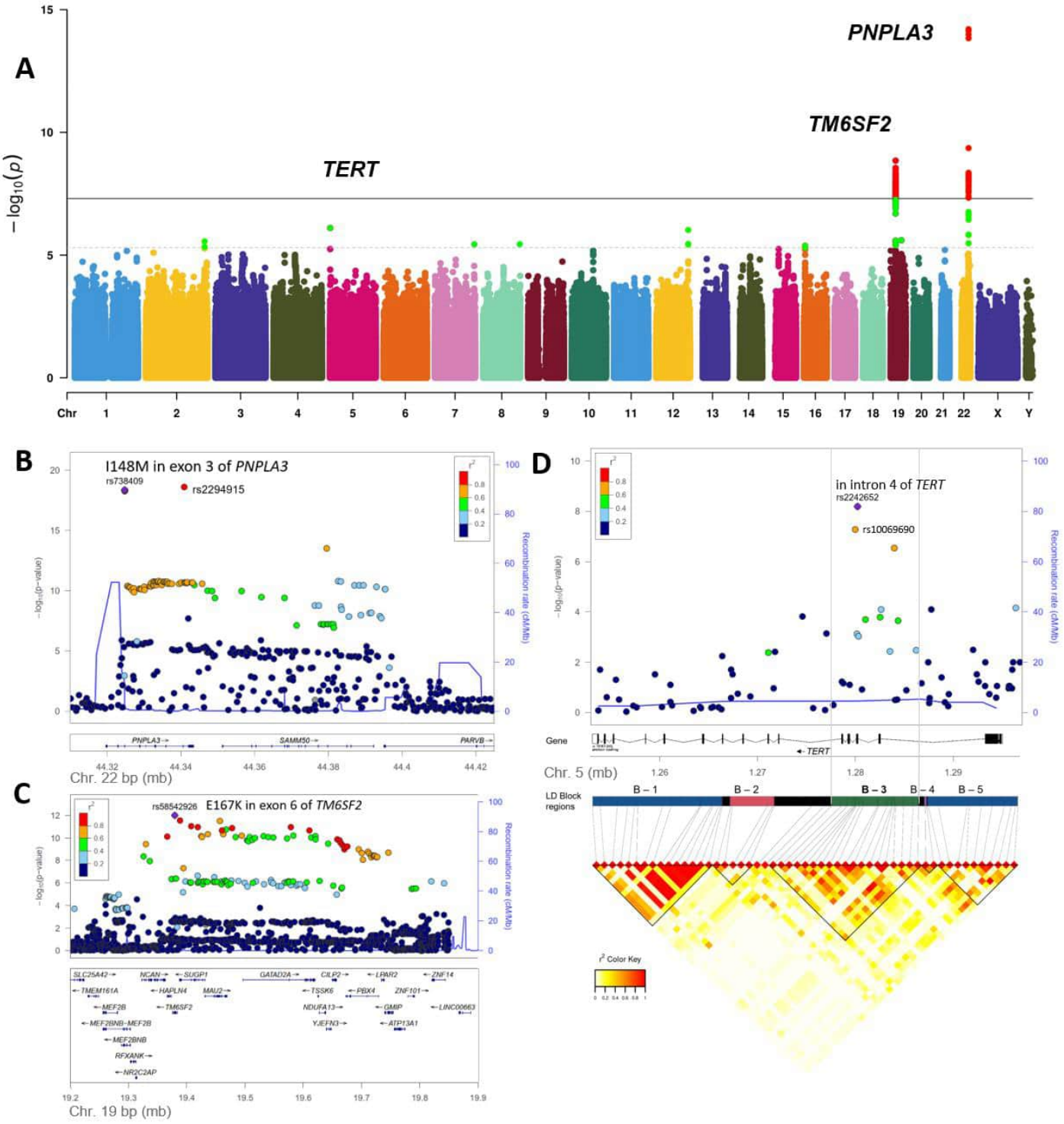
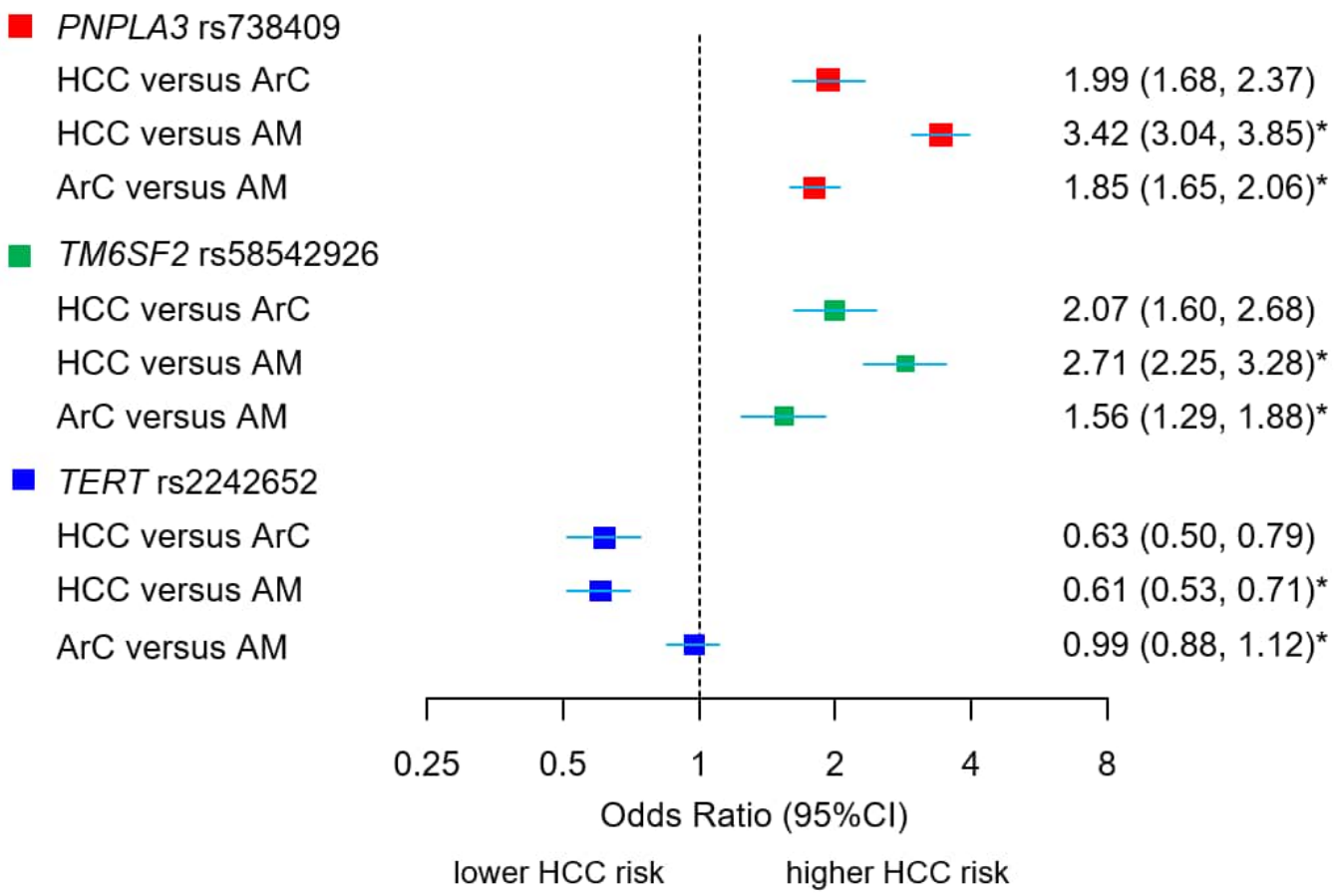


Figure 2: Association between new (*TERT*) and known loci (*PNPLA3*, *TM6SF2*) with HCC and cirrhosis phenotypes



Supplementary information

SUPPLEMENTARY METHODS A

Patient cohorts

Patients with alcohol-related cirrhosis referred to university centres in Germany (Dresden, Bonn, Rostock, Heidelberg, Munich, Leipzig, Halle, Homburg, Hannover, Hamburg, Kiel, Magdeburg, Frankfurt), Switzerland (Berne, Zürich, Lausanne), Austria (Salzburg/Oberndorf, Graz, Vienna), United Kingdom (London and UK Biobank), and Italy (Milan), were included and assigned to respective groups, as detailed in Table 1.

Ethics

The study protocol was approved by the ethics committees of the participating institutions; all included subjects provided written informed consent prior to inclusion into the study.

Germany / Switzerland / Austria Alcohol Cohort (Discovery cohort)

The diagnosis of alcohol-related cirrhosis was established as previously described [1]. In a substantial fraction of subjects included, transient elastography was performed to include patients with a liver stiffness measurement (Fibroscan, Echosens, Paris) of above 19 kPa (IQR<20%) indicating cirrhosis as per current consensus [2]. Morbidly obese (body mass index ≥45kg/m^2) patients were excluded. The diagnosis of HCC was established as previously described [3], [4] and based on histological examination of tumour tissue or typical evidence on magnetic resonance imaging, using liver-specific contrast enhancement of focal lesions that were hypervascular in the arterial phase with a fast wash-out in the portal venous or delayed phases, and revealed a serum-alpha-fetoprotein level of >200ng/ml [5], [6].

SUPPLEMENTARY METHODS B

ArC nested case-control dataset:

We created a nested case control study for ArC HCC using data from the UKB resource as described in the main text.

Participants were excluded from the case-control study for any of the following reasons

- non-White British ancestry (inferred via UKB field ID:22006);
- poor quality genetic sample (defined by UKB field ID: 22027);

- Second or first-degree relations to another participant in the case-control dataset (inferred via a kinship coefficient ≥0.121).

Individual-level data for approximately 6.2 million genetic variants were available in the version 3 UKB imputed genetic data sets, after exclusion of variants with (a) minor allele frequency <1%, (b) gross deviation from the Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-10}$), (c) imputation information score <0.8, (d) high level of missing data (>10%), and (e) non-biallelic or duplicate variants.

Evidence for alcohol-related cirrhosis was determined as:

A hospital admission for alcohol cirrhosis of liver.

- K70.3 (Alcoholic cirrhosis of liver)

We also excluded Individuals with other liver disease aetiologies (viral hepatitis, hemochromatosis and autoimmune liver disease).

Viral hepatitis and autoimmune liver disease were defined as the presence of one or more of the following ICD codes in any diagnostic position within an in-patient hospital admission record

- K754 (Autoimmune hepatitis)
- K743 (primary biliary cirrhosis)
- K744 (secondary biliary cirrhosis)
- K745 (biliary cirrhosis, unspecified)
- B16 (acute hepatitis B)
- B17 (other acute viral hepatitis)
- B18 (Chronic viral hepatitis)
- B19 (unspecified viral hepatitis)

Hemochromatosis was defined as homozygous carriage of the C282Y variant (rs18005762), as determined from the version 3 UKB genetic dataset (downloaded May 2019).

These nested case-control data were then pooled with additional patients recruited from the Centre for Hepatology at the Royal Free Hospital, London (N=306). These patients were similarly of self-reported English, Scottish, Welsh or Irish descent, and had a history of prolonged alcohol misuse as described previously [1]. All were examined by two experienced, senior clinicians for signs of liver injury. The diagnosis of HCC was based on histological examination of tumour tissue. Histological examination was undertaken, whenever possible, of liver biopsy material obtained by

percutaneous, ultrasound-guided or transjugular routes or else of explant or postmortem liver tissue. Blood was screened for antibodies to hepatitis B, hepatitis C, mitochondrial, nuclear, smooth muscle, liver and kidney autoantibodies; iron, total iron-binging capacity and ferritin; copper and caeruloplasmin; α1 antitrypsin and tissue transglutaminase. Patients were excluded if they had any other potential cause of liver injury such as chronic viral hepatitis or autoimmune liver disease; genetic haemochromatosis; Wilson's disease; alpha-1 antitrypsin deficiency or celiac disease. Patients with ArC (with no evidence of HCC) were diagnosed on the basis of a history of alcohol dependence and histological examination of liver tissue or on the basis of compatible historical, clinical, laboratory, radiological and endoscopic features.

University of Bonn (Germany) Alcohol Cohort (Replication cohort 2)

The replication cohorts included 238 Caucasian patients with ArC (42 with HCC) from the Hepatology/Gastroenterology department of the University Bonn, as detailed previously [7]**.**

University of Milan (Italy) Alcohol Cohort (Replication cohort 3)

The replication cohorts included 72 Caucasian patients with ArC (36 with HCC) from the department of Pathophysiology and Transplantation of the State University of Milan.

SUPPLEMENTARY METHODS C

Validation cohorts

For post-hoc risk assessment additional patients with alcohol misuse, but without cirrhosis were included. Non-cirrhotic control patients (n=1080) with alcohol misuse but no evidence of significant liver injury were recruited at psychiatry centers specialized in addiction medicine in Regensburg, Munich and Mannheim (all in Germany) as described, in detail, previously [1]. In brief, patients had a background of alcohol consumption of at least 60 g/d for women and 80 g/d for men for ≥10 years, all patients received a diagnosis of alcohol dependence (according to DSM-IV criteria). None had historical, clinical or laboratory evidence of liver disease, and its absence was confirmed either by a liver stiffness measurement (Fibroscan, Echosens, Paris) of below 6 kPa (IQR<20%) or by the absence of histological liver damage. These patients were then pooled together with additional non-cirrhotic patients with alcohol abuse (n=99) recruited from the Salem Medical Center, Heidelberg as described, in detail, previously [8].

Additional, non-cirrhosis control patients (n=341) recruited from the Centre for Hepatology at the Royal Free Hospital, London were diagnosed on the basis of a history of alcohol dependence and the absence of liver injury on histology or the absence of historical, clinical, radiological or

endoscopic features suggestive of significant liver injury either at presentation of during prolonged follow-up. All patients underwent abdominal ultrasound and/or abdominal computed tomography/magnetic resonance imaging, as indicated. All underwent routine upper gastrointestinal endoscopy.

SUPPLEMENTARY METHODS D

DNA preparation and genotyping

Discovery cohort

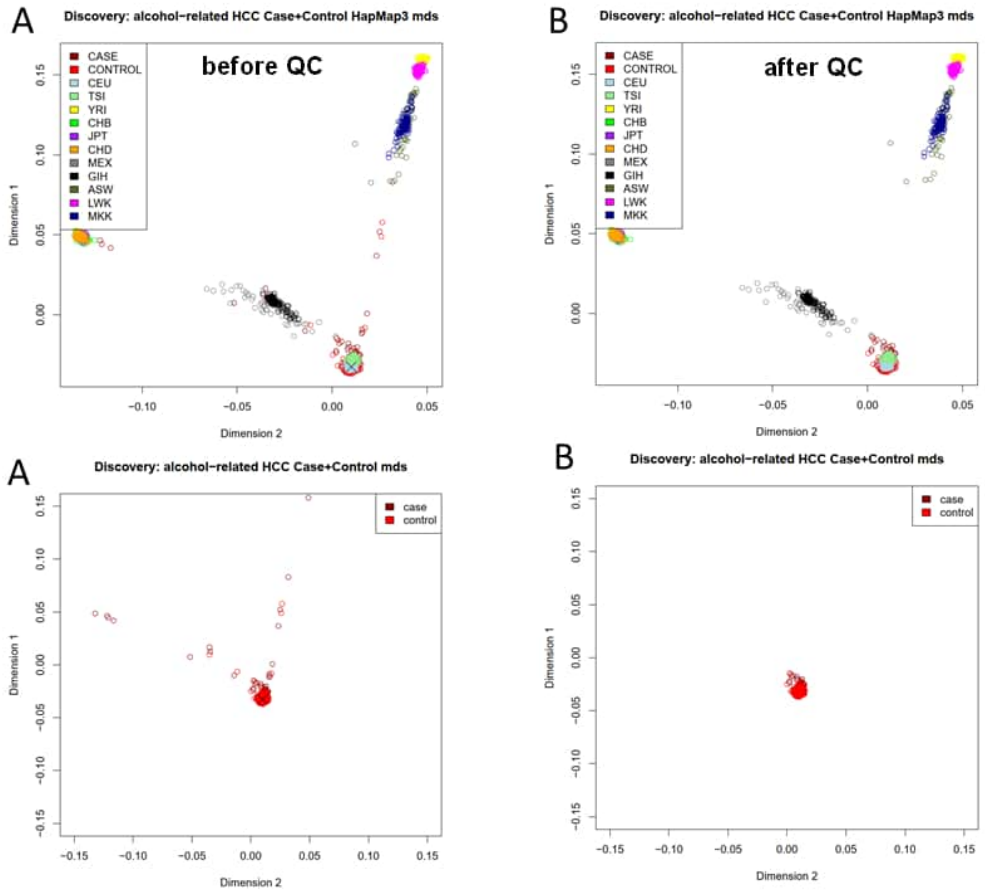
Genomic DNA was extracted from peripheral blood samples according to standard procedures, quantified using the PicoGreen dsDNA Assay kit (Invitrogen) and normalized to a concentration of 50 ng/µl. Genotyping on Illumina BeadChip arrays was performed according to the manufacturer's instructions, as described before [1].

For the present study, >665,000 SNPs (662,835 with assigned rs-numbers) were genotyped in a clinical case-control panel of 1910 patients with ArC from Germany and Switzerland with a total genotyping rate of 0.998. The discovery GWAS included 1,066 patients with ArC and HCC and 844 patients with ArC with no evidence of HCC from Germany and Switzerland (Table 1) genotyped on the Infinium®Global Screening Array (GSA) (version 24v2, Illumina). Samples were called using GenomeStudio (v2.0, Illumina), exported to Plink file format and imputed as described below.

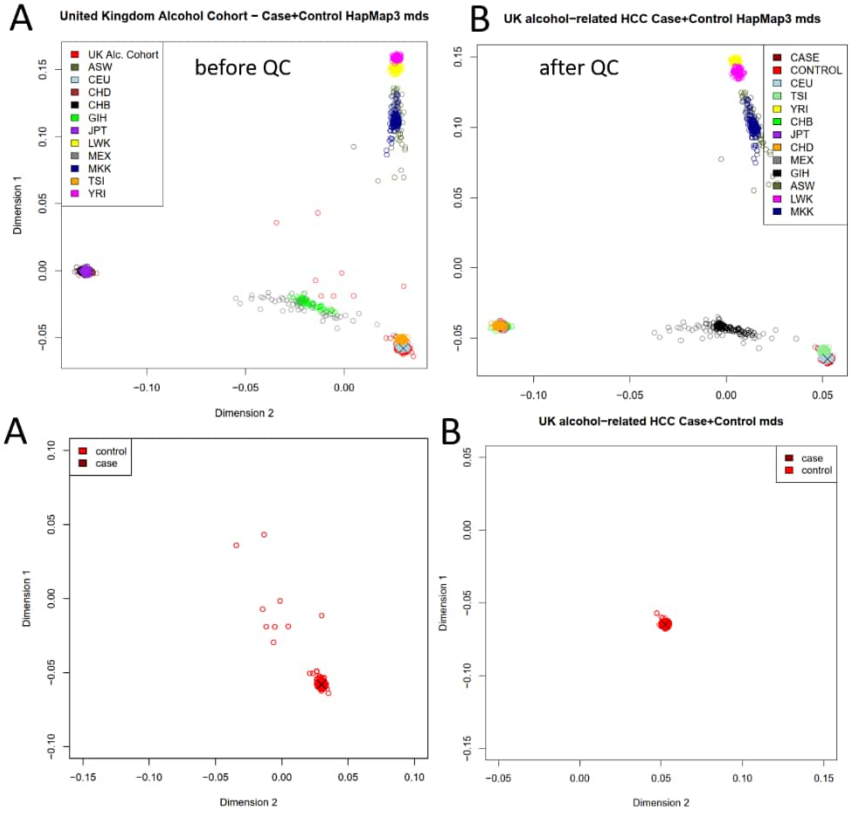
Genotype data quality control

In all data sets, individuals with genotyping success <97%, outlying autosomal heterozygosity (more than 3 SD from the mean) or a kinship coefficient (pˆ) <0.185 and those failing gender checks were excluded from analysis. Multidimensional scaling (MDS) analysis was performed on a cleaned, LD-pruned data set (indep-pairwise; excluding the human leukocyte antigen (HLA) region at chr. 6: 28,477,797–33,448,354; minor allele frequency >0.01, Hardy-Weinberg equilibrium P > 1×10^{-6}, genotyping rate threshold for each marker >98% and genotyping rate threshold for each individual >97%) that was merged with HapMap Phase 3 data from 11 different populations. Individuals deviating by more than 3 SD from the median European MDS cluster were excluded as population outliers. All quality control filtering was performed using PLINK (v1.9).

MDS plot of discovery cohort (non-European ancestry outlier removal)



MDS plot of United Kingdom replication cohort (non-European ancestry outlier removal)



Replication cohorts

Germany:

Replication samples from Germany (cohort 2) were genotyped on the OmniExpress array (version 24v1-0a), exported to Plink format and imputed as described below.

Italy:

Replication samples from Italy (cohort 3) were genotyped on the Infinium®Global Screening Array (GSA) (version 24v2, Illumina), exported to Plink format and imputed as described below.

