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ORIGINAL ARTICLE

Causal relationship between body mass index and fetuin-A level in the asian population: a bidirectional mendelian randomization study

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Summary

Objective Fetuin-A is associated with body mass index (BMI) as well as components of the metabolic syndrome. However, it is unclear if fetuin-A affects BMI or the other way around. We therefore assessed the causal association between fetuin-A and BMI or *vice versa*, utilizing a bidirectional Mendelian randomization approach.

Design and Methods This was a study of 2558 subjects from the Electricity Generating Authority of Thailand (EGAT) cohort. Two polymorphisms, that is, *rs2248690* in the *alpha2-Hereman-Schmid glycoprotein (AHSG)* gene and *rs9939609* in the fat mass and obesity-associated *(FTO)* gene were genotyped. Bidirectional causal models were constructed using a two-stage least-square instrumental variable (IV) regression. First, *rs2248690* locus was used as the instrumental variable for the effect of circulating fetuin-A on BMI, and then, the *FTO rs9939609* locus was used as the instrumental variable for the effect of BMI on circulating fetuin-A.

Results Among the 2558 subjects, the prevalence of the minor *AHSG (T)* and *FTO (A)* alleles was 17·9% and 22·1%, respectively. The *AHSG rs2248690* locus was highly related to serum fetuin-A levels ($P < 0{\cdot}001$). Likewise, the *FTO rs9939609* locus and BMI were highly associated ($P < 0{\cdot}001$). Mendelian randomization analyses showed that circulating fetuin-A, instrumented by the *AHSG rs2248690* locus, was associated with BMI (coefficient = 2·26; 95% CI: 0·39, 4·12). In contrast, BMI, instrumented by the *FTO rs9939609* locus, was not associated with circulating fetuin-A (coefficient = 0·0007; 95% CI: −0·0242, 0·0256).

Conclusion Our findings suggest a causal association leading from circulating fetuin-A to BMI. There was no evidence of reverse causality from BMI to fetuin-A.

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Fetuin-A is a multifunctional protein of hepatic origin. With regard to glucose and energy metabolism, fetuin-A inhibits insulin receptor autophosphorylation and tyrosine kinase activity.[1] Association studies in humans have found a relationship between circulating fetuin-A levels and BMI, components of the metabolic syndrome,[2] and liver fat content.[3] Nevertheless, the causal role of fetuin-A in obesity or *vice versa* is uncertain. Although no effect of weight reduction on fetuin-A was found in obese women,[4] weight loss resulted in reduced levels of fetuin-A in obese children.[5] Moreover, the markedly elevated levels of fetuin-A in morbidly obese subjects decreased after weight loss induced by gastric bypass surgery.[6] On the other hand, fetuin-A-null mice are protected against obesity associated with ageing,[7] and high baseline fetuin-A is associated with marked increase in visceral adipose tissue over 5 years in older persons. [8]

Genetic variation in the fat mass and obesity-associated *FTO rs9939609* ($T > A$) variant, located on 16q12·2 (OMIM # 612 460), has been identified from genome-wide association studies as a determinant of adiposity in children and adults,[9] and its causal role on bone mineral density has been reported.[10] Likewise, the *alpha2-Hereman-Schmid glycoprotein (ASHG)* at *rs2248690* ($A > T$) variant, located on 3q27·3 (OMIM #

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138 680), codes for the fetuin-A protein and has been found to account for a large part of the variation in circulating fetuin-A levels.[11] These genetic variations provide an opportunity to conduct a Mendelian randomization study to clarify the causal relationship between fetuin-A and obesity. The aim was to determine the direction of causal relationships, fetuin-A and obesity or *vice versa*, utilizing the bidirectional Mendelian randomization approach.[12,13]

Materials and methods

This cross-sectional study used baseline data from the Electricity Generating Authority of Thailand (EGAT 3) cohort and was started in 2009, by recruiting subjects aged 24–54 from the headquarters of EGAT in the Bangkok metropolitan area.[14] The cohort was designed to assess risk factors for cardiovascular diseases, psychological distress, health status, functional status and health-related quality of life. Subjects are followed up every 5 years and the next follow-up will be in 2014. The study was approved by the Institutional Review Board of the Faculty of Medicine at Ramathibodi Hospital. All subjects gave informed consent for genetic testing. Data were collected using a self-administered questionnaire, physical examination, electrocardiography, chest radiography and blood tests.

