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Attempt to replicate the published osteoarthritis-associated genetic variants in the Newfoundland& Labrador Population

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Abstract

Objective: Over 200 genes have been reported to be associated with osteoarthritis (OA), but most of them have not been replicated in an independent sample. Using the newly collected cohort from a genetically isolated population - the Newfoundland and Labrador population, we attempted to replicate 105 previously reported OA-associated SNPs.

Methods: A case-control study design was utilized in this study. Patients undergoing total hip/knee joint replacements due to severe OA were collected as cases. A group of healthy individuals with no evidence of OA was used as control.105 SNPs were genotyped either by Sequenom iPLEX Gold method or Illumina GWAS genotyping platform. The cross-reference was performed on both methods in a subset of samples for genotyping quality control. A logistic regression model was used to test for associations between the SNPs and OA.

Results: A total of 126 cases and 348 healthy controls were included in the final analysis. OA Patients were on average 9 years older than healthy controls ($p<0.0001$), but there was no difference in BMI. We were unable to replicate the previously reported associations. Two SNPs, rs2294995 (*COL9A3*), and rs1049007 (*BMP2*) showed an association with $p<0.05$, but the significance did not survive the Bonferroni multiple testing correction.

Conclusion: A lack of replication might be due to study design, complexity of OA, method of OA ascertainment, populations studied, or false positives in the original publications. A study with larger sample is needed to confirm the two possible SNPs with OA.

Introduction

Osteoarthritis (OA) is the most common form of arthritis causing joint pain, stiffness, limited range of motion, joint deformity, and disability [1]. Its prevalence is on the rise due to population aging and increasing prevalence in obesity. Knee, hip, hand, spine, and foot are the most affected joints while the greatest public health burden results from hip and/or knee OA [2]. To date, there are no drugs available for rebuilding the damaged cartilage, nor is there a clear understanding of the pathogenesis of the disease. Total joint replacement therapy is the only choice for people with advanced OA. In the US alone, the total number of hip and/or knee joint replacement surgery due to OA is 350,000 each year [3], and the annual per person cost of those living with OA has been estimated to be around $5700 [4]. Arthritis, mostly OA, costs $128 million per year in medical care and other indirect expenses including those resulting from work limitation and loss of productivity in the US [5].

Although the etiology of OA is not completely understood, it is believed that OA is a multifactorial condition developing and progressing as a result of a combination of different environmental and genetic factors [6]. The main environmental risk factors include age, gender, obesity, previous joint injury, and joint mal-alignments [7]. Evidence suggests a strong genetic component to OA; from twin studies this genetic influence has been estimated to be between 40% and 65% for knee, hip [8], and hand OA [9], and first-degree relatives of individuals with spine, hand, hip, or polyarticular OA have a two- to three-fold increased risk of the disease [10,11].

To date, 9 Genome-Wide Association Studies (GWAS), along with a large number of candidate gene studies and linkage analyses, have been performed on OA. Although some OA-associated genes such as *GDF5* have been replicated in independent studies, the majority of the studied loci have inconsistent results [12]. This might be due to genetic heterogeneous nature of the disease or false positive findings in the initial studies. Genetically isolated populations have advantages for complex disease gene mapping because of their reduced genetic heterogeneity and extended LD [13]. The Newfoundland and Labrador population is a young isolated founder population with a high degree of both genetic and cultural homogeneity exhibiting extended linkage disequilibrium and an increased kinship coefficient, which provides a unique source for investigating both single gene diseases and complex traits [14,15]. Using this unique population, the aim of the present study was to replicate previously reported OA-associated genes.

Methods and Materials

Subjects

The study was part of the Newfoundland Osteoarthritis Study (NFOAS) that was initiated in 2011 and aimed at identifying

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novel genetic, epigenetic, and biochemical markers for OA. OA patients were recruited from those who underwent total knee or hip replacement surgery due to primary OA between Nov. 2011 to Jun. 2013 in St. Clare's Mercy Hospital and Health Science Centre General Hospital in St. John's, the capital city of Newfoundland and Labrador (NL) province of Canada. A group of healthy people who do not have any evidence of either knee or hip OA were used as controls. The controls were selected from previous genetic association study for type 2 diabetes and obesity. The controls completed a questionnaire regarding any ongoing symptoms, previous diagnosis and medication history. Each patient was also examined by a rheumatologist. The controls were selected for this study if they had no musculoskeletal pain, a prior diagnosis of osteoarthritis, were not taking acetaminophen or NSAIDs and had a normal physical examination, as it relates to the skeletal system. All cases and controls in this study were from NL. The study was approved by Health Research Ethics Authority (HREA) of Newfoundland and Labrador and a written consent was obtained from all the participants.

