Bilirubin and Stroke Risk Using a Mendelian Randomization Design

Sun Ju Lee, MPH; Yon Ho Jee, BS; Keum Ji Jung, PhD; Seri Hong, MD; Eun Soon Shin, PhD; Sun Ha Jee, PhD

Background and Purpose—Circulating bilirubin, a natural antioxidant, is associated with decreased risk of stroke. However, the nature of the relationship between the two remains unknown. We used a Mendelian randomization analysis to assess the causal effect of serum bilirubin on stroke risk in Koreans.

Methods—The 14 single-nucleotide polymorphisms (SNPs) ($<10^{-7}$) including rs6742078 of uridine diphosphoglucuronyltransferase were selected from genome-wide association study of bilirubin level in the KCPS-II (Korean Cancer Prevention Study-II) Biobank subcohort consisting of 4793 healthy Korean and 806 stroke cases. Weighted genetic risk score was calculated using 14 SNPs selected from the top SNPs.

Results—Both rs6742078 (F statistics=138) and weighted genetic risk score with 14 SNPs (F statistics=187) were strongly associated with bilirubin levels. Simultaneously, serum bilirubin level was associated with decreased risk of stroke in an ordinary least-squares analysis. However, in 2-stage least-squares Mendelian randomization analysis, no causal relationship between serum bilirubin and stroke risk was found.

Conclusions—There is no evidence that bilirubin level is causally associated with risk of stroke in Koreans. Therefore, bilirubin level is not a risk determinant of stroke. **(*Stroke*. 2017;48:1154-1160. DOI: 10.1161/STROKEAHA.116.015083.)**

Key Words: bilirubin ■ causality ■ epidemiology ■ genes ■ stroke

Bilirubin (a heme oxygenase-1 metabolite), the end product of heme catabolism, is a potent endogenous antioxidant.[1,2] Numerous studies have shown an inverse association between bilirubin levels and several oxidative stress–mediated diseases such as coronary artery disease, diabetes mellitus, metabolic syndrome, cardiovascular disease, and colorectal cancer.[3–6] According to the first report on the association between bilirubin level and stroke in 2003,[7] plasma bilirubin concentration might serve as a useful marker of oxidative stress in patients with hemorrhagic stroke. Since then, similar results have been reported in both Western[8,9] and Asian[10,11] countries. However, Arsalan et al[12] have reported that higher serum bilirubin levels are associated with increased stroke severity, longer hospitalization, and poor prognosis. Therefore, the role of bilirubin as a risk factor or prognostic factor for stroke is uncertain because of potential confounding factors. Given the limitations of observational studies, whether the association between bilirubin and stroke is causal or because of residual confounding remains unknown.

Mendelian randomization uses genetic variants randomly allocated according to Mendel's second law without any preconception.[13,14] As instrumental variables (IV) for Mendelian randomization analysis, studies on genetic polymorphisms in major bilirubin related genes such as uridine diphosphoglucuronyltransferase (UGT1A1), solute carrier organic anion transporter family member 1B1, and solute carrier organic anion transporter family member 1B3 have been reported.[15–17] In particular, rs6742078 in UGT1A1, a single-nucleotide polymorphism (SNP) that explains 18% of the variation in total serum bilirubin levels is well known as a critical region related to bilirubin.[15] In addition, data from numerous studies have suggested that stroke is markedly heritable. The proportion of variance explained by a shared genetic background ranges from 0.37 to 40.3.[18] However, to the best of our knowledge, Mendelian randomization on bilirubin with stroke has not been reported yet.

With the assumption that bilirubin is a strong mediator between genes and stroke, the causal effects of bilirubin on stroke can be determined using a genetic marker through Mendelian randomization analysis. Using the KCPS-II (Korean Cancer Prevention Study-II) Biobank Cohort, we hypothesized that lower levels of serum bilirubin would increase stroke because of bilirubin gene variations.

Methods

Participants

The KCPS-II Biobank was initiated in April 2004. It was supported by the Seoul City Government in December 2005 as a project of the Korean Metabolic Syndrome Research Initiative study. Blood sample collection from participants in the KCPS-II Biobank was initiated in

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From the Department of Public Health, Graduate School (S.J.L.), Institute for Health Promotion (K.J.J., S.H., S.H.J.), and Department of Epidemiology (S.H.J.), Yonsei University, Seoul, Republic of Korea; MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, United Kingdom (Y.H.J.); and DNA Link Inc, Seoul, Republic of Korea (E.S.S.).

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Correspondence to Sun Ha Jee, PhD, Department of Epidemiology and Health Promotion, Graduate School of Public Health, Yonsei University, Yonsei 50–1, Saedaemun-ku, Seoul, Korea. E-mail jsunha@yuhs.ac

2004 at 2 hospitals (Severance Hospital, Bundang Cha Hospital) on a small scale. It has been expanded to 11 hospitals since April 2006. These 11 health promotion centers located in Seoul and Gyeonggi province consist of a population of 18 879 351 (≈40.9% of the total South Korea's population of 46 136 101) based on a 2000 report.[19] Study members were participants in routine health assessments at health promotion centers across Seoul and Kyong-gi province, South Korea. Specifically, the number of participants who had provided informed content in the 11 health promotion centers between 2004 and 2013 was 159 844. Five thousand participants were randomly selected as a subcohort of KCPS-II Biobank participants. A total of 850 stroke cases were reported from main cohort. Final study participants were 5599 men and women in 2016 (Figure I in the online-only Data Supplement). The Severance Medical Ethics Committee of the Korean approved this study (no. 4-2011-0277). All participants gave written informed consent.