United Kingdom:

In total, 652 patients with a history of prolonged alcohol misuse (306 patients with alcohol-related cirrhosis and 346 non-cirrhotic controls from the UK) were genotyped using the OmniExpress array (version 24v1-0a, Illumina) as described previously [1]. Samples were called using GenomeStudio (v2.0, Illumina). Genotype data was then imputed to HRC reference panel as described below. For replication of lead variants, imputed VCF format probability data was exported to Plink format and merged with Plink format genotype data from 606 patients from the United Kingdom (UK) Biobank Resource based on data from the version 3 release. Imputed genotype data from 606 patients genotyped on the UK BiLEVE and the UK Biobank Axiom array were obtained from the UK Biobank Resource [9] (under application number 8764).

The merged data set was subjected to quality control procedures described below. Association tests were performed on the merged data set on discrete genotypes.

The UK Biobank analysis was based on data from the version 3 release of the UKB imputed genetic data set. Participants were excluded from the case-control study for any of the following: non-White British ancestry (inferred via UKB field ID:22006); poor quality genetic sample (defined by UKB field ID: 22027); Second or first-degree relations to another participant in the case-control dataset (inferred via a kinship coefficient ≥0.121). Individual-level data for approximately 6.2 million genetic variants were available in the version 3 UKB imputed genetic data sets, after exclusion of variants with (a) minor allele frequency <1%, (b) gross deviation from the Hardy-Weinberg equilibrium ($P < 1.0\times10^{-10}$), (c) imputation information score <0.8, (d) high level of missing data (>10%), and (e) non-biallelic or duplicate variants.

Validation samples

Control subjects drinking excessively but without evident alcohol-related liver disease (AM) were recruited at psychiatry centers specialized in addiction medicine in Regensburg, Munich and Mannheim (all in Germany). All patients received a diagnosis of alcohol dependence (according to DSM-IV criteria). These patients were genotyped on the HumanHap550 (n = 407), Human610Quad (n = 329) and Human660w-Quad (n = 383) Illumina BeadChip arrays. Individuals with genotyping success <97% were excluded from further analysis, as described in detail before [10]. To harmonize the German data sets, genotype probabilities were generated from signal intensity data for each array. Sample phasing and genotype imputation were performed using IMPUTE2 to the1000 Genomes Project Phase 3 reference (October 2014 release). Expected genotypes were calculated from the genotype posterior probabilities using the software SNPTEST (v2.5.6; snptest (ox.ac.uk) [11].

Additional 99 AM control subjects were recruited from the Salem Medical Center, Heidelberg and were genotyped on the Infinium®Global Screening Array (GSA) (version 24v2, Illumina), exported to Plink format and imputed as described below.

SUPPLEMENTARY METHODS E

Imputation and GWAS meta-analysis

Genotype imputation was performed with Minimac4 to the HRC r1.1 (hg19) reference panel using the Michigan Imputation Server[12], [13]. In total 7,946,762 variants with imputation r^2>0.3, MAF>0.01 and HWE ($P>1x10^{-6}$) were tested for association with HCC using linear regression on allele dosages adjusted for top 15 principal components of genetic ancestry. Quality filtered plink files from the discovery and replication cohorts were prepared for imputation to the Haplotype Reference Consortium reference panel (HRC.r1-1.GRCh37) using the “HRC-1000G-check-bim” tool (Version 4.3.0) [14]. Phasing and genotype imputation was performed using the Michigan Imputation Server [15]. After imputation 7,778,317 variants were available for the discovery GWAS, after exclusion of variants with a minor allele frequency <0.01, deviation from Hardy-Weinberg equilibrium $P < 1\times10^{-6}$ and imputation information score <0.3.

SUPPLEMENTARY METHODS F

Replication analysis

In stage 2, the selected SNPs were validated in independent samples from the UK (n=860), Germany (n=238) and Italy (n=72). Study-specific β estimates and standard errors were derived from stage 2 samples and further analyzed using fixed-effect meta-analysis. Cochran's Q and I2 statistics were employed to assess consistency of effect and to quantify heterogeneity between sample sets. Two criteria were required to demonstrate replication: a) P value < 5.55×10^{-3} (corresponding to P < 0.05 after Bonferroni correction for 9 tests); and b) and consistency of allelic effect direction between discovery and replication samples. Four different multiplicative allelic models were analyzed: model (1) included the top 15 PCs, model (2) included sex, age and the top 15 PCs, model (3) included sex, age, BMI and the top 15 PCs and model (4) included age, sex, BMI, diabetes type 2 status and the top 15 PCs using Plink 2.0 for. Model 1 results were used in the primary replication analysis, model 2 results in the secondary replication analysis, model 3 and 4 estimates in *post-hoc* risk assessment (Suppl. Table 5).

Additional replication analysis of *TERT* variants in population-based cohorts

FinnGen Biobank

FinnGen is a public–private partnership project, combining genotyping data from Finnish biobanks with electronic health record data derived from national health registries. Genome-wide association study (GWAS) summary statistics for more than 1,800 phenotypes/endpoints, including for primary liver cancer, have been publicly released.

This study utilized the latest R6 data released (autumn 2020) pertaining to a sample size of 260,405 individuals. Cases were individuals with a history/diagnosis of primary liver cancer (ICD-10: C22 and ICD-9: 155), while controls were all individuals without a diagnosis of primary liver cancer (excluding all other cancers), largely comprised of individuals without any preexisting liver disease. GWAS summary statistics relating specifically to HCC were not available.

United Kingdom Biobank

As UKB data were incorporated into the discovery analysis, further interrogation of liver cancer phenotypes from UKB does not constitute independent validation. However, for reference purposes, we calculated the association between variants in *TERT* and liver-related cancer at the level of the entire UKB population.

Associations with three specific phenotypes were assessed.

1) HCC (ICD 10: C22.0).
2) Intrahepatic bile duct cancer (ICD 10: C22.1)
3) All liver related neoplasms (ICD10: C22)

N.B. the latter phenotype was selected in order to align with data from the FinnGen cohort.

As with previous analyses, UKB participants were restricted to those of white British ancestry (UKB field ID: 22006) and excluded those with a poor-quality genetic sample (defined by UKB field ID: 22027); and excluded related participants (inferred by a kinship coefficient ≥0.1).

Cases were defined as participants with the selected ICD code(s) in a hospital admission, death, or cancer registration record, either before or after UKB enrollment. The control group comprised UKB participants who did not meet the above definition of a case. All analyses were adjusted for sex, age at UKB enrolment, and the top 5 principal components of genetic ancestry.

BioBank Japan

BBJ is a prospective genome biobank that collaboratively collected DNA and serum samples from 12 medical institutions in Japan, managed by the Institute of Medical Science, the University of Tokyo. BBJ has recruited approximately 260,000 patients, mainly of Japanese ancestry. All study participants had been diagnosed with one or more of 47 target diseases. RIKEN Center for Integrative Medical Sciences contributed to genotyping of the BBJ samples.

Cases were participants with a history of hepatic cancer, defined by cancer registration with HCC (ICD-10: **C22.0**, or ICD-9: **155.0**). The control group included all BBJ participants without a history of HCC, of which most individuals would have had no history of chronic liver disease.

Additional replication analysis of *TERT* variants

In total, the STOP-HCV cirrhosis study comprises 1,059 patients with hepatitis C-related cirrhosis. Participants were recruited from 31 specialist liver clinics in the UK between January 2015 and July 2016[16]. Cirrhosis was defined through histologic assessment, imaging, or a validated serum biomarker consistent with liver cirrhosis (i.e., aspartate aminotransferase [AST]-to-platelet ratio index >2, FibroTest >0.73, or enhanced liver fibrosis score >10.48). Blood specimens collected at enrollment were used to generate host-genotyping data through the Affymetrix UK Biobank array. Imputation was performed in March 2022 using the Topmed imputation server. Missing hard called

genotypes were filled from dosage information to obtain the Euclidean-nearest best-guess genotypes.To generate prospective phenotype data, participants from England have been linked to national hospital admission, cancer registrations, and mortality data, in a similar way to the UKB.

The present analysis was restricted to participants from England to ensure complete data on hospital admissions, cancer registrations, and mortality. No restrictions were made for ethnicity/European ancestry. Participants with missing genotype data for the lead variant rs2242652 were also excluded. As with the UKB, cases were defined on the basis of an in-patient hospital admission, death, or cancer registration indicating HCC (ICD-10: C22.0; ICD-9: 155.0) either before or after study enrollment. Controls were all participants without a history of HCC. Allelic odds ratios were calculated from 2 × 2 tables on allele counts. Significance was calculated as one degree of freedom Chi-squared test.

Association with non-liver related cancers (pleiotropy analysis)

The association of *TERT* variant rs2242652 with non-liver related cancers were tested in the UKB and FinnGen population-based cohorts. Specifically, we quantified rs2242652's and rs10069690´s association individually with each of the 10 most frequent non-liver related cancers observed in the UKB population: C43 Malignant melanoma of skin; C44 Other malignant neoplasms of skin; C50 Malignant neoplasm of breast; C61 Malignant neoplasm of prostate; C18 Malignant neoplasm of colon; C20 Malignant neoplasm of rectum; C34 Malignant neoplasm of bronchus and lung; C67 Malignant neoplasm of bladder; C54 Malignant neoplasm of corpus uteri; C85 Other and unspecified types of non-Hodgkin's lymphoma. As before, each cancer phenotype was defined using data from: 1) hospital admissions; 2) mortality; and 3) cancer registries.

SUPPLEMENTARY METHODS G

SNP Heritability Analysis

The proportion of phenotypic variance explained by the additive genetic effect of common genome-wide significant SNPs (h^2_{SNP}: SNP heritability) was estimated by genome-based restricted maximum likelihood analysis using GCTA-GREML[17]. To obtain A SNP-based estimate of relatedness for each pair of individuals in the discovery GWAS cohort was obtained using imputed autosomal SNPs (N=7,585,576) with MAF > 1%, P_{HWE} >1×10^{-6} and imputation r2 > 0.3, were used to calculate the

genetic relationship matrix (GRM) between pairs of individuals. Missing hard called genotypes were filled from dosage information to obtain the Euclidean-nearest best-guess genotypes. The heritability (proportion of variance) explained by variants in the *PNPLA3* / *TM6SF2* / *TERT* LD regions that are associated with HCC in ArC with genome-wide significance were calculated using, a two genetic variance components model was established using a set of n=2026 variants from these regions as variance component 1 to allow the full genetic signal of these regions to be captured and the remaining >7.5 million GWAS variants (MAF >1%) as variance component 2, with sex, age and top 15 PCs as environment variance component. Obtained h^2 estimates were then transformed to the liabitly scale valid for binary traits assuming HCC frequencies of 1%-2.5% in ArC using the formula implemented in GCTA.

$$h2_{liability} = h2_{observed} \frac{K(1-K)}{\varphi(\Phi^{-1}[K])^2} \frac{K(1-K)}{P(1-P)}$$

KK is the frequency of the binary trait in the population, PP is the frequency of the binary trait in the observed sample. The denominator of the first fraction is the squared probability density function evaluated at the KK quantile of the inverse cumulative density function of the standard normal distribution.

The proportion of the total SNP heritability due to variance component 1 (*PNPLA3* / *TM6SF2* / *TERT* variants) was calculated $\frac{h2(vc1)}{h2(vc0)+h2(vc1)}$ =%(h^2).

The three lead variants at the HCC risk loci (viz rs738409 in *PNPLA3*, rs58542926 in *TM6SF2* and rs2242652 in *TERT*) were included as covariates when estimating the additive genetic SNP heritability (h^2 SNP) in the joint analysis in order to validate the heritability explained by component 1.

SUPPLEMENTARY METHODS H

United Kingdom Biobank cohort:

Liver fat content and telomere length data

Leukocyte telomere length was available for 471,172 participants in UKB (Field ID: 22191), whilst liver imaging fat content data was available for 8315 imaging sub-study participants (Field ID: 22436). The analysis was based on data from the version 3 release of the UKB imputed genetic data set. Participants were excluded from these analyses for any of the following reasons: non-White British ancestry (inferred via UKB field ID:22006); poor quality genetic sample (defined by UKB field ID: 22027); Second or first-degree relations to another participant in the case-control dataset (inferred via a kinship coefficient ≥0.121).

Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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SUPPLEMENTARY FIGURES

Supplementary Figure 1: QQ plot for Discovery GWAS 1 (pc adjusted)

Quantile-quantile (QQ) plot: A λ of 1.03 was obtained for the primary analysis. Y axis observed -log10P values, Y axis expected -log10P values.

Supplementary Figure 2: Manhattan Plot of Discovery GWAS 2 (sex, age and pc adjusted, secondary analysis)

Genome-wide association analysis of 1066 with ArC and HCC and 844 controls with ArC without HCC. GWAS analysis was adjusted for sex, age and top 15 principal components of genetic ancestry. P values are shown for SNPs that passed quality control. The genome-wide significance threshold ($P=5\times10^{-8}$) is shown as a solid line. The threshold for replication follow-up ($P=1\times10^{-5}$) is shown as a dashed line. The nearest gene is annotated for replicating loci. Variants with significance $P<5\times10^{-8}$ are highlighted in red, those with $P<1\times10^{-5}$ are highlighted in green.

Supplementary Figure 3: QQ plot of secondary GWAS 2: sex, age, PC adjusted

Quantile-quantile (QQ) plot: A λ of 1.02 was obtained for the secondary analysis. Y axis observed -log10P values, Y axis expected -log10P values.

Supplementary Figure 4: Forest plot of confirmed HCC risk locus *PNPLA3* for discovery and replication cohorts

Forrest plot showing the fixed-effects model (k=4) meta-analysis of allelic ORs of the *PNPLA3* rs738409:G allele, for regression model 1 adjusted for top principal components.

Supplementary Figure 5: Forest plot of confirmed HCC risk locus *TM6SF2* for discovery and replication cohorts

Forrest plot showing the fixed-effects model (k=4) meta-analysis of allelic ORs of the *TM6SF2* rs58542926:T allele, for regression model 1 adjusted for top principal components.

Supplementary Figure 6: Forest plot of the novel HCC associated locus *TERT* for discovery and replication cohorts

Forrest plot showing the fixed-effects model (k=4) meta-analysis of allelic ORs of the *TERT* rs2242652:A allele, for regression model 1 adjusted for top principal components.

Supplementary Figure 7: Regional plot *TERT* (Discovery and Replication cohorts combined)

Fine-mapping analysis of the *TERT* association signals. Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the Discovery GWAS cohort. The lead association signal is located in intron 4 of the *TERT* gene (annotated on the reverse strand), located in LD block B-3 spanning from intron 4 to intron 2 of *TERT* (see Suppl. Table 12). The plot was generated using LocusZoom and R package ”gpart”.

Supplementary Figure 8: Regional plot *TM6SF2* (Discovery and Replication cohorts combined)

Fine-mapping analysis of the *TM6SF2* association signals. The −log10 (P values) are plotted against SNP genomic position based on NCBI Build 37. SNPs are colored to reflect correlation with the most significant SNP, with red denoting the highest LD (r2>0.8) with the lead SNP. Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the Discovery GWAS cohort.

The lead association signal is located in exon 6 of the *TM6SF2* gene (annotated on the reverse strand). Estimated recombination rates from the 1000 Genomes Project (hg19/genomes March 2012 release, EUR population) are plotted in blue to reflect the local LD structure. Gene annotations were obtained from the UCSC Genome Browser.

Supplementary Figure 9: Regional plot *PNPLA3* (Discovery and Replication cohorts combined)

Fine-mapping analysis of the *PNPLA3* association signals. The −log10 (P values) are plotted against SNP genomic position based on NCBI Build 37. SNPs are colored to reflect correlation with the most significant SNP, with red denoting the highest LD (r2 >0.8) with the lead SNP. Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the Discovery GWAS cohort. The lead association signal is located in exon 6 of the *PNPLA3* gene (annotated on the forward strand). Estimated recombination rates from the 1000 Genomes Project (hg19/genomes March 2012 release, EUR population) are plotted in blue to reflect the local LD structure. Gene annotations were obtained from the UCSC Genome Browser.

Supplementay Figure 10: Forest plots for the associations of *TERT* lead variant rs2242652 [A allele] with the 10 most common cancer in UK Biobank and FinnGen biobank populated-based cohorts

Forest plot showing association between lead variant rs2242652:A in *TERT* associated with HCC in ArC in the present study and the top 10 most frequent cancers observed in the UK Biobank and FinnGen cohort. In addition, the primary liver cancer phenotypes are highlighted in red. Associations are presented in terms of the LOR. An LOR of 0 indicates that the frequency of rs2242652:A is the same for cases as for controls. LORs were calculated using logistic regression under an additive genetic model.

Supplementay Figure 11: The relative proportion of patients with HCC grouped by the number of risk alleles in *PNPLA3*, *TM6SF2*, and *TERT*

Percentage of patient with HCC in the discovery and validation cohorts stratified by the sum of crude risk alleles of *PNPLA3* rs738409 ‘G’, *TM6SF2* rs58542926 ‘T’ and *TERT* rs2242652 ‘G’ carried by each patient, grouped into 3 categories.

Supplementay Figure 12: Association between the number of risk alleles in *PNPLA3*, *TM6SF2* and *TERT* and alcohol-related hepatocellular carcinoma

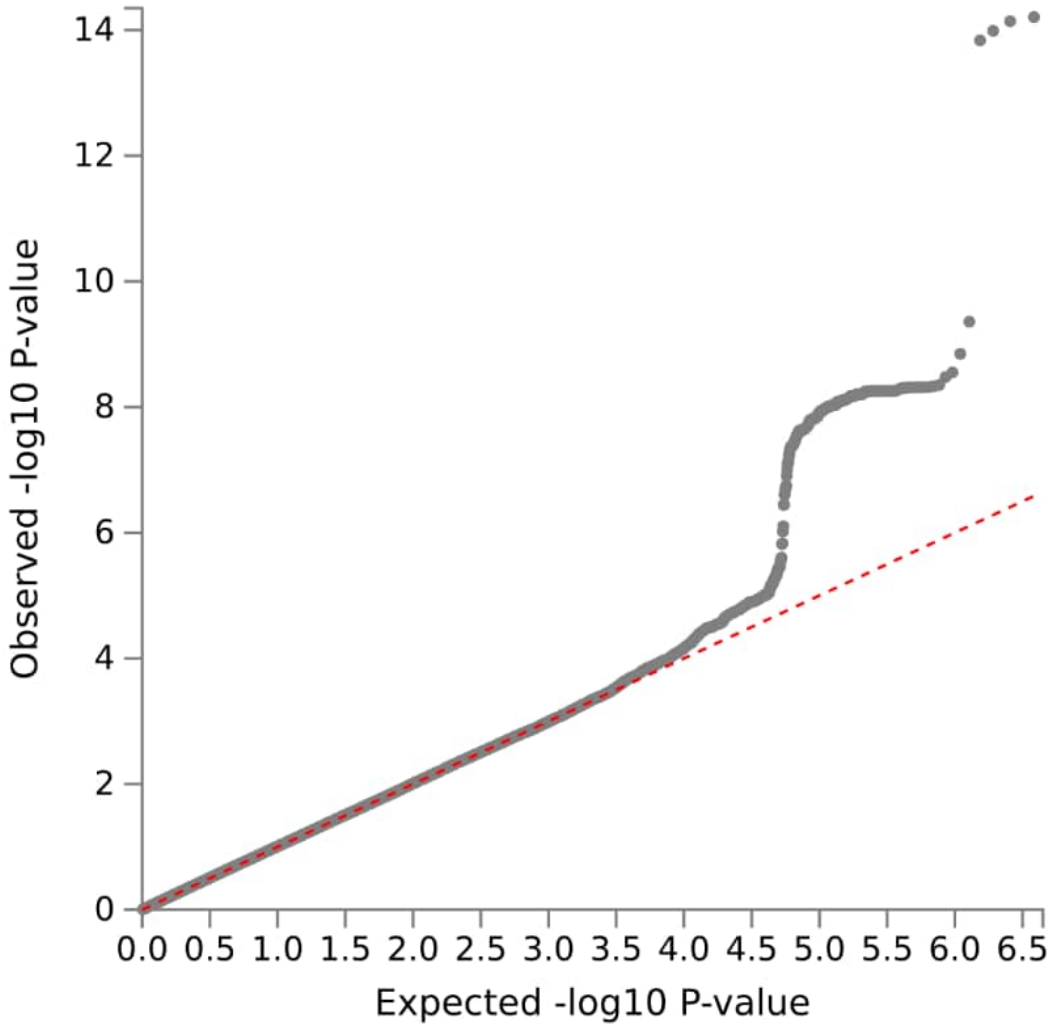
The association between the sum of crude risk alleles of *PNPLA3* rs738409 ‘G’, *TM6SF2* rs58542926 ‘T’ and *TERT* rs2242652 ‘G’ carried by each patient and alcohol-related hepatocellular carcinoma in the discovery and validation cohorts.

