• Investigation •

# Evaluating correlation between the ocular biometry and genetic variants of *MYOC* and *ABCA1* with primary angle-closure glaucoma in a cohort from northern China

*Shao-Lin Wang[1,2], Shun-Yu Piao[1], Man-Yun Xu[2], Wen Zhang[2], Jian-Qing Ma[3], Juan Hao[4], Hao Chi[5], Zhong-Qi Xue[2], Shao-Ping Ha[2], Wen-Juan Zhuang[2]*

[1]Clinical Medical College of Ningxia Medical University, Yinchuan 750001, Ningxia Hui Autonomous Region, China
[2]Ningxia Eye Hospital, People's Hospital of Ningxia Hui Autonomous Region (First Affiliated Hospital of Northwest University For Nationalities), Yinchuan 750011, Ningxia Hui Autonomous Region, China
[3]Department of Ophthalmology, Wuzhong People's Hospital, Wuzhong 751100, Ningxia Hui Autonomous Region, China
[4]Department of Ophthalmology, Taiyuan Central Hospital, Taiyuan 030000, Shanxi Province, China
[5]Shandong Academy of Medical Sciences, Jinan University, Jinan 250000, Shandong Province, China
**Co-first authors:** Shao-Lin Wang and Shun-Yu Piao
**Correspondence to:** Wen-Juan Zhuang and Shao-Ping Ha. Ningxia Eye Hospital, People's Hospital of Ningxia Hui Autonomous Region, 936 Huang He East Road, Yinchuan 750011, Ningxia Hui Autonomous Region, China. zh_wenj@163.com; hashaoping@163.com
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## Abstract

**• AIM: To investigate whether the gene variants in *MYOC* and *ABCA1* are associated with primary angle-closure glaucoma (PACG) and anterior chamber depth (ACD) and axial length (AL) in samples from northern China.**

**• METHODS: The present case-control association study consisted of 500 PACG patients and 720 unrelated controls. Each participant was genotyped for eleven single nucleotide polymorphisms (SNPs) in *MYOC* and *ABCA1* genes (rs12076134, rs183532, rs235875 and rs235913 in *MYOC,* rs2422493, rs2487042, rs2472496, rs2472493, rs2487032, rs2472459 and rs2472519 near *ABCA1*) using an improved multiplex ligation detection reaction (iMLDR) technique. The genetic association analyses were performed by PLINK using a logistic regression model. The association between genotypes and ocular biometric parameters was performed by SPSS using generalized estimation equation. Bonferroni corrections were implemented and the statistical power was calculated by the Power and Sample Size Calculation.**

**• RESULTS: Two SNPs rs183532 and rs235875 as well as a haplotype TTC in *MYOC* were nominally associated with PACG despite the significance was lost after Bonferroni correction. No association was observed between *ABCA1* and PACG, neither did the association between these variants and ACD as well as AL.**

**• CONCLUSION: The present study suggests *MYOC* and *ABCA1* do not play a part in the pathogenesis of PACG as well as the regulation of ocular biometric parameters in a northern Chinese population. Further investigations with large sample size are needed to verify this consequence.**

**• KEYWORDS:** *MYOC*; *ABCA1*; primary angle-closure glaucoma; anterior chamber depth; axial length; single nucleotide polymorphisms

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## INTRODUCTION

Glaucoma is a common eye disease characterized by the progressive degeneration of retinal ganglion cells and optic nerve axons, for which, intraocular pressure (IOP) is the primary modifiable risk factor[1]. Meanwhile, glaucoma is considered to be the most common disease leading to irreversible blindness worldwide[2]. In 2013, the glaucoma patients in the elderly population (aged 40-80y) worldwide was estimated to be 64.3 million, which will increase to 76.0 million in 2020 and 111.8 million in 2040[3]. According to the anatomical features, glaucoma is mainly subdivided into two major forms: primary open angle glaucoma (POAG) and primary angle-closure glaucoma (PACG). The prevalence of POAG is highest in Africa while most PACG cases are in Asia[4-5], especially in China[6]. Epidemiological studies

have found that PACG is the most common cause of bilateral glaucoma blindness worldwide[7].