Data Collection

Baseline information was obtained from health examinations performed from 2004 to 2013. Each participant completed systemized questionnaires including smoking habit (never smoker, ex-smoker, or current smoker), alcohol habit (nondrinker or drinker of any amount of alcohol), regular exercise (yes or no), and other characteristics such as medical history and any medications. Weight, height, and blood pressure were measured for participants in a standardized manner.

The body mass index was calculated as weight in kilograms divided by height in meters squared. Systolic and diastolic blood pressures were measured in a seated position with a mercury sphygmomanometer or automatic manometer. Fasting blood glucose, serum cholesterol, and serum bilirubin concentrations were measured through automated biochemical profiling (Hitachi-7600 analyzer; Hitachi Ltd). Bilirubin concentrations were collected in milligram per deciliter units.

Measurement of Outcome

Outcome variables were incidence of stroke and its subtypes (ischemic, hemorrhagic, and all stroke types) as recorded in hospital admission discharge records from 2005 to 2014 (median follow-up duration, 7.0 years). We ascertained these outcomes from health insurance claim data from the National Health Insurance Service.[10] We checked fatal cases from the national death certification data.[10] Ischemic, hemorrhagic, and all stroke types were defined according to the *International Classification of Diseases*-Tenth Revision codes: ischemic stroke, I63–I639; hemorrhagic stroke, I60–I629; and all stroke types, I60–I699. For those individuals with more than 1 stroke event during the follow-up period, only the first event was considered in our statistical analyses. The accuracy rate of the *International Classification of Diseases* codes for cerebrovascular diseases in medical insurance claims for men in Korea was reported to be 83.0% in 2000.[20]

DNA Extraction and SNP Analysis

Biological samples for DNA extraction used in the present study were obtained from KCPS-II Biobank at baseline. UGT1A1 genotyping was conducted for 5000 men and women in 2016. These participants were randomly selected as a subcohort of KCPS-II Biobank participants. Genotype data were produced using the Korean Chip (K-CHIP) available through the K-CHIP consortium. K-CHIP was designed by Center for Genome Science, Korea National Institute of Health, Korea (4845-301, 3000–3031). The Korean chip contains 830 000 SNPs with no imputation of SNPs.

Statistical Analysis

The association between bilirubin and SNP through GWAS was tested by an additive model of linear regression analysis after adjusting for age and sex using PLINK 1.07.[21] We used Haploview (v4.1) to generate Manhattan plots of −log10 P, linkage disequilibrium structures, and haplotype block plots. Weighted genetic risk score (WGRS) was calculated using 14 SNPs after excluding the use of r^2>0.9 in SNPs obtained from the genome-wide association study (GWAS) to investigate causal effect of bilirubin on stroke (Figures II and III in the online-only Data Supplement). The estimate is analyzed by the linear regression between the association of the number of risk allele and bilirubin. And then, the WGRS was calculated by multiplying each estimated β-coefficient by the number of corresponding risk alleles (0, 1, or 2). Observational multivariable cox proportional hazard model was performed using for association between bilirubin and stroke, while adjusting for confounding variables.

Mendelian randomization analysis was performed in three steps. First, linear regression was used to assess the strength of the association of UGT1A1 variants (rs6742078) and WGRS with bilirubin levels and determine whether the association was fully mediated by bilirubin levels. The strength of the association was expressed as F statistics. We used linear regression under the assumption of an additive genetic model. Second, we examined the association of rs6742078 and WGRS with participants' characteristics. Third, the association between stroke and bilirubin through GWAS was tested by cox proportional hazard model. A 2-sided significance level of α=0.05 was used. All statistical analysis was performed using SAS 9.2 (SAS Institute Inc, Cary, NC) and STATA/IC 13.1 (Stata Corp LP, College Station, TX).

Results

Total Serum Bilirubin Levels Among Study Participants

The characteristics of the study participants are summarized in Table 1. Bilirubin levels were measured for 5599 participants (4793 subcohort and 806 incident stroke) from the KCPS-II Biobank. Mean±SD of bilirubin concentration was 0.91±0.35 mg/dL (0.98±0.35 mg/dL in men [n=3745] and 0.77±0.30 mg/dL in women [n=1854]). During a 7-year follow-up, a total of 806 men and women were hospitalized because of stroke (Table 1).

Table 1. Baseline Characteristics of Study Subjects and Their Associations With Incident Stroke Using Cox Proportional Hazard Model

	Subcohort (n=4793)	Incident Stroke* (n=806)	HR (95% CI)
Age, y	42.4 (8.9)	52.2 (11.4)	1.073 (1.065–1.081)
Total bilirubin, mg/dL	0.92 (0.35)	0.84 (0.35)	0.685 (0.530–0.887)
Systolic BP, mm Hg	118.2 (14.2)	125.0 (16.1)	1.012 (1.007–1.017)
Fasting glucose, mg/dL	90.7 (18.5)	98.0 (25.6)	1.003 (0.999–1.006)
Total cholesterol, mg/dL	190.3 (33.2)	197.0 (35.1)	1.003 (1.001–1.006)
HDL-cholesterol, mg/dL	50.8 (10.0)	49.9 (11.4)	0.992 (0.984–1.000)
Sex (female), %	33.2	32.6	1.028 (0.831–1.299)
Smoking status, %			
Ex-smoker	19.8	22.1	0.942 (0.736–1.206)
Current smoker	31.6	30.2	1.237 (1.016–1.596)

Data are expressed as mean (SD) unless otherwise indicated. BP indicates blood pressure; CI, confidence interval; HR, hazard ratio; and HDL, high-density lipoprotein.

*Stroke incidence cases in subcohort included incident stroke group.

