Genomic Variants in *NEURL*, *GJA1* and * CUX2* Significantly Increase Genetic Susceptibility to Atrial Fibrillation

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Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia with an incidence rate of 1–2% in the general population. AF is characterized by fast and irregular abnormal atrial electrophysiological activities, which can lead to >15% of strokes, blood clots and heart failure and increases the rate of sudden death. AF is caused by genetic factors, environmental factors and interactions among these factors. The heritability of AF is 0.62. Mutations in ion channels such as KCNQ1 and Na,1.5 and non-ion channels such as NUP155 and ANF are

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Table 1. Clinical and demographic characteristics of study subjects. Data are shown as mean ±/− standard deviation (SD) for quantitative variables and percentage (%) for qualitative variables. *The differences between cases and controls for qualitative variables such as gender, hypertension, type 2 diabetes and CAD were analyzed by a Chi-square (χ²) test. The difference for quantitative variables such as means of age was analyzed with a student t test. AF, atrial fibrillation.

| Characteristics                  | AF Cases (n = 1,164) | Controls (n = 1,460) | P* |
|----------------------------------|----------------------|----------------------|----|
| Age (years, mean ± SD)           | 61.27 ± 11.33        | 63.8 ± 13.54         | <0.01 |
| Male (%)                         | 46.37%               | 42.18%               | 0.03 |
| Coronary artery disease (CAD) (%)| 24.32%               | 33.60%               | <0.01 |
| Hypertension (HTN) (%)           | 44.31%               | 48.62%               | 0.14 |
| Type 2 diabetes (DM) (%)         | 14.86%               | 14.26%               | 0.31 |

rare, but can cause AF in isolated AF families10,11. On the other hand, genome-wide association studies (GWAS) have been effective in identification of common single nucleotide polymorphisms (SNPs) that increase risk of AF. Early series of GWAS and meta-GWAS in European ancestry populations identified 10 AF-susceptibility loci, including SNPs rs2200733 and rs10033464 near PITX2c gene, rs2106261 and rs7193343 in ZFHX3, rs13376333 in KCNQ3, rs593479 in PRRX1, rs3807989 in CAV1, rs679562 in C9orf3, rs10824026 in SYNPO2L, rs1152591 in SYNE2, rs7164883 located in HCN4 and rs2040862 in WNT8A10,11. In 2014, meta-GWAS in the European ancestry populations identified additional AF susceptibility variants, including NEURL SNPs rs12415501, CAND2 SNP rs4642101, GJA1 SNP rs13216675 and TRX5 SNP rs1050724812. Also in 2014, GWAS in a Japanese population identified two risk variants for AF, including NEURL SNP rs6584555 and CUX2 SNP rs649002913.

To date, no GWAS were reported for AF in the Chinese population despite the fact that there are over 10 million AF patients in China. Our group previously analyzed the potential association between AF and the 10 AF loci identified in the early series of meta-GWAS in the European ancestry populations. We found that three of them, including the PITX2c, ZFHX3 and CAV1 loci, showed significant association with AF in the Chinese Han population, but other loci were not replicated in the Chinese Han population15–20. For the TBX5 locus, we reported in 2013 that a genomic variant in TBX5, rs3825214, showed a significant association with AF in the Chinese population21. In this study, we assessed association between AF with other meta-GWAS SNPs identified in European ancestry populations and the Japanese population, including SNP rs6584555 in NEURL, rs13216675 near GJA1, rs4642101 in CAND2 and rs6490029 in CUX2, in the Chinese GeneID population. We identified significant allelic and genotypic association between NEURL rs6584555 and GJA1 SNP rs13216675 and AF, significant genotypic association between CUX2 SNP rs6490029 and AF, but no association between CAND2 SNP rs4642101 and AF.

