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Clinical and genetic definition of serum bilirubin levels for the diagnosis of Gilbert syndrome and hypobilirubinemia

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Abstract

Background and Aims: Gilbert syndrome (GS) is genotypically predetermined by *UGT1A1**28 homozygosity in Europeans and is phenotypically defined by hyperbilirubinemia using total bilirubin (TB) cutoff ≥1mg/dL (17 µmol/L). The prevalence of illnesses associated with GS and hypobilirubinemia has never been studied prospectively. As TB varies with *UGT1A1**28 genotyping, sex, and age, we propose stratified definitions of TB reference intervals and report the prevalence of illnesses and adjusted 15 years survival.

Methods: UK Biobank with apparently healthy liver participants (middle-aged, n = 138,125) were analyzed after the exclusion of of nonhealthy individuals. The stratified TB was classified as GS when TB >90th centile; <10th centile indicated hypobilirubinemia, and between the 10th and 90th centile was normobilirubinemia. We compared the prevalence and survival rates of 54 illnesses using odds ratio (OR), logistic regression, and Cox models adjusted for confounders, and causality by Mendelian randomizations.

Results: In women, we identified 10% (7,741/76,809) of GS versus 3.7% (2,819/76,809) using the historical cutoff of ≥1 mg/dL ($P < 0.0001$). When GS and hypobilirubinemia participants were compared with normobilirubinemia, after adjustment and Mendelian randomizations, only cholelithiasis prevalence was significantly higher (OR = 1.50; 95% CI [1.3–1.7], $P = 0.001$) in men with GS compared with normobilirubinemia and in causal association with bilirubin ($P = 0.04$). No adjusted survival was significantly associated with GS or

Abbreviations: ALT, alanine aminotransferase; AF, Atrial fibrillation; AST, aspartate aminotransferase; apoA1, apolipoprotein A1; BMI, body mass index; CC, absence of the allele; CHS, Coronary Heart Disease; COPD, Chronic obstructive pulmonary disease; CRP, C-reactive protein; CT genotype, heterozygoty; CVD, Cardio vascular disease; FIB4, serum liver fibrosis index; GS, Gilbert syndrome; GGT, gamma-glutamyl transpeptidase; GWAS, genome-wide association studies; IQR, interquartile range; MR, Mendelian randomization; OS, overall survival; PLT, platelets; TB, total bilirubin; TIA, Transient Ischemic Attack; TSMR, two-sample Mendelian randomizations; TT genotype, allele homozygosity; UGT1A1, uridine-diphosphoglucuronate-glucuronosyltransferase-family-1-member-A1; UKB, UK Biobank.

Thierry Poynard and Olivier Deckmyn authors shared the lead authorship.

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hypobilirubinemia.

Conclusions: In middle-aged Europeans, the stratified TB demonstrates a careless GS underestimation in women when using the standard unisex 1 mg/dL cutoff. The prevalence of illnesses is different in GS and hypobilirubinemia as well as survivals before adjusting for confounding factors. With the exception of cholelithiasis in men, these differences were no more significant after adjustment and Mendelian randomization.

INTRODUCTION

In 1901, the first description of common benign hyperbilirubinemia, "cholémie simple familiale," was published by Gilbert and Lereboullet, which included Napoleon Bonaparte and his mother as early proof of genetic origin.[1] Gilbert syndrome (GS) affects ~3%–7% of individuals worldwide and 5%–10% in Europe, and it is associated with reduced morbidity, whereas hypobilirubinemia has the opposite association.[2–6] Diagnosis of GS is often made during routine health examinations when mildly elevated levels of serum total bilirubin (TB) are detected. In the absence of specific symptoms, TB is the only measurable phenotypic trait of GS. There is a consensual definition that GS in individuals is determined by the presence of hyperbilirubinemia in the absence of both hemolysis and liver damage, including the fibrosis stage.[7,8]

Few studies have investigated the prevalence of illnesses in large cohorts of subjects with GS, and none have investigated its association with hypobilirubinemia. The largest study without genetic variants involved 23,925 participants and did not observe more symptoms in possible GS versus controls.[9] Recently, the associations between elevated TB and 19 illnesses with a putative protective signal of TB were analyzed in 61,281 inpatients with genetic variants, without causality proven by Mendelian randomization (MR).[10]

The current responses available to individuals with GS from health authorities or charities are insufficient (Supplemental Table S1, http://links.lww.com/HC9/A457). Prevalence of symptoms has varied from "most patients with GS have no symptoms" to "1 in 3 people don't experience any symptoms at all". Historically, bilirubin $\geq$ 1 mg/dl (17.1 μmol/L) has been the standard cutoff for GS.[2–4]

Sex and age are strongly correlated with bilirubin levels in healthy subjects, suggesting that appropriate personalized bilirubin levels should be defined.[11,12] We chose to personalize bilirubin levels (subsequently referred to as "bilirubin centiles") according to the adjusted distribution: 10th centile defining hypobilirubinemia, 90th centile defining hyperbilirubinemia, and in between representing normobilirubinemia.[13] The exclusion of liver damage requires normal alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT),[14,15] which are not sufficiently sensitive to exclude significant liver fibrosis, in comparison with available noninvasive tests validated in general populations.[7,8] Therefore, clinicians cannot accurately diagnose GS or non-benign hyperbilirubinemia using unadjusted liver tests.[2–4]

The genetic cause of GS is a decrease in uridine-diphosphoglucuronate glucuronosyltransferase family 1 member A1 (*UGT1A1*),[16–20] the only isoform that significantly contributes to the bilirubin conjugationof.[18] In Europeans, the major genetic variant responsible for GS is a TA insertion in the *UGT1A1* gene promoter region, altering the TATA repeat from its usual length of 6 TA repeats. Homozygosity for this short insertion, (TA)7TAA, designated the *UGT1A1**28*rs887829* (ClinVariation ID:12275), defines genotypic GS, the *"UGT1A1*28 rs887829 allele homozygosity* here named TT genotype; other genotypes that are not *UGT1A1*28rs887829 allele* homozygotes were named the CT for the heterozygotes and CC for those without *UGT1A1*28rs887829*. In Europeans, the TT genotype has variable expressivity and incomplete penetrance in 30%–50% of carriers.[16–20] Therefore, when GS is suspected, the UGT1A genotyping alone is insufficient to indicate GS, which is partly dependent on bilirubin production, geographical origin, the involvement of environmental factors, such as adiposity, and other variants that regulate glucuronidation.[19–21] *UGT1A1* variants may influence drug-induced toxicities, including numerous medications used in oncology. Genotyping is mandatory for patients with possible GS when drugs that interact with bilirubin metabolism are prescribed as well as the metabolizer state.[22]

For the diagnosis of hypobilirubinemia, there is no associated genotype or symptoms.[2–6] There is no consensus on the appropriate lower bilirubin level; the most frequently used cutoff is <10.0 μmol/L.[3–6,23–25]

Our first aim was to propose new personalized definitions of GS and hyperbilirubinemia. Second, we

aimed to assess the prevalence of illnesses possibly associated with GS or hypobilirubinemia. Third, we aimed to assess whether participants with GS or hypobilirubinemia have different overall survival (OS) rates (referred to as "survival") than those with normobilirubinemia when adjusted for confounders.

METHODS

Study design

This was a retrospective analysis of the prospective UK Biobank cohort (UKB-ID 670334), for which measurements and data collection characteristics of the participants are described in detail elsewhere.[6,26–28] The UKB involved ~500,000 participants in their middle age (40–70 years, 54% women) recruited between 2006 and 2010. The UKB study was approved by the North-West Multicenter Research Ethics Committee (reference[11]/NW/0382). Of the 502,386 cases (Fig. 1A), 240,426 subjects were excluded due to missing data or non-European ancestry. The remaining 261,960 participants represent the general population of the United Kingdom with European ancestry, referred to in this study as the "general population." A further 32,802 nonhealthy participants were excluded due to HIV infection, alcohol disorder, nonmetabolic liver disease, significant liver fibrosis, disease with poor prognosis, or existing cancer. Finally, 2 main subsets were separately analyzed. Among the "apparently healthy liver" subset, characteristics were compared according to bilirubin centiles (Table 1). This subset of 138,125 participants had nonelevated ALT and GGT, nonsignificant fibrosis using FIB4,[7] no confounders of metabolic syndrome, and C-reactive protein (CRP) <10 IU/L, enabling to respond to the study aims. The remaining 91,033 participants, named the "at risk of NAFLD" subset (Table 2 and Supplemental Table S2, http://links.lww.com/HC9/A458), were considered in sensitivity analyses to assess the prognostic value of bilirubin centiles associated with this emerging disease, and they represented 34% of the "general population" subset (Supplemental Table S3, http://links.lww.com/HC9/A459).

Outcomes

We first determined whether previous studies on GS or hypobilirubinemia had used the grouping of 43 morbidities proposed by Barnett et al in addition to the 11 conditions detailed in gallstone and treated dyspepsia main groups, that is, 54 conditions according to the International Classification of Diseases (named illnesses here) prospectively assessed in the UKB (last connection on PubMed March 31, 2023, Supplemental Table S4, http://links.lww.com/HC9/A460).[29–31] The secondary end points were the prevalence of the frailty phenotype and multimorbidity count. Participants were deemed frail if they met at least 3 of the 5 frailty criteria.[29] For, The survival end point was overall 15 year survival adjusted for confounders of GS and hypobilirubinemia compared with the population with normobilirubinemia.

Procedures

Full methodological details, including *UGT1A1 genotyping*, are available elsewhere[26–28] and on the UK Biobank website (https://www.ukbiobank.ac.uk/). At baseline, all participants provided informed consent for the study and completed a self-administered questionnaire and a computer-assisted interview. Baseline data included the frailty phenotype and morbidity count (Supplemental Table S4, http://links.lww.com/HC9/A460).[6,26–28] Bilirubin was measured by colorimetric assay with a unisex reference interval of 5.1–17.1 μmol/L. ALT, aspartate aminotransferase (AST), and GGT were analyzed by enzymatic rate. FIB4 score was computed using the cutoff of 2.67 for significant fibrosis.[7] Clinical investigations were conducted according to the principles of the Declaration of Helsinki. All authors had access to the study data and reviewed and approved the final manuscript.

Statistical analyses

All analyses were planned before the inspection of the data in accordance with STROBE guidelines.

Personalized definitions

To elucidate the relationships between bilirubin, *UGT1A1* genotype, sex, and age, we plotted bilirubin levels against age separately for *UGT1A1* genotype (TT, TC, and CC) and sex (Fig. 1B). According to Figure 1B, the corresponding median values and centile distributions demonstrate that the usual unisex cutoff of 17.1 μmol/L induced an imbalance between sensitivity and specificity for bilirubin for the diagnosis of hyperbilirubinemia, regardless of the *UGT1A1* genotype. When applied to the general population, such an imbalance presents important limitations related to the classical dilemma of balance between sensitivity and positive predictive value.[32] Supplemental Figure S1, http://links.lww.com/HC9/A461, shows the expected variability according to 95, 90, and 80% limits of the distribution of total bilirubin by age, stratified by genotype and sex in the "General population" subset.

We applied the method developed by Ritchie et al to address the issue of reference ranges of serum measurements, which varied according to age and

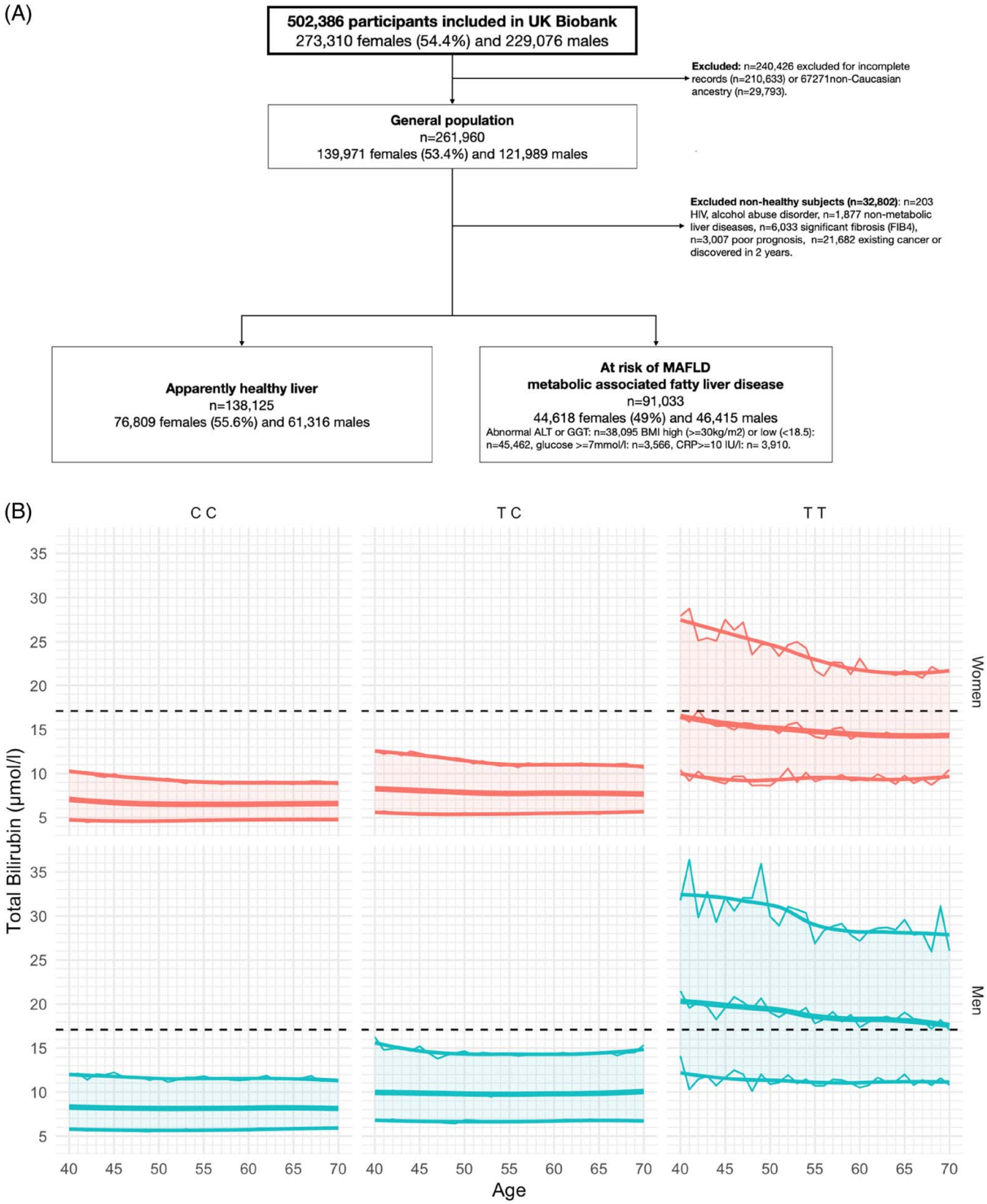


FIGURE 1 1B 10th and 90th centiles of total bilirubin levels by age and sex. Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CC, absence of the allele; CRP, C-reactive protein; CT genotype, heterozygoty; GGT, gamma-glutamyl transpeptidase TT genotype, allele homozygosity.

sex.[33] When values were expressed as multiples of age-specific and sex-specific median levels, the resulting distributions fitted a log Gaussian distribution, which can be used to assign an individual's measurement to the corresponding centile. Ideally, such a population ("apparently healthy liver") should include healthy individuals without a high risk of false positives or negatives; however, care should be taken to avoid the use of excessively stringent definitions of "normal" or "healthy" that lead to the paradox of normality becoming a rarity.[34] A simplified table of bilirubin centiles is presented in Table 3 and detailed in Supplemental Table S5, http://links.lww.com/HC9/A462.