Measurements of fetuin-A level and body mass index

Serum fetuin-A level was measured by sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN, USA). Precisions of intra- and interassays were 4·9% and 7·3%, respectively. Weight and height were measured using standard techniques. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$.

Genotyping

Genomic DNA was isolated from peripheral blood leucocytes using a standard phenol–chloroform method. The *AHSG (rs2248690)* and *FTO (rs9939609*) single polymorphisms (SNP) were genotyped using a TaqMan assay with allele-specific probes on the ABI Prism 7500 Real-time PCR system (Applied Biosystems, Foster City, CA). The genotyping call rate was higher than 99%. When the SNP calling was in doubt, direct sequencing was used to provide the correct genotypes.

Statistical analysis

Data were described as mean and frequency for continuous and categorical data, respectively. The genotype frequencies for *AHSG and FTO* SNPs observed the Hardy–Weinberg equilibrium (HWE) rule using the exact test. Relationships between these SNPs and variables were assessed using linear regression and chi-squared tests for continuous and categorical data, respectively.

Bidirectional causal equations, as described by the directed acyclic graphs[13] in Fig. 1a–b, were constructed using the Mendelian randomization approach[12] with a two-stage least-square instrumental variable (IV) regression. For model A (*AHSG*, circulating fetuin-A level and BMI), the *rs2248690* SNP was considered as the IV, circulating fetuin-A level was considered as the intermediate phenotype and BMI was the outcome of interest.[13,15] As circulating fetuin-A was quite skewed, it was transformed using a natural log scale for all analyses. For model B (*FTO*, BMI and circulating fetuin-A level), *FTO* at *rs9939609* SNP was the IV, BMI was an intermediate phenotype and circulating fetuin-A level was the outcome of interest. For both models A and B, the two-stage analysis was carried out as follows[13]: first, the association between the IV polymorphism and the intermediate phenotype was assessed. The predicted phenotype value was then estimated from the first equation. In the first-stage regression, an *F* statistic (hereafter called *F*-First) was applied to assess whether the SNP (i.e. *AHSG at rs2248690* or *FTO at rs9939609)* qualified as the IV (i.e. *F*-First > 10).[16] Second, the outcome variable was fitted onto the predicted phenotype value. The two-stage models were also adjusted for possible confounders, which might be associated with either intermediate phenotype or outcome. These confounders included age, gender, serum glucose, serum triglyceride and serum alanine aminotransferase (ALT).

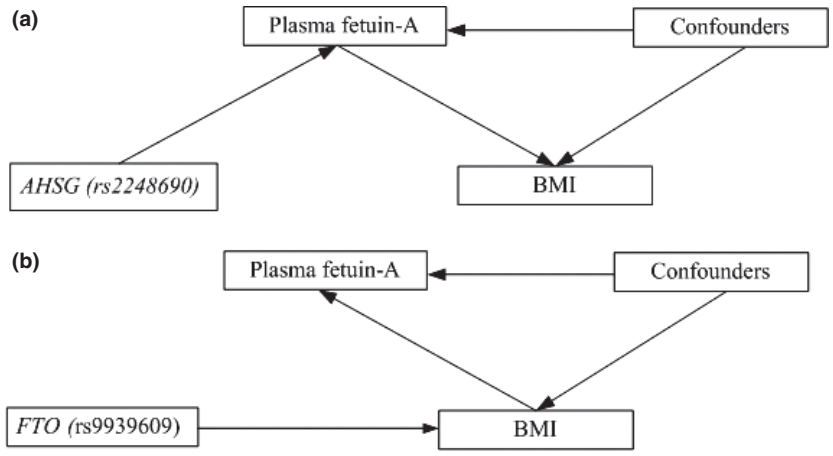

Fig. 1 Directed acyclic graphs for (a) the effects of plasma fetuin-A level on BMI using *AHSG (rs2248690)* as the instrumental variable and (b) the effects of BMI on plasma fetuin-A level using *FTO*(rs9939609) as the instrumental variable.

Two ordinary least-square (OLS) linear regression models were also constructed in one stage. The first model had its outcome variable as BMI and independent variables as *rs2248690* SNP and circulating fetuin-A, whereas the second model used circulating fetuin-A as the outcome and *rs9939609* SNP and BMI as independent variables. Results of the IV and OLS analyses were then compared using the Durbin-Wu-Hausman statistic. All analyses were performed using STATA version 12.0. A *P*-value of <0·05 was considered statistically significant.