Results

Significant allelic association between GJA1 SNP rs13216675 and NEURL SNP rs6584555 and AF. We carried out a case control association study for AF with four SNPs, including SNP rs4642101 within the CAND2 gene on chromosome 3p25.2, rs13216675 close to the GJA1 gene on chromosome 6q22.3, rs6584555 near the NEURL gene on chromosome 10q24.33 and rs6490029 within the CUX2 gene on chromosome 12q24.11. Our study population included 1,164 AF patients and 1,460 non-AF controls from the Chinese Han GeneID population. The average age of the case group was 2.6 years younger than the control group (61.27 ± 11.33 vs. 63.8 ± 13.54, P < 0.01). The other characteristics of the case group and the control group are summarized in Table 1. In the control population, the genotypic frequencies for all four SNPs did not deviate from the Hardy-Weinberg equilibrium (P > 0.01). The minor allele frequency (MAF) of each SNP in our control population is similar to the data for the Chinese Han population from the HapMap database (Table 2).

The GJA1 SNP rs13216675 showed significant association with AF (observed Pobs = 3.9 × 10−5, OR = 1.2) (Table 2). After adjusting for covariates of age, gender, hypertension (HTN), diabetes mellitus (DM) and coronary artery disease (CAD), the association remained significant (Padj = 0.01, OR = 1.19) (Table 2). The common allele T of SNP rs13216675 is the risk allele in the Chinese Han population (Table 2). The significant association between SNP rs13216675 and AF remained after adjusting for multiple testing with Bonferroni correction (corrected P = 0.04) (Table 2).

The NEURL SNP rs6584555 showed significant association with AF (Pobs = 5.05 × 10−5, OR = 1.38) (Table 2). After adjusting for covariates of age, gender, HTN, DM and CAD, the association remained significant (Padj = 9.06 × 10−5, OR = 1.39) (Table 2). The minor allele C of SNP rs6584555 is the risk allele in the Chinese Han population (Table 2). The significant association between SNP rs6584555 and AF remained after adjusting for multiple testing with Bonferroni correction (corrected P = 3.62 × 10−4) (Table 2).

The two remaining SNPs, CAND2 SNP rs4642101 and CUX2 SNP rs6490029 did not show significant allelic association with AF in the Chinese Han population before or after adjustment for covariates (Padj > 0.05) (Table 2).

Significant genotypic association between NEURL SNP rs6584555, GJA1 rs13216675 and CUX2 SNP rs6490029 and AF. We also performed the case control association analysis for genotypic frequencies, which may pinpoint potential genetic models under which a significant association is found for a genetic variant in contrast to allelic association analysis. We analyzed the genotypic association for each SNP under three common genetic models: an additive model, a dominant model, or a recessive model. The results are summarized in Table 3.
Significant genotypic association was identified between NEURL SNP rs6584555 and AF under all three models, although most significant associations were obtained under the dominant and recessive models before and after adjusting for covariates of age, gender, HTN, DM and CAD (P_{obs} = 4.03 \times 10^{-4}, \text{P}_{adj} = 4.85 \times 10^{-5} under an additive model; \text{P}_{obs} = 4.38 \times 10^{-4}, \text{P}_{adj} = 4.26 \times 10^{-4} under a dominant model; \text{P}_{obs} = 5.3 \times 10^{-3}, \text{P}_{adj} = 1.51 \times 10^{-3} under a recessive model) (Table 3). The significant genotypic association between SNP rs6584555 and AF remained after adjusting for multiple testing with Bonferroni correction (corrected \text{P} < 0.05) (Table 3).

For CUX2 SNP rs64990029, although no significant allelic association was found for AF, significant genotypic association was identified for AF under both the additive and the dominant models, but not under the recessive model (\text{P}_{obs} = 7.97 \times 10^{-4} under a dominant model; \text{P}_{adj} = 0.02 under an additive model) (Table 3). The significant genotypic association between SNP rs64990029 and AF remained under the dominant model and after adjusting for covariates of age, gender, HTN, DM and CAD (\text{P}_{adj} = 8.28 \times 10^{-10}) and after further adjusting for multiple testing with Bonferroni correction (corrected \text{P} = 0.04) (Table 3).