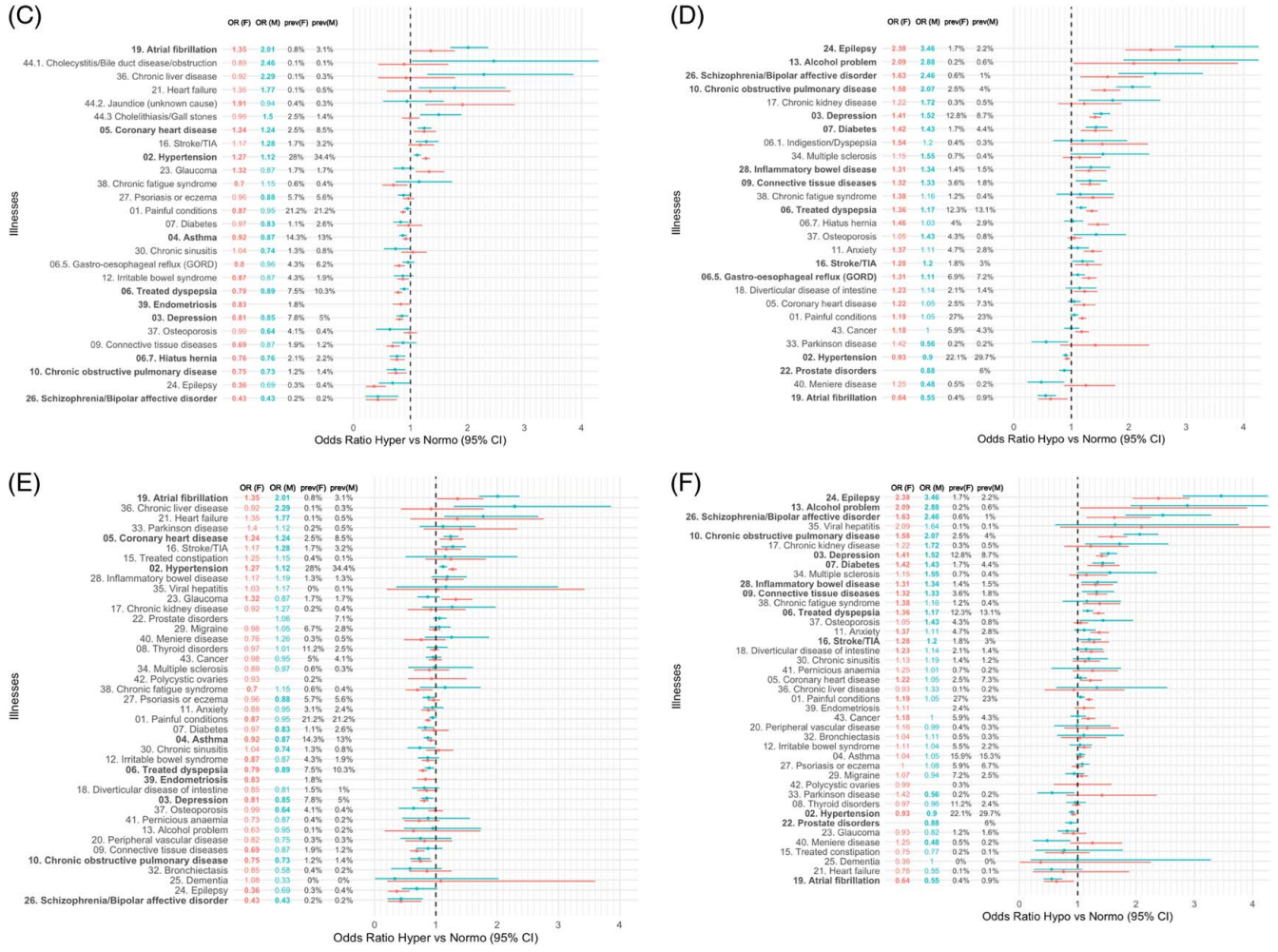


FIGURE 1 Continued.

Prevalence of illnesses and frailty phenotype

Univariate and multivariate logistic regressions were computed for each illness against all confounders and expressed as OR separately for women and men and for GS and hypobilirubinemia.

We first assessed univariate correlations between bilirubin centiles, *UGT1A1* genotyping, and age, with 17 confounders associated with bilirubin in the literature.[2–4] The confounders were analyzed in 4 groups. In addition to age, 1 group referred to environmental factors: tobacco consumption, alcohol consumption, physical activity (walking at a brisk pace), and Townsend Deprivation Index. A second group referred to metabolism: body mass index, total cholesterol (referred to as "cholesterol"), triglycerides, LDL, and apolipoprotein-A1 (apoA1). A third group referred to inflammation: CRP, albumin, and platelets. A fourth group comprised biomarkers of liver damage: ALT, AST, and GGT. The final group referred to biomarkers of hemolysis, which is a natural source of bilirubin: hemoglobin and reticulocytes (Supplemental Figure S2, http://links.lww.com/HC9/A463). After 4 regression analyses in each group (round 1), the remaining significant confounders were analyzed in a final regression analysis (round 2).

The characteristics were compared according to sex and bilirubin centiles using a Q-test and Bonferroni correction, considering the number of comparisons. Significant differences between illnesses were defined when the p-value was < 0.005 and $p < 0.05$ for OR comparison. We summarized the literature analyzing causal factors by MR (Table 4) detailed references (Supplemental Table S6, http://links.lww.com/HC9/A464).

Causality

To assess the effect of bilirubin on illnesses and confounders, we performed two-sample Mendelian randomizations (TSMR) using published databases (Table 5). TSMR is a method for strengthening causal inference in observational studies using genetic variants (*UGT1A1* genotyping) and genome-wide association studies associated with exposure (bilirubin) as instrumental variables. TSMR, using bilirubin (after inverse rank normalized transformation) as the exposure, was

TABLE 1 Main characteristics and comparisons of participants in the "apparently healthy liver" subset, (N = 138,125) according to bilirubin centiles, adjusted by *rs887829* and age

	Females (n = 76,809)					Males (n = 61,316)				
Characteristic	**hyper, N = 7741**	**hypo, N = 7653**	**normal, N = 61,415**	***p*[a]**	***q*[b]**	**hyper, N = 6179**	**hypo, N = 6149**	**normal, N = 48,988**	***p*-value[a]**	**q-value[b]**
Age (y), median (IQR)	58 (50–63)	58 (50–63)	58 (50–63)	> 0.99	> 0.99	59 (51–64)	59 (51–64)	59 (51–64)	0.98	> 0.99
Total bilirubin (μmol/l), median (IQR)	11.7 (10.3–13.5)	4.6 (4.2–5.1)	7.4 (6.2–8.8)	**< 0.001**	**< 0.001**	15.1 (13.0–17.6)	5.6 (5.1–6.3)	9.2 (7.8–11.1)	**< 0.001**	**< 0.001**
Total bilirubin (μmol/l), n (%)	—	—	—	**< 0.001**	**< 0.001**	—	—	—	**< 0.001**	**< 0.001**
< 17.1	6737 (87)	7653 (100)	59,600 (97)	—	—	4474 (72)	6149 (100)	45,995 (94)	—	
> = 17.1	1004 (13)	0 (0)	1815 (3.0)	—	—	1705 (28)	0 (0)	2993 (6.1)	—	
rs887829, n (%)	—	—	—	0.98	> 0.99				0.99	> 0.99
CC	3620 (47)	3573 (47)	28,763 (47)	—	—	2894 (47)	2880 (47)	22,974 (47)	—	
TC	3330 (43)	3296 (43)	26,473 (43)	—	—	2661 (43)	2647 (43)	21,144 (43)	—	
TT	791 (10)	784 (10)	6179 (10)	—	—	624 (10)	622 (10)	4870 (9.9)	—	
Smoker status, n (%)	—	—	—	**< 0.001**	**< 0.001**	—	—	—	**< 0.001**	**< 0.001**
Current	317 (4.1)	1297 (17)	5011 (8.2)	—	—	292 (4.7)	1665 (27)	5173 (11)	—	
Never	4822 (62)	4081 (54)	36,654 (60)	—	—	3546 (58)	2298 (37)	24,976 (51)	—	
Previous	2577 (33)	2242 (29)	19,554 (32)	—	—	2319 (38)	2170 (35)	18,683 (38)	—	
Missing	25	33	196	—	—	22	16	156	—	
Alcohol intake, n (%)	—	—	—	**< 0.001**	**< 0.001**	—	—	—	**< 0.001**	**< 0.001**
Daily or almost daily	1837 (24)	1013 (13)	10,994 (18)	—	—	1685 (27)	1465 (24)	13,041 (27)	—	
Three or 4 times a week	1978 (26)	1361 (18)	14,249 (23)	—	—	1870 (30)	1462 (24)	13,752 (28)	—	
Once or twice a week	1980 (26)	2057 (27)	16,337 (27)	—	—	1466 (24)	1638 (27)	12,580 (26)	—	
One to 3 times a month	793 (10)	1113 (15)	7686 (13)	—	—	506 (8.2)	597 (9.7)	4151 (8.5)	—	
Special occasions only	760 (9.8)	1240 (16)	7695 (13)	—	—	358 (5.8)	543 (8.8)	3048 (6.2)	—	
Never	391 (5.1)	860 (11)	4429 (7.2)	—	—	289 (4.7)	439 (7.1)	2380 (4.9)	—	
Missing	2	9	25	—	—	5	5	36	—	
Physical activity in last 4 weeks, n (%)	—	—	—	**< 0.001**	**< 0.001**	—	—	—	**< 0.001**	**< 0.001**
Heavy DIY (eg, weeding, lawn mowing, carpentry, digging)	99 (1.3)	142 (2.0)	820 (1.4)	—	—	161 (2.7)	303 (5.3)	1,734 (3.7)	—	
Light DIY (eg, pruning, watering the lawn)	347 (4.7)	565 (8.0)	3398 (5.8)	—	—	284 (4.7)	509 (8.9)	2963 (6.3)	—	
Other exercises (eg, swimming, cycling, keep fit, bowling)	877 (12)	777 (11)	6632 (11)	—	—	854 (14)	753 (13)	6282 (13)	—	
Strenuous sports	28 (0.4)	14 (0.2)	221 (0.4)	—	—	100 (1.7)	71 (1.2)	619 (1.3)	—	
Walking for pleasure (not as a means of transport)	6104 (82)	5554 (79)	47,385 (81)	—	—	4618 (77)	4079 (71)	35,651 (75)	—	
(Missing)	286	601	2959	—	—	162	434	1739	—	
ALT (UI/l), median (IQR)	16.5 (13.5–20.5)	16.0 (13.0–19.8)	16.2 (13.3–20.1)	**< 0.001**	**< 0.001**	22 (18–27)	21 (17–26)	21 (17–27)	**< 0.001**	**< 0.001**
AST (UI/l), median (IQR)	23.0 (20.2–26.4)	21.7 (19.0–24.9)	22.4 (19.7–25.5)	**< 0.001**	**< 0.001**	25.9 (22.7–29.8)	24.0 (21.0–27.6)	25.1 (22.1–28.7)	**< 0.001**	**< 0.001**
GGT (UI/l), median (IQR)	20 (15–26)	19 (15–26)	19 (15–26)	**< 0.001**	**< 0.001**	28 (21–38)	28 (22–39)	28 (22–38)	0.10	> 0.99

Platelet count (10^9 cells/l), median (IQR)	246 (214–280)	277 (242–320)	259 (226–296)	< **0.001**	< **0.001**	221 (193–252)	254 (220–292)	234 (204–269)	< **0.001**	< **0.001**
Albumin (g/l), median (IQR)	46.08 (44.50–47.77)	44.12 (42.54–45.70)	45.19 (43.57–46.82)	< **0.001**	< **0.001**	46.36 (44.72–48.06)	44.66 (43.04 – 46.38)	45.59 (43.96–47.25)	< **0.001**	< **0.001**
Glucose (mmol/l), median (IQR)	4.88 (4.59–5.19)	4.90 (4.59–5.25)	4.87 (4.57–5.20)	< **0.001**	< **0.001**	4.91 (4.59–5.25)	4.91 (4.57–5.28)	4.90 (4.57–5.25)	0.064	> 0.99
Total cholesterol (mmol/l), median (IQR)	5.70 (5.02–6.46)	5.79 (5.09–6.54)	5.85 (5.14–6.60)	< **0.001**	< **0.001**	5.28 (4.58–6.01)	5.45 (4.74–6.19)	5.49 (4.78–6.21)	< **0.001**	< **0.001**
Triglycerides (mmol/l), median (IQR)	1.04 (0.80–1.41)	1.55 (1.12–2.17)	1.24 (0.92–1.70)	< **0.001**	< **0.001**	1.32 (0.96–1.82)	1.83 (1.27–2.66)	1.55 (1.11–2.19)	< **0.001**	< **0.001**
HDL cholesterol (mmol/l), median (IQR)	1.69 (1.45–1.95)	1.51 (1.31–1.75)	1.62 (1.39–1.87)	< **0.001**	< **0.001**	1.33 (1.13–1.56)	1.22 (1.05 –1.42)	1.29 (1.11–1.49)	< **0.001**	< **0.001**
LDL direct (mmol/l), median (IQR)	3.45 (2.91–4.05)	3.55 (3.01–4.14)	3.58 (3.03–4.17)	< **0.001**	< **0.001**	3.33 (2.78–3.92)	3.45 (2.88 – 4.01)	3.48 (2.92–4.05)	< **0.001**	< **0.001**
Apolipoprotein-A1 (g/l), median (IQR)	1.69 (1.52–1.88)	1.59 (1.44–1.76)	1.64 (1.49–1.82)	< **0.001**	< **0.001**	1.46 (1.32–1.62)	1.39 (1.25–1.53)	1.43 (1.30–1.58)	< **0.001**	< **0.001**
Reticulocyte count (10^12 cells/l), median (IQR)	0.052 (0.040–0.068)	0.049 (0.037–0.062)	0.049 (0.038–0.063)	< **0.001**	< **0.001**	0.060 (0.046–0.078)	0.053 (0.041–0.067)	0.056 (0.043–0.071)	< **0.001**	< **0.001**
Creatinine (μmol/l), median (IQR)	64 (58–70)	62 (56–69)	63 (57–70)	< **0.001**	< **0.001**	81 (74–89)	78 (71 – 87)	80 (73–88)	< **0.001**	< **0.001**
C-reactive protein (mg/l), median (IQR)	0.76 (0.41–1.39)	1.56 (0.80 – 2.95)	1.03 (0.54–1.97)	< **0.001**	< **0.001**	0.79 (0.43–1.43)	1.42 (0.77–2.72)	1.00 (0.55–1.86)	< **0.001**	< **0.001**
Frailty phenotype, n (%)	—	—	—	< **0.001**	< **0.001**	—	—	—	< **0.001**	< **0.001**
Frail	70 (0.9)	207 (2.7)	905 (1.5)	—	—	50 (0.8)	160 (2.6)	573 (1.2)	—	
Not frail	5129 (66)	4436 (58)	39,332 (64)	—	—	4192 (68)	3709 (60)	32,436 (66)	—	
Prefrail	2,42 (33)	3,10 (39)	21,178 (34)	—	—	1,937 (31)	2,80 (37)	15,979 (33)	—	
Multimorbidities count, n (%)	—	—	—	< **0.001**	< **0.001**	—	—	—	< **0.001**	< **0.001**
0	1452 (19)	1158 (15)	10,814 (18)	—	—	1163 (19)	1024 (17)	8930 (18)	—	
1	3417 (44)	3053 (40)	26,571 (43)	—	—	2715 (44)	2588 (42)	21,747 (44)	—	
2	1788 (23)	1906 (25)	14,475 (24)	—	—	1419 (23)	1473 (24)	11,206 (23)	—	
3	680 (8.8)	903 (12)	5988 (9.8)	—	—	588 (9.5)	667 (11)	4700 (9.6)	—	
4+	404 (5.2)	633 (8.3)	3567 (5.8)	—	—	294 (4.8)	397 (6.5)	2405 (4.9)	—	
Overall death, n (%)	268 (3.5)	396 (5.2)	2239 (3.6)	< **0.001**	< **0.001**	384 (6.2)	569 (9.3)	3311 (6.8)	< **0.001**	< **0.001**

[a]Kruskal-Wallis rank sum test; Pearson chi-squared test; Fisher exact test.
[b]Bonferroni correction for multiple testing.
Abbreviations: ALT, alanine aminotransferase; CC, absence of the allele; CRP, C-reactive protein; CT genotype, heterozygoty; GGT, gamma-glutamyl transpeptidase; IQR, interquartile range; TT genotype, allele homozygosity.