Supplementay Figure 13: HCC association results overlayed with leukocyte telomere length association results and regulation for gene *TERT*

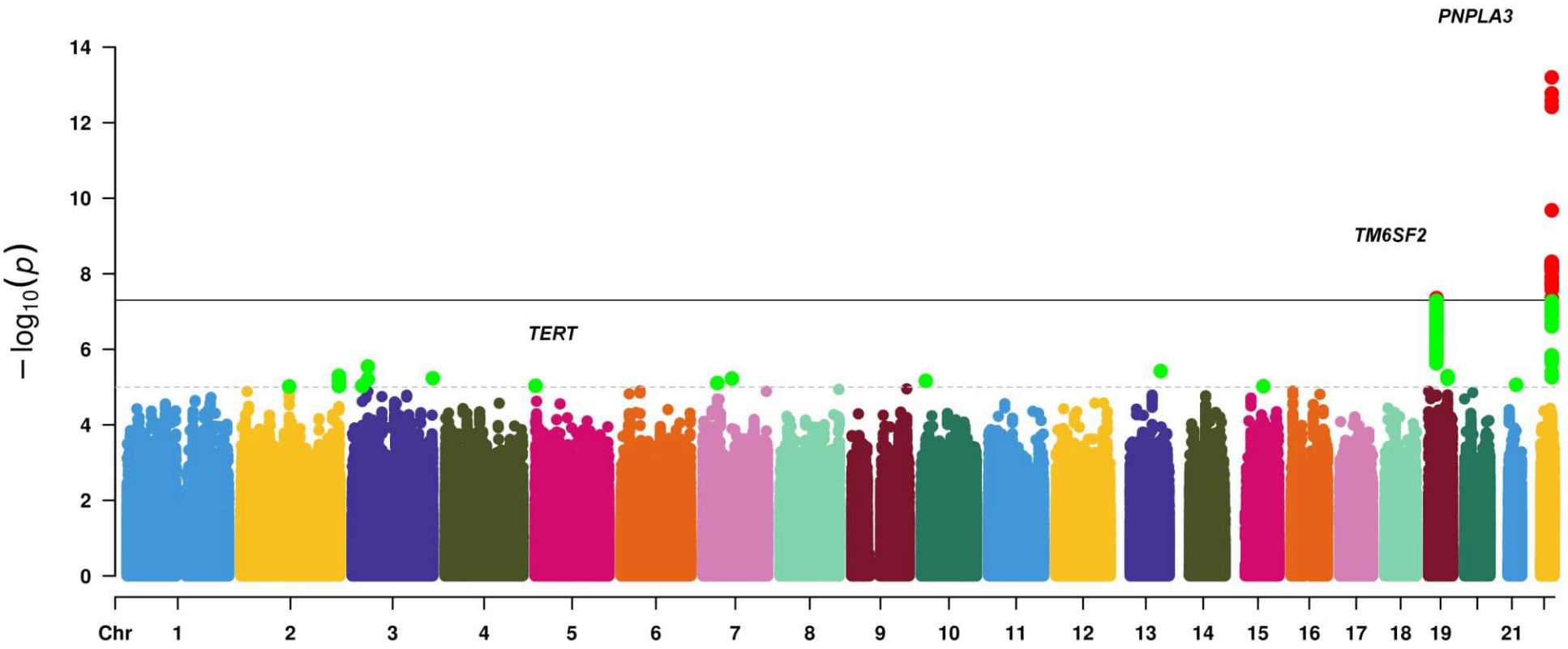
GWAS association signals (-log10 P values) of the combined of discovery and replication samples plotted for the entire *TERT* gene in comparison to association results (-log10 P values) for leukocyte telomere length observed in the total European UK-Biobank population. Annotated LD-Blocks reflect the LD pattern in the discovery GWAS cohort. The lead association signal is located in intron 4 of the *TERT* gene (annotated on the reverse strand), located in LD block B-3 spanning from intron 4

to intron 2 of *TERT*. Strongest association signals for leukocyte telomere length are also located to LD block B-3. (for further details see Suppl. Table 12). The plot was generated using Ensembl.

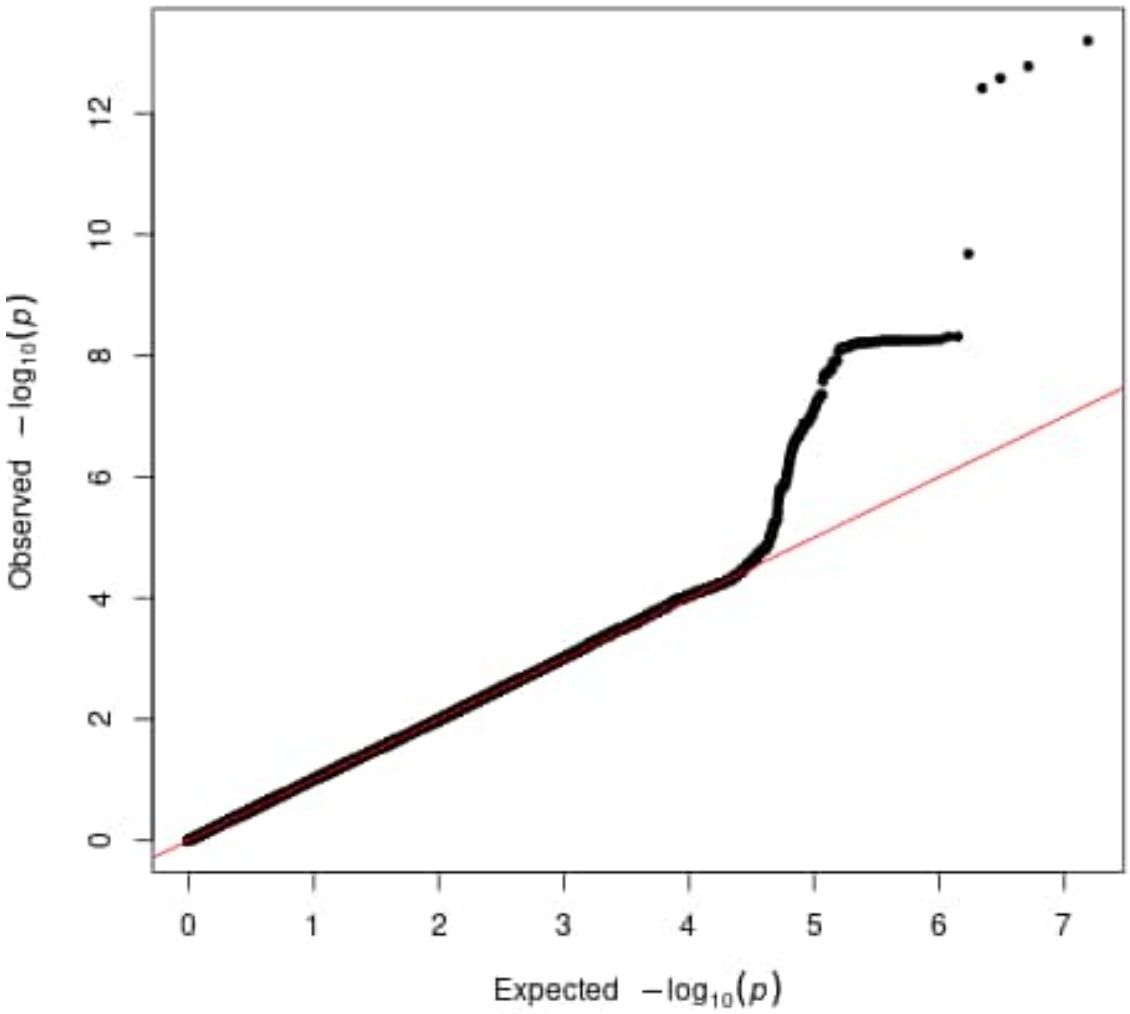
Supplementary Figure 1: QQ plot for Discovery GWAS 1 (PC adjusted)



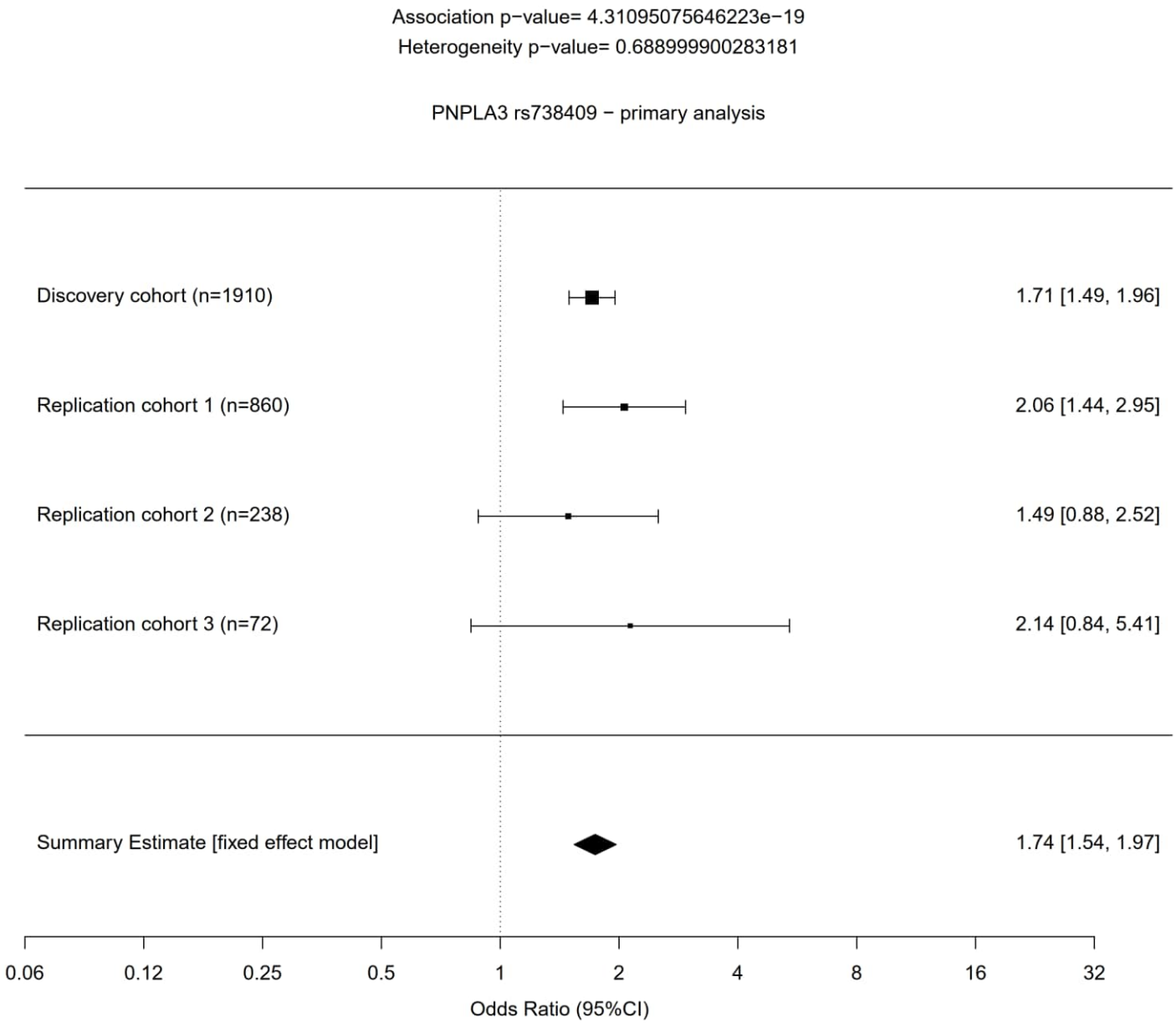
Supplementary Figure 2: Manhattan Plot of Discovery GWAS 2 (sex, age and PC adjusted)



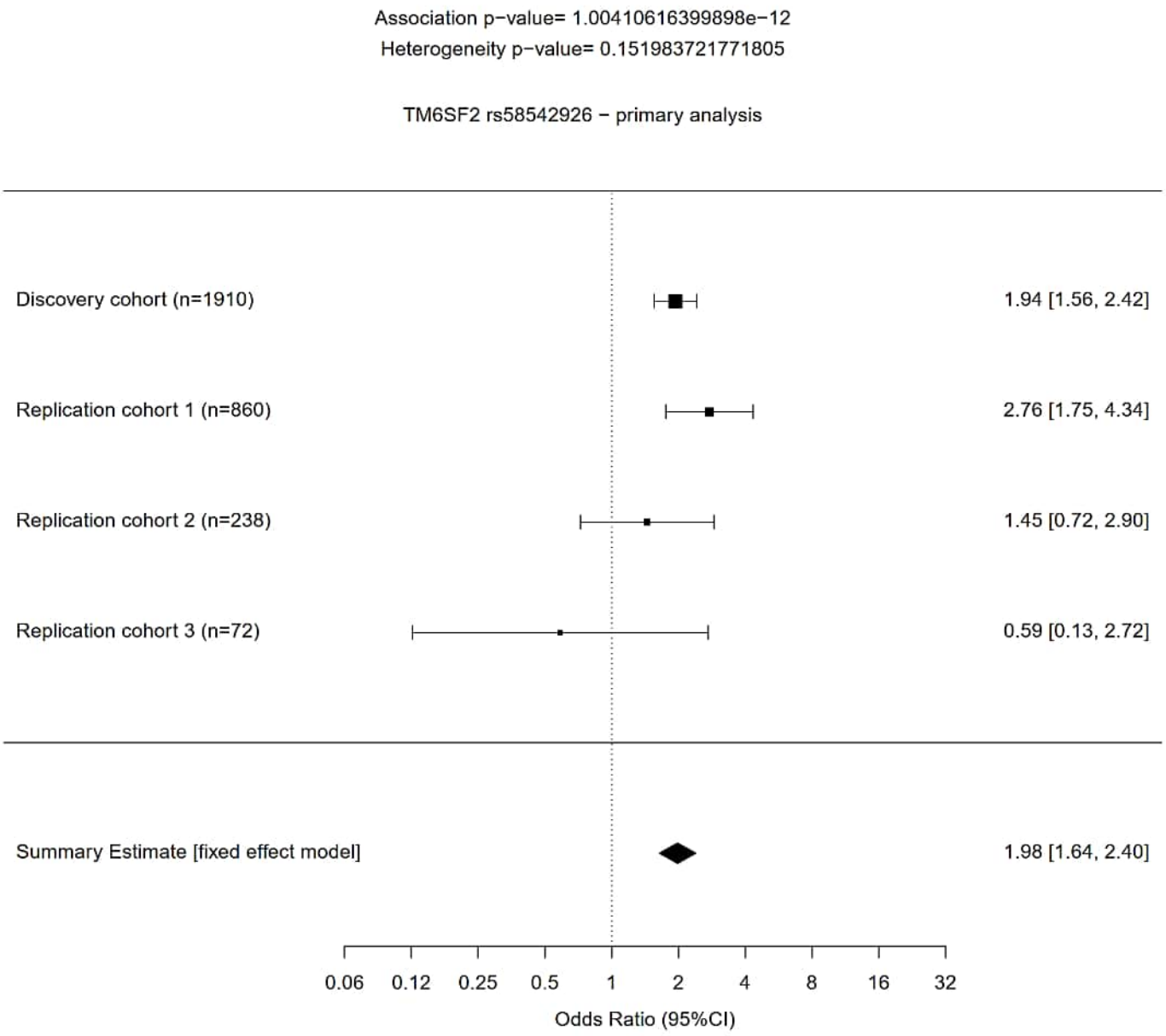
Supplementary Figure 3: QQ plot of secondary GWAS 2 (sex, age and PC adjusted)



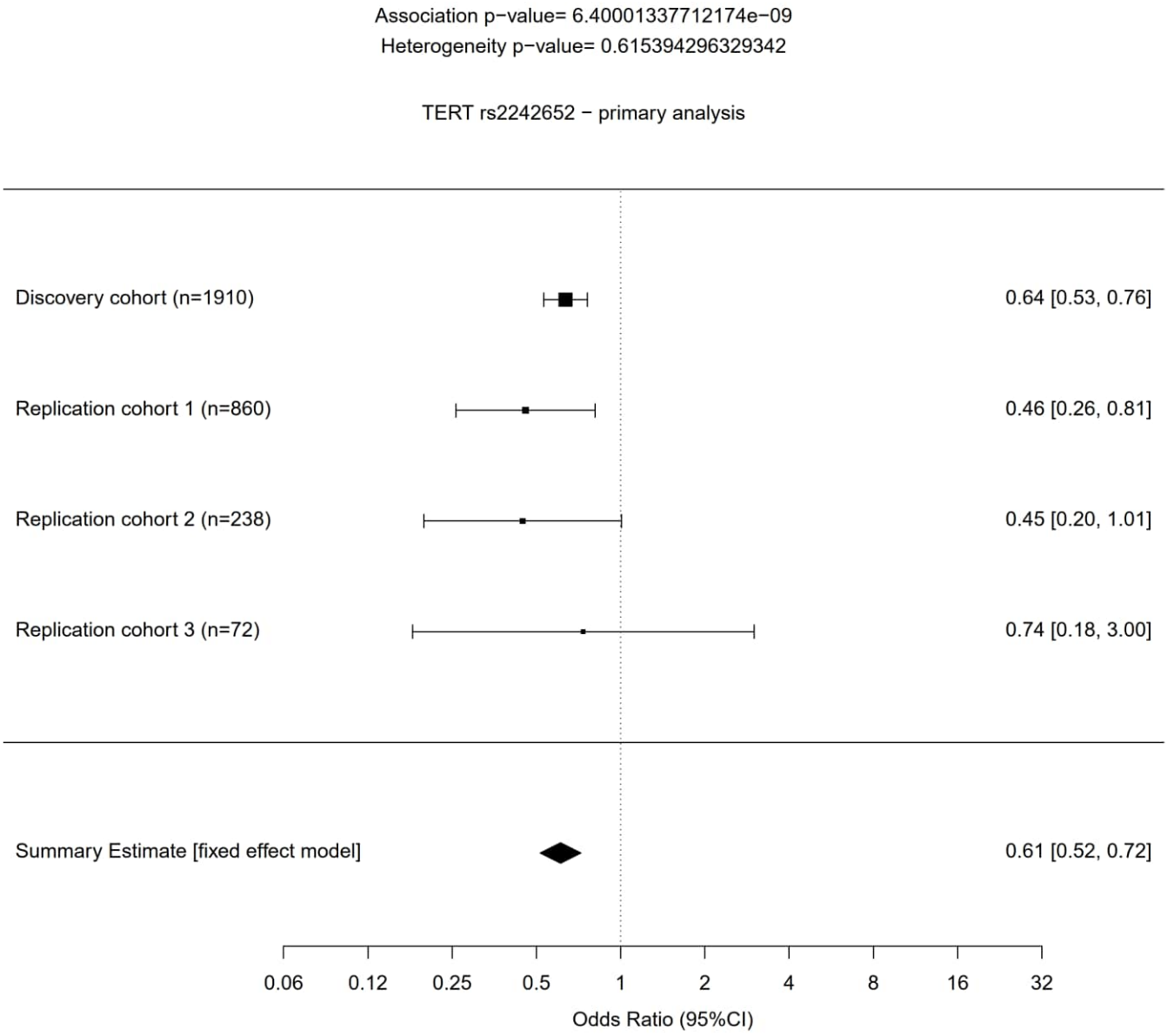
Supplementary Figure 4: Forest plot of confirmed HCC risk locus *PNPLA3* for discovery and replication cohorts



