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Analysis of the interaction effect of 48 SNPs and obesity on type 2 diabetes in Chinese Hans

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ABSTRACT

Introduction Both environmental and genetic factors contribute to type 2 diabetes (T2D) risk. Dozens of T2D susceptibility loci have been identified by genome-wide association study. However, these loci account for only a small fraction of the familial T2D risk. We hypothesized that the gene-obesity interaction may contribute to the missing heritability.

Research design and method Forty-eight T2D-associated variants were genotyped using the TaqMan OpenArray Genotyping System and iPLEX Sequenom MassARRAY platform in two separate studies. Obesity was defined according to multiple indexes (body mass index (BMI), waist circumference and waist-hip ratio). Multiplicative interactions were tested using general logistic regression to assess the gene-obesity interaction effect on T2D risk among a total of 6206 Chinese Hans.

Results After adjusting for the main effects of genes and obesity, as well as covariates (age, sex, smoking and alcohol consumption status), robust multiplicative interaction effects were observed between rs10811661 in *CDKN2A/CDKN2B* and multiple obesity indices (p ranged from 0.001 to 0.043 for BMI, waist circumference and waist-hip ratio). Obese individuals with the TT genotype had a drastically higher risk of T2D than normal weight individuals without the risk allele (OR=17.58, p<0.001). There were no significant differences between subgroups in the stratification analysis. Plausible biological explanations were established using a public database. However, there were no significant interaction effects between the other 47 single nucleotide polymorphism (SNPs) and obesity.

Conclusion Our findings indicated that the *CDKN2A/CDKN2B* gene-obesity interaction significantly increases T2D risk in Chinese Hans. The interaction effect identified in our study may help to explain some of the missing heritability in the context of T2D susceptibility. In addition, the interaction effect may play a role in the precise prevention of T2D in Chinese individuals.

Significance of this study

What is already known about this subject?

- The prevalence of type 2 diabetes (T2D) in China rapidly increased in recent years. Several risk factors have been found including obesity and genetic variants.

What are the new findings?

- In our current research, we focused on the interaction effect between SNPs and obesity for T2D. We found that *CDKN2A/CDKN2B* gene-obesity interaction significantly increases T2D risk in Chinese Hans.

How might these results change the focus of research or clinical practice?

- The interaction effect identified in our study may help us to explain some of the missing heritability in the context of T2D susceptibility. In addition, the interaction effect may play a role in the precise prevention of T2D in Chinese individuals.

INTRODUCTION

Type 2 diabetes (T2D) is a common disorder characterized by hyperglycemia, insulin resistance and deficient pancreatic beta-cell function, which accounts for approximately 90% of all diabetes cases.[1] According to the estimation of the International Diabetes Federation, 463 million adults worldwide were affected by diabetes in 2019, and this number will increase to 700 million in 2045. In addition, 4.2 million deaths worldwide were attributed to diabetes in 2019. Notably, approximately 79% of patients with diabetes live in low-income and middle-income countries including China.[2] The prevalence of T2D in China rapidly increased from less than 1% in 1980 to 11.6% in 2010.[3] Diabetes has become an urgent public health problem worldwide, and it is essential to understand its pathogenesis to slow down the rapidly increasing rate.

Obesity is an underlying cause of chronic noncommunicable disease, including T2D. Over the past few decades, rapid economic changes have drastically affected lifestyle and obesity rates, contributing to an epidemic of T2D.[4] Approximately 90% of T2D cases are attributed to excess weight.[5] Wilson *et al*[6] found that obesity was a significant predictor of T2D in the prospective Framingham Offspring Study (OR=2.50, p=0.001). These

findings together revealed the critical role of obesity in T2D.

Genetic factors also contribute to the pathogenesis of T2D.[1] T2D has an estimated rate of heritability of 30%–70%.[7] Over the past several years, genome-wide association studies (GWAS) have provided new susceptibility loci for T2D.[8–14] In our previous study, we successfully validated the association between single nucleotide polymorphism (SNPs) and T2D risk as well as lipid levels in Chinese Hans.[15 16] However, these common variants account for only a small fraction of the familial risk of T2D, with a modest effect contributed by each locus (OR for each allele ranging from 1.1 to 1.5).[17]

To explain the missing heritability, many hypotheses have been proposed, including gene-environment interactions.[18] However, no systematic study focusing on multiple genes has been performed to evaluate the interaction between SNPs and obesity in context of T2D risk in Chinese Hans. In this current study, we attempted to evaluate the gene-environment interaction in 2925 T2D cases and 3281 matched controls in Chinese Hans.