TABLE 2 Main characteristics and comparisons of participants for subsets "apparently healthy liver" and "at risk of MAFLD"

Characteristic	Female				Male			
	Apparently healthy liver, N = 76,809	At risk of MAFLD, N = 44,618	P-value[a]	q-value[b]	Apparently healthy liver, N = 61,316	At risk of MAFLD, N = 46,415	P-value[a]	q-value[b]
Age (y), median (IQR)	58 (50–63)	59 (52–64)	**< 0.001**	**< 0.001**	59 (51–64)	59 (51–64)	**< 0.001**	**< 0.001**
Total bilirubin (μmol/l), median (IQR)	7.4 (6.0–9.3)	6.9 (5.6–8.7)	**< 0.001**	**< 0.001**	9.2 (7.5–11.7)	8.9 (7.1–11.3)	**< 0.001**	**< 0.001**
Total bilirubin (μmol/l), n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
< 17.1	73,990 (96)	43,646 (98)	—	—	56,618 (92)	43,455 (94)	—	—
> = 17.1	2819 (3.7)	972 (2.2)	—	—	4698 (7.7)	2960 (6.4)	—	—
Bilirubin centiles, n (%) adjusted by *rs887829* and age	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
hyper	7741 (10)	3287 (7.4)	—	—	6179 (10)	4016 (8.7)	—	—
hypo	7653 (10.0)	6990 (16)	—	—	6149 (10)	6273 (14)	—	—
normal	61,415 (80)	34,341 (77)	—	—	48,988 (80)	36,126 (78)	—	—
rs887829, n (%)	—	—	0.14	> 0.99	—	—	0.83	> 0.99
CC	35,956 (47)	20,783 (47)	—	—	28,748 (47)	21,684 (47)	—	—
TC	33,099 (43)	19,449 (44)	—	—	26,452 (43)	20,109 (43)	—	—
TT	7754 (10)	4386 (9.8)	—	—	6116 (10.0)	4622 (10.0)	—	—
BMI (kg/m2), n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
Normal	76,809 (100)	12,620 (28)	—	—	61,316 (100)	16,261 (35)	—	—
Obese	0 (0)	31,197 (70)	—	—	0 (0)	29,942 (65)	—	—
Underweight	0 (0)	801 (1.8)	—	—	0 (0)	212 (0.5)	—	—
Smoker status, n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
Current	6625 (8.7)	3909 (8.8)	—	—	7130 (12)	5728 (12)	—	—
Never	45,557 (60)	25,203 (57)	—	—	30,820 (50)	19,650 (43)	—	—
Previous	24,373 (32)	15,324 (34)	—	—	23,172 (38)	20,822 (45)	—	—
(Missing)	254	182	—	—	194	215	—	—
Alcohol intake, n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
Daily or almost daily	13,844 (18)	5720 (13)	—	—	16,191 (26)	12,110 (26)	—	—
Three or 4 times a week	17,588 (23)	7431 (17)	—	—	17,084 (28)	11,543 (25)	—	—
Once or twice a week	20,374 (27)	11,150 (25)	—	—	15,684 (26)	12,173 (26)	—	—
One to 3 times a month	9592 (12)	6682 (15)	—	—	5254 (8.6)	4349 (9.4)	—	—

Special occasions only	9695 (13)	8657 (19)	—	—	3949 (6.4)	3558 (7.7)	—	—
Never	5680 (7.4)	4937 (11)	—	—	3108 (5.1)	2637 (5.7)	—	—
(Missing)	36	41	—	—	46	45	—	—
Physical activity in last 4 weeks, n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
Heavy DIY (eg, weeding, lawn mowing, carpentry, digging)	1061 (1.5)	794 (2.0)	—	—	2,198 (3.7)	2,147 (5.1)	—	—
Light DIY (eg, pruning, watering the lawn)	4310 (5.9)	3964 (10)	—	—	3756 (6.4)	4346 (10)	—	—
Other exercises (eg, swimming, cycling, keep fit, bowling)	8286 (11)	5170 (13)	—	—	7889 (13)	5831 (14)	—	—
Strenuous sports	263 (0.4)	87 (0.2)	—	—	790 (1.3)	455 (1.1)	—	—
Walking for pleasure (not as a means of transport)	59,043 (81)	29,513 (75)	—	—	44,348 (75)	29,529 (70)	—	—
(Missing)	3846	5090	—	—	2335	4107	—	—
Systolic blood pressure (mm Hg), median (IQR)	134 (121–149)	140 (128–154)	**< 0.001**	**< 0.001**	140 (129–153)	144 (133–157)	**< 0.001**	**< 0.001**
ALT (UI/l), median (IQR)	16 (13–20)	22 (17–32)	**< 0.001**	**< 0.001**	21 (17–27)	30 (22–41)	**< 0.001**	**< 0.001**
AST (UI/l), median (IQR)	22.4 (19.7–25.6)	24.5 (20.8–29.9)	**< 0.001**	**< 0.001**	25 (22 – 29)	28 (24 – 34)	**< 0.001**	**< 0.001**
GGT (UI/l), median (IQR)	19 (15–26)	30 (21–53)	**< 0.001**	**< 0.001**	28 (22–38)	49 (32–78)	**< 0.001**	**< 0.001**
Platelet count (10^9 cells/l), median (IQR)	259 (226–297)	270 (234–311)	**< 0.001**	**< 0.001**	235 (204–270)	236 (204–273)	**< 0.001**	**< 0.001**
Fib4 stage, n (%)	—	—	—	—	—	—	—	—
(0,1.3]	44,608 (58)	29,431 (66)	—	—	28,826 (47)	24,661 (53)	—	—
(1.3,2.67]	32,201 (42)	15,187 (34)	—	—	32,490 (53)	21,754 (47)	—	—
(2.67,3.25]	0 (0)	0 (0)	—	—	0 (0)	0 (0)	—	—
(3.25,Inf]	0 (0)	0 (0)	—	—	0 (0)	0 (0)	—	—
Albumin (g/l), median (IQR)	45.17 (43.54–46.83)	44.48 (42.76–46.22)	**< 0.001**	**< 0.001**	45.58 (43.92–47.25)	45.45 (43.68–47.19)	**< 0.001**	**< 0.001**
Glucose (mmol/l), median (IQR)	4.88 (4.57–5.20)	5.03 (4.68–5.52)	**< 0.001**	**< 0.001**	4.90 (4.58–5.25)	5.08 (4.69–5.66)	**< 0.001**	**< 0.001**

TABLE 2. (*continued*)

Characteristic	Female				Male			
	Apparently healthy liver, N = 76,809	At risk of MAFLD, N = 44,618	P-value[a]	q-value[b]	Apparently healthy liver, N = 61,316	At risk of MAFLD, N = 46,415	P-value[a]	q-value[b]
Total cholesterol (mmol/l), median (IQR)	5.83 (5.12–6.58)	5.80 (5.02–6.61)	**< 0.001**	**< 0.001**	5.46 (4.75–6.19)	5.32 (4.49–6.17)	**< 0.001**	**< 0.001**
Triglycerides (mmol/l), median (IQR)	1.24 (0.92–1.72)	1.69 (1.22–2.33)	**< 0.001**	**< 0.001**	1.55 (1.11–2.20)	2.02 (1.42–2.87)	**< 0.001**	**< 0.001**
HDL cholesterol (mmol/l), median (IQR)	1.61 (1.39–1.86)	1.41 (1.21–1.65)	**< 0.001**	**< 0.001**	1.28 (1.11–1.49)	1.16 (1.00–1.35)	**< 0.001**	**< 0.001**
LDL Direct (mmol/l), median (IQR)	3.56 (3.01–4.16)	3.63 (3.01–4.26)	**< 0.001**	**< 0.001**	3.46 (2.90–4.04)	3.36 (2.73–4.01)	**< 0.001**	**< 0.001**
Apolipoprotein-A1 (g/l), median (IQR)	1.64 (1.48–1.82)	1.54 (1.39–1.71)	**< 0.001**	**< 0.001**	1.43 (1.30–1.58)	1.36 (1.23–1.52)	**< 0.001**	**< 0.001**
Reticulocyte count (10^12 cells/l), median (IQR)	0.049 (0.038–0.064)	0.063 (0.049–0.080)	**< 0.001**	**< 0.001**	0.056 (0.043–0.072)	0.070 (0.055 –0.089)	**< 0.001**	**< 0.001**
Creatinine (μmol/l), median (IQR)	63 (57–70)	64 (57–71)	**< 0.001**	**< 0.001**	80 (73–88)	80 (72–89)	0.84	> 0.99
C-reactive protein (mg/l), median (IQR)	1.03 (0.54 – 2.01)	2.89 (1.45–5.80)	**< 0.001**	**< 0.001**	1.01 (0.55–1.90)	1.95 (1.04–3.84)	**< 0.001**	**< 0.001**
Frailty phenotype, n (%)	—	—	**< 0.001**	**< 0.001**			**< 0.001**	**< 0.001**
Frail	1182 (1.5)	2843 (6.4)	—	—	783 (1.3)	1728 (3.7)	—	—
Not frail	48,897 (64)	19,349 (43)	—	—	40,337 (66)	24,587 (53)	—	—
Prefrail	26,730 (35)	22,426 (50)	—	—	20,196 (33)	20,100 (43)	—	—
Multimorbidities count, n (%)	—	—	**< 0.001**	**< 0.001**	—	—	**< 0.001**	**< 0.001**
0	13,424 (17)	4469 (10)	—	—	11,117 (18)	4972 (11)	—	—
1	33,041 (43)	15,560 (35)	—	—	27,050 (44)	17,151 (37)	—	—
2	18,169 (24)	11,851 (27)	—	—	14,098 (23)	12,846 (28)	—	—
3	7571 (9.9)	7035 (16)	—	—	5955 (9.7)	6727 (14)	—	—
4+	4604 (6.0)	5703 (13)	—	—	3096 (5.0)	4719 (10)	—	—
Overall death, n (%)	2903 (3.8)	2761 (6.2)	**< 0.001**	**< 0.001**	4264 (7.0)	5069 (11)	**< 0.001**	**< 0.001**

[a]Wilcoxon rank sum test; Pearson chi-squared test.
[b]Bonferroni correction for multiple testing.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CC, absence of the allele; CT genotype, heterozygoty; GGT, gamma-glutamyl transpeptidase; IQR, interquartile range; TT genotype, allele homozygosity.

TABLE 3 Simplified reference interval for hypobilirubinemia, normobilirubinemia and hyperbilirubinemia (μmol/l and mg/dl) adjusted by *rs887829*, sex and age

		Female				Male			
		Low 10th (Hypo)		High 90th (Hyper)		Low 10th (Hypo)		High 90th (Hyper)	
Genotype	Age range	μmol/l	mg/dl	μmol/l	mg/dl	μmol/l	mg/dl	μmol/l	mg/dl
CC	40–44	4.6	0.27	10.0	0.58	5.8	0.34	11.9	0.70
	45–49	4.6	0.27	9.7	0.57	5.6	0.33	11.7	0.68
	50–54	4.6	0.27	9.2	0.54	5.7	0.33	11.5	0.67
	55–59	4.7	0.27	9.0	0.53	5.7	0.33	11.5	0.67
	60–64	4.8	0.28	9.0	0.53	5.8	0.34	11.6	0.68
	65–70	4.8	0.28	9.0	0.53	5.9	0.35	11.5	0.67
TC	40–44	5.5	0.32	12.4	0.73	6.7	0.39	15.0	0.88
	45–49	5.3	0.31	11.9	0.70	6.6	0.39	14.4	0.84
	50–54	5.4	0.32	11.2	0.65	6.7	0.39	14.4	0.84
	55–59	5.4	0.32	11.0	0.64	6.6	0.39	14.3	0.84
	60–64	5.5	0.32	11.0	0.64	6.8	0.40	14.4	0.84
	65–70	5.6	0.33	11.0	0.64	6.8	0.40	14.4	1.84
TT	40–44	9.4	0.55	26.2	1.53	11.1	0.65	31.6	1.85
	45–49	9.1	0.53	25.6	1.50	11.7	0.68	32.6	1.91
	50–54	9.5	0.56	24.4	1.43	11.2	0.65	30.2	1.77
	55–59	9.4	0.55	21.8	1.27	11.0	0.64	28.6	1.67
	60–64	9.3	0.54	21.8	1.27	11.0	0.64	28.5	1.67
	65–70	9.2	0.54	21.5	1.26	11.3	0.66	28.1	1.64

Notes: Low (10th centile) and high (90th centile) values for bilirubin in « apparently healthy liver » subset (n = 138,125), per sex, age group, and genotype. Subjects below low are defined as hypobilirubinemia, between low and high as normobilirubinemia, and above high as hyperbilirubinemia. The extensive version of this table is available as Supplemental Table S5.

Abbreviations: CC, absence of the allele; CT genotype, heterozygoty; TT genotype, allele homozygosity

performed sequentially for the illnesses (outcomes) and confounders (Supplemental Table S3, http://links.lww.com/HC9/A459). The selected variants in the genome-wide association studies databases were those associated with Europeans from the UKB. For each TSMR, the effect and the *p*-value were reported; *p*-value <0.05 demonstrated a causal effect.

Adjusted prognostic value

Kaplan-Meier curves were compared using first the log-rank test. Then, the primary end point of adjusted 15-year survival was assessed using the 17 confounders and multivariate Cox proportional hazards analyses. The proportional hazard assumption was evaluated by visual inspection of curves and using the Schoenfeld residual plots versus time. Survminer libraries and R software were used.

Repeated assessment

The intra-participant variability of bilirubin was assessed at 6 years, by sex, and by *UGT1A1* genotype using repeated ANOVA with Bonferroni adjustment. The correlations between confounders were analyzed using Pearson correlation coefficient (Supplemental Figure S2, http://links.lww.com/HC9/A463).