Results

Two thousand five hundred and ninety-two subjects were enrolled, of which 2558 subjects were genotyped for *rs2248690* and *rs9939609* SNPs. Characteristics of these subjects have been described in Table 1. The mean age was 40·2 (SD = 7·1) years and most of them were men (73·5%). The average serum glucose and triglyceride levels were 5·2 (SD = 1·4) and 1·5 (SD = 1·0) mmol/l, respectively. The average BMI and serum fetuin-A level were 24·0 (SD = 3·7) kg/m^2 and 559·2 (SD = 111·5) mg/l, respectively. Results of the bidirectional Mendelian randomization analyses were as follows.

Causal model A: Fetuin-A is causally related to BMI (AHSG–>*fetuin-A*–>*BMI)*

The prevalence of the minor *T* allele for the *rs2248690* SNP was 17·9% (95% CI: 16·8%, 19·1%). Genotype frequencies were 69 (2·7%), 641 (25·1%) and 1848 (72·2%) for *TT*, *AT* and *AA*, respectively. The genotype distribution did not deviate from the Hardy–Weinberg equilibrium ($P = 0·145$).

There was an association between fetuin-A levels and the *AHSG rs2248690* SNP (shown in Table 2). Fetuin-A levels were lower in the *TT* and *AT* genotypes than the *AA* genotype with mean values of 413·9, 501·9 and 584·8 mg/l, respectively ($P < 0·001$). On the other hand, our results did not detect an association between the *AHSG rs2248690* SNP and BMI, although there was a graded response in BMI across genotypes (*TT*: 23·5, *AT*: 23·8 and *AA*: 24·1 kg/m^2, respectively ($P = 0·067$)).

The association between BMI and fetuin-A can be confounded by variables such as age, gender and phenotype related to the metabolic syndrome. To be a valid IV, the *AHSG* SNP should not be associated with these potential confounders. Therefore, associations between these characteristics and the *AHSG* were assessed. As shown in Table 3, none of these potential confounders were associated with the *AHSG rs2248690* SNP.

The one-stage OLS regression model was constructed by having BMI as the outcome, with both ln(Fetuin-A) and the *AHSG rs2248690* genotypes as independent variables with adjustment for other covariates (Table 4, Model A). Factors independently associated with BMI included gender, fasting plasma glucose, serum triglyceride levels and serum alanine transferase (ALT). Ln(fetuin-A) was independently associated with BMI with a coefficient of 1·16 (95% CI: 0·29, 2·04; $P = 0·009$) after adjusting for these confounders. This could be interpreted that every increase in ln (fetuin-A) would result in an increase in BMI by 1·16 kg/m^2. The OLS model did not show direct association between the *rs2248690* SNP and BMI ($P = 0·607$) after adjusting for fetuin-A and other confounders.

To address causal model A, an IV regression analysis was carried out by treating BMI as the outcome, ln(fetuin-A) as the intermediate phenotype and the *AHSG rs2248690* SNP as the IV (Table 4, Model A). The *rs2248690* SNP was a very strong IV of circulating ln(fetuin-A) with an *F*-First of 187·33 ($P < 0·001$). The ln(fetuin-A) as predicted by the *rs2248690* IV (see Fig. 1a) was strongly correlated with BMI with a coefficient of 2·26 (95% CI: 0·39, 4·12, $P = 0·018$), suggesting that an increase in predicted ln(fetunin-A) 1 mg/l would increase BMI by 2·26 kg/m^2. Although this IV estimate of effect was much higher than the OLS estimate, it was not significantly different (Durbin-Wu-Hausman test = 0·846, $P = 0·358$).

Causal model B: BMI is causally related to fetuin-A (FTO–>*BMI*–>*fetuin-A)*

The estimated minor *A* allele prevalence for the FTO *rs9939609* variant was 22·1% (95% CI: 20·9%, 23·3%). Genotype frequencies for *AA*, *TA* and *TT* were 131 (5·1%), 869 (34·0%) and 1558 (60·9%), respectively, and the distribution conformed to Hardy–Weinberg equilibrium ($P = 0·46$).

There was an association between the *rs9939609* variant and BMI, with higher BMI in the *AA* and the *TA* genotypes than the *TT* genotype ($P < 0·001$, see Table 2). The FTO *rs9939609* genotypes were not associated with circulating fetuin-A levels ($P = 0·245$).

As shown in Table 3, none of the potential confounders of the association between the *FTO* SNP and serum fetuin-A, including age, gender and phenotypes related to the metabolic syndrome, were associated with the *FTO rs9939609* SNP. This fulfilled one of the requirements for a valid IV for the Mendelian randomization analysis.