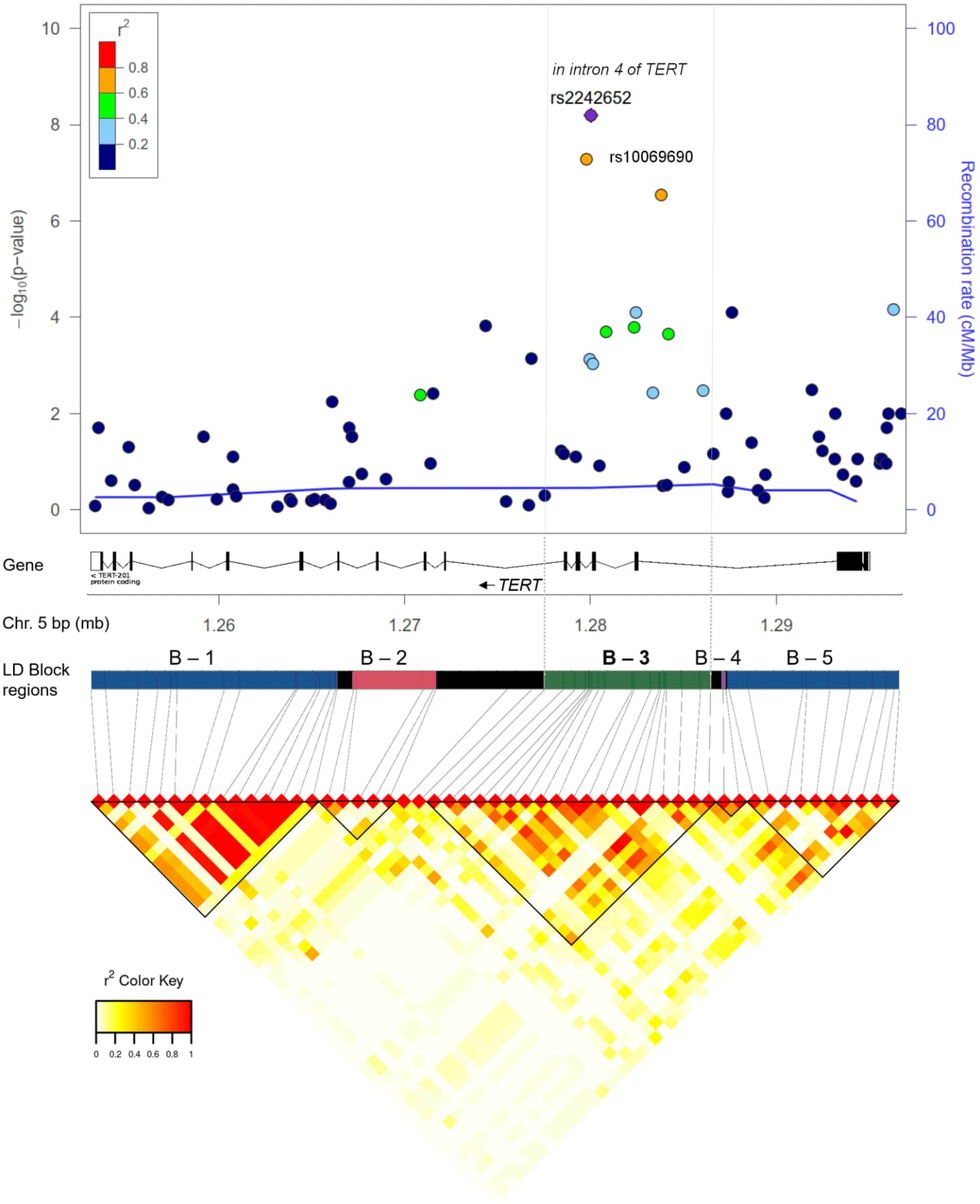
Supplementary Figure 5: Forest plot of confirmed HCC risk locus *TM6SF2* for discovery and replication cohorts



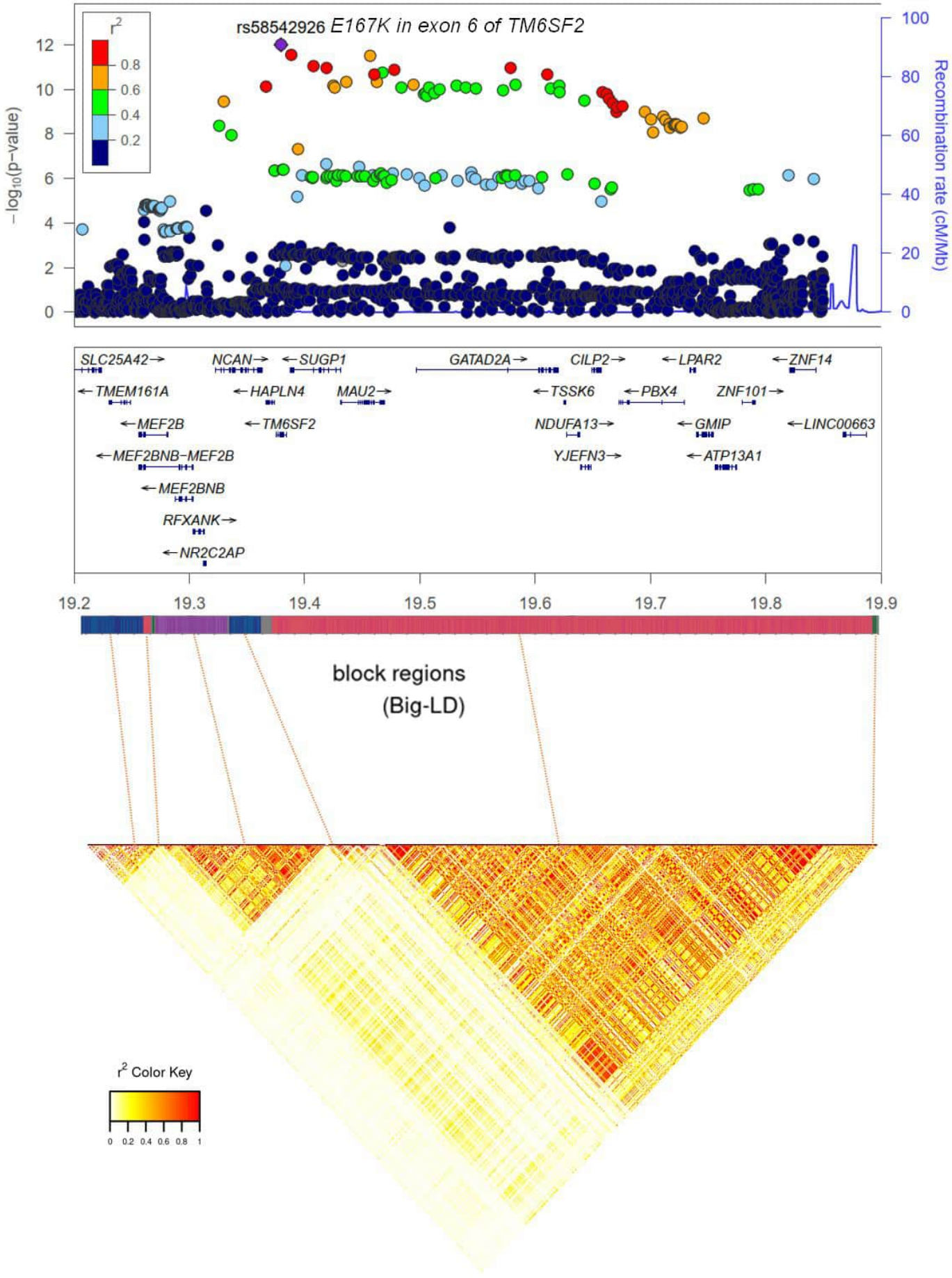
Supplementary Figure 6: Forest plot of the novel HCC associated locus rs2242652:A in *TERT* for discovery and replication cohorts



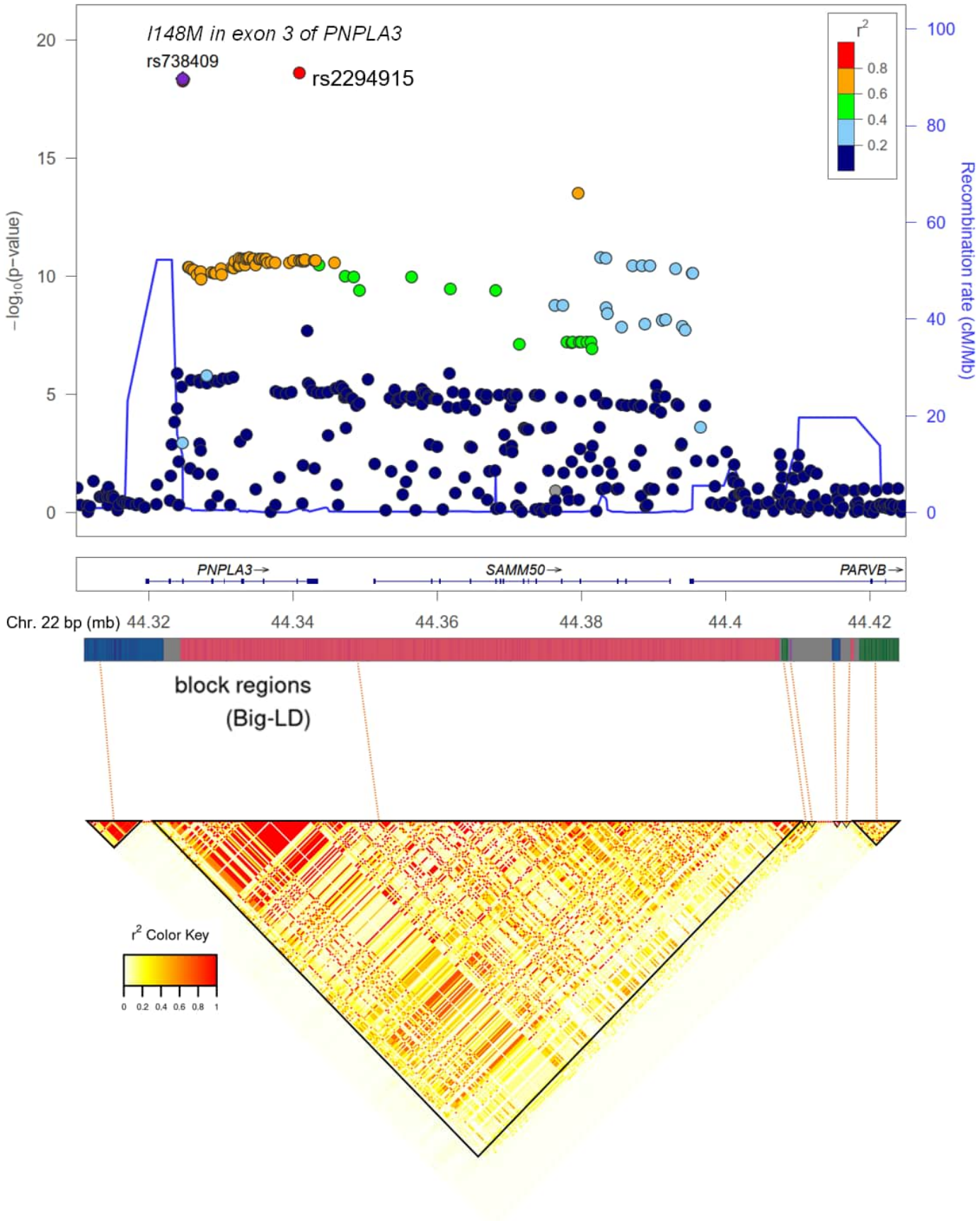
Supplementary Figure 7: Regional plot *TERT* (Discovery and Replication cohorts combined)



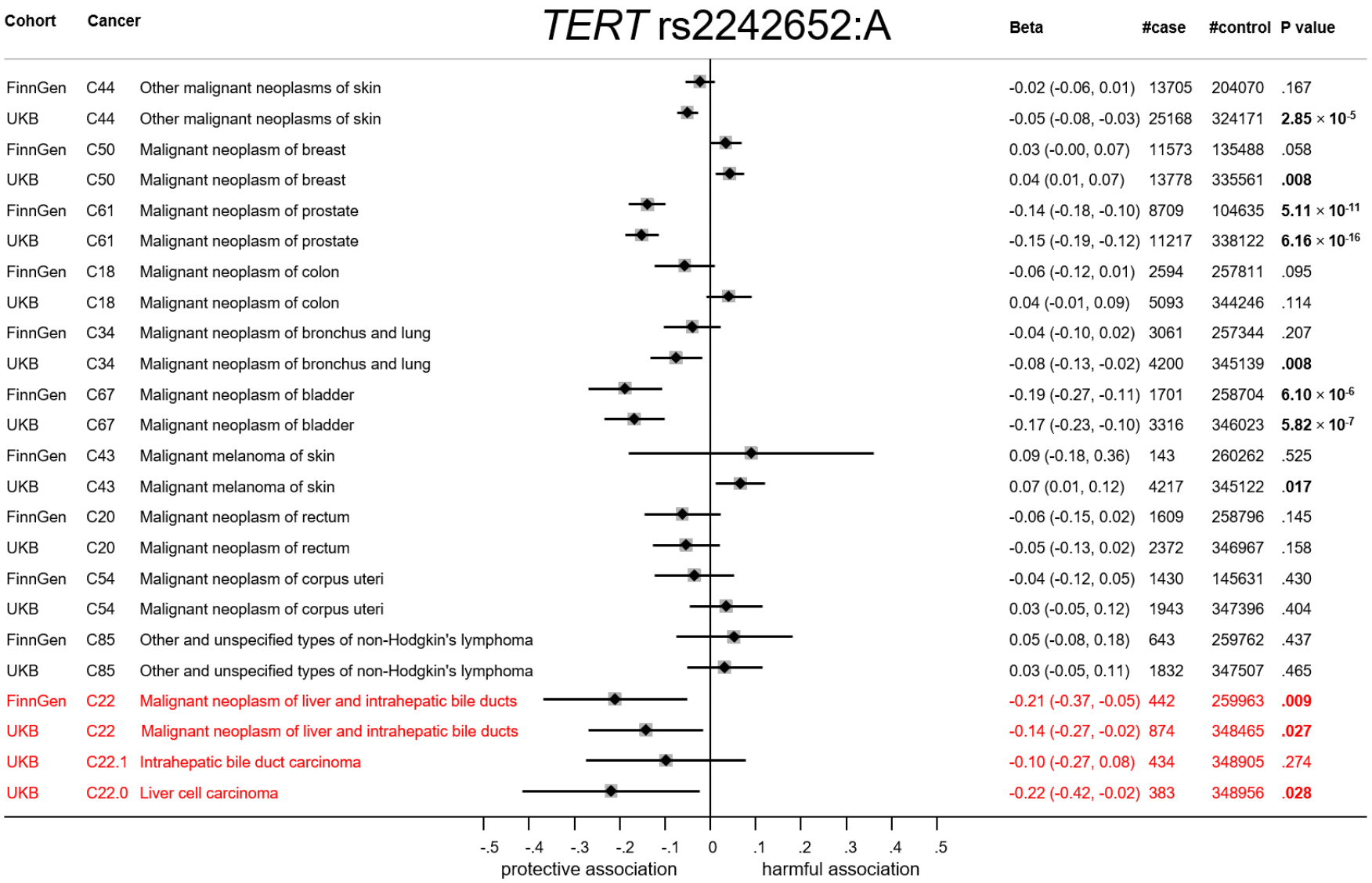
Supplementary Figure 8: Regional plot *TM6SF2* (Discovery and Replication cohorts combined)



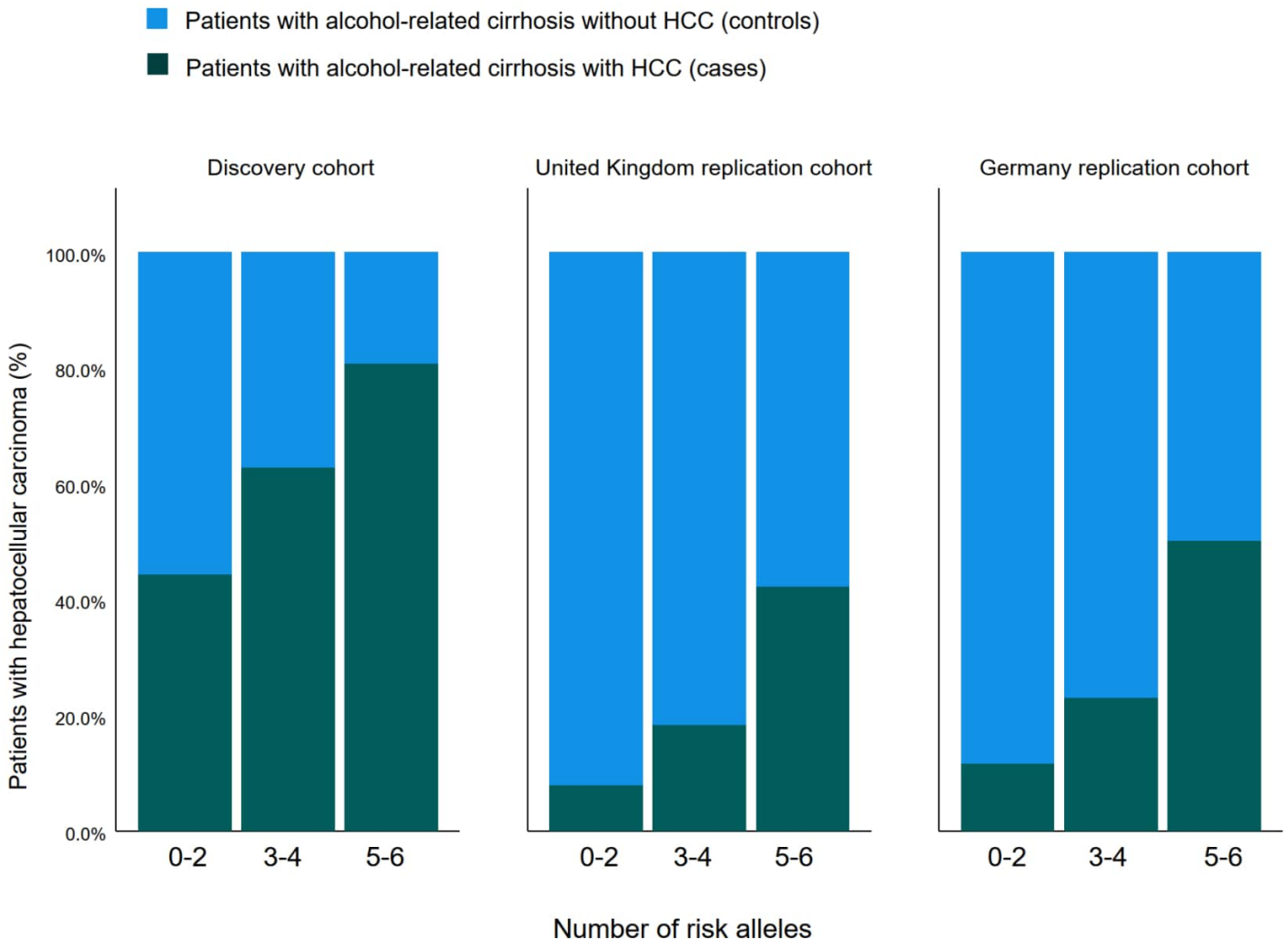
Supplementary Figure 9: Regional plot *PNPLA3* (Discovery and Replication cohorts combined)



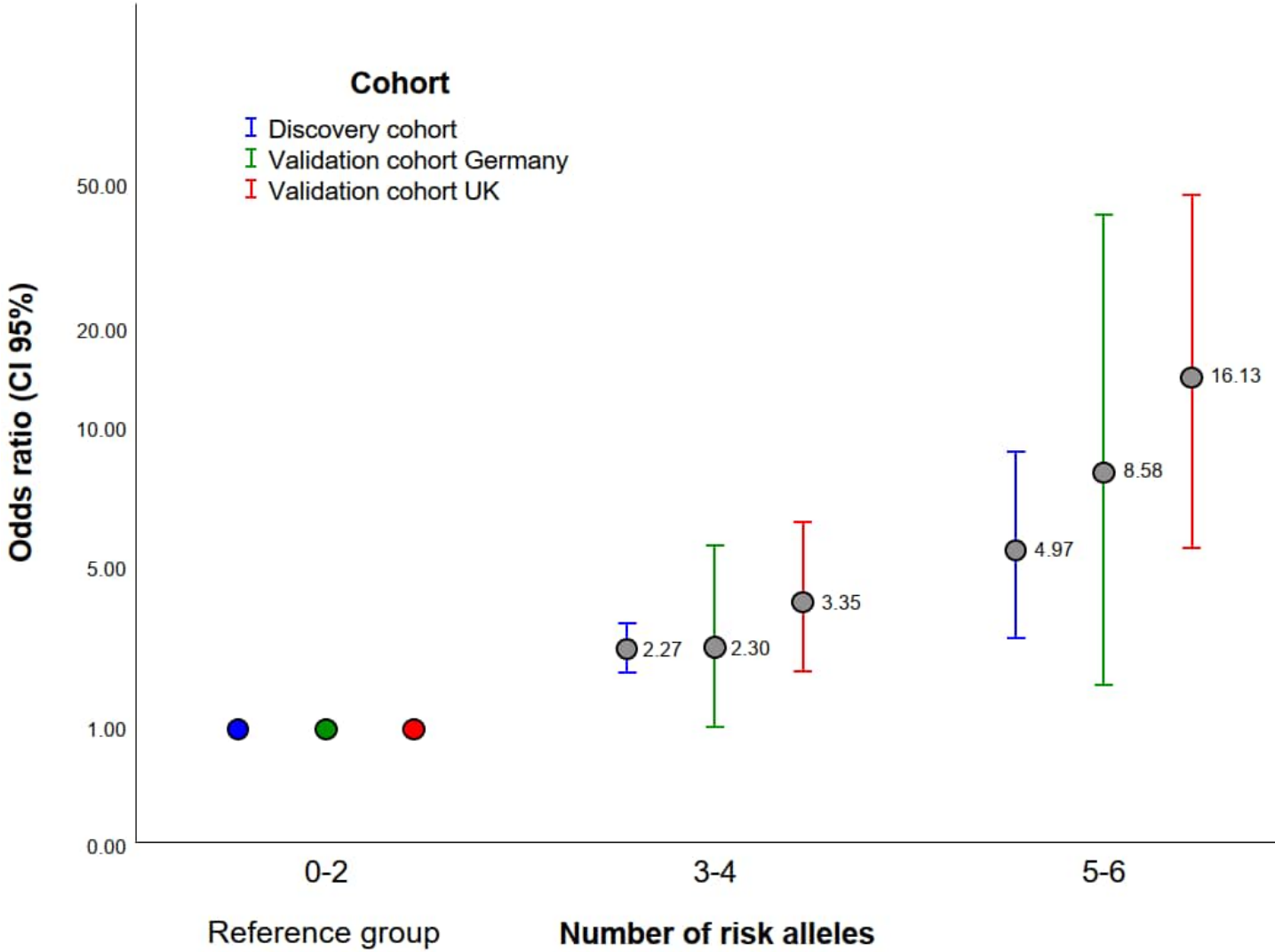
Supplementay Figure 10: Forest plots for the associations of *TERT* variant rs2242652[A allele] with the 10 most common cancer in UK Biobank and FinnGen biobank populated-based cohorts