RESULTS

In the "apparently healthy liver" subset, the 10th and 90th centiles of bilirubin, stratified by sex, age, and *UGT1A1* genotype (Fig. 1B), allowed us to readjust the GS prevalence among women and identify several significant differences in illness prevalence between GS, normobilirubinemia, and hypobilirubinemia (Figure 1, Table 2, and Supplemental Table S2, http://links.lww.com/HC9/A458). Almost all these differences were explained by confounders (Supplemental Table S6 references, http://links.lww.com/HC9/A464) as well as the differences in OS observed after (Figure 2A) and before adjustments (Figure 2B).

Personalized definitions

The centile distributions of bilirubin according to age, separately for *UGT1A1* genotype and sex, graphically in females (upper lines), demonstrated the lack of sensitivity

TABLE 4 Long-term morbidity (n = 54 illnesses) and confounders of bilirubin centiles adjusted by *rs887829* and age

	This study						Literature results
	Bilirubin association Yes if OR $P < 0.05$; NS if not						Confounders of bilirubin (TB) were validated as causal by MR or significantly associated with illness without causality. Positive means an increase of bilirubin increases the prevalence or severity of the illness Inverse means the increase of bilirubin decreases the prevalence or severity of the illness
	Gilbert syndrome		Hypobilirubinemia				
Morbidity grouping	**W**	**M**	**W**	**M**	**TB Causal by Mendelian Randomization**		**Significant confounders (including UGT1A1) in logistic regression or stratification**
Gallstone disease	—	—	—	—	—	—	Positive. UGT1A1/UGT1A4 had causal effects on gallbladder disorders by regulating TB (Yin 2022). Higher incidence of symptomatic gallstone could be due to raised TB and several gene variants, including UGT1A1 (Buch 2010, Stender 2013, Pérez-Palma 2020), and independently with ATP-binding cassette subfamily G member 8 [ABCG8] (Lim 2022, Lammert 2016, Lammert 2022) and genes of lipid metabolism (Joshi 2016, Yuan 2023). BMI and genetic associations were stronger in women compared with men. TB is associated with smoking. The risk of gallstone disease, cholelithiasis, and cholecystitis is increased by genetic liability to smoking initiation (Larsson 2022, Yuan 2023).
44.1 Cholelithiasis-gallstone	NS	1.5	ns	ns	Causal	Causal	—
44.2 Cholecystitis	NS	2.5	ns	ns	Not tested	—	—
44.3 Jaundice	1.9	NS	NS	NS	Not tested	—	—
35 Viral hepatitis	NS	NS	NS	NS	Not tested	Excluded from the “apparently healthy liver” subset	
36 Chronic liver disease	NS	NS	NS	NS	Not tested	Excluded from the “apparently healthy liver ” subset	
13 Alcohol problems	NS	ns	1.6	2.5	Not tested	Severe alcohol-associated disease excluded of “ apparently healthy liver” subset	
Treated dyspepsia	0.8	0.9	1.4	1.2			In subjects with GS, jaundice was associated with abdominal pain and dyspepsia (Kamal 2019). TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023).
6.1 Indigestion/dyspepsia	NS	NS	1.5	NS	Not tested	Not tested	
6.2 Gastric erosions	NS	NS	NS	NS	Not tested	Not tested	
6.3 Duodenal ulcer	NS	NS	NS	NS	Not causal	Not tested	
6.4 *Helicobacter pylori*	NS	NS	NS	NS	Not causal	Not tested	
6.5 Gastroesophageal reflux	0.8	NS	1.3	1.1	Not causal	Not tested	
6.6 Esophagitis	NS	NS	NS	NS	Not causal	Not tested	
6.7 Hiatus hernia	NS	NS	1.5	NS	Not causal	Not tested	
6.8 Gastric ulcers	NS	NS	ns	NS	Not causal	Not tested	
28 inflammatory bowel disease (IBD), Crohn, ulcerative colitis	NS	NS	1.3	1.3	Not causal	Not tested	TB is decreased in IBD (Lenicek 2014, Schieffer 2017). Analysis of human gut metagenomes identified a new bilirubin reductase having a decreased prevalence in patients with IBD (Hall 2023, USC bioRxiv preprint doi: 10.1101/2023.02.07.527579; posted February 8, 2023). TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023).
12 Irritable bowel syndrome	ns	ns	ns	ns	Not causal	not tested	TB associated with small intestinal bacterial overgrowth in diarrhea, irritable bowel syndrome, and decrease after rifaximin treatment (Rodriguez 2016); high proteolytic activity patients with IBS had lower fecal β-glucuronidase activity and end-products of bilirubin deconjugation (Edwinson 2022). TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023).

18 Diverticular disease of intestine	NS	NS	1.2	ns	Not causal	not tested	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Larsson 2022, Yuan 2023).
Cardio vascular disease (CVD)	—	—	—	—	—	Not causal McArdle 2012, Hou 2021	Blood pressure, cholesterol, CRP, alcohol, white blood cell count may play important roles in the pathway from bilirubin to CVD. Genetic liability to lifetime smoking was associated with increased risk of the 13 circulatory system disease (Larsson 2022)
19 Atrial fibrillation (AF)	1.4	1.8	ns	ns	Not causal	Not causal Lind 2021 Meng 2022	TB increases with atrial-appendage thrombosis, TB decreases in patients who were in sinus rhythm after cardioversion. TB may reflect an increased central venous pressure and liver congestion occurring in AF, rather than a direct effect of AF (Meyre 2022,). TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023).
5 Coronary Heart Disease (CHD)	1.2	1.2	1.2	ns	Not causal	Not causal Zanussi* 2021	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023).TB is positively associated with CHD but could be due to T2D pathology (Hou 2021).
16 Stroke and Transient Ischemic Attack (TIA)	ns	1.3	1.3	1.2	Not causal	Not causal Hou 2021, Zanussi[a] 2021	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Larsson 2022, Yuan 2023).
2 Hypertension	1.3	1.1	0.9	0.9	Not causal	Not causal Zanussi[a] 2021	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Larsson 2022). Possible vascular response to Autosomal Hypertension (Hou 2021)
7 Diabetes	ns	0.8	1.4	1.4	Not causal	Not causal Hou 2021, Zanussi[a] 2021	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023). T2-Diabetes could be one cause of TB level fluctuation (Hou 2021).
10 Chronic obstructive pulmonary disease COPD	1.6	2.1	0.8	0.7	Not causal	Not causal Dai 2022	Nonlinear does-response pattern between TB and COPD. Little evidence for the linear causal associations of TB with airflow limitation. Inverse TB association with lung function (Wen 2023).
37 Osteoporosis	ns	0.6	ns	1.4	Not causal	Not causal Zhao 2021	Negative on bone mineral density estimated by heel quantitative ultrasound
9 Connective tissue disease	0.7	ns	1.3	1.3	Not causal	Not causal Zanussi[a] 2021	In rheumatoid arthritis TB increase in GS after sarilumab treatment, a IL-6R-inhibitor, interacting with UGT1A1 (Lee 2011)
26 Schizophrenia, psychosis bipolar disorder	0.4	0.4	1.6	2.5	Not causal	Not causal Zanussi[a] 2021	Drugs interacting with UGT1A1 can be confounders. fda.gov/.. tablepharmacogenetic-associations 2023. Elevated TB can be the consequence of epilepsy treatment. (Thompson 1969)
34 Multiple sclerosis	ns	ns	ns	1.6	Not causal	Not causal Zanussi[a] 2021	TB increase and decrease by corticoid (Obradovic 2021); Reverse correlation (Miller 2021).
8 Thyroid disorders	ns	ns	ns	ns	Not causal	Causal Kjaergard 2021	High-normal free-thyroxine regulated by *DIO1/DIO2* variants is causally associated with decreased bilirubin.
3 Depression	0.8	0.9	1.4	1.5	Not causal	Causal Lu 2022	Inverse for major depression and attention-deficit/hyperactivity disorder
1 Painful condition	0.9	ns	1.2	ns	Not causal	not tested	Drugs interacting with UGT1A1 can be confounders. fda.gov/medical-devices/ precision-medicine/ tablepharmacogenetic-associations 2023.
4 Asthma	0.9	0.9	ns	ns	Not causal	not tested	Inverse TB association with lung function (Wen 2023).
23 Glaucoma	1.3	ns	ns	0.8	Not causal	not tested	TB increase (Shao 2023).
39 Endometriosis	0.8	-	ns	-	Not causal	not tested	TB increase associated with MMP7 variant in endometriosis (Liu 2022). Childhood obesity may reduce the incidence of endometriosis in adults (Yan 2022).
11 Anxiety, other neurotic	ns	ns	1.4	ns	Not causal	not tested	TB is an independent risk factor of alcohol dependance relapse in a randomized trial (Hu 2022)
22 Prostate disorders	ns	ns	ns	0.9	Not causal	not tested	Reverse association with prostate volume in non-obese, positive in obese (Ling 2022)

TABLE 4. (*continued*)

	This study						Literature results
	Bilirubin association Yes if OR $P < 0.05$; NS if not						Confounders of bilirubin (TB) were validated as causal by MR or significantly associated with illness without causality. Positive means an increase of bilirubin increases the prevalence or severity of the illness Inverse means the increase of bilirubin decreases the prevalence or severity of the illness
	Gilbert syndrome		Hypobilirubinemia				
Morbidity grouping	W	M	W	M	TB Causal by Mendelian Randomization		Significant confounders (including UGT1A1) in logistic regression or stratification
24 Epilepsy	0.4	ns	2.4	3.5	Not causal	not tested	Drugs interacting with UGT1A1 can be confounders. fda.gov/medical-devices/precision-medicine/ tablepharmacogenetic-associations 2023. Elevated TB can be the consequence of epilepsy treatment (Thompson 1969). When BMI, smoking status and tobacco were used as confounders, the number of phenotypes associated with TB decreased from 461 to 260 with epilepsy and seizure disorders (Zanussi[a]2021). TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Larsson 2022, Yuan 2023).
27 Psoriasis or eczema	ns	0.9	ns	ns	Not causal	not tested	TB is associated with the enhancement of inflammatory response in psoriasis vulgaris (Zhou 2016, Dobrica 2022). Decrease by smoking. No difference prevalence according to UGT1A1 (Beranek 2016)
30 Chronic sinusitis	ns	0.7	ns	ns	Not causal	not tested	
38 Chronic fatigue syndrome	0.7	ns	1.4	ns	Not causal	not tested	Hand grip strength associated with bilirubin and hemoglobin suggest low inflammation and hypoperfusion as potential pathomechanisms (Kedor 2022). Smoking increase severity (Jain 2017).
40 Meniere disease	ns	ns	ns	0.5	Not causal	not tested	Small decrease. In men, smoking increase, alcohol consumption decrease (Kim 2022).
29 Migraine	ns	ns	ns	ns	Not causal	not tested	Reverse. Protection by coffee, worsening by smoking. Inverse causality migraine alcohol (Yuan 2022,).
32 Bronchiectasis	ns	ns	ns	ns	Not causal	not tested	Reverse but only prognostic (Lee 2017). Inverse TB association with lung function (Wen 2023).
33 Parkinson disease	ns	ns	ns	0.6	Not tested	Not causal Zanussi[a] 20211	TB increase in Parkinson disease (Albillos 2021). Positive suggested by machine learning analysis in UKB (Lam 2022)
21 Heart failure	ns	1.8	ns	ns	Not tested	Not causal Guan 2023	TB is associated with hepatic venous pressure and hepatic function.
25 Dementia	ns	ns	ns	ns	Not tested	Not causal Zanussi[a] 20211	Positive but due to Alzheimer which is causal (Wang 2022).
20 Peripheral vascular disease	ns	ns	ns	ns	Not tested	Not causal Rantner 2008 Zanussi[a] 2021	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Yuan 2023). Not associated with UGT1A1.
17 Chronic kidney disease	ns	ns	ns	1.7	Not tested	Not causal Zanussi[a] 2021 Possible Park 2022	rs4149056 associated with higher bilirubin levels and associated with better kidney function.
43 Cancer	ns	ns	1.2	ns	Not tested	Not causal Zanussi[a] 2021 Culliford 2021	Reverse possible. Genetically raised bilirubin levels associated with low risk of squamous cell lung cancer and Hodgkin's lymphoma. Bilirubin gallstone not associated colon cancer Genetic predisposition to smoking initiation associated with increased risk of esophageal and gastric cancer (Larsson 2022, Yuan 2023)
15 Constipation	ns	ns	ns	ns	Not tested	not tested	TB is associated with smoking. The risk is increased by genetic liability to smoking initiation (Larsson 2022, Yuan 2023).

14 Other psychoactive, Opioid dependency	Not applicable, too small sample size	Not tested	Not tested	Positive. Less than in alcohol (Quraishi 2021). Therapeutic drugs interacting with UGT1A1 can be confounders. fda.gov/.. tablepharmacogenetic-associations 2023. Elevated TB can be the consequence of epilepsy treatment.
31 Anorexia abulia eating disorder	Not applicable, too small sample size	Not tested	not tested	TB positively associated with eating disorder and normalized after parenteral nutrition (Tamura 2015).
41. Pernicious anemia	Not applicable, too small sample size	Not tested	not tested	Reverse indirect association in euthyroid individuals, regulation of thyroid hormones by deiodinases plays a role in erythropoiesis (Kjaergaard 2022)
42. Polycystic ovaries	Not applicable, too small sample size	Not tested	not tested	TB positively associated with polycystic ovaries syndrome (Jędrzejuk 2019)

Notes: Summary of risk in hyperbilirubinemia and hypobilirubinemia in the present study in "apparently healthy liver" subset of a general population and in the literature. Causal was identified in a single illness, cholelithiasis-gallstone, 34 were not causal, and 19 not tested.
[a]Zanussi et al explored the association between TB and 19 illnesses using Mendelian randomization (MR) in 16,281 participants in a hospital-based biobank, not a general population.
Abbreviations: AF, Atrial fibrillation; UKB, UK Biobank; CHS, Coronary Heart Disease; COPD, Chronic obstructive pulmonary disease; CVD, Cardio vascular disease; MR, Mendelian randomization; TIA, Transient Ischemic Attack.

of the usual cutoff (horizontal dotted black line) for hyperbilirubinemia. In females with the TT genotype, the median bilirubin level was 17 μmol/L at 40 years of age, which decreased to a plateau of 14 μmol/L between 60 and 70 years of age (Fig. 1B). The hypobilirubinemia cutoff varied from 4.6 to 9.4 μmol/L, and the hyperbilirubinemia cutoff ranged from 9.0 to 26.2 μmol/L (Supplemental Table S5, http://links.lww.com/HC9/A462). The characteristics of bilirubin centiles were compared in the 3 subsets: "apparently healthy liver" (Table 2), "at risk of NAFLD" (Table 2 and Supplemental Table S2, http://links.lww.com/HC9/A458), and "general population" (Supplemental Table 3, http://links.lww.com/HC9/A459).

Associations between bilirubin centiles and confounders were described in Table 4 and Supplemental Table S6 (Table 4 and Supplemental Table S6, http://links.lww.com/HC9/A464)

For 9 hepatobiliary or digestive illnesses and 13 other illnesses, the correlations were not significant for causality. For depression and thyroid disorders, we did not observe significant causality, in contrast with previous MRs.

Aging resulted in an expected significant ($P < 0.001$) decrease in TB in 7249 participants with repeated measurements at 6 years, which was demonstrated for the first time according to *UGT1A1* genotype in both sexes. The median decrease ranged from −0.24 μmol/L in CC women to −0.97 μmol/L in TT men (Figure 2C).