Height and weight measurements were obtained from patient's hospital medical record. Body mass index (BMI) was calculated as weight in kilogram divided by squared height in meters. Age was calculated for the time of surgery or visit date.

Genotyping

HuGE Navigator [16], a continuously updated database in human genome epidemiology, indexes all of the genetic association studies for a given disease or trait. There were 231 genes reported to be associated with OA as of June 2013. Due to limited budget, we chose to replicate all the SNPs that were reported in OA GWAS studies plus some SNPs from candidate studies.

Blood samples were obtained from all study participants and DNA was extracted by using the standard protocol. All the OA cases were genotyped by Sequenom iPLEX Gold method [17]. Briefly, 384-well plate chip on Sequenom using mass spectrometry was used for genotyping. Each multiplex PCR was done using 30ng of DNA (n=1: 1.25X PCR buffer Roche, 2mM MgCl2 Roche, 0.5M dNTPs, 0.11uM PCR primer pool oligos ordered from IDT, 0.15U/uL Roche FastStart) and the amplification was done following this cycling protocol: [95c 15min, 45x (95c 20sec, 58c 30sec, 72c 60sec), 72c 3min]. The SAP reaction was done to clean the PCR product and is followed by the extension reaction. After extension, the Salt Adduct Removal Step was run using 6mg of resin. The product was then spot on a Sequenom 384-well chip using a Nanodispenser, and loaded onto the Mass Spectrometer for reading.

All the controls were previously genotyped using Illumina HumanHap550-Duo BeadChip at Centrillion Biosciences at Palo Alto, California.

Cross-validation of genotyping quality was carried out on 31 controls that were genotyped by both Sequenom iPLEX Gold method and IlluminaHumanHap550-Duo BeadChip.

Statistical analysis

Distribution of age, gender, and BMI was examined and tested between OA cases and controls by either Chi-squared test or Student t-test wherever appropriate. Genotyping accuracy between two genotyping methods was evaluated by calculating genotype concordance rate in those subjects who were genotyped by both methods. Hardy-Weinberg Equilibrium (HWE) test was performed for each of the SNPs by exact Chi-squared test and removed in the subsequent analysis if p value <0.05. Chi-squared test was utilized to test the association between each of the SNPs and OA and logistic regression modeling was used to adjust for potential confounders including age, sex, and BMI. Significance level was set at alpha level of 0.0007 after correcting multiple testing with Bonferroni method. All analyses were done using STATA/SE 11.2 (Stata Corp, College Station, Texas, USA) except for HWE test which was performed by PLINK version 1.07 [18].

Results

A total of 126 OA cases and 348 controls were included in this study. Among OA cases, 50 were males and 76 were females, 42 had total hip replacement and 84 had total knee replacement due to primary OA. Controls (146 males and 202 females) are healthy subjects who did not have evidence of joint OA. Characteristics of the study participants are presented in Table 1. OA cases were significantly older than healthy controls, and had a slightly higher BMI, which was not significant.

105 SNPs located in 71 genes were genotyped by Sequenom iPLEX Gold method for all cases and 52 healthy controls. 69 of these 105 SNPs were included in Illumina HumanHap550-Duo BeadChip and genotyped for all controls and therefore the subsequent analysis was focused on these 69 SNPs. 31 healthy controls were genotyped by both methods and were used to cross-validate the genotyping quality and accuracy between two genotyping methods. The genotype concordance rates for all 69 SNPs were 100%, indicating the comparability and accuracy of the two genotyping methods.

Two SNPs were deviated from HWE and excluded from the subsequent analyses. Table 2 presents the results of the univariable and multivariable analysis of the association of each of the SNPs between OA and healthy controls. We found that minor alleles of rs2294995 located in *COL9A3* and rs1049007 located in *BMP2* were associated with one-third reduced risk for OA, but the significance did not survive the Bonferroni correction for multiple testing.