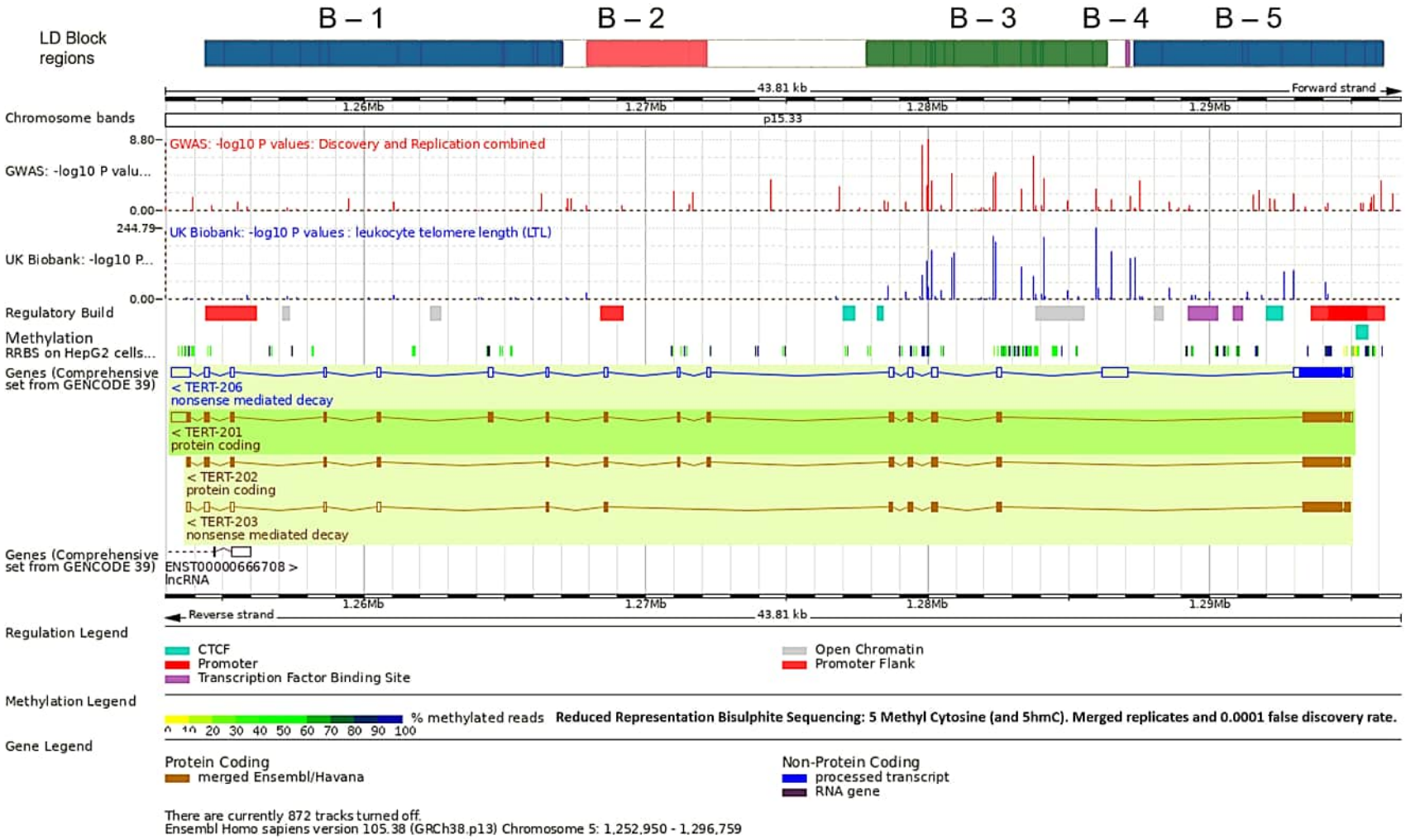
Supplementay Figure 11: The relative proportion of patients with HCC grouped by the number of risk alleles in *PNPLA3*, *TM6SF2*, and *TERT*



Supplementay Figure 12: Association between the number of risk alleles in *PNPLA3*, *TM6SF2* and *TERT* and alcohol-related hepatocellular carcinoma.



Supplementay Figure 13: HCC association results overlayed with leukocyte telomere length association results and regulation for gene *TERT*



SUPPLEMENTARY TABLES

Supplementary Table 1: Power analysis for discovery GWAS (expected power to reject the null hypothesis)

Relative Risk (~OR)	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3
Power (p-value threshold 5×10^{-6})										
Disease Allele Frequency*: 5%	0.019	0.066	0.167	0.329	0.525	0.709	**0.847**	**0.931**	**0.973**	**0.991**
Disease Allele Frequency: 10%	0.122	0.342	0.626	**0.846**	**0.995**	**0.991**	**1**	**1**	**1**	**1**
Disease Allele Frequency: 20%	0.453	**0.803**	**0.962**	**0.996**	1	**1**	**1**	**1**	1	**1**
Disease Allele Frequency: 30%	0.667	**0.933**	**0.994**	1	**1**	**1**	**1**	**1**	**1**	**1**
Disease Allele Frequency: 40%	0.750	**0.961**	**0.997**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
Power (p-value threshold 5×10^{-8})										
Disease Allele Frequency: 5%	0	0.008	0.032	0.092	0.205	0.369	0.555	0.725	**0.852**	**0.931**
Disease Allele Frequency: 10%	0.020	0.098	0.554	0.554	**0.791**	**0.928**	**1**	**1**	**1**	**1**
Disease Allele Frequency: 15%	0.069	0.252	0.537	**0.790**	**0.930**	**0.983**	**0.997**	**1**	**1**	**1**
Disease Allele Frequency: 20%	0.158	0.486	**0.813**	**0.962**	**0.996**	**1**	**1**	**1**	**1**	**1**
Disease Allele Frequency: 30%	0.325	0.729	**0.947**	**0.995**	**1**	**1**	**1**	**1**	**1**	**1**
Disease Allele Frequency: 40%	0.416	**0.810**	**0.971**	**0.998**	**1**	**1**	**1**	**1**	**1**	**1**

Power of study design. Estimate of the power of the study, with 1066 cases and 844 controls using for SNPs above 5% MAF using the software Genetic Association Study (GAS) Power Calculator (https://csg.sph.umich.edu/abecasis/gas_power_calculator/index.html). *Disease allele frequency of the risk associated allele in controls. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 1.4 to 2.3, we will be able to reject the null hypothesis that this odds ratio equals 1 with probability (power) see values in table, probabilities ≥0.80 are shown in bold print. The Type I error probability associated with this test of this null hypothesis is 5×10^{-6} for suggestive association or 5×10^{-8} for genome-wide evidence of association.

Supplementary Table 2: Summary results for SNP rs738409 in *PNPLA3* for Discovery and Replication cohorts

Cohort	Disease status		*PNPLA3* rs738409 C>G (p.I148M)						*P*-value *	Allelic OR (95%CI)
Discovery (Germany/Switzerland/Austria)		**N**	**CC**	**CG**	**GG**	**Freq. C**	**Freq. G**	**HWE *P***	Chi-Square test	(Allele G)
Cases (HCC)	Cirrhosis (with HCC)	1066	302	503	261	0.519	0.481	0.073	-	-
Controls (ArC)	Cirrhosis (without HCC)	844	361	371	112	0.648	0.352	0.282	1.62×10^{-15}	1.70 (1.49-1.94)
Replication (United Kingdom)										
Cases (HCC)	Cirrhosis (with HCC)	70	18	36	16	0.514	0.486	0.805	-	-
Controls (ArC)	Cirrhosis (without HCC)	790	393	319	78	0.699	0.301	0.264	6.31×10^{-6}	2.20 (1.55-3.11)
Controls (AM) #1	Alcohol misusers +	340	223	108	9	0.815	0.185	0.337	2.60×10^{-14}	4.15 (2.83-6.10)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									1.26×10^{-8}	1.89 (1.51-2.36)
Replication (Germany)										
Cases (HCC)	Cirrhosis (with HCC)	42	12	18	12	0.500	0.500	0.355	-	-
Controls (ArC)	Cirrhosis (without HCC)	196	73	93	30	0.610	0.390	0.966	0.064	1.56 (0.97-2.51)
Replication (Italy)										
Cases (HCC)	Cirrhosis (with HCC)	36	7	19	10	0.458	0.542	0.706		
Controls (ArC)	Cirrhosis (without HCC)	36	13	16	7	0.583	0.417	0.607	0.133	1.65 (0.86-3.20)
Risk validation in joined discovery (Germany/Switzerland) & replication (Germany) cohort										
Cases (HCC)	Cirrhosis (with HCC)	1108	314	521	273	0.519	0.481	0.052		
Controls (ArC)	Cirrhosis (without HCC)	1040	434	464	142	0.640	0.360	0.312	6.35×10^{-16}	1.65 (1.46-1.87)
Controls (AM) #2	Alcohol misusers +	1105	664	387	54	0.776	0.224	0.804	4.56×10^{-64}	3.22 (2.82-3.66)
Population controls #3	European population controls	25362	15344	8784	1234	0.778	0.222	0.610	2.15×10^{-176}	3.26 (2.99-3.55)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									6.59×10^{-23}	1.99 (1.74-2.29)

* Significance was calculated as one degree of freedom Chi-squared test of allelic counts; + heavy drinkers with no cirrhosis and no HCC; Association results for alcohol-related HCC (cases) in comparison to alcohol-related cirrhosis (controls); and in comparison, to alcohol misusers with cirrhosis (non-cirrhosis controls) and to population controls. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Allelic odds ratios were calculated from 2 × 2 tables on rounded genotype counts. **Analysis of cirrhosis risk performed in individual cohorts by comparing ArC vs. alcohol misuser without cirrhosis. HWE P: Hardy Weinberg Equilibrium P value; #1 Alcohol misusers (ALC) with a clinical diagnosis of alcohol dependence recruited from Centre for Hepatology at the Royal Free Hospital, London without clinical or laboratory evidence of liver cirrhosis or HCC (data from Buch et al. Nat Genet. 2015 Dec;47(12):1443-8). #2 Alcohol misusers with a clinical diagnosis of alcohol dependence from Germany/Switzerland without clinical or laboratory evidence of liver disease or HCC (data from Buch et al. Nat Genet. 2015 Dec;47(12):1443-8); #3 North-western European population controls from gnomAD (v2.1.1).

Supplementary Table 3: Summary results for SNP rs58542926 in *TM6SF2* for Discovery and Replication cohorts

Cohort	Disease status	*TM6SF2* rs58542926 C>T (p.E167K)							*P*-value *	Allelic OR (95%CI)
Discovery (Germany/Switzerland/Austria)		**N**	**CC**	**CT**	**TT**	**Freq. C**	**Freq. T**	**HWE *P***	Chi-Square test	(Allele T)
Cases (HCC)	Cirrhosis (with HCC)	1066	781	253	32	0.851	0.149	0.041	-	-
Controls (ArC)	Cirrhosis (without HCC)	844	710	125	9	0.915	0.085	0.191	1.61×10^{-9}	1.89 (1.53-2.33)
Replication (United Kingdom)										
Cases (HCC)	Cirrhosis (with HCC)	70	45	20	5	0.786	0.214	0.205	-	-
Controls (ArC)	Cirrhosis (without HCC)	790	648	135	7	0.906	0.094	0.991	8.35×10^{-6}	2.55 (1.65-3.95)
Controls (AM) #1	Alcohol misusers +	340	296	43	1	0.934	0.066	0.668	3.09×10^{-8}	3.85 (2.32-6.37)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									0.0286	1.47 (1.04-2.08)
Replication (Germany)										
Cases (HCC)	Cirrhosis (with HCC)	42	33	7	2	0.869	0.131	0.083	-	-
Controls (ArC)	Cirrhosis (without HCC)	196	159	32	5	0.893	0.107	0.040	0.529	1.26 (0.62-2.55)
Replication (Italy)										
Cases (HCC)	Cases (HCC)	36	31	5	0	0.931	0.069	0.654		
Controls (ArC)	Controls (ArC)	36	28	8	0	0.889	0.111	0.453	0.383	0.60 (0.19-1.92)
Risk validation in joined discovery (Germany/Switzerland) & replication (Germany) cohort										
Cases (HCC)	Cirrhosis (with HCC)	1108	814	260	34	0.852	0.148	0.021		
Controls (ArC)	Cirrhosis (without HCC)	1040	869	157	14	0.911	0.089	0.027	2.42×10^{-9}	1.78 (1.47-2.15)
Controls (AM) #2	Alcohol misusers +	1115	987	124	4	0.941	0.059	0.960	2.41×10^{-22}	2.76 (2.24-3.41)
Population controls #3	European population controls	23816	20633	3082	101	0.931	0.069	0.218	9.57×10^{-45}	2.35 (2.08-2.65)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									1.44×10^{-4}	1.58 (1.25-2.01)

* Significance was calculated as one degree of freedom Chi-squared test of allelic counts. + heavy drinkers with no cirrhosis and no HCC; Association results for alcohol-related HCC (cases) in comparison to alcohol-related cirrhosis (controls); and in comparison, to alcohol misusers with cirrhosis (non-cirrhosis controls) and to population controls. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Allelic odds ratios were calculated from 2 × 2 tables on rounded genotype counts. **Analysis of cirrhosis risk performed in individual cohorts by comparing ArC vs. alcohol misuser without cirrhosis. HWE P: Hardy Weinberg Equilibrium P value; #1 Alcohol misusers (ALC) with a clinical diagnosis of alcohol dependence recruited from Centre for Hepatology at the Royal Free Hospital, London without clinical or laboratory evidence of liver cirrhosis or HCC (data from Buch et al. Nat Genet. 2015 Dec;47(12):1443-8). #2 Alcohol misusers with a clinical diagnosis of alcohol dependence from Germany/Switzerland without clinical or laboratory evidence of liver disease or HCC (data from Buch et al. Nat Genet. 2015 Dec;47(12):1443-8); #3 North-western European population controls from gnomAD (v2.1.1).

Supplementary Table 4: Summary results for SNP rs2242652 in *TERT* for Discovery and Replication cohorts

Cohort	Disease status		*TERT* rs2242652 G>A						*P*-value *	Allelic OR (95%CI)
Discovery (Germany/Switzerland/Austria)		**N**	**GG**	**GA**	**AA**	**Freq. G**	**Freq. A**	**HWE *P***	Chi-Square test	2 × 2 table (A allele)
Cases (HCC)	Cirrhosis (with HCC)	1066	803	243	20	0.867	0.133	0.746	-	-
Controls (ArC)	Cirrhosis (without HCC)	844	555	256	33	0.809	0.191	0.610	1.07×10^{-6}	0.65 (0.55-0.77)
Replication (United Kingdom)										
Cases (HCC)	Cirrhosis (with HCC)	70	57	11	2	0.893	0.107	0.135	-	-
Controls (ArC)	Cirrhosis (without HCC)	783	500	253	30	0.800	0.200	0.775	7.64×10^{-3}	0.48 (0.28-0.83)
Controls (AM) #1	Alcohol misusers +	341	221	112	8	0.812	0.188	0.154	2.20×10^{-2}	0.52 (0.29-0.92)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									0.503	1.08 (0.86-1.36)
Replication (Germany)										
Cases (HCC)	Cirrhosis (with HCC)	42	32	10	0	0.881	0.119	0.381	-	-
Controls (ArC)	Cirrhosis (without HCC)	196	119	71	6	0.788	0.212	0.233	0.052	0.50 (0.25-1.02)
Replication (Italy)										
Cases (HCC)	Cirrhosis (with HCC)	36	27	9	0	0.875	0.125	0.391	-	-
Controls (ArC)	Cirrhosis (without HCC)	36	27	8	1	0.861	0.139	0.670	0.806	0.89 (0.34-2.33)
Risk validation in joined discovery (Germany/Switzerland) & replication (Germany) cohort										
Cases (HCC)	Cirrhosis (with HCC)	1108	835	253	20	0.868	0.132	0.869		
Controls (ArC)	Cirrhosis (without HCC)	1040	674	327	39	0.805	0.195	0.932	2.87×10^{-8}	0.63 (0.53-0.74)
Controls (AM) #2	Alcohol misusers +	1179	760	367	52	0.800	0.200	0.366	9.43×10^{-10}	0.61 (0.52-0.72)
Population controls #3	European population controls	4290	2797	1344	149	0.809	0.191	0.423	9.64×10^{-11}	0.64 (0.56-0.74)
Analysis of cirrhosis risk**: Cirrhosis (without HCC) vs. Alcohol misusers +									0.511	0.95 (0.82-1.11)

* Significance was calculated as one degree of freedom Chi-squared test of allelic counts. + heavy drinkers with no cirrhosis and no HCC; Association results for alcohol-related HCC (cases) in comparison to ArC (controls); and in comparison, to alcohol misusers with cirrhosis (non-cirrhosis controls) and to population controls. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Allelic odds ratios were calculated from 2 × 2 tables on rounded genotype counts. **Analysis of cirrhosis risk performed in individual cohorts by comparing ArC vs. alcohol misuser without cirrhosis. HWE P: Hardy Weinberg Equilibrium P value; #1 Alcohol misusers (ALC) with a clinical diagnosis of alcohol dependence recruited from Centre for Hepatology at the Royal Free Hospital, London without clinical or laboratory evidence of liver cirrhosis or HCC (data from Buch et al. Nat Genet. 2015 Dec;47(12):1443-8). #2 Alcohol misusers with a clinical diagnosis of alcohol dependence from Germany/Switzerland without clinical or laboratory evidence of liver disease or HCC (data from Buch et al. Nat Genet. 2015); #3 North-western European population controls from gnomAD (v2.1.1).

Supplementary Table 5: Replicated and known HCC risk loci successively adjusted for sex, age, BMI and type 2 diabetes status

			Discovery cohort				Combined (Discovery and Replication cohorts)			
Covariate adjustments			PCs	PCs, sex, age	PCs, sex, age, BMI	PCs, sex, age, BMI, Diabetes	PCs	PCs, sex, age	PCs, sex, age, BMI	PCs, sex, age, BMI, Diabetes
N (cases)			1066	1066	740	616	1214	1214	857	731
N (controls)			844	844	765	423	1866	1866	1684	1337
Effective sample size*			1884	1884	1505	1003	2352	2352	1889	1382
Locus	Chr.	SNP ID (effect allele)	Adjusted OR[a] (*P value*)	Adjusted OR[b] (*P value*)	Adjusted OR[c] (*P value*)	Adjusted OR[d] (*P value*)	Adjusted OR[a] (*P value*)[a,e]	Adjusted OR[b] (*P value*)[e]	Adjusted OR[c] (*P value*)[e]	Adjusted OR[d] (*P value*)[e]
PNPLA3	22	rs738409	1.71 (1.49-1.96)	1.75 (1.51-2.03)	1.87 (1.58-2.22)	2.07 (1.69-2.54)	1.74 (1.54-1.97)	1.77 (1.55-2.02)	1.86 (1.60-2.16)	1.99 (1.68-2.37)
p.I148M		(G)	7.23×10^{-15}	1.67×10^{-13}	3.22×10^{-13}	3.21×10^{-12}	4.31×10^{-19}	5.35×10^{-17}	4.93×10^{-16}	5.62×10^{-15}
TM6SF2	19	rs58542926	1.94 (1.56-2.42)	1.93 (1.51-2.45)	2.03 (1.55-2.65)	1.98 (1.43-2.74)	1.98 (1.64-2.40)	1.99 (1.61-2.44)	2.08 (1.66-2.61)	2.07 (1.60-2.68)
p.E167K		(T)	2.81×10^{-9}	1.21×10^{-7}	2.01×10^{-7}	3.43×10^{-5}	1.00×10^{-12}	8.80×10^{-11}	2.24×10^{-10}	3.63×10^{-8}
TERT	5	rs2242652	0.64 (0.53-0.76)	0.64 (0.52-0.78)	0.68 (0.55-0.85)	0.69 (0.54-0.90)	0.61 (0.52-0.72)	0.60 (0.50-0.72)	0.64 (0.52-0.78)	0.63 (0.50-0.79)
		(A)	7.87×10^{-7}	9.28×10^{-6}	5.19×10^{-4}	5.89×10^{-3}	6.40×10^{-9}	4.08×10^{-8}	9.11×10^{-6}	7.94×10^{-5}
WNT3A-WNT9A	1	rs708113	0.97 (0.85-1.11)	0.97 (0.83-1.13)	0.93 (0.79-1.10)	0.94 (0.77-1.16)	0.99 (0.87-1.14)	0.98 (0.86-1.13)	0.92 (0.79-1.07)	0.91 (0.77-1.09)
		(T)	0.666	0.680	0.402	0.579	0.932	0.800	0.279	0.321
APOE	19	rs429358	0.74 (0.60-0.91)	0.76 (0.60-0.96)	0.74 (0.57-0.96)	0.68 (0.50-0.93)	0.71 (0.58-0.86)	0.72 (0.58-0.89)	0.68 (0.54-0.87)	0.63 (0.47-0.83)
		(C)	5.44×10^{-3}	2.35×10^{-2}	2.50×10^{-2}	1.59×10^{-2}	5.76×10^{-4}	2.72×10^{-3}	2.17×10^{-3}	1.07×10^{-3}
HSD17B13	4	rs72613567	0.81 (0.69-0.95)	0.84 (0.71-1.01)	0.85 (0.69-1.03)	0.79 (0.63-1.01)	0.85 (0.73-0.99)	0.82 (0.71-0.95)	0.83 (0.69-0.99)	0.79 (0.64-0.96)
		(TA)	8.95×10^{-3}	5.91×10^{-2}	0.102	5.91×10^{-2}	3.63×10^{-2}	7.22×10^{-3}	3.73×10^{-2}	1.96×10^{-2}

Multivariate logistic regression analyses of susceptibility loci for alcohol-related HCC in comparison to ArC, under the additive genetic model; PCs: principal components of of genetic ancestry; all analyses were adjusted by top 15 PCs; Chr., chromosome; OR, odds ratio. Odds ratios are provided with 95% confidence interval; Phenotypic information for BMI and type 2 diabetes status was available for cases and controls accordingly to Table 1.