The one-stage OLS regression model was constructed by having ln(fetuin-A) as the outcome, and both BMI and the FTO

Table 1. Characteristics of the study population

Characteristics	Mean	SD
Number of subjects	2558	
Age	40·6	7·1
Men*	1887	73·5
Cholesterol, mM	56·1	10·2
Triglyceride, mM	1·5	1·0
HDL, mM	13·4	3·2
LDL, mM	38·4	9·6
Fasting glucose mM	5·2	1·4
ALT, U/l	47·5	22·1
Creatinine, μM	80·4	17·7
BMI, kg/m^2	24	3·7
Fetuin, mg/l	559·2	111·5

*Number and percentage.

Table 2. Associations of *AHSG/FTO* genotypes with circulating fetuin-A and BMI

	AHSG									
	AA			AT			TT			
Outcomes	N	Mean	SD	N	Mean	SD	N	Mean	SD	*P* value
Fetuin-A, mg/l	1264	584·8	106·0	433	501·9	95·6	51	413·9	74·5	<0·001*
BMI, kg/m^2	1826	24·1	3·8	633	23·8	3·6	68	23·5	3·8	0·067
	FTO									
	TT			TA			AA			
Fetuin-A, mg/l	1051	556·3	111·6	618	565·2	112·3	79	554·3	105·5	0·245 *
BMI, kg/m^2	1540	23·8	3·7	856	24·3	3·7	131	25·4	4·2	<0·001

*Based on geometric means.

Table 3. Associations of *AHSG* and *FTO* genotypes with confounders

	AHSG						
Characteristics	*AA*		*AT*		*TT*		*P* value
Age, years, mean, SD	40·8	7·1	40·2	7·2	40·6	6·6	0·142
Men, no, %	1350	73·1	476	74·3	51	73·9	0·833
Triglyceride, mM, mean, SD	1·5	1·0	1·4	1·1	1·6	1·2	0·623*
Blood glucose, mM, mean, SD	5·2	1·5	5·1	1·3	5·0	0·55	0·141
ALT, U/l, mean, SD	47·7	22·6	46·8	21	45·8	18·2	0·606 *
	FTO						
	TT		*TA*		*AA*		
Age, year, mean, SD	40·7	7·1	40·4	7	41	7·2	0·509
Men, no,%	1138	73·0	643	73·9	96	73·3	0·879
Triglyceride, mM, mean, SD	1·5	1·0	1·5	1·0	1·6	1·0	0·692
Blood glucose, mM, mean, SD	5·2	1·4	5·2	1·4	5·4	1·5	0·349
ALT, U/l, mean, SD	47	22·2	47·9	22·1	49·9	21·4	0·269

*Based on geometric means.

rs9939609 genotypes as independent variables with adjustment for other covariates (Table 4B). Factors independently associated with serum fetuin-A levels included gender, fasting plasma glucose, serum triglyceride levels and serum ALT. BMI was independently associated with ln(fetuin-A), with a coefficient of 0·0048 (95% CI: 0·0020, 0·0077; $P = 0·001$) after adjustment for covariates. No independent association was found between the FTO gene variant and serum ln(fetuin-A).

We then addressed the causal model B to examine if BMI was causally related to serum fetuin-A by performing an IV regression analysis with ln(fetuin-A) as the outcome, BMI as the intermediate phenotype and the FTO *rs9939609* as the IV (Table 4, Model B). Although the *rs9939609* variant could be considered a strong IV of BMI (F-First = 11·005, $P < 0·001$), predicted BMI was not causally related to fetuin-A levels, which was in contrast to the finding of causal model A that fetuin-A was causally related to BMI. There was no difference between the OLS and IV estimates in model B (Durbin-Wu-Hausman test = 1·105, $P = 0·745$).

Discussion

Our cohort study results suggested that the *AHSG* locus plays a large role in explaining the variance of circulating fetuin-A, with significant correlation between fetuin-A and BMI. Using the Mendelian randomization approach and IV analyses further suggested a causal relationship of circulating fetuin-A on BMI. However, the reverse pathway, that is, BMI influencing fetuin-A, using *FTO* at *rs9939609* locus as the IV, did not show any association.