MATERIALS AND METHODS

Study subjects

This study was approved by the Institutional Review Board of Wuxi Center for Disease Control and Prevention. The details of the study subjects have been described previously.[19 20] In brief, one part of samples (1200 T2D cases and 1200 orthoglycemic controls) were recruited from a community-based noncommunicable disease screening program conducted in Wuxi, Jiangsu, China from April to July in 2007 (Wuxi study). The other portion of samples (1725 cases and 2081 controls) were recruited from a population-based cohort study conducted in Changzhou and Nantong cities in Jiangsu Province during 2004 and 2008 (Changzhou-Nantong study). T2D cases were defined according to fasting plasma glucose (FPG)≥7.0 mmol/L or a history of T2D.[21] Controls were selected from the subjects with FPG level <5.6 mmol/L and without a history of diabetes, coronary heart disease, hypertension, stroke or cancer. Controls were frequently matched to the cases by age, sex and residential area. Subjects were interviewed in person by trained interviewers to collect personal information and demographic data after signing informed consent forms. Personal information including name, ID number, address, career, history of diabetes, hypertension, stroke and cancer, smoking and drinking status were collected. Physical examinations including height, weight, waist circumference (WC), hip circumference and blood pressure were performed at the same time. Body mass index (BMI) was divided based on the Chinese criteria or WHO criteria:[21] Chinese criteria: underweight: BMI<18.50 kg/m^2; normal: 18.50≤BMI<24.00 kg/m^2; overweight: 24.00≤BMI<28.00 and obesity: BMI≥28.00 kg/m^2. Individuals were defined as smokers if they had smoked at an average of one cigarette or more per day and for at least 1 year in their lifetime. Similarly, participants were defined as drinkers if they drunk at least once per day and ≥4 days per week. Approximately 5 mL venous blood was collected from each subject after fasting for more than 8 hours. FPG and lipid levels (triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C)) levels were measured in a standard method by Biochemistry Auto-analyzer (Olympus C2734-Au640).

SNP selection

The details of the selection process have been described previously.[15] Briefly, 76 SNPs were reported in the GWAS catalog, and we included SNPs based on the following criteria: (1) minor allele frequency ≥0.05 in Chinese Han and (2) SNPs with strong linkage disequilibrium (r^2>0.8); potential functional variants were retained as priorities. As a result, 48 SNPs from 34 genes were included in this study (online supplemental table 1).

DNA isolation, genotyping and quality control (QC)

Genomic DNA was isolated from the leukocyte pellets of venous blood by proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. All of the DNA samples were checked for quality by DNA electrophoresis. For the participants in Wuxi study, genotyping was performed using TaqMan OpenArray Genotyping System (Life Technologies, Carlsbad, California, USA). The overall call rates ranged from 98.5% to 99.3%. For Changzhou-Nantong study, SNPs were genotyped by iPLEX Sequenom MassARRAY platform (Sequenom, San Diego, California, USA). For quality control, there were two nontemplate controls in each plate. Based on the duplicate samples, the concordance rate was 100% for this two-genotyping platform.

Statistical analysis

The distributions of continuous variables (age, BMI, FPG level, WC, hip circumference and lipid levels) were described using the mean±SD or median value (P25, P75). Categorical variables (sex, smoking status and alcohol consumption status) were defined as counts (percentages). Multiplicative interactions were tested using a general logistic regression model by applying the equation:

$$Y = \beta_0 + \beta_G \times G + \beta_E \times E + \beta_{GE} \times (G \times E) + \Sigma\beta_i \times Covar_i$$

where Y is the logit of case-control status, G is the selected SNPs and E is the environment factor (obesity). β_0 is the constant, and β_G and β_E represent the main effects of SNPs and obesity, respectively. β_{GE} is the interaction term and $Covar_i$ are the covariates for adjustment, including age, sex, smoking status and alcohol consumption status. The effect size directions of interaction should be consistent in Wuxi study and Changzhou-Nantong study for further analysis. To examine the differences between subgroups, the χ^2-based Q-test was used to test the heterogeneity of effect sizes (ORs and 95% CIs) derived from