[a] adjusted for top 15 principal components of genetic ancestry

[b] adjusted for age, sex, and top 15 principal components of genetic ancestry

[c] adjusted for age, sex, BMI, and top 15 principal components of genetic ancestry

[d] adjusted for age, sex, BMI, type II diabetes mellitus and top 15 principal components of genetic ancestry

[e] derived from fixed effect model summary estimates of discovery and replication cohorts

* Effective sample size = 4 / (1/number of cases + 1/number of controls); in the combined analysis the sum of the effective sample sizes of each cohort is reported

Supplementary Table 6: Meta-analysis GWAS association results for loci with P<1×10^{-7}

				EA	Meta-analysis of stage 1 and 2 cohorts (pc adjusted)			
*Locus**	CHR	Position	SNP		P-value [a]	OR	HetISq	Direction
TERT	5	1280028	rs2242652	A	6.40E-09	0.61	0	----
TERT	5	1279790	rs10069690	T	5.19E-08	0.66	0	----
TM6SF2	19	19379549	rs58542926	T	1.00E-12	1.98	43.25	+++-
TM6SF2	19	19388500	rs8107974	A	2.93E-12	1.94	44.39	---+
TM6SF2	19	19456917	rs58489806	T	3.04E-12	1.88	32.17	+++-
TM6SF2	19	19407718	rs10401969	T	8.98E-12	1.89	40.74	---+
TM6SF2	19	19419071	rs739846	A	1.09E-11	1.9	41.15	+++-
TM6SF2	19	19578743	rs73002956	A	1.09E-11	1.88	1.57	---+
TM6SF2	19	19477877	rs56255430	A	1.28E-11	1.88	4.72	---+
TM6SF2	19	19467545	rs2285626	T	1.83E-11	1.75	54.01	+++-
TM6SF2	19	19610596	rs3794991	T	2.07E-11	1.86	3.28	+++-
TM6SF2	19	19460541	rs73001065	C	2.19E-11	1.96	28.52	+++-
TM6SF2	19	19436229	rs111234557	C	4.46E-11	1.75	59.43	---+
TM6SF2	19	19462702	rs11672355	C	4.67E-11	1.75	59.19	+++-
TM6SF2	19	19582992	rs73002960	T	6.20E-11	1.72	44.85	+++-
TM6SF2	19	19494483	rs150268548	A	6.32E-11	1.95	0	+++-
TM6SF2	19	19621004	rs56273306	T	6.60E-11	1.72	44.48	---+
TM6SF2	19	19531910	rs11668386	A	6.61E-11	1.72	43.7	---+
TM6SF2	19	19425025	rs57962361	T	6.88E-11	1.74	59.86	+++-
TM6SF2	19	19366632	rs72999033	T	7.69E-11	1.97	10.34	+++-
TM6SF2	19	19539891	rs8182472	T	8.03E-11	1.72	44.23	---+
TM6SF2	19	19426181	rs11668104	A	8.06E-11	1.73	60.03	+++-
TM6SF2	19	19484008	rs59148799	A	8.25E-11	1.72	44	---+
TM6SF2	19	19508013	rs10424702	A	8.34E-11	1.71	42.5	---+
TM6SF2	19	19613622	rs57009615	A	8.91E-11	1.7	28.05	---+
TM6SF2	19	19548643	rs79954596	T	9.47E-11	1.71	43.81	---+
TM6SF2	19	19517169	rs188552254	A	9.61E-11	1.71	41.81	---+
TM6SF2	19	19572220	rs28720066	T	1.09E-10	1.71	45.39	+++-
TM6SF2	19	19621197	rs113365218	A	1.38E-10	1.72	49.71	+++-
TM6SF2	19	19658472	rs16996148	T	1.38E-10	1.82	0	+++-
TM6SF2	19	19512657	rs10408596	A	1.48E-10	1.7	44.39	---+
TM6SF2	19	19505087	rs10415849	T	1.51E-10	1.7	44.45	+++-
TM6SF2	19	19503573	rs10408875	T	1.61E-10	1.7	43.6	---+
TM6SF2	19	19662220	rs17216525	T	1.65E-10	1.83	0	+++-
TM6SF2	19	19506092	rs56241616	T	1.93E-10	1.7	44.7	+++-
TM6SF2	19	19664077	rs17216588	T	2.69E-10	1.81	0	+++-
TM6SF2	19	19642795	rs56397647	T	3.22E-10	1.7	9.17	+++-
TM6SF2	19	19329924	rs2228603	T	3.47E-10	1.85	0	+++-
TM6SF2	19	19667254	rs143988316	T	4.28E-10	1.8	0	+++-
TM6SF2	19	19675696	rs73004933	T	5.83E-10	1.8	0	+++-
TM6SF2	19	19671266	rs73004926	T	6.08E-10	1.8	0	+++-
TM6SF2	19	19670610	rs150824230	A	9.71E-10	1.78	0	+++-
TM6SF2	19	19695228	rs73004951	T	9.80E-10	1.78	0	+++-
TM6SF2	19	19711139	rs73004959	T	1.57E-09	1.76	0	+++-
TM6SF2	19	19746151	rs2304128	T	2.00E-09	1.77	0	++++
TM6SF2	19	19700552	rs12608729	T	2.26E-09	1.75	0	+++-
TM6SF2	19	19713069	rs73004962	A	2.47E-09	1.75	0	---+
TM6SF2	19	19721722	rs12610185	A	3.56E-09	1.73	0	+++-
TM6SF2	19	19716558	rs73004966	T	3.57E-09	1.74	0	+++-

TM6SF2	19	19720399	rs57504626	T	3.81E-09	1.74	0	+++-
TM6SF2	19	19720788	rs16996185	T	3.81E-09	1.74	0	---+
TM6SF2	19	19721976	rs12610191	T	3.81E-09	1.74	0	+++-
TM6SF2	19	19723215	rs10500212	T	3.81E-09	1.73	0	+++-
TM6SF2	19	19325963	rs3761077	T	4.38E-09	1.68	0	+++-
TM6SF2	19	19727152	rs73004975	A	4.66E-09	1.72	0	---+
TM6SF2	19	19726022	rs58847337	A	5.05E-09	1.72	0	+++-
TM6SF2	19	19717056	rs73004967	A	5.40E-09	1.82	0	---+
TM6SF2	19	19702384	rs17217098	A	8.56E-09	1.81	0	+++-
TM6SF2	19	19336608	rs2238675	T	1.18E-08	1.62	0	+++-
TM6SF2	19	19394368	rs138295924	A	4.64E-08	1.92	0	---+
PNPLA3	22	44340904	rs2294915	T	2.44E-19	1.75	0	++++
PNPLA3	22	44324727	rs738409	G	4.31E-19	1.74	0	++++
PNPLA3	22	44324855	rs3747207	A	4.71E-19	1.77	0	++++
PNPLA3	22	44324730	rs738408	T	5.62E-19	1.77	0	++++
PNPLA3	22	44379565	rs2294922	C	3.11E-14	1.62	16.85	++++
PNPLA3	22	44333968	rs2896020	T	1.62E-11	1.56	0	----
PNPLA3	22	44382684	rs2294927	T	1.63E-11	1.51	0	----
PNPLA3	22	44332570	rs2281135	A	1.70E-11	1.55	0	++++
PNPLA3	22	44333694	rs2896019	T	1.70E-11	1.55	0	----
PNPLA3	22	44383400	rs6006602	T	1.73E-11	1.51	0	++++
PNPLA3	22	44334476	rs4823176	T	1.86E-11	1.56	0	----
PNPLA3	22	44333172	rs2072906	A	1.87E-11	1.56	0	----
PNPLA3	22	44333479	rs2072905	C	1.87E-11	1.56	0	----
PNPLA3	22	44333945	rs2401512	C	1.87E-11	1.56	0	----
PNPLA3	22	44335331	rs16991175	T	1.87E-11	1.56	0	----
PNPLA3	22	44335406	rs35621602	A	1.87E-11	1.56	0	++++
PNPLA3	22	44335416	rs34352134	T	1.87E-11	1.56	0	++++
PNPLA3	22	44335744	rs2073081	T	1.87E-11	1.56	0	----
PNPLA3	22	44334529	rs4823178	T	1.87E-11	1.56	0	----
PNPLA3	22	44335453	rs34376930	T	1.87E-11	1.56	0	++++
PNPLA3	22	44334486	rs4823177	T	1.88E-11	1.56	0	----
PNPLA3	22	44332878	rs34879941	T	1.90E-11	1.56	0	++++
PNPLA3	22	44336310	rs1010022	A	1.91E-11	1.56	0	----
PNPLA3	22	44336098	rs1010023	T	1.96E-11	1.56	0	----
PNPLA3	22	44341666	rs13055900	A	2.00E-11	1.55	0	----
PNPLA3	22	44341672	rs13055874	T	2.04E-11	1.55	0	----
PNPLA3	22	44340086	rs36069781	T	2.10E-11	1.55	0	++++
PNPLA3	22	44341193	rs4823179	T	2.15E-11	1.55	0	----
PNPLA3	22	44340922	rs2294916	T	2.16E-11	1.55	0	----
PNPLA3	22	44343151	rs1810508	A	2.18E-11	1.55	0	----
PNPLA3	22	44342969	rs2008451	T	2.18E-11	1.55	0	----
PNPLA3	22	44341606	rs4823181	T	2.28E-11	1.55	0	----
PNPLA3	22	44341298	rs4823180	A	2.28E-11	1.55	0	++++
PNPLA3	22	44331943	rs1883349	A	2.35E-11	1.55	0	++++
PNPLA3	22	44336957	rs73176497	A	2.49E-11	1.55	0	++++
PNPLA3	22	44339526	rs13056555	C	2.66E-11	1.55	0	----
PNPLA3	22	44336496	rs8142145	T	2.66E-11	1.55	0	----
PNPLA3	22	44337533	rs926633	A	2.66E-11	1.55	0	++++
PNPLA3	22	44345771	rs13054885	A	2.73E-11	1.55	0	++++
PNPLA3	22	44343626	rs12484795	A	3.26E-11	1.55	0	----
PNPLA3	22	44334842	rs2281293	T	3.29E-11	1.55	0	----
PNPLA3	22	44333370	rs2076207	A	3.33E-11	1.55	0	----
PNPLA3	22	44332653	rs2072907	C	3.37E-11	1.55	0	----
PNPLA3	22	44387108	rs1986095	A	3.55E-11	1.5	0	----
PNPLA3	22	44389514	rs2235778	T	3.57E-11	1.5	0	----

PNPLA3	22	44388417	rs3788604	A	3.58E-11	1.5	0	----
PNPLA3	22	44332477	rs2281138	T	3.65E-11	1.55	0	----
PNPLA3	22	44332493	rs2281137	T	3.65E-11	1.55	0	----
PNPLA3	22	44331513	rs1997693	C	4.24E-11	1.54	0	----
PNPLA3	22	44325631	rs12484809	T	4.26E-11	1.56	0	++++
PNPLA3	22	44325565	rs12484801	T	4.26E-11	1.56	0	++++
PNPLA3	22	44325516	rs12485100	T	4.28E-11	1.56	0	++++
PNPLA3	22	44331778	rs13056638	C	4.29E-11	1.54	0	----
PNPLA3	22	44331815	rs1883348	C	4.29E-11	1.54	0	----
PNPLA3	22	44330031	rs1977080	T	4.76E-11	1.55	0	++++
PNPLA3	22	44393075	rs6006473	T	4.91E-11	1.49	0	++++
PNPLA3	22	44325996	rs12483959	A	5.14E-11	1.56	0	++++
PNPLA3	22	44326272	rs9625962	T	5.36E-11	1.56	0	----
PNPLA3	22	44327179	rs16991158	A	6.36E-11	1.54	0	++++
PNPLA3	22	44327192	rs36055245	A	6.36E-11	1.54	0	----
PNPLA3	22	44328730	rs4823173	A	7.00E-11	1.54	0	++++
PNPLA3	22	44329078	rs2076211	T	7.14E-11	1.54	0	++++
PNPLA3	22	44395451	rs1007863	T	7.26E-11	1.49	0	----
PNPLA3	22	44329275	rs2294433	A	7.29E-11	1.54	0	++++
PNPLA3	22	44395389	rs2281292	A	7.29E-11	1.48	0	----
PNPLA3	22	44330128	rs1977081	T	8.72E-11	1.54	0	----
PNPLA3	22	44326700	rs11090617	T	8.76E-11	1.54	0	++++
PNPLA3	22	44347251	rs2092501	A	1.02E-10	1.54	0	++++
PNPLA3	22	44348446	rs34912062	T	1.06E-10	1.54	0	++++
PNPLA3	22	44356468	rs56373884	A	1.09E-10	1.53	0	++++
PNPLA3	22	44327273	rs12484700	A	1.31E-10	1.53	0	----
PNPLA3	22	44361842	rs2294921	T	3.46E-10	1.51	6.21	+++-
PNPLA3	22	44349236	rs1474745	T	3.81E-10	1.51	7.06	---+
PNPLA3	22	44368122	rs3761472	A	3.84E-10	1.51	10.13	---+
PNPLA3	22	44376335	rs67450864	T	1.67E-09	1.44	28.91	++++
PNPLA3	22	44377442	rs4823182	A	1.68E-09	1.44	28	----
PNPLA3	22	44391686	rs2143571	A	6.56E-09	1.47	0	++++
PNPLA3	22	44391234	rs2281298	A	7.20E-09	1.47	0	++++
PNPLA3	22	44388817	rs3827385	T	1.02E-08	1.46	0	---+
PNPLA3	22	44394019	rs2401514	A	1.31E-08	1.46	0	+++-
PNPLA3	22	44385594	rs2073079	A	1.42E-08	1.46	0	---+
PNPLA3	22	44394402	rs2073080	T	1.87E-08	1.45	0	+++-
PNPLA3	22	44341986	rs2294917	T	2.00E-08	0.65	0	++++
PNPLA3	22	44378809	rs2235777	T	5.95E-08	1.45	45.46	+++-
PNPLA3	22	44380767	rs12167845	T	5.95E-08	1.45	44.77	---+
PNPLA3	22	44380009	rs9626079	A	6.07E-08	1.44	44.82	---+
PNPLA3	22	44379740	rs2294923	A	6.08E-08	1.44	44.81	+++-
PNPLA3	22	44377999	rs2235776	T	6.12E-08	1.44	45.71	+++-
PNPLA3	22	44381340	rs4823108	T	6.21E-08	1.44	45.54	---+
PNPLA3	22	44378672	rs4823183	A	6.70E-08	1.44	45.69	+++-

Abbreviations: SNP: single nucleotide polymorphism; Chr: chromosome; OR: odds ratio; HetISq: I^2-measure of percentage of between cohort heterogeneity Meta; P value: Significance derived from a fixed effect meta-analysis.[a] Odds ratio and *P* value adjusted for top 15 principal components of genetic ancestry; * risk loci *TM6SF2* and *PNPLA3* annotate to multiple genes not listed in detail

Supplementary Table 7: Known alcohol- and HCV/HBV-related HCC associated variants detailed in the current study analyses cohorts

Previously known HCC disease associations				Discovery cohort (Current study)			Replication cohort 1 UK (Current study)			Replication cohort 2 Germany (Current study)		
Study	Study type	Gene, Chrom.	SNP (Effect Allele)	AF in Ca|Co	Odds Ratio	P value	AF in Ca|Co	Odds Ratio	P value	AF in Ca|Co	Odds Ratio	P value
Trepo et al./ Stickel et al.	GWAS / CGS	*PNPLA3* (Chr 22)	rs738409 (G)	0.48|0.35	1.71 (1.49-1.96)	7.23×10^{-15}	0.49|0.30	2.20 (1.55-3.11)	6.31×10^{-6}	0.49|0.38	1.57 (0.97-2.56)	0.068
Trepo et al./ Stickel et al.	GWAS / CGS	*TM6SF2* (Chr 19)	rs58542926 (T)	0.15|0.08	1.94 (1.56-2.42)	2.81×10^{-9}	0.21|0.09	2.55 (1.65-3.95)	8.35×10^{-6}	0.13|0.11	1.21 (0.63-2.35)	0.565
Trepo et al.	GWAS	*WNT3A* (Chr 1)	rs708113 (T)	0.37|0.38	0.97 (0.85-1.11)	0.678	0.37|0.38	0.95 (0.70-1.29)	0.741	0.43|0.35	1.48 (0.89-2.47)	0.132
Stickel, Lutz, Buch et al.	CGS	*HSD17B13* (Chr 4)	rs72613567 (TA)	0.19|0.22	0.83 (0.71-0.97)	0.020	0.23|0.26	0.86 (0.61-1.21)	0.381	0.18|0.22	0.81 (0.47-1.41)	0.457
Innes et al.	CGS	*APOE* (Chr 19)	rs429358 (C)	0.09|0.12	0.74 (0.60-0.92)	6.35×10^{-3}	0.07|0.14	0.51 (0.28-0.93)	0.027	0.13|0.15	0.84 (0.42-1.70)	0.633
Innes et al.	CGS	*TM6SF2* (Chr 19)	rs187429064 (G)	0.02|0.01	1.82 (1.10-3.00)	0.019	0.08|0.02	3.21 (1.67-6.18)	4.79×10^{-4}	0.01|0.02	0.71 (0.05-9.78)	0.799
Miki et. al	GWAS (HCV)	*DEPDC5* (Chr 22)	rs1012068 (G)	0.27|0.24	1.15 (0.99-1.35)	0.069	0.27|0.23	1.36 (0.91-2.02)	0.134	0.28|0.28	1.00 (0.59-1.68)	1
Jiang et al.	GWAS (HBV)	*STAT4* (Chr 2)	rs7574865 (G)	0.77|0.78	0.97 (0.83-1.14)	0.730	0.76|0.77	0.88 (0.58-1.32)	0.529	0.76|0.81	0.74 (0.42-1.30)	0.316
Jiang et al.	GWAS (HBV)	*HLA-DQ* (Chr 6)	rs9275319 (A)	0.88|0.87	1.03 (0.83-1.27)	0.799	0.86|0.89	0.78 (0.43-1.43)	0.443	0.86|0.86	0.94 (0.49-1.80)	0.859

AF: Allele frequency, Ca: Cases (ArC with HCC), Co: Controls (ArC without HCC). Trepo et al.: Common genetic variation in alcohol-related HCC: a case-control genome-wide association study. The Lancet Oncology Dec 2021; Stickel, Lutz, Buch et al.: Genetic Variation in *HSD17B13* Reduces the Risk of Developing Cirrhosis and HCC in Alcohol Misusers. Hepatology Jul 2020; Innes et al.: The rs429358 locus in APOE is associated with HCC in patients with cirrhosis. Hepatology Communications Dec 2021; Stickel et al.: Genetic variants in PNPLA3 and TM6SF2 predispose to the development of HCC in individuals with ArC. Am J Gastroenterol. 2018 Oct; Miki et.al.: Variation in the DEPDC5 locus is associated with progression to HCC in chronic hepatitis C virus carriers. Nature Genetics Jul 2011.; Jiang et al.: Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related HCC. Nature Genetics Jan 2013.