For GJA1 SNP rs13216675, significant genotypic association was identified for AF under an additive model and a dominant model, but not under a recessive model (\text{P}_{obs} = 6.72 \times 10^{-3}, \text{P}_{adj} = 0.01 under an additive model; \text{P}_{obs} = 2.28 \times 10^{-3}, \text{P}_{adj} = 3.04 \times 10^{-3} under a dominant model; \text{P}_{obs} = 0.07, \text{P}_{adj} = 0.14 under a recessive model).
Locus               | AF heritability explained |
-------------------|----------------------------|
rs13216675 (near the GJA1) | 1.8%                      |
rs6584555 (NEURL)       | 3.7%                      |
rs6490029 (CUX2)        | 2.6%                      |
rs2200733 (near PITX2)  | 1.8%                      |
rs2106261 (ZFHX3)       | 1.7%                      |
rS3807989 (CAV1)        | 2.9%                      |
Total                | 14.5%                     

Table 4. Estimation of AF heritability explained by SNPs showing significant association in the Chinese Han population.

(Table 3). The significant genotypic association between SNP rs13216675 and AF remained after adjusting for multiple testing with Bonferroni correction ($P < 0.05$) (Table 3).

For CAND2 SNP rs4642101, similar to the data from allelic association analysis, we did not find any significant genotypic association with AF in the Chinese Han population.

Estimation of AF heritability explained by SNPs significantly associated with AF in the Chinese population. For the three SNPs showing significant association with AF in the Chinese Han population (GJA1 SNP rs13216675, NEURL SNP rs6584555 and CUX2 SNP rs6490029), we estimated the heritability of AF explained by each of them. As shown in Table 4, GJA1 SNP rs13216675, NEURL SNP rs6584555 and CUX2 SNP rs6490029 explained 1.8%, 3.7% and 2.6% of AF heritability, respectively. Together, these three variants explained approximately 8.1% of AF heritability. Previously, we reported three other SNPs which also showed significant association with AF in the Chinese Han population, including SNP rs2200733 on 4q25 and near PITX2, rs2106261 on ZFHX3 locus and rs3807989 on CAV1. SNP rs2200733, rs2106261 and rs3807989 explained about 6.4% of AF heritability (1.8% for rs2200733, 1.7% for rs2106261 and 2.9% for rs3807989). Together, these six SNPs explained 14.5% of AF heritability.

Discussion
In this study, we analyzed four genomic variants associated with AF in either the European ancestry populations or the Japanese population for their association with AF in the Chinese Han population. These variants include NEURL SNP rs6584555, GJA1 SNP rs13216675, CUX2 SNP rs6490029 and CAND2 SNP rs4642101. Our study population consisted of 1,164 AF patients and 1,460 non-AF controls. Three of the four loci, the NEURL locus, GJA1 locus and CUX2 locus, were successfully replicated in the Chinese population (Tables 2 and 3). The CAND2 locus was not replicated in the Chinese population (Tables 2 and 3), which may be due to an insufficient power of the sample size for this variant.

The 2014 meta-GWAS in the European ancestry populations reported four loci for AF, including NEURL (rs12415501), GJA1 (rs13216675), TBX5 (rs10507248) and CAND2 (rs4642101). The TBX5 locus was reported in 2013 by us by studying a Chinese AF population before the GW AS report. For the three remaining loci, the NEURL and GJA1 loci were significantly associated with AF in the Chinese population, whereas the CAND2 locus did not show any significant association with AF (Tables 2 and 3). Previously, we showed that only three of the 10 AF GWAS loci identified in the European ancestry populations before 2014 were significantly associated with AF in the Chinese populations. Interestingly, the two AF loci reported in the 2014 GWAS in a Japanese population, namely NEURL SNP rs6584555 and CUX2 SNP rs6490029, were both replicated in the Chinese population (Tables 2 and 3). This may be related to the fact that the evolution distance between the Japanese population and the Chinese population is closer that that between the European ancestry populations and the Chinese population.

Our study has a limitation. Our study population of 1,164 AF patients and 1,460 non-AF controls has a sufficient power of 97% and 91% for genomic variants rs6584555 in NEURL and rs6490029 in CUX2, respectively. However, its power for rs13216675 near GJA1 and rs4642101 in CAND2 was 0.40 and 0.39, respectively. Therefore, lack of association between rs4642101 in CAND2 and AF may be due to the small sample size. Future studies with larger AF case control populations may be needed to further clarify the association between rs4642101 in CAND2 and AF in the Chinese Han population.