In the joint specific analyses, three SNPs were associated with knee OA [*COL9A3* rs2294995 (OR:0.51, 95%CI: 0.32-0.8, P=0.004), *HFE* rs1799945 (OR:1.67, 95%CI: 1.06-2.6, P=0.025), and *PACE4* rs900414 (OR:0.63, 95%CI: 0.41-0.97, P=0.036)], and five SNPs with hip OA [*EDG2* rs10980705 (OR:0.4, 95%CI: 0.19-0.89, P=0.024), *IL1RN* rs315952 (OR:0.5, 95%CI: 0.26-0.93, P=0.03), *BMP2* rs1049007 (OR:0.56, 95%CI: 0.33-0.95, P=0.03), *IL1RN* rs9005 (OR:1.69, 95%CI: 1.03-2.78, P=0.038), and *COX2* rs5277 (OR:1.74, 95%CI: 1-3.01,

Table 1: Descriptive statistics of the study population*.

Variables	OA (n=126)	Healthy Controls (n=348)	P-value¶
Age (yr)	63.8±0.87	54.8±0.81	<0.0001
BMI (kg/m²)	33.05±0.67	31.7±0.39	0.07
Sex (% female)	60.3%	58.1%	0.65

* Figures stand for Mean ± SE unless stated. ¶P-value for comparison between OA cases and healthy controls.

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Table 2: Results of the association test for each SNP between OA cases and healthy controls.*