Supplementary Table 8: Univariate analyses for association of *TERT* rs2242652 with alcohol-related cirrhosis and HCC in the whole cohort

Cohorts	*TERT* (rs2242652)	Comparative groups		Genotypic OR (95% CI), P value	Allelic OR (95% CI), P value	Cases / Controls (n)
		HCC	**ArC**			
Alcoholic-related cirrhosis and HCC **(HCC)**	G|G	919	1201			
	A|G	273	588	0.61 (0.51-0.72)	0.62 (0.53-0.71)	1214 | 1859
	A|A	22	70	0.41 (0.25-0.67)		
	MAF	0.131	0.196	2.32×10^{-10}	2.81×10^{-11}	
		ArC	**AM**			
Alcohol-related cirrhosis without HCC **(ArC)**	G|G	1201	981			
	A|G	588	479	1.00 (0.87-1.16)	0.99 (0.88-1.12)	1859| 1520
	A|A	70	60	0.95 (0.67-1.36)		
	MAF	0.196	0.197	0.963	0.899	
		HCC	**AM**			
Alcohol misusers **(AM)**	G|G	919	981			
	A|G	273	479	0.61 (0.51-0.72)	0.61 (0.53-0.71)	1214 | 1520
	A|A	22	60	0.39 (0.24-0.64)		
	MAF	0.131	0.197	6.38×10^{-10}	6.13×10^{-11}	

CI: confidence intervals, MAF: minor allele frequency, OR: odds ratio. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Genotypic odds ratios for heterozygous and homozygous carriership of the rare allele and allelic odds ratios were calculated from 2 × 2 tables on genotype counts. Significance was calculated as one degree of freedom Chi-squared test of allelic counts and 2 degree of freedom Chi-squared test of genotype counts.

Supplementary Table 9: Univariate analyses for association of *PNPLA3* rs738409 with alcohol-related cirrhosis and HCC in the whole cohort

Cohorts	*PNPLA3* (rs738409)	Comparative groups		Genotypic OR (95% CI), P value	Allelic OR (95% CI), P value	Cases/ Controls (n)
		HCC	**ArC**			
Alcoholic-related cirrhosis and HCC **(HCC)**	C|C	339	840			
	C|G	576	799	1.79 (1.51-2.11)	1.85 (1.67-2.06)	1214 | 1866
	G|G	229	227	3.26 (2.64-4.04)		
	MAF	0.484	0.336	3.28×10^{-28}	4.15×10^{-31}	
		ArC	**AM**			
Alcohol-related cirrhosis without HCC **(ArC)**	C|C	840	887			
	C|G	799	495	1.70 (1.47-1.97)	1.85 (1.65-2.06)	1866 | 1455
	G|G	227	63	3.80 (2.83-5.11)		
	MAF	0.336	0.215	1.99×10^{-25}	2.52×10^{-27}	
		HCC	**AM**			
Alcohol misusers **(AM)**	C|C	339	887			
	C|G	576	495	3.04 (2.56-3.62)	3.42 (3.04-3.85)	1214 | 1455
	G|G	229	63	12.42 (9.21-16.75)		
	MAF	0.484	0.215	6.21×10^{-85}	1.29×10^{-94}	

CI: confidence intervals, MAF: minor allele frequency, OR: odds ratio. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Genotypic odds ratios for heterozygous and homozygous carriership of the rare allele and allelic odds ratios were calculated from 2 × 2 tables on genotype counts. Significance was calculated as one degree of freedom Chi-squared test of allelic counts and 2 degree of freedom Chi-squared test of genotype counts.

Supplementary Table 10: Univariate analyses for association of *TM6SF2* rs58542926 with alcohol-related cirrhosis and HCC in the whole cohort

Cohorts	*TM6SF2* (rs58542926)	Comparative groups		Genotypic OR (95% CI), P value	Allelic OR (95% CI), P value	Cases/ Controls (n)
		HCC	**ArC**			
Alcoholic-related cirrhosis and HCC **(HCC)**	C|C	890	1545			
	C|T	285	300	1.65 (1.37-1.98)	1.74 (1.49-2.04)	1214 | 1866
	T|T	39	21	3.22 (1.88-5.52)		
	MAF	0.150	0.092	1.02×10^{-10}	3.14×10^{-12}	
		ArC	**AM**			
Alcohol-related cirrhosis without HCC **(ArC)**	C|C	1545	1283			
	C|T	300	167	1.49 (1.22-1.83)	1.56 (1.29-1.88)	1866 | 1455
	T|T	21	5	3.49 (1.31-9.28)		
	MAF	0.092	0.061	2.19×10^{-5}	3.44×10^{-6}	
		HCC	**AM**			
Alcohol misusers **(AM)**	C|C	890	1283			
	C|T	285	167	2.46 (2.00-3.03)	2.71 (2.25-3.28)	1214 | 1455
	T|T	39	5	11.24 (4.41-28.64)		
	MAF	0.150	0.061	5.09×10^{-24}	1.02×10^{-26}	

CI: confidence intervals, MAF: minor allele frequency, OR: odds ratio. Genotype counts reflect the rounded sum of imputed genotype probabilities for individuals in the sample. Genotypic odds ratios for heterozygous and homozygous carriership of the rare allele and allelic odds ratios were calculated from 2 × 2 tables on genotype counts. Significance was calculated as one degree of freedom Chi-squared test of allelic counts.

Supplementary Table 11: Finemapping, conditional analysis and expression analysis of the *TERT* Locus

GWAS	*TERT* Gene Variants Annotation				Discov.	Meta-analysis stage 1&2				MAF	Imputation		Conditional A.		LD	Expression		Leukocyte telomere length		
TOP	**SNP:ALT**	**Pos Chr 5**	**Type**	**Location**	**Pgwas**	**Pfix**	**ORfix**	**Prand**	**ORrand**	**Ca | Co**	**Type**	**Rsq**	**Pcon**	**R2Lead**	**Block**	**eQTL_P**	**Zscore**	**Beta**	**P_Telo**	**TOP**
	rs35535053:T	1252972	downstr.	DIST=315	0.396	0.49	0.83	0.69	0.86	0.016 | 0.018	Imp	0.74	0.918	0.016	B-1	0.71	0.37 (T)	-	-	
	rs2853690:A	1253744	3´UTR	EX=16/16	0.531	0.83	0.98	0.83	0.98	0.173 | 0.179	Imp	0.90	0.277	0.005	B-1	0.71	0.37 (A)	-0.0010	7.99E-08	
	rs35033501:T	1253918	synonym	EX=16/16	9.1E-3	0.02	1.67	0.02	1.67	0.035 | 0.021	Imp	0.85	0.023	0.003	B-1	0.44	0.77 (T)	0.0001	0.885	
	rs35719940:T	1254594	missense	EX=15/16	0.386	0.25	1.31	0.25	1.31	0.021 | 0.017	Imp	0.85	0.612	0.004	B-1	0.17	1.37 (T)	0.0021	8.17E-06	
	rs33954691:A	1255520	synonym	EX=14/16	0.084	0.05	1.24	0.05	1.24	0.103 | 0.088	Imp	0.88	0.155	0.008	B-1	5.0E-5	-4.05 (A)	0.0004	0.119	
	rs35387865:T	1255844	intronic	IN=13/15	0.280	0.31	1.33	0.31	1.33	0.017 | 0.013	Imp	0.86	0.448	0.004	B-1	0.87	0.17 (T)	0.0055	5.58E-16	
	rs2853687:A	1256585	intronic	IN=13/15	0.857	0.94	1.01	0.94	1.01	0.265 | 0.273	Imp	0.96	0.335	0.023	B-1	0.72	-0.35 (A)	-0.0009	5.81E-08	
	rs35041195:T	1257288	intronic	IN=13/15	0.801	0.55	0.94	0.55	0.94	0.119 | 0.128	Imp	0.90	0.573	0.002	B-1	0.96	0.05 (T)	-0.0018	7.79E-12	
	rs2736122:A	1257621	intronic	IN=13/15	0.389	0.62	0.97	0.62	0.97	0.261 | 0.280	Gen	0.99	0.133	0.014	B-1	0.60	-0.52 (A)	-0.0007	1.54E-05	
	rs144704378:T	1259489	intronic	IN=12/15	0.028	0.03	1.39	0.03	1.39	0.054 | 0.041	Imp	0.94	0.072	0.007	B-1	0.01	-2.44 (T)	0.0008	0.021	
	rs2736118:C	1260195	intronic	IN=12/15	0.530	0.60	0.96	0.60	0.96	0.262 | 0.278	Imp	0.98	0.204	0.014	B-1	0.73	-0.35 (C)	-0.0006	4.62E-05	
	rs34041736:T	1261051	intronic	IN=11/15	0.518	0.38	1.17	0.38	1.17	0.036 | 0.033	Imp	0.90	0.739	0.005	B-1	0.21	1.24 (T)	-0.0033	5.87E-15	
	rs11133715:A	1261052	intronic	IN=11/15	0.063	0.08	0.88	0.08	0.88	0.329 | 0.344	Imp	0.90	0.452	0.051	B-1	0.13	1.51 (A)	-	-	
	rs36077395:A	1261220	intronic	IN=11/15	0.679	0.53	1.18	0.53	1.18	0.016 | 0.015	Imp	0.87	0.854	0.002	B-1	0.25	1.14 (A)	-0.0012	0.020	
	rs34529095:C	1263408	intronic	IN=11/15	0.749	0.87	0.97	0.97	1.01	0.031 | 0.030	Imp	0.80	0.492	0.026	B-1	0.07	1.84 (C)	0.0011	0.014	
	rs2736115:T	1264068	intronic	IN=11/15	0.420	0.61	0.97	0.61	0.97	0.259 | 0.276	Imp	0.95	0.143	0.015	B-1	0.77	-0.29 (T)	-0.0008	1.16E-06	
	rs2853685:G	1264152	intronic	IN=11/15	0.463	0.68	0.97	0.68	0.97	0.270 | 0.285	Imp	0.94	0.152	0.017	B-1	0.80	-0.25 (G)	-0.0009	7.74E-09	
	rs2736114:T	1265204	intronic	IN=10/15	0.422	0.65	0.97	0.65	0.97	0.260 | 0.277	Imp	0.95	0.142	0.015	B-1	0.75	-0.32 (T)	-0.0008	3.69E-07	
	rs2736113:A	1265373	intronic	IN=10/15	0.376	0.60	0.96	0.60	0.96	0.259 | 0.277	Imp	0.95	0.120	0.015	B-1	0.77	-0.29 (A)	-0.0008	4.05E-07	
	rs2736111:A	1265935	intronic	IN=10/15	0.404	0.63	0.97	0.63	0.97	0.259 | 0.277	Imp	0.95	0.133	0.015	B-1	0.78	-0.28 (A)	-0.0008	8.65E-07	
	rs2853684:C	1266226	intronic	IN=10/15	0.515	0.75	0.98	0.75	0.98	0.262 | 0.278	Imp	0.95	0.182	0.016	B-1	0.76	-0.3 (C)	-0.0008	1.67E-06	
	rs2075786:G	1266310	intronic	IN=10/15	5.8E-3	5.7E-03	0.82	5.7E-03	0.82	0.623 | 0.659	Imp	0.88	0.061	0.035	B-1	0.49	-0.7 (A)	-	-	
	rs3891054:G	1267202	intronic	IN=9/15	0.374	0.27	1.11	0.27	1.11	0.147 | 0.146	Imp	0.87	0.974	0.025	B-2	N/A	N/A	-0.0014	2.16E-08	
	rs34194491:C	1267213	intronic	IN=9/15	0.024	0.02	1.61	0.02	1.61	0.033 | 0.022	Gen	1.00	0.051	0.004	B-2	0.01	-2.5 (C)	0.0010	0.042	
	rs4246742:A	1267356	intronic	IN=9/15	0.042	0.03	1.22	0.03	1.22	0.179 | 0.159	Imp	0.88	0.259	0.030	B-2	N/A	N/A	-	-	
	rs35812074:G	1267881	intronic	IN=9/15	0.218	0.18	1.42	0.18	1.42	0.020 | 0.015	Imp	0.75	0.379	0.004	B-2	N/A	N/A	0.0061	3.86E-25	
	rs114401494:T	1269161	intronic	IN=8/15	0.248	0.23	0.74	0.23	0.74	0.016 | 0.027	Imp	0.88	0.124	0.005	B-2	0.31	1.03 (T)	-	-	
	rs11742908:G	1270983	intronic	IN=8/15	5.0E-3	4.1E-03	0.77	4.1E-03	0.77	0.154 | 0.185	Imp	0.87	0.306	0.516	B-2	N/A	N/A	-	-	
	rs11133719:C	1271524	intronic	IN=7/15	0.172	0.11	0.86	0.11	0.86	0.832 | 0.849	Imp	0.84	0.631	0.030	B-2	0.02	-2.37 (T)	-	-	
	rs13172201:C	1271661	intronic	IN=7/15	4.9E-3	3.9E-03	1.25	3.9E-03	1.25	0.284 | 0.252	Imp	0.91	0.099	0.055	B-2	0.75	-0.31 (C)	-	-	

4	rs35517815:A	1274445	intronic	IN=6/15	1.7E-4	1.5E-04	5.11	1.5E-04	5.11	0.020 | 0.006	Imp	0.73	0.0004	0.002		0.37	0.9 (A)	-	-	
	rs4975605:A	1275528	intronic	IN=6/15	0.505	0.68	0.97	0.68	0.97	0.462 | 0.469	Imp	0.92	0.060	0.061		0.04	-2.04 (A)	-	-	
	rs145685051:G	1276736	intronic	IN=6/15	0.869	0.80	1.07	0.80	1.07	0.018 | 0.016	Imp	0.88	0.038	0.109		0.68	0.41 (G)	0.0033	3.74E-09	
6	rs56345976:A	1276873	intronic	IN=6/15	8.7E-4	7.2E-04	0.80	7.2E-04	0.80	0.569 | 0.624	Gen	0.99	0.070	0.107		0.002	-3.13 (G)	-	-	
	rs33961405:A	1277577	intronic	IN=6/15	0.682	0.51	1.05	0.51	1.05	0.533 | 0.534	Imp	0.93	0.100	0.150	B-3	0.74	-0.34 (A)	-	-	
	rs144020096:A	1278447	intronic	IN=6/15	0.116	0.06	0.54	0.06	0.54	0.008 | 0.015	Imp	0.95	0.740	0.066	B-3	0.43	-0.78 (A)	-0.0008	0.278	
	rs2075785:T	1278584	intronic	IN=6/15	0.199	0.07	1.18	0.07	1.18	0.135 | 0.128	Imp	0.94	0.691	0.029	B-3	0.37	0.90 (T)	-0.0034	6.56E-48	
	rs35241335:G	1279224	intronic	IN=5/15	0.260	0.08	1.23	0.08	1.23	0.077 | 0.075	Imp	0.94	0.697	0.016	B-3	0.55	0.59 (G)	-0.0036	4.40E-29	
Top 2	**rs10069690:T**	**1279790**	**intronic**	**IN=4/15**	**5.7E-6**	**5.2E-08**	**0.66**	**5.2E-08**	**0.66**	**0.183 | 0.242**	**Gen**	**1.00**	**0.485**	**0.696**	**B-3**	**0.003**	**2.96 (T)**	**0.0031**	**4.08E-84**	
9	rs10054203:C	1279964	intronic	IN=4/15	3.4E-3	7.6E-04	0.79	0.03	0.76	0.352 | 0.392	Imp	0.95	0.967	0.328	B-3	0.04	2.01 (C)	0.0036	8.83E-133	10
Top1	**rs2242652:A**	**1280028**	**intronic**	**IN=4/15**	**7.9E-7**	**6.4E-09**	**0.61**	**6.4E-09**	**0.61**	**0.133 | 0.191**	**Gen**	**1.00**	**NA**	**1.000**	**B-3**	**1.4E-5**	**4.35 (A)**	**0.0026**	**2.12E-44**	
	rs7734992:C	1280128	intronic	IN=4/15	0.019	9.3E-04	0.81	5.9E-03	0.78	0.367 | 0.401	Imp	0.96	0.555	0.315	B-3	0.04	2.03 (C)	0.0041	2.15E-168	5
	rs13167280:A	1280477	intronic	IN=3/15	0.099	0.12	1.15	0.12	1.15	0.158 | 0.139	Imp	0.95	0.482	0.033	B-3	0.26	-1.13 (A)	0.0026	3.28E-32	
10	rs4975538:C	1280830	intronic	IN=3/15	3.9E-3	2.0E-04	0.78	2.0E-04	0.78	0.316 | 0.356	Imp	0.95	0.826	0.383	B-3	0.01	2.57 (C)	0.0039	5.02E-145	8
	rs7726159:A	**1282319**	**intronic**	**IN=3/15**	**5.2E-3**	**1.6E-04**	**0.78**	**9.2E-03**	**0.72**	**0.292 | 0.329**	**Imp**	**0.99**	**0.795**	**0.354**	**B-3**	**0.02**	**2.32 (A)**	**0.0048**	**1.16E-219**	**Top2**
8	rs7725218:A	1282414	intronic	IN=3/15	3.2E-3	8.1E-05	0.77	4.4E-03	0.73	0.302 | 0.342	Gen	1.00	0.997	0.334	B-3	0.05	1.96 (A)	0.0045	2.77E-198	4
	rs7713218:G	1283312	intronic	IN=2/15	0.018	3.7E-03	1.20	3.7E-03	1.20	0.554 | 0.523	Imp	0.95	0.830	0.185	B-3	0.004	2.87 (A)	0.0033	5.31E-114	
Top3	**rs72709458:T**	**1283755**	**intronic**	**IN=2/15**	**3.9E-5**	**2.9E-07**	**0.66**	**1.1E-03**	**0.58**	**0.156 | 0.208**	**Imp**	**0.97**	**0.771**	**0.749**	**B-3**	**3.4E-5**	**4.14 (T)**	**0.0034**	**1.10E-81**	
	rs6420019:C	1283841	intronic	IN=2/15	0.656	0.32	0.91	0.32	0.91	0.845 | 0.852	Imp	0.89	0.595	0.034	B-3	0.57	-0.57 (A)	-0.0019	4.95E-20	
	rs6420020:C	1284046	intronic	IN=2/15	0.651	0.31	0.91	0.32	0.90	0.845 | 0.852	Imp	0.89	0.600	0.034	B-3	0.62	-0.5 (T)	-0.0019	3.03E-20	
	rs4449583:T	**1284135**	**intronic**	**IN=2/15**	**5.3E-3**	**2.2E-04**	**0.78**	**1.5E-03**	**0.77**	**0.291 | 0.329**	**Imp**	**0.99**	**0.796**	**0.353**	**B-3**	**0.02**	**2.27 (T)**	**0.0048**	**9.93E-215**	**Top3**
	rs35029535:T	1284976	intronic	IN=2/15	0.165	0.13	1.10	0.13	1.10	0.406 | 0.377	Imp	0.95	0.687	0.125	B-3	0.02	-2.31 (T)	-0.0017	9.77E-30	
	rs7705526:A	**1285974**	**intronic**	**IN=2/15**	**0.046**	**3.3E-03**	**0.82**	**6.6E-03**	**0.81**	**0.295 | 0.322**	**Gen**	**1.00**	**0.647**	**0.210**	**B-3**	**0.27**	**1.1 (A)**	**0.0052**	**1.64E-245**	**Top1**
	rs2736100:A	1286516	intronic	IN=2/15	0.333	0.07	1.12	0.12	1.18	0.536 | 0.528	Gen	1.00	0.230	0.162	B-3	0.11	-1.61 (A)	-0.0039	1.47E-166	6
	rs2853677:A	1287194	intronic	IN=2/15	0.022	0.01	1.18	0.01	1.18	0.596 | 0.571	Gen	0.99	0.349	0.077	B-4	0.11	1.58 (G)	0.0036	1.31E-139	9
	rs35838177:T	1287290	intronic	IN=2/15	0.554	0.43	1.14	0.43	1.14	0.04 | 0.031	Gen	0.99	0.492	0.000	B-4	0.87	-0.17 (T)	0.0001	0.891	
	rs2736099:G	1287340	intronic	IN=2/15	0.504	0.27	1.08	0.35	1.11	0.661 | 0.655	Imp	0.94	0.686	0.047	B-4	0.78	0.28 (A)	0.0040	8.35E-146	7
5	rs7710703:C	1287505	intronic	IN=2/15	6.6E-4	7.9E-05	1.47	0.02	1.57	0.888 | 0.859	Imp	0.89	0.146	0.164	B-5	0.002	3.16 (T)	0.0015	3.16E-12	
	rs2853676:C	1288547	intronic	IN=2/15	0.168	0.04	1.15	0.04	1.15	0.753 | 0.740	Gen	1.00	0.642	0.132	B-5	0.31	1.02 (T)	0.0020	1.71E-37	
	rs34677523:A	1288883	intronic	IN=2/15	0.165	0.40	0.79	0.92	1.08	0.012 | 0.020	Imp	0.87	0.107	0.001	B-5	N/A	N/A	-	-	
	rs72709460:A	1289220	intronic	IN=2/15	0.571	0.57	1.22	0.57	1.22	0.012 | 0.010	Imp	0.77	0.696	0.001	B-5	0.39	-0.85 (A)	-	-	
	rs115451758:A	1289277	intronic	IN=2/15	0.223	0.19	1.61	0.19	1.61	0.012 | 0.007	Imp	0.94	0.302	0.001	B-5	0.005	-2.79 (A)	-	-	
	rs796501027:T	1291530	intronic	IN=2/15	0.052	0.02	1.41	0.71	1.24	0.127 | 0.113	Imp	0.43	0.192	0.008	B-5	N/A	N/A	-	-	