In conclusion, we found significant associations between AF and NEURL SNP rs6584555, GJA1 SNP rs13216675 and CUX2 SNP rs6490029, but not CAND2 SNP rs4642101. Together with our earlier reports, we show that among the 15 GWAS loci for AF reported in the European ancestry populations and Japanese population, seven loci (PITX2c, ZFHX3, CAV1, NEURL, GJA1, TBX5 and CUX2 loci) also confer a significant risk of AF in the Chinese Han population. Our findings provide an important understanding of the detailed genomic landscape for AF susceptibility in the Chinese Han population. Our data also suggest that although the European ancestry populations share some common susceptibility loci for AF with the Chinese population, different populations may contain their own unique susceptibility loci for AF.

Materials and Methods
Study subjects. The study subjects for this study were from the large GeneID database, which has over 80,000 study subjects with cardiovascular diseases in the Chinese Han population. To minimize stratification of population heterogeneity, only study subjects of Han ethnic origin (by self-description) were included. A total
SNP genotyping. Human genomic DNA was extracted from peripheral blood samples using the Wizard Genomic DNA Purification Kit as described previously by us. Each SNP was genotyped using a Rotor-Gene 6000 High Resolution Melt system as described by us previously. The HRM technology is based on the different molecular physical properties of DNA molecules on the fragment length, GC content and GC distribution, which makes DNA molecules with different genotypes (two different homozygotes and the heterozygote) have different shapes and positions of its dissolution curves when heated at different temperatures. The three different genotypes for a genomic variant can then be distinguished based on their different dissolution curves. The polymerase chain reactions (PCR) for genotyping was performed in a 25 μl mixture with 2.5 μl of 10 × PCR buffer, 10 mM dNTP (0.5 μl), 25 mM Mg2+ (1.5 μl), 5 pmol of each primer, 25 ng of genomic DNA and 0.7 μl of 5 mM SYTO9. PCR was performed on an ABI9700 System (Applied Biosystems, Foster City, CA) with a thermal profile of 95 °C for 5 minutes, 40 cycles of 95 °C for 15 seconds and 72 °C for 20 seconds and 72 °C for 10 minutes. Primers for PCR are listed in Table 5. PCR products were directly genotyped using the high resolution melting (HRM) analysis on a Rotor-Gene 6000 System (Corbett Life Science, Australia). DNA samples from 100 study subjects were randomly selected for each SNP for direct Sanger sequencing analysis and the sequencing data completely matched the HRM genotyping data.

Statistical analysis. We used PLINK version 1.07 to perform the Hardy–Weinberg linkage equilibrium test in the control group as described by us previously. Pearson 2 × 2 and 2 × 3 contingency table χ2 tests were performed with SPSS (version 17.0; SPSS, Inc., Chicago, IL) to analyze allelic association and genotype association, respectively and to compute odds ratios (ORs) and corresponding 95% confidential intervals (CIs). Multiple logistic regression analysis was used to adjust significant covariates of age, gender, hypertension (HTN), coronary artery disease (CAD) and diabetes mellitus (DM) for AF using SPSS (version 17.0; SPSS, Inc., Chicago, IL).

We estimated the heritability of AF explained by each significant SNP using the multifactorial liability threshold model based on OR estimates using the R package as described previously. The computation of heritability was based on the frequency of the risk allele, relative risk of one risk allele (Aa) over that of no risk allele (aa) (OR: Aa/aa), relative risk of two risk alleles (AA) over that of no risk allele (aa) (OR:AA/aa) and the overall prevalence rate of AF in the population. We assumed a disease prevalence estimate of 0.73% for AF in the Chinese population.

Statistical power analysis of the study population was conducted using program PS (Power and Sample size Calculations, version 3.0.43). For power analysis, we utilized the reported OR values in the European ancestry populations (GIAF SNP rs1321667 and CAND2 SNP rs4642101) or the Japanese population (NEURL SNP rs6584555 and CUX2 SNP rs6490029) as the minor allele frequencies for the studied variants in the Chinese population from the HapMap database and a type I error of 0.05.