Table 1 Demographic and clinical characteristics of study subjects

	Wuxi study		Changzhou-Nantong study		Combined	
Variables	**Case (n=1200)**	**Control (n=1200)**	**Case (n=1725)**	**Control (n=2081)**	**Case (n=2925)**	**Control (n=3281)**
Gender						
Male (%)	478 (39.83)	478 (39.83)	620 (35.94)	756 (36.33)	1098 (37.53)	1234 (37.61)
Smoke						
Ever (%)	320 (26.73)	279 (23.37)	401 (23.46)	548 (26.55)	721 (24.81)	827 (25.38)
Alcohol consumption						
Ever (%)	208 (17.72)	128 (10.73)	330 (19.31)	507 (24.60)	538 (18.66)	635 (19.51)
Age (years)	57.43±9.77	56.43±8.02	58.77±10.31	56.66±10.81	58.21±10.11	56.57±9.88
BMI (kg/m^2)	24.92±3.42	22.64±2.87	25.13±3.55	21.82±2.44	25.05±3.50	22.12±2.63
Waist circumference (cm)	85.46±9.27	81.42±9.48	87.18±10.29	76.08±7.96	86.47±9.92	78.04±8.93
Hip circumference (cm)	95.62±7.36	91.21±7.93	95.27±7.31	90.77±6.25	95.46±7.33	90.99±7.16
FPG (mmol/L)	8.00 (6.70, 10.3)	4.50 (4.20, 4.80)	8.10 (7.09, 10.50)	4.52 (4.11, 4.99)	8.05 (7.00, 10.42)	4.50 (4.11, 4.90)
TG (mmol/L)	1.77 (1.22.2.82)	1.28 (0.95, 1.66)	1.73 (1.16, 3.00)	0.90 (0.68, 1.19)	1.76 (1.19, 2.92)	1.00 (0.74, 1.37)
TC (mmol/L)	5.29 (4.58, 5.98)	4.62 (4.14, 5.15)	4.60 (3.91, 5.26)	4.21 (3.70, 4.80)	4.86 (4.17, 5.62)	4.39 (3.85, 4.95)
HDL-C (mmol/L)	1.36 (1.15, 1.56)	1.48 (1.29,1.71)	1.44 (1.21, 1.73)	1.63 (1.40, 1.88)	1.40 (1.19, 1.65)	1.57 (1.35, 1.83)

Data are expressed as number (per cent), mean±SD or median value (P25, P75).
BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

the corresponding subgroups. All of the statistical analyses were performed with R software (V.3.5.1; The R Foundation for Statistical Computing, http://www.cran.r-project.org/) and Stata V.12.0 software (Stata, College Station, Texas, USA).

RESULTS

The demographic and clinical characteristics of study population are summarized in table 1. No significant differences of sex, smoking status and alcohol consumption status were observed in either group or the combined population (p>0.05). The age of the T2D case group was approximately 1 year older than that of the control group, although the age group (5 years) was matched for the case and control groups. As expected, patients with T2D had significantly higher obesity indexes (BMI, WC, hip circumference), FPG, TGs and TC and lower levels of HDL-C than the control group in the combined population and the two separate groups.

The associations results between selected SNPs, obesity indices and T2D risk are shown in online supplemental table 2. First, we focused on the effect of the interaction between selected SNPs and BMI level on T2D risk. Of the 48 selected SNPs, multiplicative interaction analysis revealed that the interaction effect directions of seven SNPs were consistent in Wuxi study and Changzhou-Nantong study. As shown in table 2, of these seven SNPs, rs10811661 in *CDKN2A/CDKN2B* had a significant interaction effect with BMI level on T2D risk (p<0.05). Of interest, effects of the interactions between rs10811661 and BMI level were significant in both studies (Wuxi study: OR=1.22, p=0.033; Changzhou-Nantong study:

Table 2 Interaction between selected SNPs and BMI levels on T2D risk

				Wuxi study		Changzhou-Nantong study		Combined		
Gene	**SNP**	**Allele***	**MAF**	**OR†**	**P value†**	**OR†**	**P value†**	**OR (95% CI)†**	**P value†**	**FDR-P**
CDKN2A/CDKN2B	rs10811661	T/C	0.487	1.22	0.033	1.21	0.035	1.22 (1.07 to 1.39)	0.003	0.021
CENTD2	rs1552224	A/C	0.087	1.08	0.626	1.52	0.018	1.23 (0.97 to 1.55)	0.084	0.196
KCNQ1	rs2237892	C/T	0.341	1.02	0.822	1.12	0.250	1.06 (0.92 to 1.21)	0.430	0.502
KCNQ1	rs2237895	A/C	0.312	0.94	0.555	0.82	0.035	0.88 (0.77 to 1.01)	0.061	0.196
SLC30A8	rs13266634	C/T	0.445	1.10	0.312	1.02	0.855	1.03 (0.91 to 1.17)	0.615	0.615
TP53INP1	rs896854	C/T	0.347	1.16	0.120	1.20	0.068	1.18 (0.97 to 1.25)	0.153	0.262
VEGFA	rs9472138	C/T	0.110	0.84	0.203	0.94	0.679	0.88 (0.73 to 1.06)	0.187	0.262

*Major/Minor allele.
†OR and p value were calculated by multiplicative interaction logistic regression with adjustment of SNP, BMI, age, sex, smoking and alcohol consumption.
BMI, body mass index; FDR-P, false discovery rate P value; MAF, minor allele frequency; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

Table 3 Interaction between rs10811661 and BMI level on T2D risk

BMI*	Genotype	Case N (%)	Control N (%)	OR (95% CI)†	P value†
<18.50	CC	9 (0.31%)	36 (1.10%)	0.80 (0.38 to 1.70)	0.565
18.50–24.99	CC	171 (5.93%)	575 (17.65%)	1	–
25.00–27.99	CC	236 (8.19%)	138 (4.24%)	5.67 (4.31 to 7.46)	<0.001
≥28	CC	102 (3.54%)	19 (0.58%)	18.43 (10.95 to 31.02)	<0.001
<18.50	TC	35 (1.21%)	85 (2.61%)	1.41 (0.91 to 2.18)	0.12
18.50–24.99	TC	519 (18.01%)	1238 (38.00%)	1.42 (1.16 to 1.73)	0.001
25.00–27.99	TC	628 (21.79%)	263 (8.07%)	8.13 (6.49 to 10.19)	<0.001
≥28	TC	294 (10.20%)	47 (1.44%)	22.01 (15.37 to 31.51)	<0.001
<18.50	TT	23 (0.80%)	62 (1.90%)	1.21 (0.72 to 2.02)	0.467
18.50–24.99	TT	364 (12.63%)	612 (18.78%)	1.98 (1.60 to 2.46)	<0.001
25.00–27.99	TT	352 (12.21%)	153 (4.70%)	7.51 (5.80 to 9.71)	<0.001
≥28	TT	149 (5.18%)	30 (0.93%)	17.58 (11.38 to 27.16)	<0.001
P for multiplicative interaction					0.003

*BMI was divided into four groups based on Chinese criterion: underweight: <18.50; normal: 18.50–23.99; overweight: 24.00–27.99; obesity: ≥28.00.
†OR and p value were calculated by multiplicative interaction logistic regression with adjustment of rs10811661, BMI, age, sex, smoking and alcohol consumption.
BMI, body mass index; T2D, type 2 diabetes.

OR=1.21, p=0.035). After false discovery rate (FDR) correction, rs10811661 still had a significant interaction effect with BMI level on T2D risk in the combined population (OR=1.22, p=0.021). Besides, in consideration of the small number of obesity, we combined the overweight and obesity group to conduct a sensitive analysis. The sensitive analysis showed that the interaction effect was robust (OR=1.27, p=0.002). However, there were no significant interaction effects between the other SNPs and BMI on T2D risk (online supplemental table 1).

Compared with normal weight individuals without risk allele T, obese individuals with the TT genotype had a substantially increased risk of T2D (OR=17.58, p<0.001) (table 3).