		Risk Allele	Univariable		Multivariable		Risk allele Freq.	
Gene	SNP		OR (95% CI)	P-value	OR (95% CI)	P-value	Controls	Cases
BMP2	rs1049007 (A/G)	A	0.69 (0.5 - 0.94)	0.021*	0.64 (0.45 - 0.9)	0.011*	0.43	0.35
COL9A3	rs2294995 (A/G)	A	0.64 (0.46 - 0.9)	0.010*	0.65 (0.46 - 0.94)	0.021*	0.33	0.24
TLR8	rs5744080 (T/C)	T	1.2 (0.94-1.53)	0.130	1.31 (1.00-1.71)	0.040*	0.41	0.48
PACE4	rs900414 (A/G)	A	0.76 (0.54 - 1.06)	0.109	0.7 (0.48 - 1.01)	0.060	0.31	0.26
MTHFR	rs1801133 (A/G)	A	1.32 (0.98 - 1.78)	0.068	1.36 (0.98 - 1.89)	0.065	0.31	0.38
ADAM12	rs1871054 (A/G)	G	1.2 (0.9 - 1.61)	0.219	1.33 (0.96 - 1.85)	0.085	0.46	0.50
IL18	rs1946518 (A/C)	A	1.15 (0.85 - 1.55)	0.363	1.33 (0.95 - 1.85)	0.097	0.40	0.44
DIO3	rs945006 (A/C)	C	0.81 (0.52 - 1.26)	0.356	0.69 (0.43 - 1.13)	0.139	0.15	0.12
ADAM12	rs3740199 (C/G)	C	1.26 (0.95 - 1.68)	0.107	1.25 (0.91 - 1.7)	0.163	0.47	0.53
ADAM12	rs1044122 (A/G)	A	1.21 (0.88 - 1.67)	0.234	1.26 (0.89 - 1.78)	0.196	0.23	0.27
IL4R	rs2234895 (C/T)	T	0.7 (0.38 - 1.28)	0.244	0.65 (0.34 - 1.25)	0.199	0.08	0.06
PTGS2/COX-2	rs20417 (C/G)	G	1.23 (0.82 - 1.83)	0.319	1.33 (0.86 - 2.06)	0.201	0.15	0.18
ASTN2	rs4836732 (C/T)	C	1.19 (0.89 - 1.58)	0.243	1.22 (0.9 - 1.66)	0.208	0.48	0.53
HFE	rs1799945 (C/G)	C	1.3 (0.89 - 1.91)	0.178	1.3 (0.86 - 1.96)	0.214	0.15	0.19
DIO2	rs225014 (C/T)	T	1.27 (0.95 - 1.69)	0.108	1.22 (0.89 - 1.67)	0.214	0.36	0.42
IL6	rs1800797 (A/G)	A	0.78 (0.58 - 1.05)	0.098	0.82 (0.6 - 1.13)	0.228	0.41	0.35
IL6	rs1800796 (G/C)	G	0.7 (0.35-1.38)	0.310	0.6 (0.32-1.31)	0.230	0.05	0.04
GLTBD1	rs6976 (C/T)	C	0.89 (0.66 - 1.2)	0.447	0.82 (0.6 - 1.13)	0.230	0.41	0.38
IL1B	rs1143633 (A/G)	A	1.18 (0.88 - 1.58)	0.272	1.2 (0.87 - 1.64)	0.260	0.34	0.38
COX2	rs5277 (C/G)	G	1.14 (0.77 - 1.67)	0.518	1.26 (0.82 - 1.93)	0.285	0.15	0.17
IL1B	rs1143634 (C/T)	T	0.88 (0.63 - 1.25)	0.485	0.83 (0.57 - 1.19)	0.305	0.23	0.21
VDR	rs731236 (C/T)	T	1.16 (0.87 - 1.55)	0.302	1.17 (0.85 - 1.6)	0.329	0.42	0.46
IL1RN	rs315952 (A/G)	G	0.83 (0.59 - 1.15)	0.255	0.84 (0.59 - 1.19)	0.332	0.28	0.25
ESR1/alpha	rs2234693 (G/A)	G	0.8 (0.61-1.06)	0.130	0.87 (0.64-1.18)	0.370	0.46	0.40
ANP32A	rs7164503 (A/G)	G	0.92 (0.57 - 1.49)	0.737	0.79 (0.47 - 1.33)	0.375	0.11	0.1
HLA class II/III	rs7775228 (A/G)	G	1.2 (0.77 - 1.86)	0.414	1.22 (0.75 - 1.99)	0.417	0.11	0.13
DIO2	rs12885300 (A/G)	A	0.82 (0.6 - 1.12)	0.222	0.87 (0.62 - 1.22)	0.421	0.36	0.32
EDG2	rs10980705 (A/G)	G	0.82 (0.58 - 1.16)	0.256	0.86 (0.59 - 1.25)	0.427	0.23	0.19
CHST11	rs835487 (A/G)	A	0.84 (0.62 - 1.15)	0.280	0.87 (0.62 - 1.22)	0.431	0.36	0.32
IL1A	rs1800587 (A/G)	A	0.94 (0.69 - 1.3)	0.727	0.88 (0.62 - 1.25)	0.473	0.29	0.28
Matrilin3	rs8176070 (C/T)	C	0.95 (0.7 - 1.29)	0.755	0.89 (0.63 - 1.25)	0.496	0.35	0.34
SUPT3H/CDC5L	rs10948172 (A/G)	G	0.87 (0.63 - 1.2)	0.406	0.89 (0.63 - 1.26)	0.504	0.31	0.28
pTGS2/ PLA2G4A	rs4140564 (C/T)	C	1.56 (0.87 - 2.81)	0.137	1.24 (0.65 - 2.35)	0.515	0.05	0.08
LRCH1	rs912428 (A/G)	A	1.06 (0.74 - 1.52)	0.745	1.13 (0.77 - 1.68)	0.532	0.20	0.21
IL18R1/IL18RAP	rs2287037 (C/T)	T	1.05 (0.78 - 1.42)	0.743	1.11 (0.8 - 1.54)	0.539	0.35	0.36
IL1B	rs16944 (A/G)	A	1.03 (0.76 - 1.4)	0.835	1.11 (0.79 - 1.55)	0.543	0.34	0.35
TLR-3	rs3775296 (G/T)	T	1.03 (0.72 - 1.48)	0.868	1.12 (0.77 - 1.65)	0.549	0.18	0.18
IL4	rs2070874 (C/T)	T	0.89 (0.58 - 1.37)	0.598	0.87 (0.53 - 1.42)	0.566	0.13	0.12
MCF2L	rs11842874 (A/G)	G	0.87 (0.52 - 1.46)	0.592	0.86 (0.5 - 1.48)	0.580	0.09	0.08
CALM2	rs10153674 (A/G)	A	0.98 (0.65 - 1.48)	0.917	0.88 (0.56 - 1.39)	0.587	0.15	0.14
A2BP1	rs716508 (C/T)	T	1.12 (0.82 - 1.53)	0.461	1.09 (0.77 - 1.54)	0.615	0.31	0.34
FTO	rs8044769 (C/T)	C	0.83 (0.62 - 1.11)	0.208	0.92 (0.67 - 1.27)	0.621	0.48	0.43
HLA class II/III	rs10947262 (A/G)	A	1.2 (0.68 - 2.13)	0.530	1.16 (0.63 - 2.15)	0.632	0.06	0.07
TXNDC3	rs4720262 (C/T)	T	0.88 (0.64 - 1.22)	0.452	0.92 (0.65 - 1.3)	0.634	0.28	0.26
ADAMTS5	rs2380585 (A/G)	A	0.93 (0.65 - 1.34)	0.701	0.91 (0.62 - 1.36)	0.658	0.21	0.2
TLR-9	rs187084 (A/G)	G	1.01 (0.76 - 1.35)	0.928	0.93 (0.68 - 1.28)	0.673	0.41	0.41