Genome-Wide Association With Total Serum Bilirubin Levels

A genome-wide association with total serum bilirubin levels was tested in 5599 individuals from the KCPS-II biobank to identify genetic factors affecting serum bilirubin levels. As shown in Figure IV in the online-only Data Supplement, significant associations were observed on chromosome 2 located in the promoter of UGT1A1 gene. Among the 3 most significant UGT1A1 SNPs, rs887829 showed the strongest association with bilirubin levels (P=1.29E−82). The second most significant variant in this gene was rs6742078 (P=2.33E−82). Both variants had the same effect on bilirubin levels (0.207 mg/dL per copy of the minor allele C and G, respectively; Table I in the online-only Data Supplement).

Association of rs6742078 and WGRS With Risk Factors of Stroke

To account for potential confounding bias, we tested whether rs6742078 and WGRS were associated with common stroke risk factors (Table 2). Genotype frequencies of rs6742078 for genotypes GG, GT, and TT were 77.7%, 20.8%, and 1.5%, respectively. Mean bilirubin level was the highest in rs6742078*TT carriers (1.4±0.5 mg/dL; n=82). It was lower in rs6742078*TG (1.1±0.4 mg/dL; n=1168) and rs6742078*GG (0.9±0.3 mg/dL; n=4349) carriers (P<0.0001; Table 2; Figure 1). As shown in Table 2, no significant differences across genotype groups and WGRS were found in age, sex, body mass index, systolic blood pressure, fasting serum glucose, total cholesterol level, high-density lipoprotein-cholesterol level, hypertension, or diabetes mellitus. Notably, genotypes were significantly related to smoking status. A total of 70.7% of the rs6742078*TT carriers were never smokers, whereas a total of 47.9% of the rs6742078*GG carriers were never smokers. A total of 31.8% of the rs6742078*GG carriers were current smokers, whereas 19.5% (P=0.0018) of the rs6742078*TT carriers were current smokers. Overall, both rs6742078 and WGRS were associated with bilirubin level but these were not related confounding variables.

Causal Estimates for the Effect of Bilirubin on Stroke

Using the highly associated SNP rs6742078, and WGRS of top 14 SNPs, we performed Mendelian randomization to investigate whether bilirubin levels had a causal effect on stroke

Table 2. Associations Between UGT1A1 Polymorphism rs6742078 or WGRS Using 14 Single-Nucleotide Polymorphisms From Bilirubin Genome-Wide Association Study and Total Bilirubin Level in KCPS-II (Korean Cancer Prevention Study-II) Biobank Participants

	UGT1A1 Polymorphism rs6742078				WGRS	
	GG (n=4349)	TG (n=1168)	TT (n=82)	*P* Value*	*r* or Mean (SD)	*P* Value*
Total bilirubin, mg/dL	0.9 (0.3)	1.1 (0.4)	1.4 (0.5)	<0.0001	0.369	<0.0001
Age, y	43.7(9.9)	43.9(9.8)	45.0(10.9)	0.473	0.017	0.218
Body mass index, kg/m^2	23.8 (3.0)	23.9 (3.1)	23.9 (3.4)	0.816	0.024	0.071
Systolic BP, mm Hg	119.2 (14.7)	119.4 (14.6)	118.3 (17.3)	0.738	0.007	0.611
FSG, mg/dL	91.8 (19.7)	91.7 (20.0)	90.0 (23.5)	0.689	0.019	0.166
Total cholesterol, mg/dL	191.3 (33.5)	191.3 (34.1)	189.9 (31.3)	0.935	-0.020	0.138
HDL-cholesterol, mg/dL	50.7 (10.3)	50.4 (9.8)	52.4 (10.9)	0.198	-0.012	0.365
Sex men	67.1	66.9	57.3	0.177	0.52 (0.30)	0.066
Women	32.9	33.1	42.7		0.50 (0.29)	
Smoking status, %						
Never	47.9	48.5	70.7	0.002	0.51 (0.30)	0.854
Ex-smoker	20.3	20.4	9.8		0.51 (0.29)	
Current	31.8	31.1	19.5		0.52 (0.29)	
Hypertension, %†						
No	81.8	81.6	86.6	0.523	0.51 (0.29)	0.782
Yes	18.2	18.4	13.4		0.52 (0.30)	
Diabetes mellitus, %‡						
No	94.3	94.3	96.3	0.7247	14.3 (4.6)	0.934
Yes	5.7	5.7	3.7		14.4 (4.8)	

BP indicates blood pressure; FSG, fasting serum glucose; HDL, high-density lipoprotein; UGT1A1, uridine diphosphoglucuronyltransferase; and WGRS, weighted genetic risk score.

*P value from ANOVA for continuous variables or from χ^2 test for categorical variables.

†Systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or treatment history.

‡FSG ≥126 mg/dL or treatment history.