Univariate regressions

In women (Supplemental Table S7, http://links.lww.com/HC9/A465), for GS, the confounders with the most significant ORs (all $P < 0.001$) were current smoking (negative, −), daily or almost daily alcohol consumption (positive, +), deprivation index (−), brisk walking pace (+), triglycerides (−), apoA1 (+), CRP (−), albumin (+), AST (+), and reticulocytes (+). Inverse results were observed for hypobilirubinemia.

In men (Supplemental Table S8, http://links.lww.com/HC9/A466), the same results were observed for ORs, with the exception of alcohol intake 3 to 4 times a week having the highest OR for regression for both GS and hypobilirubinemia, and there was a lack of significant association between hypobilirubinemia and body mass index and cholesterol.

Multivariate regressions

Detailed analyses of round 1 are detailed in Supplemental Table S9, http://links.lww.com/HC9/A467, for women and in Supplemental Table S10, http://links.lww.com/HC9/A468, for men, permitted to select non-colinear confounders.

TABLE 5 Two-sample Mendelian randomization.

	Grouping	Name	Phecode	OR	%95CI		SNPs	*p*-value	Sample size
Confounders	Environmental	Alcohol intake frequency	ukb-b-5779	1.0107	0.9967	1.0249	116	0.1356	462346
	—	Smoking status: Current	ukb-a-225	1.0003	0.9978	1.0029	116	0.7964	336024
	—	Smoking status: Never	ukb-d-20116_0	0.9983	0.9944	1.0022	120	0.3869	359706
	—	Smoking status: Previous	ukb-a-224	1.0013	0.9977	1.0048	116	0.4869	336024
	—	Townsend deprivation index	ukb-b-10011	0.9999	0.9938	1.0061	116	0.9811	462464
	—	Usual walking pace	ukb-b-4711	1.0002	0.9941	1.0064	116	0.9401	459915
	Hemolysis	Hemoglobin concentration	ukb-d-30020_irnt	1.0290	0.9891	1.0706	120	0.1562	350474
	—	Reticulocytes count	ukb-d-30250_irnt	1.0392	0.9945	1.0860	120	0.0864	344729
	Inflammatory	Alanine aminotransferase	ukb-d-30620_irnt	1.0104	0.9806	1.0411	120	0.4967	
	—	Albumin	ukb-d-30600_irnt	1.0103	0.9885	1.0326	120	0.3569	
	—	Aspartate aminotransferase	ukb-d-30650_irnt	1.0086	0.9758	1.0425	120	0.6111	
	—	C-reactive protein	ukb-d-30710_irnt	1.0050	0.9553	1.0573	120	0.8476	
	—	Gamma glutamyltransferase	ukb-d-30730_irnt	1.0135	0.9748	1.0537	120	0.5009	
	—	Platelets count	ukb-d-30080_irnt	0.9797	0.9494	1.0110	120	0.2007	350474
	Metabolic	Apolipoprotein-A1	ukb-d-30630_irnt	1.0032	0.9721	1.0353	120	0.8411	
	—	Body mass index	ukb-b-19953	0.9947	0.9801	1.0096	116	0.4840	461460
	—	Low-density cholesterol-direct	ukb-d-30780_irnt	0.9874	0.9522	1.0240	120	0.4950	
	—	Total cholesterol	ukb-d-30690_irnt	0.9746	0.9371	1.0135	120	0.1972	
	—	Triglycerides	ukb-d-30870_irnt	0.9980	0.9422	1.0572	120	0.9458	
Illnesses	Anxiety	Anxiety/panic attacks	ukb-b-17243	0.9999	0.9992	1.0005	97	0.7021	462933
	Asthma	Asthma	ukb-b-18113	0.9985	0.9952	1.0019	116	0.3848	462933
	Atrial fibrillation	Atrial fibrillation	ukb-b-11550	0.9997	0.9992	1.0003	88	0.3262	462933
	Bronchiectasis	Bronchiectasis	ukb-b-18163	0.9998	0.9981	1.0016	39	0.8525	462933
	COPD	COPD/chronic obstructive pulmonary disease	ukb-b-13447	1.0000	0.9997	1.0004	59	0.8911	462933

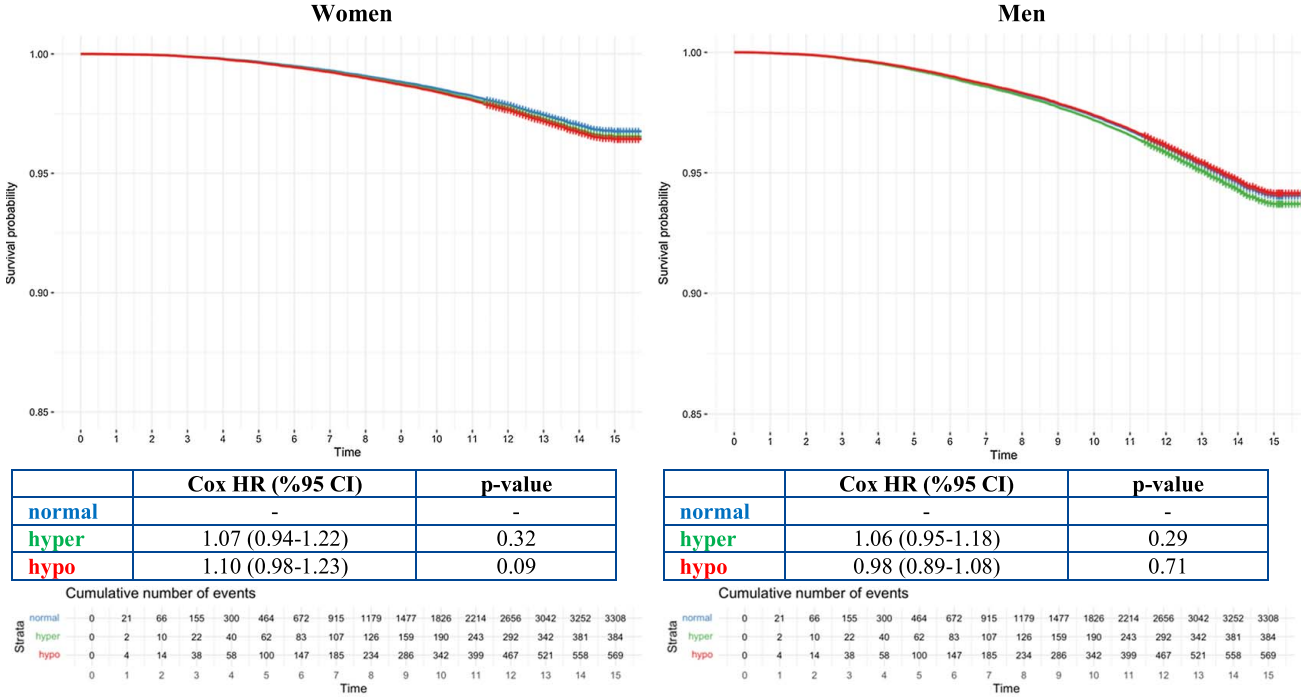
—	Emphysema/chronic bronchitis	ukb-b-7280	1.0001	0.9994	1.0008	97	0.8113	462933
Chronic fatigue syndrome	Chronic fatigue syndrome	ukb-b-8961	1.0002	0.9998	1.0007	73	0.2499	462933
Chronic sinusitis	Chronic sinusitis	ukb-b-69	1.0001	0.9997	1.0005	84	0.6586	462933
Connective tissue diseases	Rheumatoid arthritis	ukb-b-9125	0.9997	0.9991	1.0004	95	0.4277	462933
Coronary heart disease	Angina	ukb-b-8650	1.0006	0.9992	1.0020	103	0.4244	462933
—	Heart attack/MI	ukb-b-15829	0.9993	0.9979	1.0006	100	0.2947	462933
Depression	Depression	ukb-b-12064	0.9997	0.9983	1.0010	112	0.6324	462933
Diabetes	Diabetes	ukb-b-12948	1.0008	0.9987	1.0029	109	0.4511	462933
—	Type 2 diabetes	ukb-b-13806	1.0001	0.9996	1.0006	84	0.6230	462933
Diverticular disease	Diverticular disease/diverticulitis	ukb-b-14796	0.9996	0.9990	1.0002	95	0.2303	462933
Dyspepsia	Duodenal ulcer	ukb-b-4725	1.0004	1.0000	1.0008	69	0.0749	462933
—	Gastric stomach ulcers	ukb-b-20078	1.0003	0.9997	1.0008	87	0.3649	462933
—	Gastroesophageal reflux	ukb-b-16818	0.9990	0.9977	1.0003	109	0.1165	462933
—	*Helicobacter pylori*	ukb-b-531	1.0003	0.9999	1.0007	52	0.0909	462933
—	Hiatus hernia	ukb-b-6514	1.0003	0.9993	1.0013	100	0.5680	462933
—	Esophagitis/Barrett's esophagus	ukb-b-9496	1.0000	0.9997	1.0003	55	0.8462	462933
Endometriosis	Endometriosis	ukb-b-10903	1.0001	0.9995	1.0006	91	0.7523	462933
Epilepsy	Epilepsy	ukb-b-16309	0.9998	0.9993	1.0003	91	0.4857	462933
Gallstone	**Cholelithiasis/gallstone**	**ukb-b-18700**	**1.0013**	**1.0001**	**1.0025**	**97**	**0.0408**	**462933**
Glaucoma	Glaucoma	ukb-b-8398	1.0000	0.9994	1.0007	95	0.9145	462933
Hypertension	Essential hypertension	ukb-b-7582	1.0003	1.0000	1.0007	73	0.0862	462933
—	Hypertension	ukb-b-14057	0.9981	0.9927	1.0035	116	0.4853	462933
Inflammatory bowel disease	Crohn disease	ukb-b-8210	0.9997	0.9994	1.0001	55	0.1558	462933
—	Ulcerative colitis	ukb-b-7584	0.9999	0.9995	1.0004	78	0.7841	462933
Irritable bowel syndrome	Irritable bowel syndrome	ukb-b-2592	1.0003	0.9993	1.0013	101	0.5406	462933
Meniere disease	Meniere disease	ukb-b-11736	1.0001	0.9998	1.0004	51	0.6673	462933
Migraine	Migraine	ukb-b-16868	1.0000	0.9987	1.0014	103	0.9635	462933
Multiple sclerosis	Multiple sclerosis	ukb-b-17670	0.9998	0.9994	1.0002	61	0.3159	462933
Osteoporosis	Osteoporosis	ukb-b-12141	0.9999	0.9990	1.0008	97	0.7721	462933
Painful conditions	Ankylosing spondylitis	ukb-b-18194	0.9999	0.9996	1.0002	50	0.5372	462933
—	Arthritis	ukb-b-8229	0.9997	0.9992	1.0003	88	0.3346	462933
—	Back pain	ukb-b-11241	0.9999	0.9994	1.0003	78	0.6160	462933
—	Back problem	ukb-b-9306	1.0004	0.9996	1.0011	97	0.3142	462933
—	Cervical spondylosis	ukb-b-2349	0.9999	0.9994	1.0004	87	0.7730	462933
—	Gout	ukb-b-13251	0.9997	0.9986	1.0008	97	0.6324	462933
—	Headaches (not migraine)	ukb-b-12623	0.9998	0.9993	1.0004	92	0.5132	462933
—	Joint pain	ukb-b-4122	0.9998	0.9994	1.0002	59	0.2640	462933
—	Osteoarthritis	ukb-b-14486	1.0008	0.9990	1.0027	116	0.3713	462933

TABLE 5. (*continued*)

Grouping	Name	Phecode	OR	%95CI		SNPs	*p*-value	Sample size
—	Prolapsed disk/slipped disk	ukb-b-15904	0.9996	0.9987	1.0005	97	0.4040	462933
—	Sciatica	ukb-b-8194	0.9999	0.9993	1.0004	93	0.6543	462933
—	Spine arthritis spondylitis	ukb-b-5389	0.9998	0.9992	1.0003	92	0.3725	462933
Pernicious anemia	Pernicious anemia	ukb-b-8720	1.0000	0.9997	1.0003	55	0.9811	462933
Prostate disorders	Enlarged prostate	ukb-b-7469	1.0004	0.9997	1.0011	97	0.2380	462933
Psoriasis or eczema	Eczema dermatitis	ukb-b-20141	1.0002	0.9991	1.0013	103	0.6961	462933
—	Psoriasis	ukb-b-10537	1.0004	0.9996	1.0011	95	0.3498	462933
Schizophrenia/bipolar affective disorder Bipolar	Bipolar disorder	ukb-b-6906	0.9999	0.9996	1.0002	50	0.4523	462933
—	Mania	ukb-b-6906	0.9999	0.9996	1.0002	50	0.4484	462933
—	Manic depression	ukb-b-6906	0.9999	0.9996	1.0002	50	0.4484	462933
Stroke/TIA	Stroke	ukb-b-6358	0.9999	0.9992	1.0006	97	0.8213	462933
—	TIA	ukb-b-15749	1.0002	0.9998	1.0006	63	0.4130	462933
Thyroid disorders	Hyperthyroidism/thyrotoxicosis	ukb-b-20289	0.9998	0.9985	1.0011	88	0.7727	462933
—	Hypothyroidism/myxedema	ukb-b-19732	1.0012	0.9990	1.0034	110	0.2852	462933
—	Thyroid problem (not cancer)	ukb-b-13532	1.0001	0.9998	1.0004	48	0.4163	462933

Notes: Assessing the causal effect of bilirubin on self-reported illnesses and confounders. The exposure and the outcome database selected in the GWAS databases are the one for the European subjects from the UK Biobank and with available data. Results display the OR with 95% CI, number of SNPs involved, *p*-value using inverse variance weighted method, and the sample size in the UK Biobank (source GWAS).
Abbreviation: GWAS, genome-wide association studies.