	rs56023411:T	1291740	intronic	IN=2/15	7.0E-3	3.2E-03	1.32	0.13	1.38	0.804 | 0.778	Imp	0.77	0.632	0.226	B-5	0.004	2.84 (G)	-	-
	rs71595003:A	1292118	intronic	IN=2/15	0.031	0.03	1.51	0.07	1.50	0.035 | 0.022	Gen	0.99	0.073	0.005	B-5	0.63	0.48 (A)	0.0000	0.982
	rs35334674:A	1292299	intronic	IN=2/15	0.051	0.06	0.70	0.80	0.88	0.030 | 0.038	Imp	0.85	0.030	0.002	B-5	0.89	0.14 (A)	0.0010	0.014
	rs114616103:T	1292958	intronic	IN=2/15	0.294	0.09	0.72	0.10	0.29	0.025 | 0.031	Gen	1.00	0.571	0.011	B-5	0.19	-1.31 (T)	-0.0039	8.23E-21
	rs2853672:A	1292983	intronic	IN=2/15	0.044	0.01	1.17	0.15	1.21	0.527 | 0.507	Gen	1.00	0.441	0.062	B-5	0.69	-0.4 (A)	-0.0030	2.91E-99
	rs79662648:G	1293389	intronic	IN=2/15	0.392	0.19	1.24	0.19	1.24	0.040 | 0.031	Imp	0.95	0.498	0.001	B-5	N/A	N/A	-0.0009	0.027
	rs2736098:T	1294086	synonym	EX=2/16	0.398	0.26	1.08	0.40	1.10	0.271 | 0.253	Imp	0.98	0.936	0.036	B-5	0.36	-0.92 (T)	0.0026	1.48E-58
	rs61748181:T	1294166	missense	EX=2/16	0.294	0.09	0.72	0.10	0.29	0.025 | 0.031	Gen	1.00	0.571	0.011	B-5	0.47	-0.72 (T)	-0.0040	6.40E-21
	rs2853669:G	1295349	upstream	DIST=187	0.246	0.11	1.11	0.18	1.12	0.315 | 0.289	Gen	1.00	0.830	0.037	B-5	0.06	-1.88 (G)	-	-
	rs35226131:T	1295373	upstream	DIST=211	0.294	0.09	0.72	0.10	0.29	0.025 | 0.031	Gen	1.00	0.571	0.011	B-5	0.26	-1.13 (T)	-	-
	rs35161420:G	1295452	upstream	DIST=290	0.296	0.09	0.71	0.10	0.29	0.025 | 0.031	Imp	0.99	0.575	0.011	B-5	0.23	-1.19 (G)	-	-
	rs33958877:T	1295682	upstream	DIST=520	0.328	0.11	0.73	0.11	0.29	0.025 | 0.031	Gen	1.00	0.616	0.011	B-5	0.16	-1.42 (T)	-	-
	rs34768248:A	1295716	upstream	DIST=554	0.017	0.02	0.50	0.02	0.50	0.008 | 0.015	Gen	1.00	0.099	0.017	B-5	0.79	0.26 (A)	-	-
	rs34685900:C	1295803	upstream	DIST=641	7.5E-3	0.01	0.51	0.01	0.51	0.009 | 0.018	Gen	1.00	0.042	0.014	B-5	0.71	0.37 (C)	-	-
7	rs7712562:G	1296072	upstream	DIST=910	9.1E-4	6.9E-05	1.45	0.00	1.46	0.878 | 0.843	Imp	0.96	0.484	0.302	B-5	8.5E-5	3.93 (A)	-	-
	rs2735940:G	1296486	upstream	DIST=1324	0.006	0.01	1.17	0.14	1.21	0.529 | 0.507	Gen	1.00	0.409	0.061	B-5	0.65	1.54 (C)	-	-
	rs33977403:T	1296727	upstream	DIST=1565	0.931	0.67	1.12	0.67	1.12	0.013 | 0.014	Gen	0.99	0.748	0.002	B-5	0.25	-1.91 (T)	-	-

LD-Block information at the TERT locus, *cise*QTL Expression, UK Biobank Leukocyte telomere length data and conditional analysis on the lead SNP rs2242652 in the primary GWAS samples. Abbreviations: Top 10 GWAS variants ranked by P value in the discovery GWAS, top 3 in bold print.; SNP:ALT: SNP with alternative allele (reference allele for odds ratio); POS: genomic position on Chr 5; IN: intron, EX: exon; DIST:distance to *TERT* gene; Pgwas: Association P value in discovery; Pfix: fixed-effects meta-analysis and Prand random-effects meta-analysis association P value in the combined analysis of stage 1 discovery and stage 2 replication; ORfix/rand (CI95): Allelic odds ratio with 95% confidence interval; MAF Ca | Co minor allele freq in cases (HCC) and controls (CIRR); Imputation information: Rsq (imputation r2 info score); Annotated LD-Blocks are clusters of strong pairwise LD SNPs and reflect the LD pattern in the discovery GWAS cohort. Conditional Analysis: Pcon: Logistic regression P value of the respective variant conditioned on the allele dosage of the lead variant rs2242652 shown in bold print. Upon conditional analysis, the variant rs2242652 captures the association information in LD block B3 and of the entire *TERT* locus; R2Lead: R2 value of the respective variant in relation to the lead variant rs2242652:A in the discovery sample; Block: LD block assignment based on R2 value; Expression: CiseQTL P values for cis-eQTL effect on TERT expression in blood, from eQTLGen (https://eqtlgen.org/). Zscore of effect strength and effect direction of assed allele in brackets. Leukocyte telomere length information obtained from UK Biobank: P_Telo/Beta: P value and beta of association of the respective variant with leukocyte telomere length; TOP: Top 10 variants ranked by P value in the leukocyte telomere length analysis, top 3 in bold print.

Supplementary Table 12: *TERT* Locus - conditional analysis in the primary discovery samples

Conditioned on rs2242652	**OR (CI 95%)**	**P value**
rs2242652	NA	NA
rs10069690	OR = 0.90 (0.67-1.21)	0.485
rs72709458	OR = 1.05 (0.75-1.47)	
Conditioned on rs10069690		
rs2242652	OR = 0.70 (0.50-0.98)	**0.036**
rs10069690	NA	NA
rs72709458	OR = 0.91 (0.67-1.24)	

Conditional analysis of the top 3 associated variants at the *TERT* locus, located in LD block B3. Upon conditional analysis, the variant rs2242652 captures the association information at the locus, as shown in Supplementary Table 12**.** The variant rs2242652 remains significantly associated with HCC after conditioning on variant rs10069690.

Supplementary Table 13: Summary of data set used for additional replication of *TERT* variants

		Characteristics				[Minor allele] frequency (%)			
Cohort	**Phenotypes (ICD10 codes)**	HCC (n,%)	N	Age* (mean)	% male	*TERT* [A] rs2242652	*TERT* [T] rs10069690	*PNPLA3*[G] rs738409	*TM6SF2*[T] rs58542926
Stop-HCV	Cases: C22.0 Liver cell carcinoma (HCC) in HCV-related cirrhosis	169 (100)	169	60	73	0.151	0.210	0.281	0.080
	Controls: HCV-related cirrhosis without HCC		890	56	77	0.197	0.270	0.253	0.090
Trépo *et al*	Cases: C22.0 HCC in ALD (with advanced fibrosis/cirrhosis)	775 (100)	775	65	90	n/a	n/a	0.43	0.13
	Controls: alcohol-related liver disease advanced fibrosis/cirrhosis		1332	56	72	n/a	n/a	0.33	0.08
Zhang *et al*.	Cases: Chinese patients with C22.0 HCC (patients with HCV were excluded)	473 (100)	473	56	83	0.133	0.135	n/a	n/a
	Controls: non-cancer individuals from the Physical Examination Center of Haikou People's Hospital		564	54	60	0.179	0.171	n/a	n/a
Dong *et al*.	Cases: male Chinese patients with C22.0 HCC (hepatitis-induced)	181 (100)	181	n/a	100	n/a	0.072	n/a	n/a
	Controls: male, non-cancer individuals from General Hospital of PLA, Beijing		106	n/a	100	n/a	0.184	n/a	n/a
FinnGen	Cases: C22 malignant neoplasm of liver and intrahepatic bile duct	265 (60)	442	71	82	0.193	0.253	0.320	0.105
	Controls: FinnGen biobank participants without a diagnosis of primary liver cancer (excluding all other cancers)		204,070	63	43	0.230	0.296	0.227	0.064
UKBB**	Cases: C22 malignant neoplasm of liver and intrahepatic bile duct	383 (44)	874	62	78	0.169	0.226	0.264	0.109
	Controls: UKB participants who did not meet the above definition of a case		348,465	58	47	0.190	0.258	0.216	0.076
UKBB*	Cases: C22.0 Liver cell carcinoma (HCC)	383 (100)	383	62	77	0.159	0.202	0.325	0.142
	Controls: UKB participants who did not meet the above definition of a case		348,956	58	47	0.190	0.258	0.216	0.076
BBJ Japan***	Cases: C22.0 Liver cell carcinoma (HCC)	1866 (100)	1866	68	74	n/a	n/a | 0.183	0.495	0.085
	Controls: BBJ participants who did not meet the above definition of a case			62	50	n/a	n/a | 0.209	0.458	0.078

n/a: data not available; *Mean age at first event (years) ** As UKB data were incorporated into our discovery analysis, further interrogation of liver cancer phenotypes from UKB does not constitute independent validation. *** Variants rs2242652 and rs10069690 were not available in BioBank Japan summary GWAS data from Ishigaki et al.(67) (PMID: 32514122), publicly available from http://jenger.riken.jp/en/result; maf data is reported for proxy variants rs72709458 (r2=0.973 between rs72709458 and rs2242652, both variants are in high LD).

Supplementary Table 14: Information for association of *TERT* variants rs2242652, rs10069690 with other cancer from the NHGRI-EBI Catalog of human genome-wide association studies

Reported cancer trait NHGRI-EBI -GWAS Catalog	Variant	P-value	Effect allele	Association	OR	CI	First Author	PubMed ID
Prostate cancer	rs2242652	8 x 10-55	A - minor allele	protective	0.88	[0.86-0.89]	Conti DV	33398198
Prostate cancer	rs2242652	4 x 10-52	A - minor allele	protective	0.85	[0.84-0.87]	Schumacher	29892016
Prostate cancer	rs2242652	3 x 10-24	A - minor allele	protective	0.87	[0.84-0.90]	Kote-Jarai Z	21743467
Prostate cancer	rs2242652	1 x 10-15	A - minor allele	protective	0.86	NR	Emami NC	33293427
Prostate cancer	rs2242652	5 x 10-12	A - minor allele	protective	0.85	NR	Rashkin SR	32887889
Prostate cancer	rs2242652	8 x 10-6	A - minor allele	protective	0.87	NR	Takata R	31562322
Uterine leiomyoma	rs2242652	2 x 10-14	A - minor allele	protective	0.90	[0.88-0.92]	Sakai K	31988393
Multiple myeloma	rs2242652	1 x 10-3	A - minor allele	protective	0.81	[0.72-0.92]	Campa D	25066524
Breast cancer (ER negative)	rs2242652	2 x 10-14	A - minor allele	risk increasing	1.18	[1.13-1.23]	Couch FJ	27117709
Gastric cancer	rs2242652	4 x 10-4	A - minor allele	risk increasing	1.46	[1.28-2.92]	Lili M	32502020
Skin cancer	rs2242652	4 x 10-3	A - minor allele	risk increasing	1.50	[1.14-1.98]	Nan H	21116649
Esophageal cancer	rs2242652	1 x 10-3	A - minor allele	risk increasing	1.48	[1.17-1.89]	Wu Y	28060765
Lung cancer	rs2242652	0.04	A - minor allele	risk increasing	1.47	[1.02-2.13]	Gao L	25254308
Breast cancer (ER negative)	rs10069690	2 x 10-35	T - minor allele	risk increasing	1.18	[1.15-1.21]	Milne RL	29058716
Breast cancer	rs10069690	2 x 10-20	T - minor allele	risk increasing	1.06	[1.05-1.08]	Shu X	32139696
Breast cancer	rs10069690	8 x 10-17	T - minor allele	risk increasing	1.06	[1.04-1.08]	Michailidou K	29059683
Breast cancer	rs10069690	5 x 10-12	T - minor allele	risk increasing	1.15	[1.11-1.20]	Garcia-Closas	23535733
Breast cancer	rs10069690	1 x 10-10	T - minor allele	risk increasing	1.18	[1.13-1.25]	Haiman CA	22037553
Breast cancer	rs10069690	7 x 10-9	T - minor allele	risk increasing	1.06	[1.04-1.09]	Michailidou K	23535729
Breast cancer (ER negative)	rs10069690	1 x 10-7	T - minor allele	risk increasing	1.24	[1.14-1.34]	Purrington KS	24325915
Glioblastoma	rs10069690	8 x 10-74	T - minor allele	risk increasing	1.61	[1.53-1.69]	Melin BS	28346443
Glioblastoma	rs10069690	3 x 10-35	T - minor allele	risk increasing	1.64	[1.52-1.78]	Ostrom QT	29743610
Glioma	rs10069690	3 x 10-66	T - minor allele	risk increasing	1.45	[1.39-1.51]	Melin BS	28346443
Glioma	rs10069690	8 x 10-31	T - minor allele	risk increasing	1.49	[1.39-1.60]	Ostrom QT	29743610
Non-glioblastoma glioma	rs10069690	1 x 10-16	T - minor allele	risk increasing	1.27	[1.20-1.34]	Melin BS	28346443
Non-glioblastoma glioma	rs10069690	8 x 10-7	T - minor allele	risk increasing	1.25	[1.15-1.37]	Kinnersley B	26424050
Serous ovarian cancer	rs10069690	1 x 10-9	T - minor allele	risk increasing	1.22	[1.14-1.29]	Phelan CM	28346442
Epithelial ovarian cancer	rs10069690	9 x 10-9	T - minor allele	risk increasing	1.14	[1.10-1.19]	Kuchenbaecker	25581431
Epithelial ovarian cancer	rs10069690	3 x 10-8	T - minor allele	risk increasing	1.09	[1.06-1.12]	Phelan CM	28346442
Thyroid cancer	rs10069690	3 x 10-7	T - minor allele	risk increasing	1.20	[1.12-1.29]	Gudmundsson J	28195142

Supplementary Table 15: Association between the number of risk alleles in *PNPLA3*, *TM6SF2* and *TERT* and alcohol-related HCC

unweighted genetic risk score	Number of risk alleles (reference: 0-2 risk alleles) *PNPLA3*:rs738409:G; *TM6SF2*:rs58542926:T; *TERT*:rs2242652:G			
Discovery (n=1910)	**3-4 risk alleles**		**5-6 risk alleles**	
Adjustments	OR (CI95%)	P value	OR (CI95%)	P value
unadjusted	2.12 (1.76-2.56)	4.88×10^{-15}	5.24 (2.82-9.77)	1.76×10^{-07}
pc, sex, age	2.27 (1.83-2.82)	1.36×10^{-15}	4.97 (2.50-9.91)	5.01×10^{-06}
Validation UK (n=860)	**3-4 risk alleles**		**5-6 risk alleles**	
unadjusted	3.25 (1.84-5.73)	4.60×10^{-05}	17.78 (6.38-49.57)	3.78×10^{-08}
pc, sex, age	3.35 (1.84-6.08)	7.60×10^{-05}	16.13 (5.07-51.31)	2.00×10^{-06}
Validation Germany (n=238)	**3-4 risk alleles**		**5-6 risk alleles**	
unadjusted	2.28 (1.08-4.83)	0.031	7.67 (1.69-34.73)	8.23×10^{-03}
pc, sex, age	2.30 (1.03-5.15)	0.031	8.58 (1.62-45.49)	0.012

Supplementary Table 16: Analysis of factors associated with HCC in patients with alcoholic cirrhosis with liver fat content (FFD%) and leukocyte telomere length in the UK Biobank

		Liver fat content			Leukocyte telomere length			Association with HCC		
Variable	SNP	N	Adjusted Beta (se) [a,b]	Significance (*P*) [b]	N	Adjusted Beta [a,b]	Significance (*P*) [b]	GWAS *P*-value	Per-allele OR (95%CI)	MAF Cases (HCC) / Controls (CIRR)
PNPLA3	rs738409:G	8,315	0.2204	3.39×10^{-61}	471,172	-0.00013	0.458	7.23×10^{-15}	1.71 (1.49-1.96)	0.48 / 0.35
TM6SF2	rs58542926:T	8,315	0.2904	5.94×10^{-45}	62,296	-0.00053	0.475	2.81×10^{-9}	1.94 (1.56-2.42)	0.15 / 0.08
TERT	rs2242652:A	8,319	0.0209	0.1442	458,714	0.00256	2.12×10^{-44}	7.87×10^{-7}	0.64 (0.53-0.76)	0.13 / 0.19
TERT	rs10069690:T			N/A	471,172	0.00313	4.08×10^{-84}	5.73×10^{-6}	0.69 (0.58-0.81)	0.183 / 0.242
TERT	rs72709458:T			N/A	454,347	0.00344	1.10×10^{-81}	3.92×10^{-5}	0.70 (0.59-0.83)	0.156 / 0.208
TERT	rs7726159:A			N/A	471,172	0.00476	1.16×10^{-219}	5.20×10^{-3}	0.81 (0.70-0.94)	0.292 / 0.329

SNP: single nucleotide polymorphism, N/A: not analyzed

[a] adjusted for age, gender, principal component 1 to 10.

[b] derived from UK Biobank analysis of >450.000 individuals of European decent

Supplementary Table 17: Estimates of the proportion of phenotypic variance explained by additive genome-wide significant SNPs for HCC (GWAS discovery cohort)

Discovery GWAS cohort n = 1910		*total variance explained by all SNPs*				*total variance explained by replicated risk variants / remaining variants*						
		all GWAS-SNPs [a] n = 7,585,576				*GWAS* SNPs [b] n = 7,583,550		***PNPLA3 / TM6SF2 / TERT* SNPs** [b] n = 2026 (LD region variants)				
Genetic variance component		Sum of V(G1+G2) / Vp				V(G1) / Vp		V(G2) / Vp				
		observed scale	disease prevalence 1% *	disease prevalence 2.5% *	LRT	observed scale	LRT	observed scale	disease prevalence 1% *	disease prevalence 2.5% *	LRT	*Proportion of variance explained by replicated risk variants*
Method	environmental variance component	h^2 (se) [a]	h^2 (se) [a]	h^2 (se) [a]	*P* [a]	h^2 (se) [b]	*P* [b]	h^2 (se) [b]	h^2 (se) [b]	h^2 (se) [b]	*P* [b]	% of total h^2 [c]
GCTA-GREML	15 PCs [e]	0.296 (0.181)	0.204 (0.107)	0.257 (0.135)	5.55×10^{-17}	0.221 (0.181)	0.108	0.075 (0.022)	0.042 (0.013)	0.053 (0.016)	1.32×10^{-17}	25.5%
GCTA-GREML	sex, age, 15 PCs [g]	0.243 (0.185)	0.176 (0.107)	0.222 (0.136)	3.16×10^{-13}	0.188 (0.185)	0.152	0.054 (0.018)	0.030 (0.010)	0.038 (0.013)	3.16×10^{-14}	22.2%

[a] GCTA-GREML estimate of the phenotypic variance (h^2) explained by all genotyped and imputed genome-wide SNPs in the discovery cohort (termed the SNP heritability), including the environmental variance component V(e) and the residual variance Vp.

[b] GCTA-GREML estimate of the phenotypic variance (h^2) explained by HCC associated genome-wide significant SNPs (V(G2) locating to the *PNPLA3* / *TM6SF2* / *TERT* associated linkage disequilibrium region (LD region) and remaining GWAS variants (V(G1) outside these LD regions.

[c] Percentage of SNP heritability due to *PNPLA3* / *TM6SF2* / *TERT* variants calculated by $\frac{h2(\mathrm{vc1})}{h2(\mathrm{vc0})+h2(\mathrm{vc1})}$.

[d] GCTA-GREML variance components G2 estimate (h^2) after adjustment for lead variants rs738409 in *PNPLA3* / rs58542926 in *TM6SF2* / rs2242652 in *TERT*.

[e] Likelihood model adjustments for top 15 principal components of genetic ancestry (environmental variance component).

[g] Likelihood model adjusted for sex, age and top 15 principal components of genetic ancestry (environmental variance component).

* Transformed h^2 estimates from heritability on the observed scale (GWAS cohort) to heritability on the liability scale (population) assuming HCC prevalence estimates of 1%-2.5% in patients with alcohol-related liver disease.

Abbreviations: V(G1) = genetic variance component 1; V(G2) = genetic variance component 2; (Vp) residual variance; h^2, SNP heritability; PC, principal components; se, standard error; LRT, likelihood ratio test; P, likelihood ratio test significance of the predicted change in log likelihood.