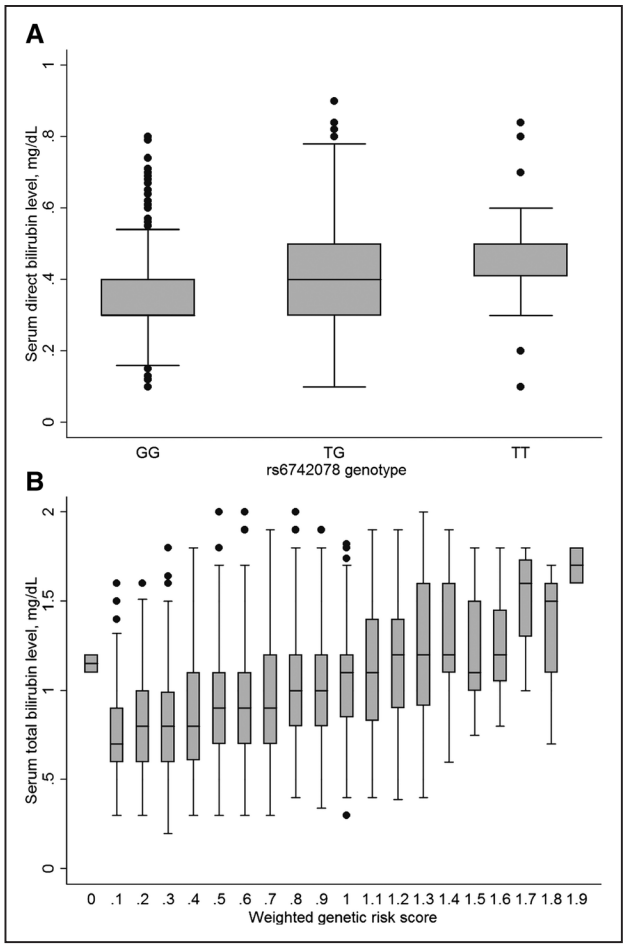

Figure 1. Associations between rs6742078 genotypes (GG, TG, and TT; **A**) or weighted genetic risk score (**B**) using 14 single-nucleotide polymorphisms related to bilirubin in chromosome 2 or 12 and total serum bilirubin levels.

(Table 3). After adjusting for age, sex, systolic blood pressure, fasting serum glucose, total cholesterol, high-density lipoprotein-cholesterol, and smoking status, the F statistic for the association of WGRS with bilirubin level was higher than of rs6742078 ($F_{rs6742078}$=138; F_{WGRS}=187), indicating that WGRS was a valid instrument for bilirubin levels. The bilirubin levels significantly decreased the risk of stroke in observational multivariate hazard ratio ($HR_{rs6742078}$=0.67; 95% confidence interval, 0.51–0.87; HR_{WGRS}=0.70; 95% confidence interval, 0.53–0.91) even after adjusting for confounding variables (Figure 2). However, such association was not significant in IV analysis ($P_{rs6742078}$=0.531; P_{WGRS}=0.240).

Discussion

Our study revealed no evidence of a causal role between serum bilirubin level and stroke risk although strong associations between serum bilirubin level and stroke risk have been reported in previous observational studies. We showed that serum bilirubin level was associated with stroke in an ordinary least-squares analysis. However, using rs6742078 and the WGRS with 14 SNPs selected from our GWAS of serum bilirubin as instrument variables in the 2-stage least-squares Mendelian randomization analysis, we observed no causal relationship between serum bilirubin level and stroke risk. A previous Mendelian randomization study[16] showed an inverse association of bilirubin with cardiovascular disease risk factors such as body mass index or cholesterol. This study is in line with our study results, confirming that bilirubin-associated genes are not causal.

Bilirubin and Stroke Risk in Observational Studies

We confirmed the previous findings that elevated bilirubin levels were associated with reduction in the risk of stroke.[9–12] Also, there was statistically significant evidence that serum total bilirubin level was independently associated with cardioembolic stroke, so that the level can be used as a measure to diagnose that stroke subtype.[22] Moreover, another study proved that serum bilirubin levels were positivity associated with severity of acute ischemic stroke with higher adjusted odds ratio of severity in the top quartile of total bilirubin and direct bilirubin.[23] The association between bilirubin levels and stroke risk gradually decreased after adjusting for age, sex, systolic blood pressure, fasting serum glucose, total cholesterol, high-density lipoprotein-cholesterol, and smoking status, suggesting that the previous observational studies showing the association between bilirubin levels and stroke risk[9,10] might have been confounded by these variables. Moreover, the association may disappear after adjusting for unknown possible confounding variables, resulting in no evidence of association between bilirubin levels and stroke. Therefore, the effect of bilirubin levels on stroke remains inconclusive in conventional observational studies.

Genetic Polymorphisms and Bilirubin Levels

UDP-glucuronosyltransferases (UGTs) are a family of membrane-bound enzymes involved in the conjugation of endogenous such as bilirubin with UDP-glucuronic acid.[24] In this study, we confirmed the large contribution of UGT1A1 to human serum bilirubin levels. Three genetic variants (rs887829, rs6742078, and rs10929302) at the UGT1A1 locus were found to be associated with bilirubin levels. The strongest association was found between variant rs887829 (P=1.29E−82) and bilirubin level, which was strongly linked to the other 2 variants.

These results are consistent with previous GWAS. SNP rs887829 at UGT1A1 has the lowest P value in Europeans[25] and blacks.[26] SNP rs6742078 is the most significant SNP located in UGT1A1 in Europeans[27] and the third most significant variants in Korean populations.[28]

Consistent with the previous GWAS performed in Korean populations,[28] we found that 2 top SNPs (rs887829 and rs6742078) were strongly linked to each other. Interestingly, Johnson et al[27] have reported that rs6742078 is in strong linkage disequilibrium (r^2=0.88) with functional promoter polymorphism UGT1A1*28, indicating that UGT1A1*28 might be polymorphism associated with serum bilirubin levels in the UGT1A1 locus. Thus, the major association of the UGT1A1 promoter region might be derived from the functional promoter polymorphism of UGT1A1*28 in which the longer TATAA element can result in a 5-fold reduction in promoter activity. Bosma has found that mutations in this gene are

Table 3. Association of Serum Total Bilirubin With Stroke Risk Using a Mendelian Randomization Analysis