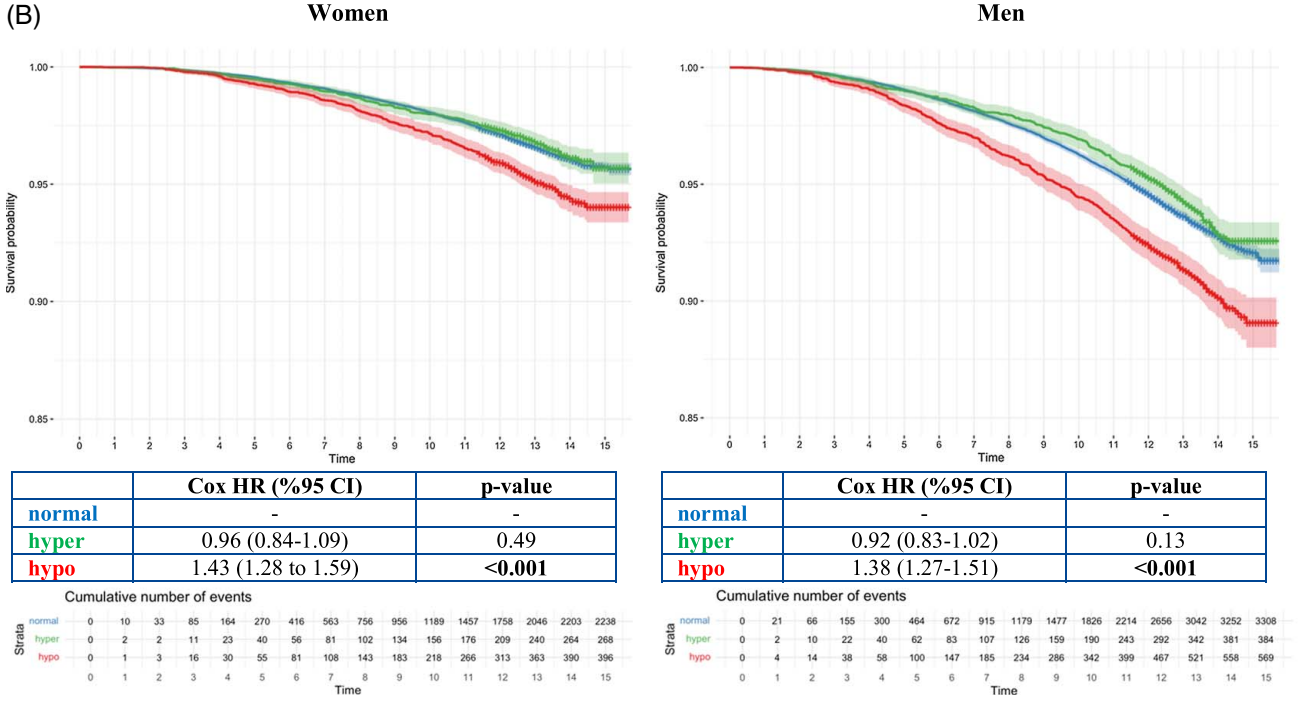
(A) Adjusted Cox curves



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.07 (0.94-1.22)	0.32
hypo	1.10 (0.98-1.23)	0.09

	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.06 (0.95-1.18)	0.29
hypo	0.98 (0.89-1.08)	0.71

(B)



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	0.96 (0.84-1.09)	0.49
hypo	1.43 (1.28 to 1.59)	**<0.001**

	Cox HR (%95 CI)	p-value
normal	-	-
hyper	0.92 (0.83-1.02)	0.13
hypo	1.38 (1.27-1.51)	**<0.001**

FIGURE 2 Adjusted and nonadjusted survivals. (A) Adjusted survivals in the « apparently healthy liver» subset. Adjusted Cox curve in women (left) and men (right) for overall survival according to bilirubin centiles; hyperbilirubinemia (green curve) and hypobilirubinemia (red curve) against the reference normobilirubinemia group (blue). The curves are adjusted for confounders (age, smoking status, alcohol intake, walking pace, deprivation index, CRP, albumin, PLT, and cholesterol). The table shows the HR and 95% CI for bilirubin centiles for the multivariate Cox regression. Abbreviation: PLT, platelets.

Round 2 allowed us to identify the most significant independent factors. In women (Supplemental Table S11, http://links.lww.com/HC9/A469) and men (Supplemental Table S12, http://links.lww.com/HC9/A470), for GS, these main confounders (ORs all $P < 0.001$) were smoking status (−), TC (−), triglycerides (−), LDL (+), apoA1 (+), CRP (−), albumin (+), AST (+), hemoglobin (+), and reticulocytes (+), with inverse results in hypobilirubinemia. Unexpected discordances were observed according to sex; alcohol intake was not

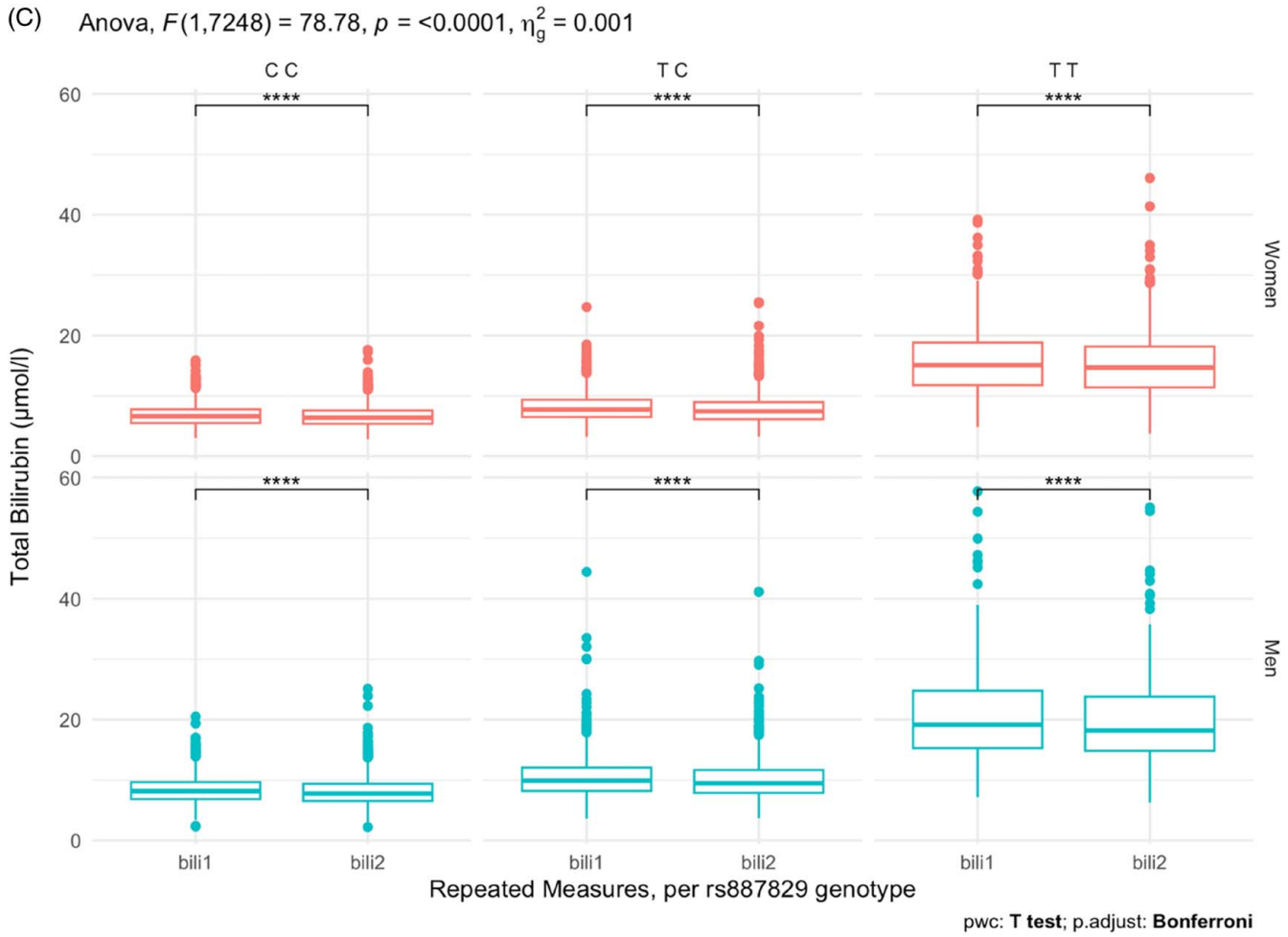


FIGURE 2 Continued.

significant in GS men, but positive in GS women, and brisk pace was not significant in GS women but negative in GS men.

Prevalence of illnesses according to bilirubin centiles

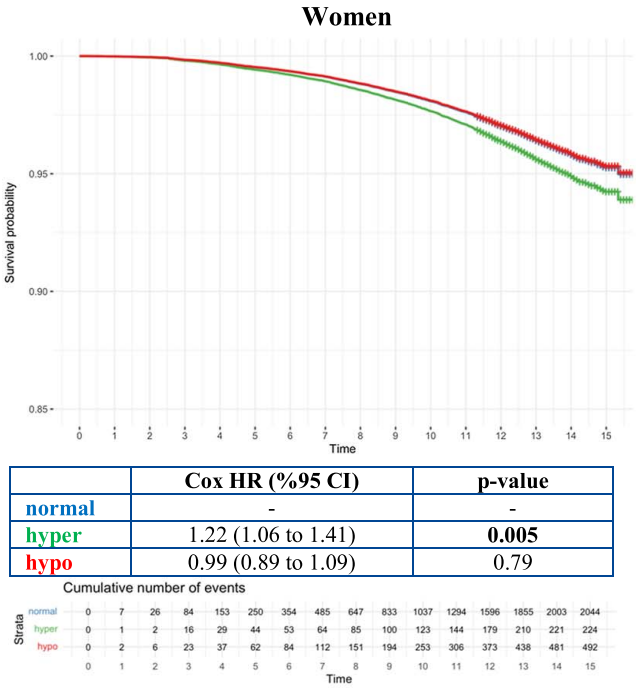
For the first time, 28 out of 54 illnesses with significant differences were identified in both GS and hypobilirubinemia compared with normobilirubinemia ($P < 0.05$) (Figs. 1C, 1D).

Cholelithiasis in men and jaundice of unknown cause in women (Fig. 1C) were the only 2 conditions out of the 10 detailed biliary and digestive illnesses that were more frequent in GS than normobilirubinemia. Two factors were different in GS: higher risk for cholecystitis/bile duct disease/obstruction and lower risk for hiatus hernia in women (Fig. 1C). In hypobilirubinemia, only gastroesophageal reflux risk was lower for both sexes (Fig. 1D).

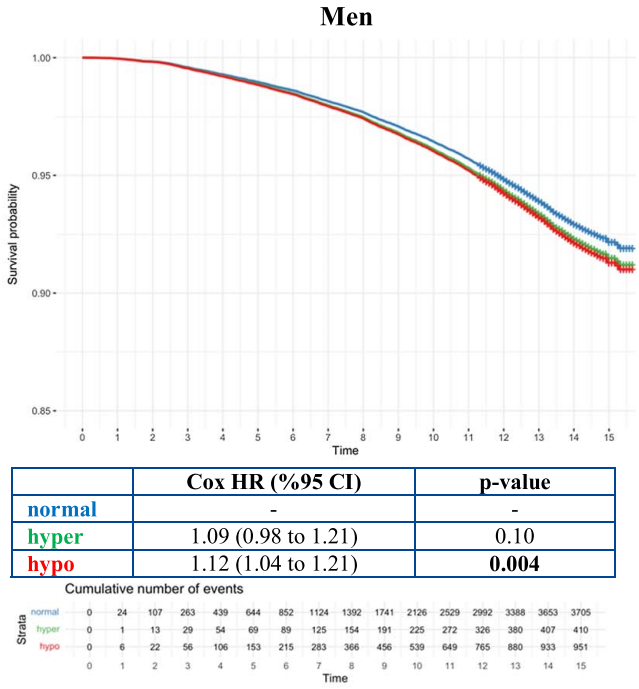
Regarding nonbiliary digestive conditions (Fig. 1E), 3 illnesses were more frequent in GS for both sexes: atrial fibrillation, coronary heart disease, and hypertension. Heart failure was more frequent only in men, and glaucoma was more frequent only in women. Four illnesses were less frequent in both sexes: schizophrenia, obstructive disease, asthma, and depression. Four illnesses were less frequent only in women: epilepsy, connective tissue disease, painful conditions, and endometriosis. Four illnesses were less frequent only in men: diabetes, sinusitis, psoriasis/eczema, and osteoporosis. For hypobilirubinemia, most results were similar and reversed (Fig. 1F).

Only 18%/18% (women/men) of participants declared no illness (Table 1). Three illnesses were declared at a frequency above 10%: hypertension (24%/32%), painful conditions (24%/22%), and asthma (15%/15%). The remaining 20 illnesses had a frequency of 5%–10%. Frailty was significantly less frequent in GS compared with normobilirubinemia in women (GS: 70/7,741 [0.9%]; normobilirubinemia: 905/(15%) (2.7%) and men (GS: 50/6179 [0.8%]; normobilirubinemia: 573/48,988 [1.2%]) ($P < 0.001$); the inverse was observed for hypobilirubinemia. Similarly, the multimorbidity counts were lower in GS compared with normobilirubinemia in both women and men and the inverse was observed for hypobilirubinemia (Table 1).

(D) Adjusted Cox curves

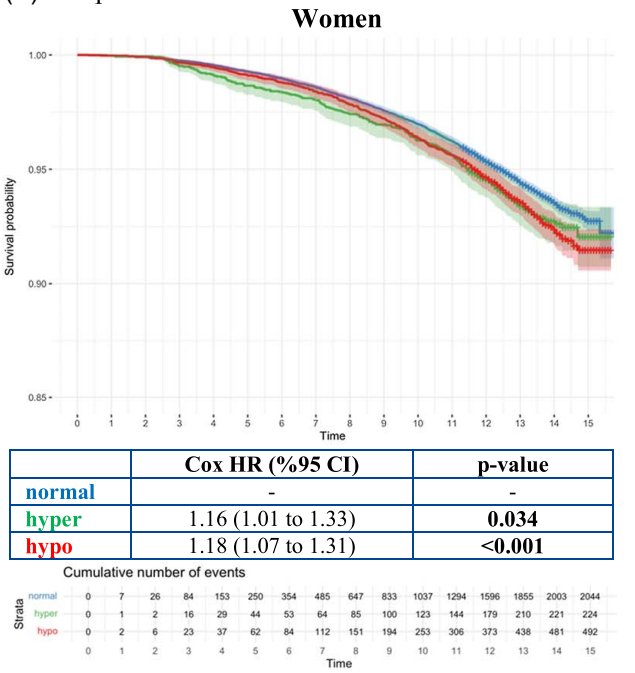


	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.22 (1.06 to 1.41)	**0.005**
hypo	0.99 (0.89 to 1.09)	0.79

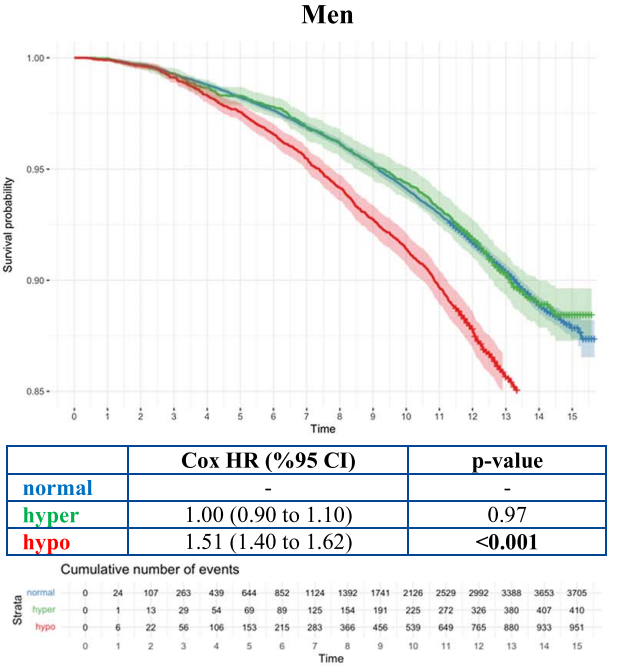


	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.09 (0.98 to 1.21)	0.10
hypo	1.12 (1.04 to 1.21)	**0.004**

(E) Kaplan Meir curves



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.16 (1.01 to 1.33)	**0.034**
hypo	1.18 (1.07 to 1.31)	**<0.001**



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.00 (0.90 to 1.10)	0.97
hypo	1.51 (1.40 to 1.62)	**<0.001**

FIGURE 2 Continued.