We further analyzed the interaction effect between rs10811661 and other obesity indices (WC and waist-hip ratio (WHR)) on T2D risk. As shown in online supplemental tables 3 and 4, significant interaction effects were also observed between rs10811661 and other obesity indices (for WC, p=0.001; for WHR, p=0.043).

In the stratification analysis, the interaction effect was evaluated in subgroups based on age, sex, smoking status and alcohol consumption status. As shown in figure 1, there were no significant differences between subgroups in regard to the interaction effect between rs10811661 and BMI level (all *P* for heterogeneity >0.05).

To reveal the biological role of the identified SNP, we performed a bioinformatic analysis of the results above by using the public database GTEX (http://www.gtexportal.org/home/). According to GTEX database, rs10811661 in *CDKN2A/CDKN2B* significantly decreased the expression of *CDKN2B* in whole blood samples (β=−0.12, p=0.016) and pancreas (β=−0.17, p=0.035) (figure 2). However, there was no significant association between rs10811661 and *CDKN2A* (data not shown).

DISCUSSION

In this study, we evaluated the effect of the interaction between 48 selected SNPs and obesity indexes on T2D risk in Han Chinese individuals. Robust interaction effects were observed between rs10811661 and BMI (Chinese criterion and WHO criterion), WC and WHR. To the best of our knowledge, this is the first study to report the effects of the interaction between rs10811661 in *CDKN2A/CDKN2B* and different obesity indexes in Han Chinese individuals. Our study provided genetic

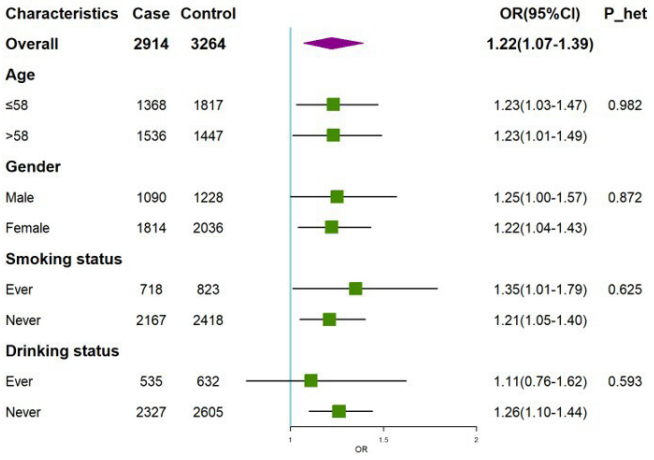

Figure 1 Stratified analysis of the interaction between rs10811661 and BMI on type 2 diabetes risk. BMI, body mass index.

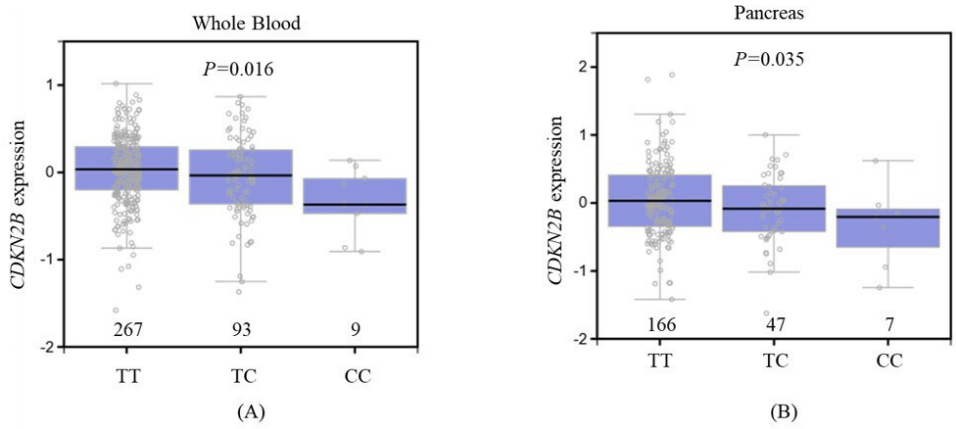

Figure 2 The association of rs10811661 with host gene expression. Data were available in public database GTEX (A: whole blood; B: pancreas).

evidence at the population level that the gene-obesity interaction may contribute to T2D risk.