The *AHSG rs2248690* locus was strongly associated with circulating fetuin-A level. Those carrying the major *A* allele had higher circulating fetuin-A levels than those carrying the minor *T* allele, consistent with a previous study.[17] The *rs2248690* variant is known to be in high linkage disequilibrium with other neighbouring polymorphisms in the *AHSG* gene, that is, *rs1071592* (D' = 0·95), *rs2077119* (D' = 95), *rs4918* (D' = 0·90) and *rs4917* (D' = 0·88).[18,19] These polymorphisms have also been identified as significantly influencing circulating fetuin-A in various general populations[17,20,21] and disease populations (e.g. chronic kidney disease[22,23] and pseudoxanthoma elasticum[24]).

Table 4. Association between Ln(fetuin-A) and BMI using *AHSG/FTO* loci as IVs

	OLS model						IV model					
	b	se	t	p	LL	UL	b	se	t	p	LL	UL
Model A: BMI outcome (kg/m^2)												
Ln(fetuin-A), mg/l	1·1645	0·4449	2·617	0·009	0·2919	2·0371	2·2572	0·9552	2·363	0·018	0·3850	4·1294
Age, years	0·0190	0·0122	1·551	0·121	−0·0050	0·0429	0·0192	0·0122	1·576	0·115	−0·0047	0·0431
Sex (male *vs* female)	1·0696	0·1878	5·694	0·000	0·7012	1·4380	1·0949	0·1885	5·807	0·000	0·7253	1·4644
Triglyceride, mM	0·6780	0·0884	7·663	<0·001	0·5045	0·8516	0·6521	0·0899	7·249	<0·001	0·4758	0·8284
Glucose, mM	0·2869	0·0665	4·315	<0·001	0·1565	0·4174	0·2748	0·0675	4·070	<0·001	0·1424	0·4071
ALT, U/L	0·0464	0·0039	11·860	0·000	0·0387	0·0541	0·0453	0·0040	11·276	0·000	0·0375	0·0532
AHSG												
AT vs AA	−0·1322	0·1954	−0·677	0·499	−0·5153	0·2510						
TT vs AA	−0·4750	0·4962	−0·957	0·339	−1·4483	0·4983						
Model B: Ln(fetuin-A) outcome (mg/l)												
BMI, kg/m^2	0·0048	0·0014	3·385	0·001	0·0020	0·0077	0·0007	0·0127	0·055	0·956	−0·0242	0·0256
Age, year	0·0003	0·0007	0·361	0·718	−0·0012	0·0017	0·0003	0·0008	0·446	0·655	−0·0012	0·0018
Sex (male *vs* female)	−0·0279	0·0113	−2·481	0·013	−0·0500	−0·0058	−0·0228	0·0173	−1·317	0·188	−0·0568	0·0111
Triglyceride, mM	0·0172	0·0053	3·240	0·001	0·0067	0·0276	0·0202	0·0102	1·964	0·050	0·0000	0·0404
Glucose, mM	0·0122	0·0039	3·092	0·002	0·0045	0·0200	0·0135	0·0055	2·454	0·014	0·0027	0·0244
ALT, U/L	0·0009	0·0002	3·572	0·000	0·0004	0·0013	0·0011	0·0006	1·620	0·105	−0·0002	0·0023
FTO												
TA vs TT	0·0092	0·0099	0·929	0·353	−0·0102	0·0287						
AA vs TT	−0·0199	0·0229	−0·871	0·384	−0·0647	0·0249						

IV, instrumental variable; OLS, ordinary least square. *F*-First test: 187·331 ($P < 0·001$) and 11·005 ($P < 0·001$) for models A and B, respectively. Durbin-Wu-Hausman = 0·846 ($P = 0·358$) and 0·105 ($P = 0·745$) for models A and B, respectively.

On the other hand, a direct association between the *AHSG rs2248690* polymorphism and BMI was not identified in our study, which was different to a previous study[17] with the same polymorphism and to other studies with other linked variants.[18,25] Also, the *AHSG* variants at *rs4917* and *rs4918* were found to be associated with obesity or overweight after adjusting for some covariables.[26] In addition, the *rs1071592 C* allele (which was in high linkage disequilibrium with *rs2248690*) has been reported to increase risk of diabetes by 27%.[19]

Our study also found that serum fetuin-A levels were positively associated with BMI. Our finding was in keeping with a number of previous studies[22,27,28] which found a modest correlation, but this was not replicated in a larger study by Stefan et al.[29] In addition, some evidence suggests that high levels of fetuin-A might increase the risk of type 2 diabetes,[27–29] cardiovascular disease[30] and arterial stiffness in men.[21] Conversely, high circulating fetuin-A was found to lower the risk of cardiovascular disease from all causes or cardiovascular disease mortality[31] and diabetes[22] in patients with end-stage renal disease. However, the magnitude of association for all causes or cardiovascular disease mortality was very modest in a subsequent updated study with larger sample size and longer follow-up by Verduijn et al.[23] The authors used the Mendelian randomization approach and suggested that neither the *AHSG rs4918* locus nor fetuin-A level was significantly associated with mortality in patients with end-stage renal disease.