	Change in Bilirubin Per Risk Allele				Observational Multivariable Cox Proportional Hazard Model*		Mendelian Randomization Analysis	
	β	SE	F	*P* Value	HR	*P* Value	HR	*P* Value
UGT1A1 (rs6742078)								
Model 1	0.21	0.010	475	<0.0001	0.52 (0.40–0.67)	<0.0001	1.19 (0.44–3.25)	0.731
Model 2	0.22	0.009	381	<0.0001	0.61 (0.47–0.79)	0.0002	0.75 (0.28–2.01)	0.562
Model 3	0.22	0.009	171	<0.0001	0.65 (0.50–0.84)	0.001	0.71 (0.26–1.94)	0.501
Model 4	0.22	0.009	138	<0.0001	0.67 (0.51–0.87)	0.003	0.72 (0.26–2.01)	0.531
WGRS using 14 SNPs								
Model 1	0.44	0.015	866	<0.0001	0.54 (0.42–0.70)	<0.0001	0.63 (0.30–1.33)	0.229
Model 2	0.43	0.014	522	<0.0001	0.63 (0.49–0.83)	0.001	0.63 (0.30–1.35)	0.234
Model 3	0.43	0.014	233	<0.0001	0.67 (0.52–0.88)	0.004	0.63 (0.30–1.36)	0.240
Model 4	0.43	0.014	187	<0.0001	0.70 (0.53–0.91)	0.007	0.63 (0.30–1.36)	0.240

Model 1: crude model. Model 2: adjusted for age and sex. Model 3: model 2 plus additional adjustment for systolic blood pressure, fasting serum glucose, total cholesterol, and high-density lipoprotein-cholesterol. Model 4: model 3 plus additional adjustment for smoking status. HR indicates hazard ratio; SNP, single-nucleotide polymorphism; UGT1A1, uridine diphosphoglucuronyltransferase; and WGRS, weighted genetic risk score.

*Cox proportional hazard model that examined bilirubin and stroke.

responsible for Crigler–Najjar syndrome and mild unconjugated hyperbilirubinemia of Gilbert syndrome.[29]

Genetic Polymorphisms and Stroke Risk

Ekblom et al[30] have found that there is no evidence for a protective effect of the UGT1A1*28 polymorphism or bilirubin against stroke. These findings suggest that other factors influencing the risk of stroke might also affect bilirubin levels.[30] However, the latter finding is inconsistent with the results of the current study or previous studies.[15–17] The risk of stroke may not be associated with UGT1A1*28. Similarly, bilirubin levels may not be influenced by UGT1A1*28. The main purpose of Mendelian randomization is to determine whether bilirubin level elevated by UGT1A1*28 can causally affect stroke. Our findings suggest no evidence that genetically elevated bilirubin level is causally associated with stroke. To date, it is uncertain whether decreased total bilirubin levels pose an independent risk above and beyond the known risk factors.

One of main strengths of the current study lies in its case–cohort study design from a large prospective cohort study. Second, we incorporated multiple bilirubin-associated SNPs identified from GWAS to generate WGRS with greater power than any single variant in isolation. Finally, we performed multivariable Mendelian randomization analysis that allowed statistical adjustment for confounders.

However, our analyses must be interpreted in the context of the limitations of available data. First, we did not perform a replication analysis in an adequately powered second group. Therefore, more cohorts are needed to verify these results in other populations and other ethnicities in the future. Second, because of restricted data, we could not confirm the exact

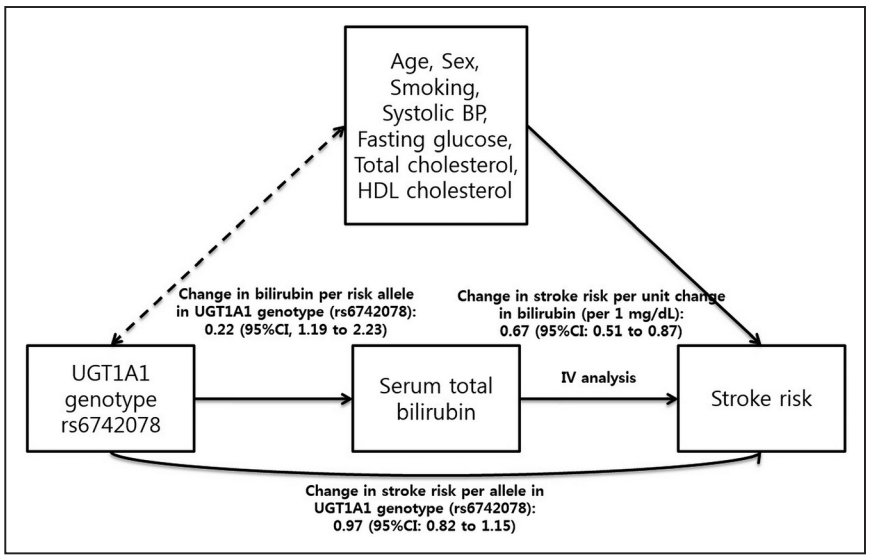

Figure 2. Mendelian randomization of bilirubin level and stroke risk from the Korean Cancer Prevention Study-II Biobank. Dotted line reflects that there was no association between confounding variables and UGT1A1. BP indicates blood pressure; CI, confidence interval; HDL, high-density lipoprotein; and IV, instrumental variable.

recurrent stroke in our analysis. Therefore, we did not further analyze the related bilirubin and recurrence of stroke. However, we conducted additional analysis between bilirubin and stroke subtype using Mendelian randomization. As a result, regardless of stroke subtypes, no causal association was found between bilirubin and stroke. Third, limitation of our study is the potential existence of pleiotropy.[31] Genetic variant used as IV in Mendelian randomization analysis is associated with multiple risk factors. The basic assumption in the analysis of Mendelian randomization is that IV should be associated with a single exposure variable. However, other unknown factors were associated with the IV.