Several studies have focused on the interaction effects of SNPs and obesity on T2D risk.[22–24] However, these studies each mainly focused on only one gene (*PPARG*, *FOXO1*, *TCF7L2*), and the number of selected SNPs was limited. In addition, no research has been reported on the *CDKN2A/CDKN2B*-obesity interaction.

The SNP rs10811661 is located upstream of *CDKN2A/CDKN2B* (cyclin-dependent kinase inhibitor 2A/2B). The effect of rs10811661 on T2D risk is well known. Li *et al*[25] performed a meta-analysis including 17 studies with 29 990 cases and 40 977 controls to evaluate the association between rs10811661 and T2D risk. The results showed that the rs10811661 T allele was significantly associated with increased T2D risk in the additive model (OR=1.51, 95% CI 1.40 to 1.63, p<0.001). In our previous study, a similar result was identified (OR=1.32, 95% CI 1.17 to 1.49, $p=1.22\times10^{-5}$).[15] Interestingly, in human populations, the rs10811661 risk allele T is also associated with reduced insulin secretory capacity.[26–28]

According to the GTEX database, rs10811661 is an eQTL (expression quantitative trait loci) for *CDKN2B*. The risk allele T was identified to be associated with increased *CDKN2B* levels in whole blood and pancreas samples. Proteins encoded by the *CDKN2B* gene regulate pancreatic beta cell replication by inhibiting cyclin-dependent kinase 4/6 (CDK4/6).[29] It has been shown that mice lacking CDK4 exhibit insulin-deficient diabetes due to a reduction in pancreatic beta cells.[30] These results together suggest that rs10811661 may increase the risk of T2D by affecting pancreatic beta cells. Recently, Kong *et al*[31] found that the risk allele of rs10811661 increased *ANRIL* expression in islet samples, which correlates with higher BMI and fat mass.[32–35] Obesity is well studied and has been shown to contribute to T2D risk mainly by reducing insulin sensitivity and promoting insulin resistance.[36] These mechanisms may together elucidate the increased risk of T2D in obese individuals with the rs10811661 TT genotype.

BMI is usually used to assess general obesity. However, general obesity, as defined by BMI, is not a fitness indicator of body fat distribution.[37] Recently, central obesity, also known as abdominal obesity, measured by WC and WHR have been widely studied.[38] Cheng *et al*[39] found that WHR was a better index than BMI for predicting the risk of T2D in Taiwanese population. Besides, Hu *et al*[40] conducted the interASIA study and found that central obesity is more related to T2D than overall obesity in the Chinese population. However, in our study, the interaction effects between SNP and general obesity, central obesity were similar (ORs for BMI, WC and WHR were 1.22, 1.31 and 1.20, respectively). There was no significant difference between these obesity indices (p for heterogeneity was 0.84). Li *et al*[24] conducted a study to explore the interaction effect of TCF7L2 and BMI/ WC on T2D. The effect was similar for BMI and WC (for BMI: OR=4.833; WC: OR=4.629). The present study is not sufficiently large to support the hypothesis that there may be difference of interaction effect between SNP-general obesity and SNP-central obesity. Further research, which focus on the difference, should be done.

There were several strengths in our present study. First, this study was well designed and included two separate study populations to ensure the authenticity of the results. Second, multiple obesity indexes were evaluated for interaction effects to ensure the robustness of the study results. Third, a public database was used, and plausible interpretations of our population results were established. However, the biological mechanisms of the identified interaction effect remain unclear. Future functional studies will be necessary to validate our findings.

In summary, our results suggested, for the first time, that rs10811661 in *CDKN2A/CDKN2B* had robust interaction effects with multiple obesity indexes. The identified gene-obesity interaction may contribute to some of the missing heritability of T2D risk.

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Contributors YQ designed and directed this study. JL and LW conducted the genotype assay and wrote this paper. QS, HM and CS directed the survey and quality control. HC and JD analyzed the data. GJ, ZH and HS directed the assay and quality control.

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Competing interests None declared.

Patient consent for publication Not required.

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