Glaucoma is a multifactorial disease, genetic factors take an important part in its pathogenesis[1], many disease-causing mutations and multiple susceptibility loci have been found to be associated with various forms of glaucoma. What's interesting is some genetic risks are shared for the two main types of glaucoma PACG and POAG, for example polymorphisms within the *ARHGEF12* and *GAS7* gene are found to be associated with both POAG and PACG[8]. Since *MYOC* is preferentially expressed in trabecular meshwork (TM)[9], which is thought to affect the outflow of aqueous humor resulting in elevation of IOP[10], while *ABCA1* has been proved to be associated with POAG and IOP by GWAS[11], in addition, mutations in *MYOC* gene as well as variants near *ABCA1* gene have been confirmed to be associated with POAG[12-15]. In view of this, this study aimed to investigate whether the POAG related genes *MYOC* and *ABCA1* are associated with PACG in a northern Chinese cohort. Meanwhile, the associations between these single nucleotide polymorphisms (SNPs) and anterior chamber depth (ACD) and axial length (AL) were also evaluated.

## SUBJECTS AND METHODS

**Ethical Approval** The present study was approved by the Ethics Committee of Ningxia People's Hospital and its implementation process strictly complied with the standards of the Declaration of Helsinki. Each participant was informed in detail of the purpose of the study and signed a written informed consent prior to the study.

**Subjects** It consisted of 500 PACG cases and 720 unrelated control subjects recruited from Ningxia Eye Hospital from the northern regions of China. The detailed ophthalmic examinations for every participant as well as the inclusion and exclusion criteria were identical to our previous study[16].

**DNA Extraction** Peripheral venous blood was drawn from all subjects, genomic DNA was extracted utilizing the Simgen DNA Blood Mini Kit (Simgen, Hangzhou, China) according to the manufacturer's protocol. The extracted DNA was eluted in TE buffer (10 mmol/L Tris-HCl, 0.5 mmol/L EDTA, pH 9.0) and was measured for the A260/A280 optical density by Nanodrop2000. DNA was then stored at -80° until use.

**Single Nucleotide Polymorphism Selection and Genotyping** Drew on the experiences of previous studies[17-18], eleven SNPs were chosen as candidate SNPs including rs12076134, rs183532, rs235875 and rs235913 in *MYOC*, rs2422493, rs2487042, rs2472496, rs2472493, rs2487032, rs2472459 and rs2472519 near *ABCA1*. Genotyping was conducted using an improved multiplex ligation detection reaction (iMLDR) technique by Genesky Biotechnologies Inc (Shanghai, China).

**Table 1 Demographic characteristic of study participants**

| Parameters | PACG | Controls | *P* |
|---|---|---|---|
| Number | 500 | 720 | |
| Age, y, mean±SD | 63.77±9.576 | 71.82±7.2 | 0.000 |
| Gender, *n* (%) | | | 0.000 |
| Male | 147 (29.4) | 332 (46.1) | |
| Female | 353 (70.6) | 388 (53.9) | |

PACG: Primary angle-closure glaucoma.

**Statistical Analysis** The comparison of demographic characteristics between cases and controls and the correlation analysis between genotypes and ocular biometric parameters were implemented by SPSS software (version 17.5: SPSS Science, Chicago, IL, USA). Linkage disequilibrium (LD) patterns were generated using Haploview 4.2 software (Daly Lab at the Broad Institute, Cambridge, MA). The genetic association analyses were performed by PLINK (version 1.07; http://pngu.mgh.harvard.edu/-purcell/plink/, in the public domain) using a logistic regression model. Bonferroni correction was used for multiple comparisons and the statistical power was evaluated by the Power and Sample Size Calculation (PS; version 3.1.232).

## RESULTS

We enrolled 500 PACG patients and 720 control subjects in this study. The general demographic characteristics of the participants are listed in Table 1. The control subjects were significantly older and included less women than the case group.