All Mendelian randomization studies are confounded by linkage disequilibrium. Therefore, confounding by population stratification cannot be completely ruled out. We used the multidimensional-scaling method for population stratification analysis.[32] Because the cluster of our data using multidimensional-scaling was not separated, the matter of population stratification in our data does not need to be concerned. The majority of the Korean population is ethnically homogenous, minimizing a negative effect.[33] Despite these limitations, Mendelian randomization approach is a useful tool to assess the nature of observed associations between putative risk factors and disease. This approach overcomes some potential limitations of observational studies such as the presence of confounding variables.[34]

In summary, the present study demonstrated a significant association between SNPs at UGT1 locus and bilirubin levels. However, genetic evidence based on Mendelian randomization approaches suggests no causal effect of bilirubin levels on the development of stroke. Further studies are warranted to determine the association between bilirubin level and stroke risk.

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Disclosures

None.

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Bilirubin and Stroke Risk Using a Mendelian Randomization Design

Sun Ju Lee, Yon Ho Jee, Keum Ji Jung, Seri Hong, Eun Soon Shin and Sun Ha Jee

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SUPPLEMENTAL MATERIAL

Title: Bilirubin and Stroke Risk Using a Mendelian Randomization Design

Contents: Supplementary Table Ⅰ and Supplementary Figure Ⅰ- Ⅳ

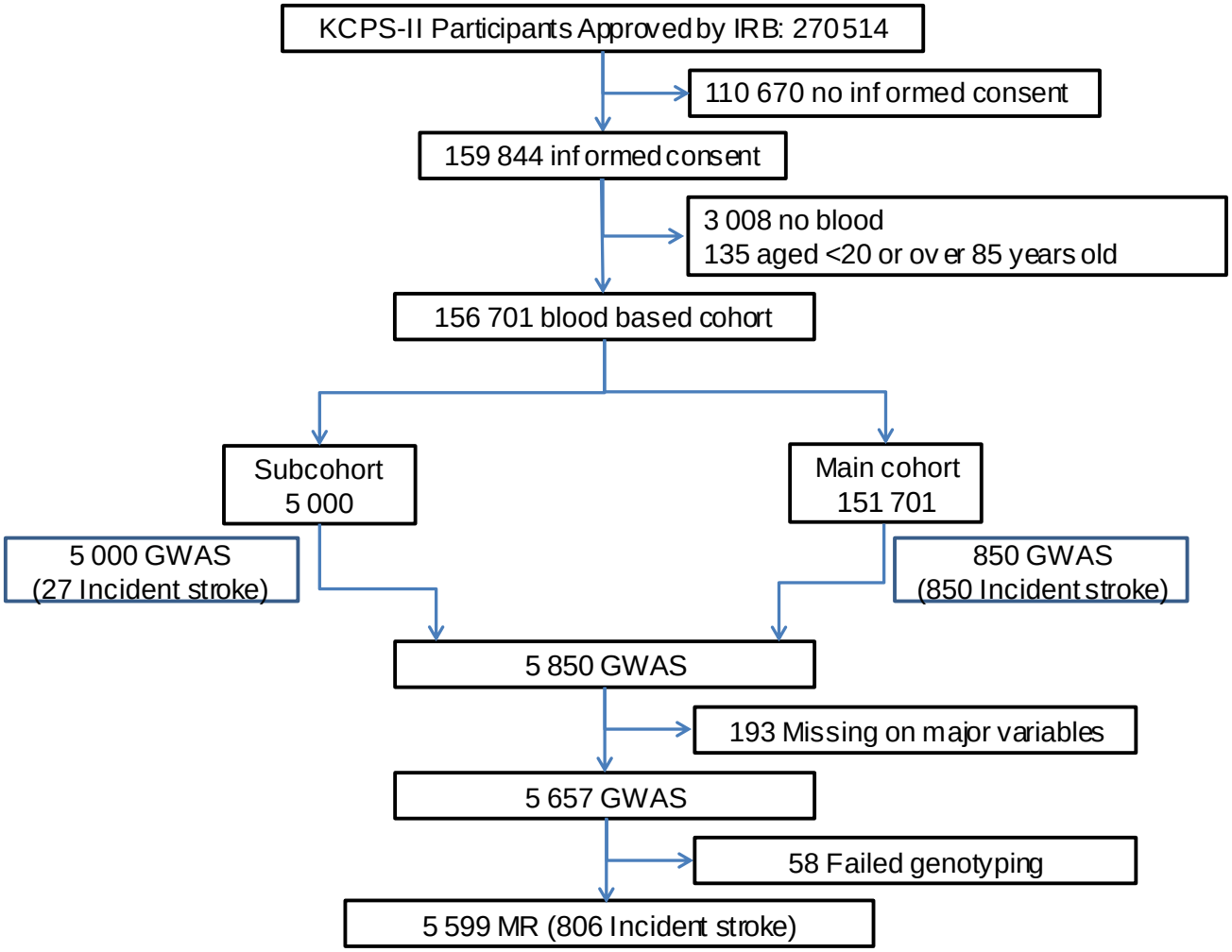
Supplementary Table Ⅰ. Association of top 14 SNPs with total serum bilirubin level based on Korean chip results