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IL1RN	rs419598 (A/G)	G	1.02 (0.74 - 1.39)	0.922	0.93 (0.66 - 1.31)	0.691	0.30	0.3
GDF5/ASPN	rs13301537 (C/T)	C	1.03 (0.75 - 1.42)	0.840	1.07 (0.76 - 1.52)	0.698	0.28	0.28
ESR2/beta	rs1256031 (C/T)	T	1.16 (0.86 - 1.56)	0.335	1.06 (0.77 - 1.47)	0.717	0.45	0.48
TRPV1	rs8065080 (A/G)	A	0.98 (0.72 - 1.32)	0.875	0.94 (0.68 - 1.31)	0.724	0.38	0.38
TNF-A	rs1800629 (A/G)	A	0.94 (0.64 - 1.39)	0.765	0.93 (0.61 - 1.41)	0.738	0.18	0.17
IL4R	rs1805013 (A/G)	A	1.09 (0.49 - 2.42)	0.838	0.87 (0.36 - 2.09)	0.760	0.03	0.04
AACT	rs4934 (C/T)	T	0.91 (0.68 - 1.21)	0.501	0.96 (0.7 - 1.31)	0.800	0.48	0.46
IL1R1	rs1465325 (C/T)	C	0.95 (0.65 - 1.4)	0.808	0.95 (0.62 - 1.44)	0.802	0.17	0.17
LEP	rs2060715 (A/G)	A	0.85 (0.63 - 1.13)	0.264	0.96 (0.7 - 1.32)	0.808	0.48	0.44
TP63	rs12107036 (A/G)	A	1.04 (0.78 - 1.38)	0.795	1.03 (0.76 - 1.42)	0.832	0.48	0.49
ESR1/alpha	rs2228480 (C/T)	T	0.89 (0.62 - 1.27)	0.513	0.96 (0.65 - 1.41)	0.835	0.22	0.2
NFKB1A	rs8904 (C/T)	C	0.96 (0.7 - 1.33)	0.820	0.97 (0.69 - 1.38)	0.871	0.31	0.31
HAPLN1	rs179851 (C/T)	C	0.92 (0.67 - 1.25)	0.588	1.03 (0.74 - 1.44)	0.873	0.32	0.31
LEP	rs12706832 (C/T)	T	0.86 (0.64 - 1.15)	0.303	0.97 (0.71 - 1.34)	0.875	0.47	0.44
IL1RN	rs9005 (C/T)	C	1.09 (0.8 - 1.49)	0.577	1.02 (0.73 - 1.43)	0.909	0.31	0.33
RAGE	rs2070600 (A/G)	A	1.39 (0.77 - 2.5)	0.280	1.04 (0.52 - 2.07)	0.921	0.05	0.07
COL2A1	rs2070739 (C/T)	T	0.92 (0.57 - 1.47)	0.720	0.98 (0.59 - 1.62)	0.922	0.11	0.1
UQCC	rs6087704 (A/G)	G	1.02 (0.75 - 1.37)	0.911	1.02 (0.73 - 1.41)	0.926	0.35	0.35
WISP1	rs2929970 (C/T)	C	0.99 (0.74 - 1.32)	0.952	0.99 (0.72 - 1.36)	0.958	0.49	0.49
ESR2/beta	rs1256049 (C/T)	T	0.69 (0.28 - 1.69)	0.418	0.98 (0.38 - 2.55)	0.973	0.03	0.02
GDF5	rs224329 (C/T)	T	1.01 (0.74 - 1.36)	0.964	1.00 (0.72 - 1.39)	1.000	0.34	0.34

P=0.048)], but all the significance did not survive the Bonferroni multiple testing correction.