Table 5. Sequences for primers used for HRM genotyping and direct Sanger sequencing analysis. HRM, high-resolution melting; SNP, single-nucleotide polymorphism.

| SNP        | HRM Primers                      | Sequencing Primers                      |
|------------|----------------------------------|----------------------------------------|
| rs6584555  | GAGATTAGAAGATGGATGCCCCCCCCTTCA   | GAGATTAGAAGATGGATGCCCCCCCCTTCA         |
| rs6490029  | AGAATGGTGTTGCGATTTCTGA           | AGAATGGTGTTGCGATTTCTGA                 |
| rs1321667  | GGAGGAGGATCAGTTGAAACCAGG         | GGAGGAGGATCAGTTGAAACCAGG              |
| rs4642101  | CCACTATTTTCCCTCTATTG             | CCACTATTTTCCCTCTATTG                  |
| rs6490029  | ACCCCCTACCTTTTTCTCTCTGA          | ACCCCCTACCTTTTTCTCTCTGA               |
References

1. Staerk, L., Sherer, J. A., Ko, D., Benjamin, E. J. & Helm, R. H. Atrial Fibrillation: Epidemiology, Pathophysiology and Clinical Outcomes. Circulation Research 120, 1501–1517 (2017).
2. Chugh, S. S. et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. Circulation 129, 837–847 (2014).
3. Bai, Y., Wang, Y. L., Shantsila, A. & Lip, G. Y. H. The Global Burden of Atrial Fibrillation and Stroke: A Systematic Review of the Clinical Epidemiology of Atrial Fibrillation in Asia. Chest (2017).
4. Reifel, J. A. Atrial fibrillation and stroke: epidemiology. American Journal of Medicine 127, e15–e16 (2014).
5. Kirchhof, P. The future of atrial fibrillation management: integrated care and stratified therapy. Lancet. S0140-6736(17)3027-3 (2017).
6. Anumonwo, J. M. & Kalifa, J. Risk Factors and Genetics of Atrial Fibrillation. Heart Failure Clinics 12, 157–166 (2016).
7. Anumonwo, J. M. & Kalifa, J. Risk factors and genetics of atrial fibrillation. Cardioiology Clinics 32, 485–494 (2014).
8. Christophersen, I. E. & Ellinor, P. T. Genetics of atrial fibrillation: from families to genomes. Journal of Human Genetics 61, 61–70 (2016).
9. Christophersen, I. E. et al. Familial aggregation of atrial fibrillation: a study in Danish twins. Circulation: Arrhythmia Electrophysiology 2, 378–383 (2009).
10. Zhang, X. et al. Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. Cell 135, 1017–1027 (2008).
11. Ren, X. et al. Identification of NPPA variants associated with atrial fibrillation in a Chinese GeneID population. Clinica Chimica Acta 411, 481–485 (2010).
12. Gudbjartsson, D. F. et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature 448, 353–357 (2007).
13. Christophersen, I. E. et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. Nature Genetics 41, 879–881 (2009).
14. Gudbjartsson, D. F. et al. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. Nature Genetics 41, 876–878 (2009).
15. Ellinor, P. T. et al. Common variants in KCNJ3 are associated with lone atrial fibrillation. Nature Genetics 42, 240–244 (2010).
16. Ellinor, P. T. et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. Nature Genetics 44, 670–675 (2012).
17. Sinner, M. F. et al. Integrating genetic, transcriptional and functional analyses to identify 5 novel genes for atrial fibrillation. Circulation 130, 1225–1235 (2014).
18. Chen, S. et al. Significant Association Between CAV1 Variant rs3807989 on 7p31 and Atrial Fibrillation in a Chinese Han Population. Journal of the American Heart Association 4, e001980 (2015).
19. Shi, L. et al. Assessment of association of rs2200733 on chromosome 4q25 with atrial fibrillation and ischemic stroke in a Chinese Han population. Human genetics 126, 843–849 (2009).
20. Li, C. et al. Significant association of SNP rs2196261 in the ZFHX3 gene with atrial fibrillation in a Chinese Han GeneID population. Human Genetics 129, 239–246 (2011).