Mapping both the *AHSG rs2248690* locus and serum fetuin-A together using the Mendelian randomization approach[12] with an IV analysis[13,15] allowed us to infer the causal association between fetuin-A and BMI, as described in the directed acyclic graph Fig. 1a. This suggested that a one unit increase in natural log-transformed serum fetuin-A, which is influenced by the *rs2248690* polymorphism, would result in a 2·26 kg/m^2 increase in BMI (i.e. Approximately 0·83 kg/m^2 per 1 mg/l of fetuin-A), even after adjusting for covariables. The estimated *F*-First of 187·331 was far above the threshold of 10, indicating that the *rs2248690* polymorphism was valid as the IV.[16] In addition, a correction for weak instrumental inference using a conditional likelihood ratio test[32] yielded a range of estimates for the fetuin-A effect on BMI of 0·567–4·018, which was narrower than the uncorrected value (0·477–4·036). This confirmed that the *rs2248690* polymorphism was a robust IV. Taken together, our analyses suggested that fetuin-A is highly likely to play a causal role in the determination of BMI. On the contrary, when we investigated the reverse causal chain leading from BMI to serum fetuin-A, we could not demonstrate any association using the same approach.

Our finding was in keeping with some studies looking at the effect of weight loss on serum fetuin-A. Weight reduction did not result in changes in circulating fetuin-A levels in obese women.[4] On the other hand, few studies have shown a reduction in fetuin-A levels after weight loss in obese children[5] or after gastric bypass surgery.[6] Based on our findings, it is less likely that adiposity would alter circulating fetuin-A directly. The regulation of fetuin-A is currently poorly understood. A recent study has implicated endoplasmic reticulum stress as one of the factors controlling fetuin-A transcription.[33] Obesity also induces endoplasmic reticulum stress leading to insulin resistance.[34] It is thus

likely that endoplasmic reticulum stress is the underlying basis for the observed reduction in circulating fetuin-A after weight reduction in some studies.

The role of hepatokine fetuin-A in type 2 diabetes and cardiovascular diseases has been studied, and findings have recently been reviewed and summarized.[35] In addition, a recent study has also suggested that hepatokine fetuin-A was strongly associated with insulin resistance, and the magnitude of association depended mainly on free fatty acid.[36] Understanding the pathogenesis of hepatokine fetuin-A on these diseases more fully should shed more light on the metabolic syndrome.

There are a number of strengths to our study. We have applied the Mendelian randomization approach to determine a causal role of circulating fetuin-A on BMI, but not *vice versa*. Assumptions behind Mendelian randomization and IV analyses were verified and confirmed[13,15]; that is, the two IVs for models A and B in Fig. 1 (i.e. *rs2248690* and *rs9939609*) were highly associated with intermediate phenotypes, but were not associated with other covariables, which might confound associations between intermediate phenotypes and outcomes (see Table 3). Furthermore, the *rs2248690* and *rs9939609* IVs were not directly associated with the outcomes of interest (i.e. BMI for model A and circulating fetuin-A for model B). However, some limitations could not be avoided. Our data were cross-sectional; thus, changes in circulating fetuin-A and BMI could not be determined. It would be more beneficial to look at associations with clinical end-points (e.g. diabetes, chronic kidney disease or cardiovascular disease), and this should be possible when the next follow-up of the cohort (every 5 years) is conducted. We considered only one locus as the IV for each model. As mentioned previously, the *AHSG rs2248690 locus* is in high linkage disequilibrium with other loci, and considering them as multiple IVs may provide more valid estimation of causal associations than a single IV[13]. We could not detect differences between the effects of *AHSG*-fetuin-A in the OLS and IV regressions. This might be due to the fact that the Mendelian randomization itself required a very large sample size (say $n > 10\,000$),[37] although the *AHSG* SNP was a strong IV, or because there was no regression dilution or confounding effects.[13]

Conclusion

Our study has confirmed a causal association between circulating fetuin-A and BMI using *AHSG rs2248690* as an IV. There was no evidence for the reverse causal association between circulating fetuin-A and BMI.

Declaration of interest

The authors declare no conflict of interest.

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