Multivariate regression analyses

The analyses for multivariate round 1 are detailed in Supplemental Table S9, http://links.lww.com/HC9/A467 for women and Supplemental Table S10, http://links.lww.com/HC9/A468 for men. Multivariate round 2 is presented in Supplemental Table S11, http://links.lww.com/HC9/A469 for women and Supplemental Table S12, http://links.lww.com/HC9/A470 for men. Eleven confounding factors were independently associated with GS and hypobilirubinemia. The deprivation index was not associated with GS in men

or women, nor was it associated with hypobilirubinemia in men. Furthermore, in men, body mass index and GGT were not associated with hypobilirubinemia.

Mendelian randomizations

We found a significant causal association between bilirubin centiles and cholelithiasis/gallstone illness. The TSMR assessing the causal effect of bilirubin on illnesses is summarized in Table 5. No other significant causal association between bilirubin centiles and illnesses was found.

Adjusted survival in "apparently healthy liver" participants

There was no significant difference between OS once adjusted for the significant 11-factor model, which combined bilirubin centiles (including three confounders: sex, age, and *UGT1A1*) and 8 other confounders: smoking, alcohol intake, walking pace, deprivation, CRP, platelets, albumin, and cholesterol (Fig. 3A). In women with GS (hyperbilirubinemia), the OS HRs were nonsignificant for normobilirubinemia (HR: 1.10, 95% CI [0.94–1.22], $P = 0.32$) or for hypobilirubinemia (HR: 1.10, 95% CI [0.98–1.23], $P = 0.09$). The differences were nonsignificant in men with GS (HR: 1.06, 95% CI [0.95–1.18], $P = 0.30$) and hypobilirubinemia (HR: 0.98, 95% CI [0.89–1.08], $P = 0.71$).

According to a nonadjusted model (Fig. 3B), there was considerably lower OS in hypobilirubinemia for both sexes compared with normobilirubinemia in women (HR: 1.43, 95% CI [1.28–1.59]) and men (HR: 1.38, 95% CI [1.27–1.51]) ($P < 0.001$). There were no significant differences in OS between GS and normobilirubinemia for women.

The main causes of mortality are presented in Supplemental Table S13, http://links.lww.com/HC9/A471. In both sexes, the most striking difference was the higher mortality associated with pulmonary cancer in hypobilirubinemia compared with normal and hyperbilirubinemia.

Sensitivity analyses in the "at risk of NAFLD" subset

Analyses against confounders are detailed for univariate regression in Supplemental Table S14, http://links.lww.com/HC9/A472, for women and Supplemental Table S15, http://links.lww.com/HC9/A473, for men and multivariate round 1 in Supplemental Table S16, http://links.lww.com/HC9/A474, for women and Supplemental Table S17, http://links.lww.com/HC9/A475, for men. Multivariate round 2 is presented in Supplemental Table S18, http://links.lww.com/HC9/A476, for women and Supplemental Table S19, http://links.lww.com/HC9/A477, for men. Compared with the "apparently healthy liver" subset, in the "at risk of NAFLD" subset, there was a significant association between a deprivation index and hyperbilirubinemia for both sexes; in men only, there was no association between CRP and hyperbilirubinemia or between apoA1 and hypobilirubinemia. Illnesses had varying prevalence according to bilirubin centile. In hyperbilirubinemia, more cholelithiasis was observed in men, but also in women (Supplemental Figure S3A, http://links.lww.com/HC9/A478), and in hypobilirubinemia, more hiatus hernias were observed in women and for men (Supplemental Figure S3B, http://links.lww.com/HC9/A478).

In contrast with the "apparently healthy liver" subset, women with normobilirubinemia (Fig. 2D, Fig. 2E blue lines) had lower risk of death (HR = 1) than women with hypobilirubinemia (HR = 1.18, 95% CI [1.07–1.33], $P < 0.001$) or hyperbilirubinemia (HR = 1.16, 95% CI [1.01–1.33], 0.03), suggesting a U-shaped curve of bilirubinemia risk. In men, there was a higher HR (1.51, 95% CI [1.40–1.62]) in hypobilirubinemia compared with normobilirubinemia, but no difference for hyperbilirubinemia (Fig. 2E). Interestingly, univariate comparisons indicated a significantly increased risk of death in hypobilirubinemia (Fig. 2E).

The main causes of mortality are indicated in Supplemental Table S13, http://links.lww.com/HC9/A471. Despite the small sample size, the most striking results in the causes of death were the ranking of COVID-19 in participants in the "at risk of NAFLD" group, where COVID-19 was in the top 6 causes of death, including 2 in the second place in women, in comparison with "apparently healthy liver" group, where COVID-19 never appeared in the top 4 causes of death.

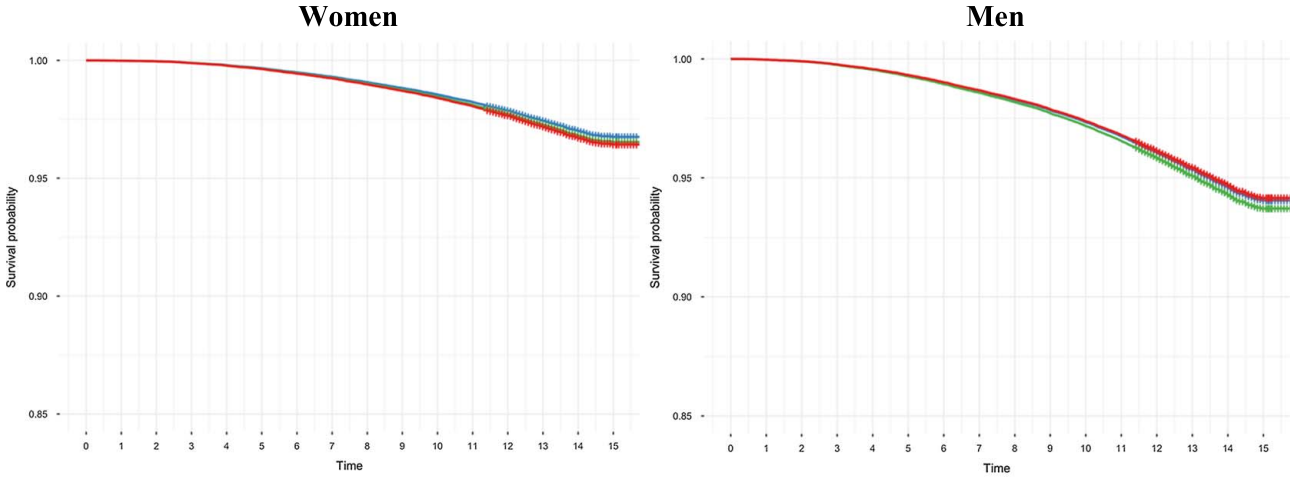
DISCUSSION

The findings addressed the 3 aims of the study. These findings must be discussed in the context of the literature, the study limitations, and the expected consequences for patients and health care services.

Personalized bilirubin centiles as hyperbilirubinemia and hypobilirubinemia definitions

We agree with Vitek that due to the many confounding factors affecting bilirubin serum concentration levels, it is hard to establish reliable decision limits and that data on women were scarce.[4] The response to the first aim was a proposal of personalized bilirubin centiles according to sex, age, and *UGT1A1* genotyping, using the 10th centile for hypobilirubinemia, 90th centile for hyperbilirubinemia,

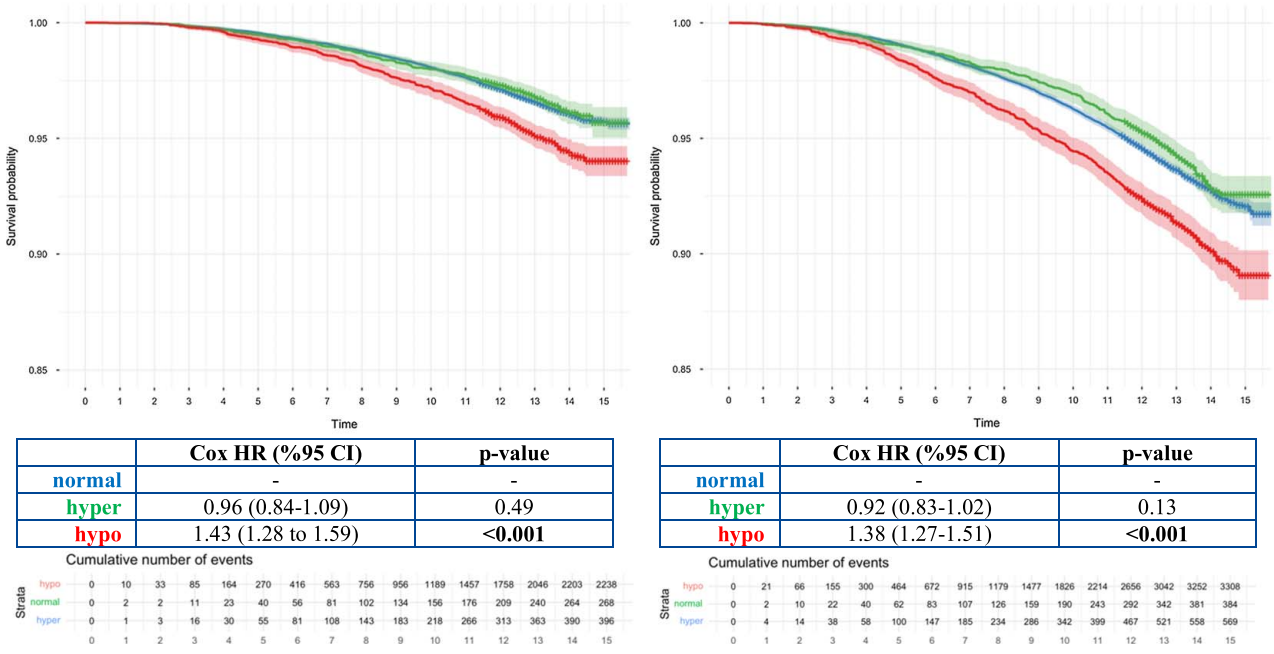
(A) Adjusted Cox curves



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.07 (0.94-1.22)	0.32
hypo	1.10 (0.98-1.23)	0.09

	Cox HR (%95 CI)	p-value
normal	-	-
hyper	1.06 (0.95-1.18)	0.29
hypo	0.98 (0.89-1.08)	0.71

(B) Kaplan Meier curves



	Cox HR (%95 CI)	p-value
normal	-	-
hyper	0.96 (0.84-1.09)	0.49
hypo	1.43 (1.28 to 1.59)	**<0.001**

	Cox HR (%95 CI)	p-value
normal	-	-
hyper	0.92 (0.83-1.02)	0.13
hypo	1.38 (1.27-1.51)	**<0.001**

FIGURE 3 Adjusted survival and Kaplan-Meier in the « apparently healthy liver » subset. (A) Adjusted Cox curve in women (left) and men (right) for overall survival according to bilirubin centiles; hyperbilirubinemia (green curve) and hypobilirubinemia (red curve) against the reference normobilirubinemia group (blue). The curves are adjusted for confounders (age, smoking status, alcohol intake, walking pace, deprivation index, CRP, albumin, PLT, and cholesterol). The table shows the HR and 95% CI for bilirubin centiles for the multivariate Cox regression. (B). Kaplan–Meier curve and 95% CI in women (left) and men (right) for overall survival according to bilirubin centiles; hyperbilirubinemia (green curve) and hypobilirubinemia (red curve) against the reference normobilirubinemia group (blue). The table shows the HR and 95% CI for bilirubin centiles for the univariate Cox regression. The horizontal time scale is displayed in years of exposure to the risk. All figures use the same scale to ease visual comparison. Cumulative number of events are shown in the lower left table for women and the lower right table for men. Abbreviation: PLT, platelets.

and values in between for normobilirubinemia—as simple decision limits that was already used by the National Institutes of Health for liver markers.[13] This choice permitted a better balance between sensitivity and specificity, especially in women, than the unisex cutoff.[32]

For women, the results demonstrated that the existing GS cutoff was not sufficiently sensitive to assess the

prognosis and illnesses associated with bilirubin centiles. GS is described as being more frequent in males than females (Supplemental Table S1, http://links.lww.com/HC9/A457), which is not true according to the age distribution of bilirubinemia (Figure 1B). A population study illustrated this risk of underestimation of GS in women. Among participants referred for genetic testing after incidental discovery of hyperbilirubinemia using the usual cutoff, a total of 1191—1150 males and only 41 females had the *UGT1A1rs8175347,rs4148323* genotyping.[35] Another implication for women with the *UGT1A1* genotyping is the increased risk of toxicity associated with chemotherapies, such as irinotecan. The largest study on colorectal cancer (n = 1362) observed a higher confounder-adjusted risk of irinotecan-induced severe neutropenia and more severe diarrhea in women compared with men.[36]

The new definitions should improve studies on the effectiveness of drugs interacting with bilirubin metabolism. The Food and Drug Administration recommendations concerning pharmacogenetic associations, seven drugs were affected by bilirubin according to *UGT1A1*-genotyping variants and metabolizer status. Three drugs require therapeutic management: belinostat (28/28 variant, poor metabolizers), irinotecan (1/6, 1/28 intermediate metabolizers; or 6/6, 6/28, 28/28 poor metabolizers), and sacituzumab govitecan-hziy (28/28 poor metabolizers). For nilotinib and pazopanib (both 28/28 poor metabolizers), there is a potential impact on safety and response, and for dolutegravir (poor metabolizers) and raletegravir (28/28 poor metabolizers), there is an impact only on pharmacokinetic properties.[37]

Regarding hypobilirubinemia, there is no rationale for using a unisex cutoff. Several mechanisms could contribute to low bilirubin levels in women despite the same prevalence of the *UGT1A1* genotype (Table 1), such as combinations of mutations, polymorphisms, estrogen, and the degradation of heme and nutrients.[23–25] For the first time, our findings allow the adjustment of personalized hypobilirubinemia definitions in the Europeans, with a range of 4.7–9.2 μmol/L in women and 5.6–12.1 μmol/L in men (Table 3). The previously recommended cutoffs of hypobilirubinemia without adjustment were 5.0 μmol/L for women [24] and 7 μmol/L for men.[2]

Prevalence of illnesses, frailty, confounders, and causality

The genetic association of *UGT1A1* and several other variants with cholelithiasis/gallstones was established in 2010 but was never assessed prospectively in a large general population. For the first time, the prevalence of cholelithiasis in men with GS was assessed at 20% of European adults together with a 20% increase of the risk of complications, significantly more frequent than in men with normobilirubinemia, after adjusting for confounders. Furthermore, our TSMR validated the causality pathway, including *UGT1A1* variants observed in 3 studies.[38–41]

Among the remaining 34 illnesses evaluated by TSMR, none had a causal association with TB. A nonsignificant causality association is not proof of an absence of causality; however, our results were concordant with previous MR and multivariate confounder analyses for 11 common illnesses (Table S4 Supplemental references, http://links.lww.com/HC9/A460). The most frequent confounders for TB are now well identified and include sex, age, smoking, alcohol consumption, and drugs interacting with the *UGT1A1* genotype. For 2 illnesses, thyroid disorder[44] and depression,[45] we did not find the significant associations reported by others (Table 4). These discordances might be explained by the lower power of our study, with a smaller panel of genetic instrumental variables and a smaller number of participants in our database. However, the association might be inverse due to the prescription of antidepressant drugs interacting with bilirubin metabolism.[37] Recent associations with gut metagenomes in patients with inflammatory bowel disease or irritable bowel syndrome suggest a potential causal role of bilirubin (Table 4). The prevalence of the frailty phenotype, estimated for the first time in GS, was very low in both sexes (< 1%), significantly less than in normobilirubinemia, and contrasting with the prevalence of the pre-fail status (33% in GS vs. 34% in normobilirubinemia) (Table 1 and Supplemental Table S2, http://links.lww.com/HC9/A458). These differences may be explained by a greater proportion of participants younger than 65 years of age and the exclusion of nonhealthy participants in our study compared with previous studies.[29–31] In contrast, the multimorbidity count revealed that 82% of the participants with normobilirubinemia in the "apparently healthy liver" subset declared at least 1 morbidity at baseline. This is a major finding for improving communication with anxious individuals after a diagnosis of GS, who have a significantly lower multimorbidity count than individuals with normobilirubinemia (Supplemental Table S1, http://links.lww.com/HC9/A457).