All eleven SNPs conformed to the Hardy-Weinburg equilibrium (HWE; $P>0.05$; Table 2), in which, rs183532 in *MYOC* was nominally associated with PACG under the allelic model as well as the dominant genetic model ($P=0.025$, 0.029, respectively; Tables 2 and 3). The frequency of the minor T allele of rs183532 was less in the PACG group than in the control group. In addition, rs235875 in *MYOC* was also nominally associated with PACG under the recessive genetic model with a *P*-value of 0.032. The frequency of the TT genotype of rs235875 was higher in PACG group than in the control group (Table 3). However, none of these SNPs remained significant after Bonferroni correction. In LD analysis, three LD blocks were identified in strong linkage disequilibrium (Figure 1). A haplotype TTC in *MYOC* (including rs12076134, rs183532, rs235875) was found to be significantly different between the two groups ($P=0.018$), but the significance was lost after 10000 permutations ($P=0.157$; Table 4). There were no differences between the two groups in the distribution of genotype and allele frequencies for the remaining 9 SNPs. Furthermore, no association between the eleven SNP genotypes and the ocular biometric parameters of AL and ACD was found using generalized estimation equation tests (Table 5).

**Table 2 Allele frequencies and the association between SNPs and PACG under the allelic model**

| Gene | SNP | CHR | BP | Minor allele | Genotype (AA/Aa/aa)[a] | | MAF | | HWE-*P* | | OR (95%CI) | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Case | Control | Case | Control | Case | Control | | |
| *MYOC* | rs12076134 | 1 | 171606054 | G | 331/150/19 | 468/218/33 | 0.188 | 0.198 | 0.662 | 0.2401 | 0.954 (0.759-1.198) | 0.6828 |
| *MYOC* | rs183532 | 1 | 171609481 | T | 295/181/24 | 402/271/47 | 0.229 | 0.254 | 0.7037 | 0.9215 | 0.781 (0.629-0.969) | 0.025 |
| *MYOC* | rs235875 | 1 | 171613756 | T | 239/204/57 | 363/300/57 | 0.318 | 0.287 | 0.1813 | 0.716 | 1.171 (0.964-1.423) | 0.1124 |
| *MYOC* | rs235913 | 1 | 171618656 | T | 193/225/82 | 237/363/120 | 0.389 | 0.419 | 0.2586 | 0.3589 | 0.883 (0.733-1.063) | 0.1895 |
| *ABCA1* | rs2422493 | 9 | 107690995 | A | 175/242/83 | 232/374/114 | 0.408 | 0.418 | 1 | 0.0782 | 0.968 (0.801-1.171) | 0.7397 |
| *ABCA1* | rs2487042 | 9 | 107694522 | T | 262/192/46 | 366/296/58 | 0.284 | 0.286 | 0.2262 | 0.9274 | 0.959 (0.783-1.173) | 0.6817 |
| *ABCA1* | rs2472496 | 9 | 107695353 | A | 169/237/94 | 223/372/125 | 0.425 | 0.433 | 0.5219 | 0.1718 | 0.979 (0.812-1.181) | 0.8267 |
| *ABCA1* | rs2472493 | 9 | 107695848 | A | 149/245/106 | 195/382/142 | 0.457 | 0.464 | 0.7871 | 0.0722 | 0.992 (0.822-1.197) | 0.9303 |
| *ABCA1* | rs2487032 | 9 | 107703934 | A | 156/239/105 | 202/374/143 | 0.449 | 0.459 | 0.4697 | 0.2295 | 0.942 (0.782-1.135) | 0.5304 |
| *ABCA1* | rs2472459 | 9 | 107710562 | T | 250/196/54 | 350/306/64 | 0.304 | 0.302 | 0.1124 | 0.8596 | 0.981 (0.804-1.196) | 0.8476 |
| *ABCA1* | rs2472519 | 9 | 107715878 | G | 236/202/62 | 333/314/71 | 0.326 | 0.318 | 0.0832 | 0.8634 | 0.984 (0.809-1.196) | 0.8691 |

SNP: Single nucleotide polymorphisms; PACG: Primary angle-closure glaucoma; CHR: Chromosome; BP: Base pair position; MAF: Minor allele frequency; HWE-*P*: The *P*-value of Hardy-Weinburg equilibrium; OR: Odds ratio; CI: Confidence intervals. *P*, OR, and CI were calculated with logistic regression model by adjusting for age and gender under the allelic model with the major allele as reference. [a]A represents the wild allele and a represents the minor allele.