21. Zhang, X. et al. SNPs rs3825214 in TBX5 is associated with lone atrial fibrillation in Chinese Han population. PLoS One 8, e64966 (2013).
22. Wang, F. et al. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. Nature Genetics 43, 345–349 (2011).
23. Xu, C. et al. Minor allele C of chromosome 1p32 single nucleotide polymorphism rs11206510 confers risk of ischemic stroke in the Chinese Han population. Stroke 41, 1587–1592 (2010).
24. Tu, X. et al. The IL-33-ST2L pathway is associated with coronary artery disease in a Chinese Han population. American Journal of Human Genetics 93, 652–660 (2013).
25. Xu, C. et al. Candidate pathway-based genome-wide association studies identify novel associations of genomic variants in the complement system associated with coronary artery disease. Circulation: Cardiovascular Genetics 7, 887–894 (2014).
26. Huang, Y. et al. Molecular Basis of Gene-Gene Interaction: Cyclic Cross-Regulation of Gene Expression and Post-GWAS Gene-Gene Interaction Involved in Atrial Fibrillation. PLoS Genetics 11, e1005393 (2015).
27. Yin, D. et al. Genomic Variant in IL-37 Confers A Significant Risk of Coronary Artery Disease. Scientific Reports 7, 42175 (2017).
28. Fuster, V. et al. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation: full text: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 guidelines for the management of patients with atrial fibrillation) developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. Europace 8, 651–745 (2006).
29. Wang, C. et al. Identification of rare variants in TNNJ3 with atrial fibrillation in a Chinese GeneID population. Molecular genetics and genomics 291, 79–92 (2016).
30. Zhou, S. et al. Loss of heterozygosity detected at three short tandem repeat locus commonly used for human DNA identification in a case of paternity testing. Legal Medicine (Tokyo) 24, 7–11 (2017).
31. Wang, P. et al. Association of SNP R9943582 in APLNR with Left Ventricle Systolic Dysfunction in Patients with Coronary Artery Disease in a Chinese Han GeneID Population. PLoS One 10, e0125926 (2015).
32. Xiong, X. et al. BRG1 variant rs11226082 on chromosome 19p13.2 confers protection against stroke and regulates expression of premRNA-splicing factor SFRS3. Human Genetics 133, 499–508 (2014).
33. Shen, G. Q., Li, L. & Wang, Q. K. Genetic variant R952Q in LRPI8 is associated with increased plasma triglyceride levels in patients with early-onset CAD and MI. Annual Human Genetics 76, 193–199 (2012).
34. Wang, A. Z., Li, L., Zhang, B., Shen, G. Q. & Wang, Q. K. Association of SNP rs17465637 on chromosome 1q41 and rs599839 on 1p13.3 with myocardial infarction in an American caucasian population. Annual Human Genetics 75, 475–482 (2011).
35. Shen, G. Q. et al. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. Arteriosclerosis Thrombosis and Vascular Biology 28, 360–365 (2008).
36. So, H. C., Gui, A. H., Cherry, S. S. & Sham, P. C. Evaluating the heritability explained by known susceptibility variants: a survey of ten complex diseases. Genetic Epidemiology 35, 310–317 (2008).
37. Dupont, W. D. & Plummer, W. D. Jr. Power and sample size calculations for studies involving linear regression. Controlled Clinical Trials 19, 589–601 (1998).

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Design of the study: Q.C., C.X. and Q.K.W. Experiments and data analysis: P.X.W., W.Q., P.Y.W., Y.H., Y.L., R.Z., S.L., Q.Y., X.W., F.C., J.L., B.Y., X.C., Y.L., Y.W., T.K., X.T., X.R., Y.Y., Y.X., X.L., M.L., J.L., Y.X., Q.C., C.X. and Q.K.W. Drafting of the manuscript: P.X.W. and C.X. Critical revision of the manuscript: H.L., C.X., Q.C. and Q.K.W. Study supervision: Q.K.W.

Additional Information
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