Our choice of defining hyperbilirubinemia and hypobilirubinemia stratified by age, sex, and *UGT1A1* genotype allowed us to interpret the confounders on the pathway from bilirubin to illness. For individuals with GS, the findings will facilitate the construction of personalized risk scores combining genetic and environmental factors for early diagnosis and prevention of cholelithiasis in men.[38]

Survival

The response to the third aim was that the prognostic value of TB published in the literature and initially retrieved in our univariate results disappeared after

adjustment for confounders in both females and males. These results are original due to the power of the cohort but are certainly not definitive.

Another original result was observed in the "at risk of NAFLD" subset, as participants with normobilirubinemia had higher adjusted survival (Figure 2D) than those with hyperbilirubinemia, which is visible in nonadjusted curves (Figure 2E). These participants were selected for having a low risk of significant liver fibrosis by FIB4; therefore, it is prudent to assess the absence of significant fibrosis with nonelevated transaminases using more accurate, noninvasive biomarkers.

To date, contrasting results have been published in various cohort studies investigating an association between genetic *UGT1A* variants and NAFLD. A likely explanation for the inconsistent data reported involves the presence of *UGT1A* polymorphisms found in isoforms other than *UGT1A1*.[42]

Limitations

These findings on definition, symptoms, and survival must be discussed in consideration of the methodological limitations and the expected consequences for patients with newly identified phenotypes. The UKB has limitations, including the middle-aged European participants with better survival than the general population of the United Kingdom,[29] which decreases the generalizability of the results. As the reference intervals start at the age of 40 years, it remains unclear if this will be useful in younger subjects. It could be the ones needing advice and or reassurance when first diagnosed with having GS.

We limited the number of confounders to 17 to balance the risk of missing an independent confounder and the risk of overfitting. We did not include several behavioral confounders: the time of the day of blood sampling, fasting hours, and coffee consumption.[43] However, we tested the intra-subject TB variability using repeated samples (n = 7249) and a sample that was small but representative of the general population. The decrease in TB with age was expected and underlined the risk of a false-negative diagnosis of GS when the *UGT1A1* genotype is not assessed in both sexes in older individuals (Fig. 2C). Many illnesses were self-reported and monitored by trained staff; other items were assessed directly by trained employees, such as grip strength.[30]

We used the simple methodology outlined by Ritchie et al[33] following the reference distributions for the 10th-90th centiles adjusted for age and sex, and not narrower intervals, in maximizing the balance between the sensitivity and specificity of bilirubin centiles for establishing nonlinear relationships with survivals and confounders. We focus on total bilirubin and not on unconjugated bilirubin, also available in the UK Biobank. Indeed, the Pearson correlation in 118,796 participants was = 0.997, and the linear regression showed no pragmatic interest in large population as unconjugated bilirubin is a less widespread biomarker (Supplemental Figure S4, http://links.lww.com/HC9/A479).

We did not analyze the variants in populations with more genetic heterogeneity, such as Asian populations, nor did we compare recessive and dominant genes. The variant rs887829 in the *UGT1A1* gene has been reported to explain at least 30%–50% of the variance in bilirubin levels, and it is in nearly complete linkage disequilibrium with the genetic polymorphisms that underlie GS in Europeans. We did not study the possible association of survival with other loci, such as the solute carrier organic anion transporter family member 1B1, which is also strongly associated with bilirubin levels.[46]

Even after MR, genetic analysis was not robust enough to detect nonlinear effects or to quantitatively estimate causal effects; therefore, further studies are required to investigate to what extent bilirubin centiles are beneficial for predicting survival through different related functions.[21]

The finding that participants who never or only occasionally drank alcohol was more likely to have the frailty genotype may be explained by abstainer bias, suggesting that these subjects drank no alcohol because they had poorer health and might have been following advice to abstain.[29] The differences in alcohol-bilirubin correlations observed between GS and hypobilirubinemia suggest more complex correlations between confounders than the U-shape (Table 5).

FIB4 was the fibrosis score computed with the data available in the UKB but has been shown to be a poor predictor of fibrosis in patients with NAFLD[46] and has worse performance in older patients, which has the potential to further skew the data over time.[15]

Expected consequences for patients and health care services

As stated in other studies, the identification of either benefits or harms would be a powerful argument for preemptive genotyping so that these individuals could enjoy the benefits and avoid the harms associated with their genotype.[10,38] Even if confounders ruled out direct and causal relationships between bilirubin centiles, the *UGT1A1* genotype, illnesses, and survival, the findings of this study should improve the awareness of GS and hypobilirubinemia among individuals in European populations. The frequencies of most illnesses in GS were less than or similar in normobilirubinemia.

In conclusion, 2 consequences of these findings are expected, the first being a better awareness of the benefits and risks associated with GS, especially in women needing chemotherapy for whom GS is underestimated. Second, the findings support the construction of a personalized cholelithiasis risk score in men with GS.

DATA AVAILABILITY STATEMENT

UK Biobank data are available through a procedure described at http://www.ukbiobank.ac.uk/using-the-resource/. Supplementary data are given in supplementary materials.

AUTHOR CONTRIBUTIONS

Thierry Poynard and Olivier Deckmyn contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing: original draft, and writing: review and editing. Valentina Peta, Mehdi Sakka, Pascal Lebray, Joseph Moussalli, and Raluca Pais, contributed to data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing: original draft, and writing: review and editing. Vlad Ratziu, Eric Pasmant, Chantal Housset, and Dominique Thabut contributed to project administration, supervision, validation, visualization, writing: original draft, and writing: review and editing.

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CONFLICTS OF INTEREST

Thierry Poynard is employed by BioPredictive and FibroTest. Olivier Deckmyn is employed by BioPredictive. Valentina Peta is employed by BioPredictive. The remaining authors have no conflicts to report.

REFERENCES

1. Gilbert NA, Lereboullet P. La cholémie simple familiale. Sem Méd (Paris). 1901;11:241–3.
2. Vitek L, Hinds TD, Stec DE, Tiribelli C. The physiology of bilirubin: health and disease equilibrium. Trends Mol Med. 2023; 29:315–28.
3. Wagner KH, Shiels RG, Lang CA, Seyed Khoei N, Bulmer AC. Diagnostic criteria and contributors to Gilbert's syndrome. Crit Rev Clin Lab Sci. 2018;55:129–39.
4. Vítek L. Bilirubin as a predictor of diseases of civilization. Is it time to establish decision limits for serum bilirubin concentrations? Arch Biochem Biophys. 2019;672:108062.
5. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994;40:18–23.
6. Seyed Khoei N, Wagner KH, Sedlmeier AM, Gunter MJ, Murphy N, Freisling H. Bilirubin as an indicator of cardiometabolic health: a cross-sectional analysis in the UK Biobank. Cardiovasc Diabetol. 2022;21:54.
7. Stern C, Castera L. Identification of high-risk subjects in NAFLD. Clin Mol Hepatol. 2022. Clin Mol Hepatol 202329:S196–206.
8. Poynard T, Munteanu M, Deckmyn O, Ngo Y, Drane F, Messous D, et al. Applicability and precautions of use of liver injury biomarker FibroTest. A reappraisal at 7 years of age. BMC Gastroenterol. 2011;11:39.
9. Bailey A, Robinson D, Dawson AM. Does Gilbert's disease exist? Lancet. 1977;309:931–3.
10. Zanussi JT, Zhao J, Dorn CA, Liu G, Feng Q, Wei W, et al. Identifying potential therapeutic applications and diagnostic harms of increased bilirubin concentrations: A clinical and genetic approach. Clin Pharmacol Ther. 2022;111:435–43.
11. Rosenthal P, Pincus M, Fink D. Sex- and age-related differences in bilirubin concentrations in serum. Clin Chem. 1984;30:1380–2.
12. Zucker SD, Horn PS, Sherman KE. Serum bilirubin levels in the U.S. Population: Gender effect and inverse correlation with colorectal cancer. Hepatology. 2004;40:827–35.
13. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the US population. Hepatology. 2016;63:1170–83.
14. Petroff D, Bätz O, Jedrysiak K, Kramer J, Berg T, Wiegand J. Age dependence of liver enzymes: An analysis of over 1,300,000 consecutive blood samples. Clin Gastroenterol Hepatol. 2022; 20:641–50.
15. McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol. 2017;112:740–51.
16. Arias IM, London IM. Bilirubin glucuronide formation in vitro ; Demonstration of a defect in Gilbert's Disease. Science. 1957; 126:563–4.
17. Powell LW, Hemingway E, Billing BH, Sherlock S. Idiopathic unconjugated hyperbilirubinemia (Gilbert's Syndrome): A Study of 42 Families. N Engl J Med. 1967;277:1108–12.
18. Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Oude Elferink RP, Chowdhury JR, et al. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. J Biol Chem. 1994;269:17960–4.
19. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's Syndrome. N Engl J Med. 1995;333:1171–5.
20. Kadakol A. Interaction of coding region mutations and the Gilbert-type promoter abnormality of the UGT1A1 gene causes moderate degrees of unconjugated hyperbilirubinaemia and may lead to neonatal kernicterus. Journal of Medical Genetics. 2001; 38:244–9.
21. Kingdom R, Wright CF. Incomplete penetrance and variable expressivity: from clinical studies to population cohorts. Front Genet. 2022;13:920390.
22. Nelson RS, Seligson ND, Bottiglieri S, Carballido E, Cueto AD, Imanirad I, et al. UGT1A1 Guided Cancer Therapy: Review of the evidence and considerations for clinical implementation. Cancers. 2021;13:1566.
23. Hinds TD, Stec DE. Bilirubin, a cardiometabolic signaling molecule. Hypertension. 2018;72:788–95.
24. Creeden JF, Gordon DM, Stec DE, Hinds TD. Bilirubin as a metabolic hormone: the physiological relevance of low levels. American Journal of Physiology-Endocrinology and Metabolism. 2021;320:E191–207.
25. Ong KL, Allison MA, Cheung BMY, Wu BJ, Barter PJ, Rye KA. the relationship between total bilirubin levels and total mortality in older adults: the United States National Health and Nutrition Examination Survey (NHANES) 1999-2004. PLoS ONE. 2014;9: e94479.
26. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12:e1001779.
27. Elliott P, Peakman TC. on behalf of UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. International Journal of Epidemiology. 2008;37:234–44.
28. Bycroft C, Freeman C, Petkova D, Petkova D, Band G, Elliott LT, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature. 2018;562:203–9.

29. Hanlon P, Nicholl BI, Jani BD, Lee D, McQueenie R, Mair FS. Frailty and pre-frailty in middle-aged and older adults and its association with multimorbidity and mortality: a prospective analysis of 493 737 UK Biobank participants. Lancet Public Health. 2018;3:e323–32.
30. Hanlon P, Jani BD, Butterly E, Nicholl B, Lewsey J, McAllister DA, et al. An analysis of frailty and multimorbidity in 20,566 UK Biobank participants with type 2 diabetes. Commun Med (Lond). 2021;1:28.
31. Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. Lancet. 2012;380:37–43.
32. Albuquerque J, Medeiros AM, Alves AC, Bourbon M, Antunes M. Comparative study on the performance of different classification algorithms, combined with pre- and post-processing techniques to handle imbalanced data, in the diagnosis of adult patients with familial hypercholesterolemia. PLoS ONE. 2022;17:e0269713.
33. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for the negative acute-phase serum proteins, albumin, transferrin and transthyretin: a practical, simple and clinically relevant approach in a large cohort. J Clin Lab Anal. 1999;13:273–9.
34. Manrai AK, Patel CJ, Ioannidis JPA. In the era of precision medicine and big data, who is normal? JAMA. 2018;319:1981–2.
35. Bale G, Avanthi US, Padaki NR, Sharma M, Duvvur NR, Vishnubhotla VRK. Incidence and Risk of Gallstone Disease in Gilbert's Syndrome Patients in Indian Population. Journal of Clinical and Experimental Hepatology. 2018;8:362–6.
36. Ichikawa W, Uehara K, Minamimura K, Tanaka C, Takii Y, Miyauchi H, et al. An internally and externally validated nomogram for predicting the risk of irinotecan-induced severe neutropenia in advanced colorectal cancer patients. Br J Cancer. 2015;112:1709–16.
37. Food and Drug Administration. Table of Pharmacogenetic Associations. https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations.
38. Lammert F, Gurusamy K, Ko CW, Miquel JF, Méndez-Sánchez N, Portincasa P, et al. Gallstones. Nat Rev Dis Primers. 2016;2:16024.
39. Buch S, Schafmayer C, Völzke H, Seeger M, Miquel JF, Sookoian SC, et al. Loci from a genome-wide analysis of bilirubin levels are associated with gallstone risk and composition. Gastroenterology. 2010;139:1942–51.
40. Stender S, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Extreme bilirubin levels as a causal risk factor for symptomatic gallstone disease. JAMA Intern Med. 2013;173:1222–8.
41. Yin X, Bose D, Kwon A, Hanks SC, Jackson AU, Stringham HM, et al. Integrating transcriptomics, metabolomics, and GWAS helps reveal molecular mechanisms for metabolite levels and disease risk. Am J Hum Genet. 2022;109:1727–41.
42. Kjaergaard AD, Teumer A, Marouli E, Deloukas P, Kuś A, Sterenborg R, et al. Thyroid function, pernicious anemia and erythropoiesis: a two–sample Mendelian randomization study. Hum Mol Genet. 2022;31:2548–59.
43. Lu Z, Pu C, Zhang Y, Sun Y, Liao Y, Kang Z, et al. Oxidative Stress and Psychiatric Disorders: Evidence from the bidirectional Mendelian Randomization Study. Antioxidants (Basel). 2022;11:1386.
44. Landerer S, Kalthoff S, Paulusch S, Strassburg CP. A Gilbert syndrome-associated haplotype protects against fatty liver disease in humanized transgenic mice. Sci Rep. 2020;10:8689.
45. Tanaka M, Budhathoki S, Hirata A, Morita M, Kono S, Adachi M, et al. Behavioral and clinical correlates of serum bilirubin concentrations in Japanese men and women. BMC Endocr Disord. 2013;13:39.
46. Bravo-Gómez A, Salvador-Martín S, Zapata-Cobo P, Sanjurjo-Sáez M, López-Fernández LA. Genotyping of *UGT1A1**80 as an Alternative to *UGT1A1**28 Genotyping in Spain. Pharmaceutics. 2022;14:2082.
47. Kjaergaard M, Lindvig KP, Thorhauge KH, et al. Using the ELF test, FIB-4 and NAFLD fibrosis score to screen the population for liver disease [published online ahead of print, 2023 Apr 21]. J Hepatol. 2023:S0168–8278.

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