**Table 3 Genotype distribution of target SNPs in cases and controls**

| Gene | SNP | Dominant model | | | | | | Recessive model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aa+aa[a] | AA[a] | Aa+aa[b] | AA[b] | OR (95%CI) | *P* | aa[a] | AA+Aa[a] | aa[b] | AA+Aa[b] | OR (95%CI) | *P* |
| *MYOC* | rs12076134 | 169 | 331 | 251 | 468 | 0.960 (0.732-1.26) | 0.769 | 19 | 481 | 33 | 686 | 0.858 (0.447-1.649) | 0.647 |
| *MYOC* | rs183532 | 205 | 295 | 318 | 402 | 0.748 (0.575-0.972) | 0.029 | 24 | 476 | 47 | 673 | 0.707 (0.403-1.242) | 0.228 |
| *MYOC* | rs235875 | 261 | 239 | 357 | 363 | 1.114 (0.861-1.441) | 0.412 | 57 | 443 | 57 | 663 | 1.595 (1.04-2.446) | 0.032 |
| *MYOC* | rs235913 | 307 | 193 | 483 | 237 | 0.776 (0.593-1.015) | 0.064 | 82 | 418 | 120 | 600 | 0.987 (0.698-1.397) | 0.942 |
| *ABCA1* | rs2422493 | 325 | 175 | 488 | 232 | 0.852 (0.649-1.12) | 0.251 | 83 | 417 | 114 | 606 | 1.168 (0.822-1.661) | 0.386 |
| *ABCA1* | rs2487042 | 238 | 262 | 354 | 366 | 0.858 (0.663-1.11) | 0.243 | 46 | 454 | 58 | 662 | 1.324 (0.831-2.112) | 0.238 |
| *ABCA1* | rs2472496 | 331 | 169 | 497 | 223 | 0.836 (0.635-1.101) | 0.203 | 94 | 406 | 125 | 595 | 1.221 (0.873-1.71) | 0.244 |
| *ABCA1* | rs2472493 | 351 | 149 | 524 | 195 | 0.861 (0.647-1.146) | 0.305 | 106 | 394 | 142 | 577 | 1.18 (0.855-1.628) | 0.314 |
| *ABCA1* | rs2487032 | 344 | 156 | 517 | 202 | 0.795 (0.599-1.055) | 0.112 | 105 | 395 | 143 | 576 | 1.126 (0.816-1.554) | 0.471 |
| *ABCA1* | rs2472459 | 250 | 250 | 370 | 350 | 0.865 (0.668-1.119) | 0.270 | 54 | 446 | 64 | 656 | 1.401 (0.899-2.183) | 0.137 |
| *ABCA1* | rs2472519 | 264 | 236 | 385 | 333 | 0.846 (0.653-1.095) | 0.204 | 62 | 438 | 71 | 647 | 1.443 (0.95-2.192) | 0.086 |

SNP: Single nucleotide polymorphisms; OR: Odds ratio; CI: Confidence intervals. [a]The genotype counts in cases; [b]The genotype counts in controls; a represents the minor allele, A represents the wild allele; Dominant model: Aa+aa compared with AA; Recessive model: aa compared with AA+Aa. *P*, OR, and CI were calculated with logistic regression model by adjusting for age and gender.

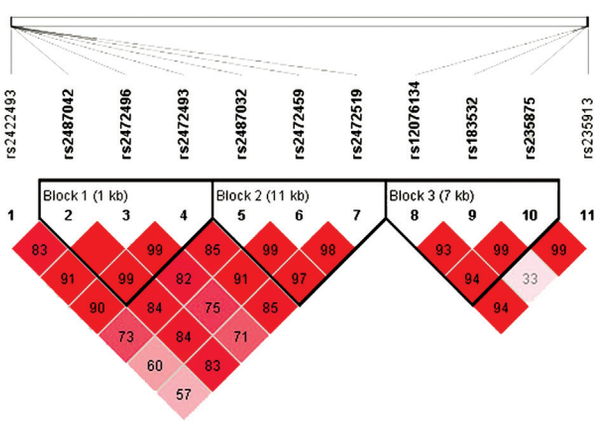


**Figure 1 The patterns of linkage disequilibrium of the eleven target SNPs with their D' value** Three haplotype blocks are presented in HapMap CHB cohort combined of PACG cases and controls.