Discussion

The study is one of the few efforts on the replication of the previously identified genetic variants in OA. Advantages of the current study include using a genetically homogeneous population and advanced OA cases. The reduced genetic heterogeneity and using advanced OA cases are believed to increase study power in genetic association test.

Although we were unable to replicate any of the SNPs that were previously reported to be associated with OA with a stringent significance level, two SNPs showed a potential to be associated with OA. One is rs2292995 located in *COL9A3,* which is a structure gene of articular cartilage. The gene has been associated with multiple epiphyseal dysplasia type 3 [19], and primary OA [20]. The second SNP is rs1049007 located in *BMP2*, which is involved in TGF-β signalling pathway that has been implicated in OA [21]. Although the associations for these SNPs did not reach the Bonferroni corrected significance level, it warrants further investigation in a large sample.

SNP rs143383 located in *GDF5* gene is by far the most replicated SNP that is associated with OA. It was initially discovered in Asian population [22], followed by replication studies in European populations [23], and several meta-analyses confirmation [24,25]. The SNP was not included in the Illumina GWAS genotyping platform we used but we have a proxy SNP rs224329 which has r^2=0.92 with rs143383. However, we could not detect any association between rs224329 and OA. Small sample size in the current study might be a possible explanation, but the results are the same as in the recent large GWAS on OA performed in the UK population [26], in which over 7410 OA cases and 11,009 unrelated population controls were included. Given the genetic similarity between Newfoundland and Labrador population and British population, the results may suggest the *GDF5*-OA association is population specific.

Data on replication of previously reported OA-associated genes are limited. GOAL study [27] utilized large number of symptomatic radiographic knee or hip OA and controls to replicate 68 variants in 12 genes including *IL1A, IL1B, IL1RN, IL4R, IL6, COL2A1, ADAM12, ASPN, IGF1, TGFB1, ESR1* and *VDR*, but they did not replicate any of these associations. All the genetic variants they studied were included in our study. Similarly, these SNPs were not associated with OA in our sample; neither did in the large genome-wide meta-analysis [28].

Failure in the replication of previously reported genetic associations is common to the point that only less than 5% of the reported associations could be replicated in an independent study [29], which along with other factors has raised criticism on the usefulness of association studies [30]. The reasons for the failures are numerous: initially reported associations might be false positives, or replications might be false negative findings resulting from a biased sampling, hidden or uncorrected population stratification, small sample sizes. The association might only hold true for the population in which the association was reported due to the differences and specificity of LD patterns across different populations. The complexity of multifactorial traits is another issue: the effect of multiple genetic variants and heterogeneity means that the presence of all of the risk alleles together may not be required for the disease to develop; therefore, distribution of risk alleles in two groups of cases from different or even same population might be different from each other. All these factors are aggravated in the study of OA due to the age dependency and the lack of a unique method for the ascertainment of the study subjects used by different investigators.

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The above-mentioned reasons could partially explain why we were not able to replicate the majority of the SNPs in our study. In addition, our study has been limited by a number of factors: sample size is relatively small. Given our sample size and assuming minor allele frequency of 35% in controls, we have 80% power to only detect an OR of 1.8 or above at an alpha level of 0.05. The minimum detectable OR would be 2.5 if we define the significance level at 0.0007 after taking into account of multiple testing. However, the study power is maximized by the optimal case-control ratio and using extreme severe OA cases. We recently found that NL population has slightly subtle population stratification (not published data), which might lead to false negatives.

In conclusion, we were unable to replicate the previously reported OA-associated genes, but two SNPs showed suggestive associations with plausible biological mechanism. Further studies are needed to confirm the results.

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