SNPID	chr	Closest Ref seq gene	A1	A2	MAF	Beta‡	p-value	KoGES*	Meta†
rs6742078	2	UGT1A1	T	G	0.118	0.207	2.33E-82	o	o
rs6759892	2	UGT1A6	T	G	0.244	0.146	1.77E-72	o	o
rs4261716	2	UGT1A7	T	G	0.244	0.145	1.09E-71	o	o
rs36075906	2	USP40	T	C	0.243	0.071	4.75E-18	x	x
rs4663580	2	DGKD	T	C	0.058	0.124	1.58E-16	x	x
rs2417940	12	SLCO1B3	T	C	0.216	0.067	1.02E-14	o	x
rs2741045	2	UGT1A_locus	T	C	0.015	0.181	1.17E-10	o	o
rs6758317	2	ATG16L1	T	C	0.125	0.062	7.01E-09	x	x
rs11562977	2	TRPM8	T	C	0.171	0.050	1.04E-07	x	x
rs12228798	12	SLCO1B3	T	C	0.165	0.050	1.60E-07	x	x
rs28898574	2	UGT1A_locus	A	G	0.013	0.155	3.54E-07	x	x
rs6431631	2	MROH2A	A	C	0.120	0.055	4.19E-07	o	x
rs16861329	3	ST6GAL1	T	C	0.167	0.047	4.36E-07	x	x
rs1042640	2	UGT1A8	C	G	0.119	0.054	8.51E-07	x	o

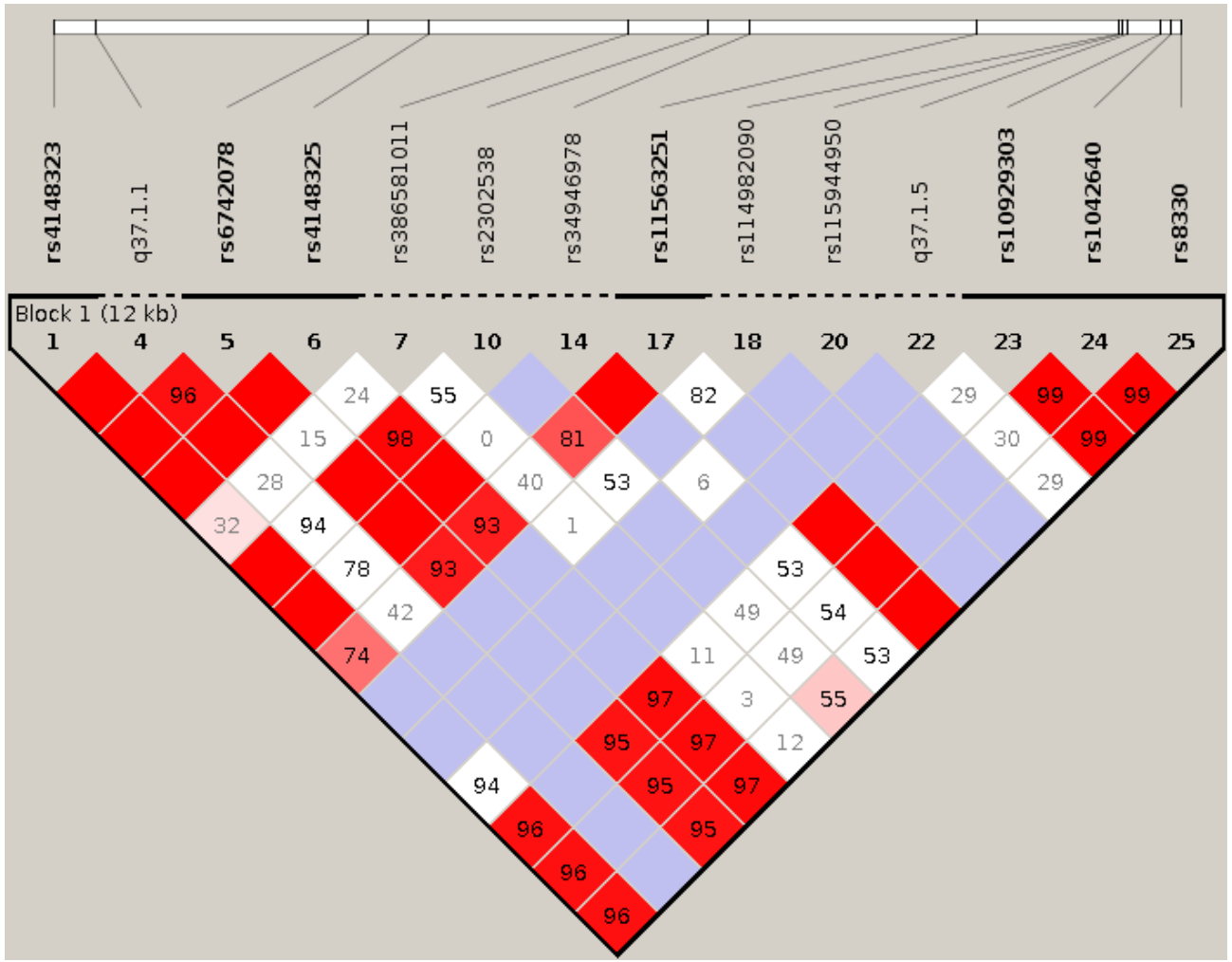
*SNPs in bold are SNPs for total bilirubin levels identified by *Kang* et al. [2010]

†The SNPs underlined are SNPs for total bilirubin levels observed in *Johnson* et al. [2009]

'o' denotes that SNP from Korean chip overlapped with SNPs reported from either KoGES or Meta-analysis. ‡ Indicated the effect of A1 allele compared with A2 allele.