Due to the differences of the minor allele frequencies (MAF), the power varies between the eleven SNPs. Therefore, assuming an allelic odds ratio (OR) of 1.5, our sample size provides more than 98% of statistical power to detect a significant association at an α level of 0.05.

## DISCUSSION

The present study evaluated the association of two POAG related genes *MYOC* and *ABCA1* with PACG in a northern China cohort and found rs183532 and rs235875 as well as a haplotype TTC in *MYOC* were nominally associated with PACG, moreover, there was no correlation between *ABCA1* and PACG. Glaucoma is a multifactorial disease, genetic factors have been found to be significant for its progression[1]. Studies have shown that PACG partly shared the genetic risks

**Table 4 Haplotype frequencies in PACG and control cohorts**

| Block | SNPs | Haplotype | $f^1$ (%) | $f^2$ (%) | OR[a] | *P*[a] | *P*-permutation[b] |
|---|---|---|---|---|---|---|---|
| Block 1 | rs2487042, rs2472496, rs2472493 | TAA | 0.283 | 0.286 | 0.958 | 0.679 | 0.9998 |
| | | CAA | 0.14 | 0.146 | 1.01 | 0.913 | 1 |
| | | CGA | 0.034 | 0.031 | 1.2 | 0.493 | 0.9967 |
| | | CGG | 0.541 | 0.537 | 0.998 | 0.98 | 1 |
| Block 2 | rs2487032, rs2472459, rs2472519 | ATG | 0.303 | 0.293 | 1.01 | 0.905 | 1 |
| | | ACG | 0.018 | 0.019 | 0.72 | 0.334 | 0.9671 |
| | | ACA | 0.127 | 0.141 | 0.948 | 0.691 | 1 |
| | | GCA | 0.546 | 0.534 | 1.06 | 0.536 | 0.9981 |
| Block 3 | rs12076134, rs183532, rs235875 | TCT | 0.315 | 0.284 | 1.18 | 0.104 | 0.6279 |
| | | TTC | 0.223 | 0.253 | 0.77 | 0.018 | 0.1576 |
| | | GCC | 0.179 | 0.196 | 0.94 | 0.598 | 0.999 |
| | | TCC | 0.274 | 0.264 | 1.1 | 0.34 | 0.9693 |

OR: Odds ratio; $f^1$: Haplotype frequencies in cases; $f^2$: Haplotype frequencies in controls. [a]OR and *P* value were calculated with logistic regression model by adjusting for age and gender. [b]A total of 10 000 permutations were performed.

**Table 5 Association between SNPs and AL and ACD**

| Gene | SNP | Minor allele | ACD (2.74±0.474; 0.67-3.51)[a] | | | AL (22.92±0.891; 20.01-25.51)[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | *β* | SE | *P* | *β* | SE | *P* |
| *MYOC* | rs12076134 | G | 0.008 | 0.0194 | 0.674 | -0.009 | 0.037 | 0.817 |
| *MYOC* | rs183532 | T | 0.027 | 0.0178 | 0.132 | -0.015 | 0.0345 | 0.667 |
| *MYOC* | rs235875 | T | -0.017 | 0.0175 | 0.330 | -0.010 | 0.0328 | 0.757 |
| *MYOC* | rs235913 | T | 0.008 | 0.0158 | 0.617 | 0.014 | 0.0290 | 0.635 |
| *ABCA1* | rs2422493 | A | 0.009 | 0.0162 | 0.598 | 0.017 | 0.0319 | 0.592 |
| *ABCA1* | rs2487042 | T | 0.009 | 0.0174 | 0.594 | -0.014 | 0.0338 | 0.688 |
| *ABCA1* | rs2472496 | A | 0.014 | 0.0161 | 0.395 | 0.006 | 0.0314 | 0.85 |
| *ABCA1* | rs2472493 | A | 0.013 | 0.0160 | 0.405 | -0.011 | 0.0313 | 0.725 |
| *ABCA1* | rs2487032 | A | -0.005 | 0.0161 | 0.758 | 0.025 | 0.0301 | 0.407 |
| *ABCA1* | rs2472459 | T | -0.0001 | 0.0178 | 0.996 | -0.013 | 0.0342 | 0.714 |
| *ABCA1* | rs2472519 | G | -0.003 | 0.0173 | 0.844 | 0.010 | 0.0337 | 0.770 |

SNP: Single nucleotide polymorphisms; AL: Axial length; ACD: Anterior chamber depth; *β*: Per-allele effect of the minor allele; SE: Standard error; *P*: *P*-value for association adjusting for age and gender. [a]The mean±SD and the range of measured values for AL or ACD.

with POAG[8]. *MYOC* is the first and the most significant gene found to be associated with POAG[9-10]. About 3% to 5% of adult POAG cases worldwide are caused by *MYOC*[19-23]. *MYOC* is preferentially expressed in the anterior segment of eye, especially in the TM[13,24]. Transgenic mouse models of POAG indicated mutant *MYOC* accumulated in the endoplasmic reticulum of TM, thereby inducing endoplasmic reticulum stress in TM, which was found to be associated with TM cell death and elevation of IOP[25-26]. The mechanism of action of *MYOC* working in POAG is still being researched deeply, since PACG and POAG share the same basic characteristics: elevated IOP and progressive degeneration of retinal ganglion cells and optic nerve axons[1]. Whether or not *MYOC* is associated with PACG is also worth exploring. Faucher *et al*[27] founded a mutation in *MYOC* was associated with PACG in the Quebec population. The association between *MYOC* gene mutation and PACG was also reported in Chinese population[28-29]. Even so, there are also some studies that did not support the association between *MYOC* mutation and PACG[30-31]. Hence. The relationship of *MYOC* gene variants with PACG is still unclear. In 2015, Jin *et al*[17] identified rs183532 in *MYOC* was associated with PACG in samples from south of China. The OR value of the A allele of rs183532 in their study was 1.541. In present study, we found rs183532 and rs235875 as well as a haplotype TTC in *MYOC* were nominally associated with PACG (uncorrected *P*=0.025, 0.032, 0.018; OR=0.781, 1.595, 0.77, respectively). The OR value of the two SNPs were in line with the haplotype, but contrary to previous Jin *et al*'s[17] finding. The explanation for this consequence is that our finding did not survive multiple testing corrections and may represent a false positive result. In addition, in view of the fact that Jin *et al*'s[17] study is relatively small sample size (212 patients and 255 controls), hence, the relationship between *MYOC* and PACG still need further study.

*ABCA1* gene encodes a membrane protein that is a major regulator of cellular cholesterol and phospholipid homeostasis[32]. It is expressed in many ocular tissues especially in the ganglion cell layer of retina[11]. Recent GWAS have indicated that genetic variants near *ABCA1* gene were significantly associated with POAG[11-12]. Variants near *ABCA1* gene were also associated with IOP in normal populations[15]. Previous studies using the DBA/2J glaucoma mouse model identified *ABCA1* was related to ganglion cell death[33]. Degeneration of retinal ganglion cells is a common sign of both PACG and POAG[1]. It also prompts us for the possible association between *ABCA1* and PACG. However, Luo *et al*[18] evaluated the correlation between *ABCA1* and PACG in a Chinese population and did not find a certain association between them, although they found two haplotypes in *ABCA1* were associated with PACG/PAC. In present study, the association between *ABCA1* and PACG has not been confirmed, thus, further research is necessary to validate the role of *ABCA1* in the progress of PACG.

Moreover, seeing that shallow ACD and short AL have been reported to be strong risk factors for PACG[34-36], the associations between these eleven SNP genotypes and the ocular biometric parameters of AL and ACD were also evaluated in our study. We found that none of the target SNPs showed significant association with ocular biometric parameters of AL and ACD. Our result suggests these POAG related genes *MYOC* and *ABCA1* do not have a role in the regulation of ocular biometric parameters of AL and ACD.

The limitation of the study is also obvious. The SNPs were chosen on the experiences of previous studies, which may not represent the gene completely in our cohort. Therefore, further study utilizing the tagger program based on our cohort should be done in the future.

In conclusion, our study investigated the association of two POAG related genes *MYOC* and *ABCA1* with PACG as well as ocular biometric parameters of AL and ACD in a northern Chinese cohort. Our result suggests these POAG related genes *MYOC* and *ABCA1* do not play a part in the pathogenesis of PACG as well as the regulation of ocular biometric parameters of AL and ACD. Additional studies are necessary to confirm this conclusion.

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