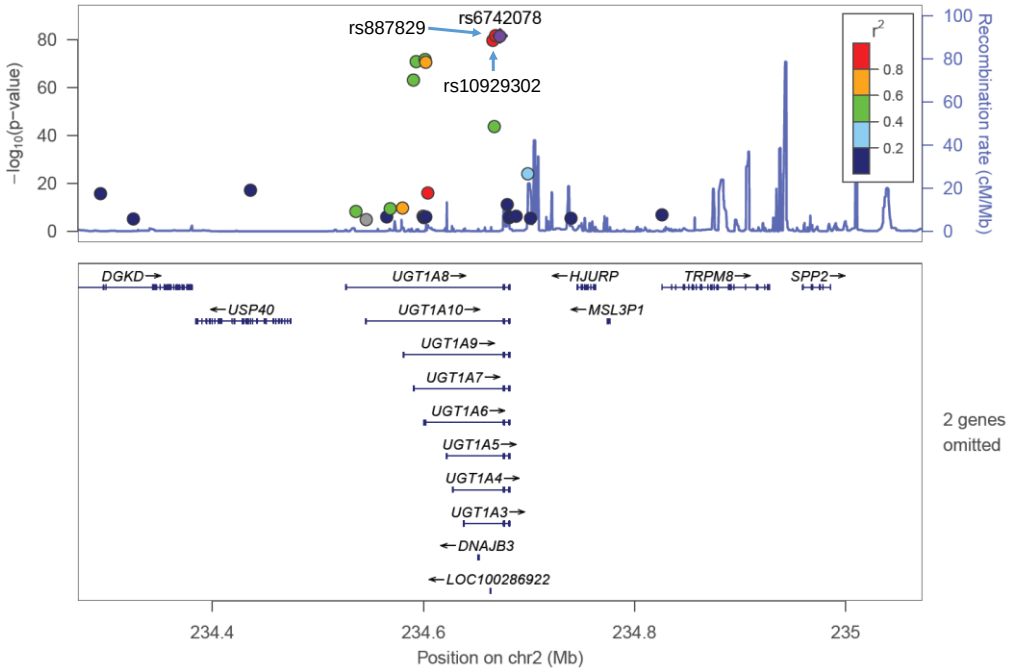


Supplementary Figure Ⅰ. Overall study design

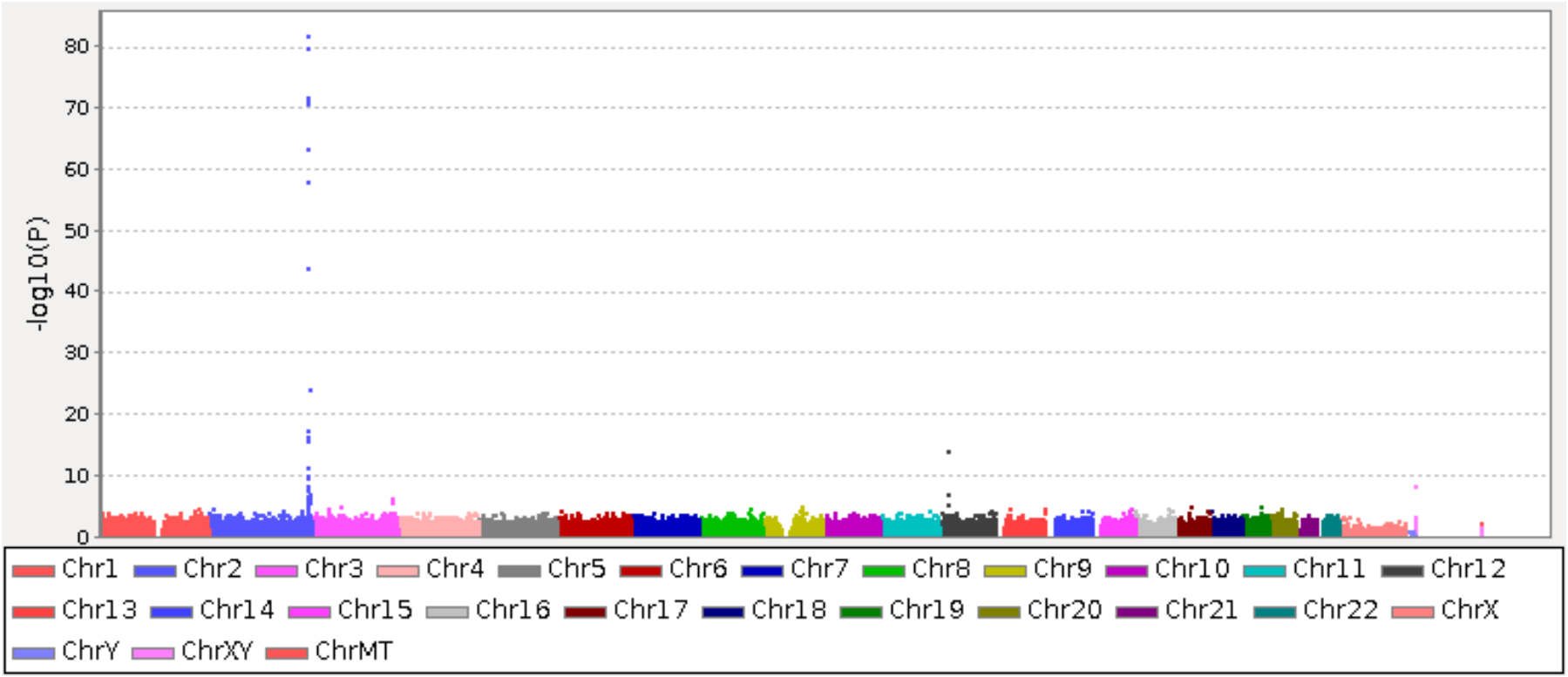


Supplementary Figure Ⅱ. Linkage disequilibrium as measured by r2 in UGT1A1 model in the study participants

White: r2=0. Shades of gray: 0<r2<1. Red: r2=1



Supplementary Figure Ⅲ. Significance of SNPs in UGT1A1 model in the study participants



Supplementary Figure Ⅳ. Summary of significance in the genome wide analysis of total serum bilirubin level in the study participants. The statistical significance [- log10 (p-value)] from a linear regression that included age and sex as covariates. Excluded SNPs that a minor allele frequency<0.01, a call rate<0.95 or a deviation from Hardy-Weinberg equilibrium (P<0.0001).