Genetic Variants at 20p11 Confer Risk to Androgenetic Alopecia in the Chinese Han Population

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Abstract

Background: Androgenetic alopecia (AGA) is a well-characterized type of progressive hair loss commonly seen in men, with different prevalences in different ethnic populations. It is generally considered to be a polygenic heritable trait. Several susceptibility genes/loci, such as AR/EDA2R, HDAC9 and 20p11, have been identified as being involved in its development in European populations. In this study, we aim to validate whether these loci are also associated with AGA in the Chinese Han population.

Methods: We genotyped 16 previously reported single nucleotide polymorphisms (SNPs) with 445 AGA cases and 546 healthy controls using the Sequenom iPLEX platform. The trend test was used to evaluate the association between these loci and AGA in the Chinese Han population. Conservatively accounting for multiple testing by the Bonferroni correction, the threshold for statistical significance was P ≤3.13×10⁻³.

Results: We identified that 5 SNPs at 20p11 were significantly associated with AGA in the Chinese Han population (1.84×10⁻¹¹ ≤P≤2.10×10⁻⁹).

Conclusions: This study validated, for the first time, that 20p11 also confers risk for AGA in the Chinese Han population and implicated the potential common genetic factors for AGA shared by both Chinese and European populations.

Citation: Liang B, Yang C, Zuo X, Li Y, Ding Y, et al. (2013) Genetic Variants at 20p11 Confer Risk to Androgenetic Alopecia in the Chinese Han Population. PLoS ONE 8(8): e71771. doi:10.1371/journal.pone.0071771

Editor: Qingyang Huang, Central China Normal University, China

Received February 1, 2013; Accepted July 3, 2013; Published August 26, 2013

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Funding: This work was supported by the General Program of the National Natural Science Foundation of China (30771941/H1106) and CMA-L’OREAL China Skin/Hair Grant (2008). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Androgenetic alopecia (AGA) is a common disease that is characterized by a distinctive pattern of progressive hair loss from the scalp. The proportion of affected males increases steadily with age, and its high prevalence in older men suggests that this form of hair loss may be a normal consequence of aging [1,2]. The prevalence of AGA is different among races. The prevalence of AGA in Chinese males is lower than in Caucasians at all ages [3,4].

The etiology of AGA was first described by Hamilton, including two essential components: genetic predisposition and hormone dependency [5,6]. So far, several genetic factors, such as the androgen receptor (AR/ the ectodysplasin A2 receptor (EDA2R) [7–10], histone deacetylase 9 (HDAC9) [11] and 20p11 [12,13], have been identified as being associated with this disorder. The present study aims to investigate whether the known susceptibility loci for the European populations are also associated with AGA in the Chinese Han population by the investigation of 8 SNPs at HDAC9 [11], 5 SNPs on 20p11 [13] and 3 SNPs at the AR/EDA2R locus [9,10].

Materials and Methods

Samples and DNA extraction

This investigation involved samples from 445 affected cases and 546 controls. All participants were males of Chinese Han descent (Table 1). The hair status of all participants was assessed by dermatologists according to the Hamilton/ Norwood (HN) classification system. Affected men were <30 years of age with AGA grades IV–VII or <40 years with AGA grades V–VII. The controls comprised 546 unaffected male controls aged >60 years. Blood samples were collected in tubes containing disodium ethylene diamine tetraacetic acid (EDTA) as an anticoagulant and stored at −80°C until extraction. Genomic DNA was extracted from peripheral blood lymphocytes using Flexi Gene DNA kits (Qiagen, Hilden, Germany) according to standard
procedures and diluted to working concentrations of 20 ng/μl for the replication study of the findings in the European population. All the samples were obtained after written informed consent. The study was approved by the Ethics Committee of Anhui Medical University and conducted according to Declaration of Helsinki Principles.

Genotyping
Genotyping was performed in multiplex reactions using the MassArray system and a Sequenom Compact MALDI-TOF device (Sequenom Inc., San Diego, CA, U.S.A.) at the Key Lab of Dermatology, Ministry of Education, Anhui Medical University, China. For the association analysis, the minor allele frequency (MAF) was ≥1% with P ≥0.05 for Hardy-Weinberg equilibrium in the controls, and SNPs with call rates higher than 95% in cases or controls were used for the SNP quality criteria.

Statistical analysis
For the association testing, the Armitage trend test was used to detect allelic and genotypic effects using Plink 1.07 software (Harvard, Boston, MA, USA). The power was calculated using the power Fisher exact test in SPSS (SPSS version 13.0). In total, 16 SNPs were genotyped in 445 cases and 546 controls. Conserva- tion was estimated from the genotypes of the SNPs on chromosome 20 using the Armitage trend test. The five SNPs that showed the highest statistical power were subsequently selected for replication (rs2180439, rs1998076, rs1131491, rs6137444 and rs201571) (Table 4). These five AGA susceptibility SNPs identified in the Chinese population were associated with one genetic locus (20p11). The SNP rs2180439 showed a stronger association (OR = 1.92, P = 1.84 × 10^{-4}) than the other four SNPs. Furthermore, controlling for the genetic effect of the SNP rs2180439 by using conditional logistic regression analysis also abolished the association of the other four SNPs (P > 0.05, Table 3). These findings indicate that the other four SNPs are correlated with the SNP rs2180439 and that the association of the other four SNPs is mainly driven by the SNP rs2180439. The difference in the distribution of the rs2180439 genotype between the AGA cases and controls was significant (P = 1.29 × 10^{-30}) (Table 4).

Discussion
This study is the first to investigate the contribution of the major AGA susceptibility loci AR/EDA2R, 20p11 and HDAC9 to the development of AGA in the Chinese Han population.

In this study, we confirmed that the susceptibility locus 20p11 for AGA in the Chinese Han population, which suggested the existence of common genetic factors shared for AGA in diverse ethnic populations. Because a gene contributing to the development of AGA would be expected to be expressed in the human scalp, Hillmer AM et al. quantified the gene expression of the 20p11 locus and the expression of the closest paired box 1 (PAX1) gene [13]. The results showed that PAX1 was expressed at very high levels in the scalp skin. Although the PAX1 gene is more than 100 kb away from the 20p11 locus, the expression data might suggest that PAX1 confers the AGA-relevant effect at this locus and that a regulatory variant within the associated LD block may modulate its expression through long-range control. However, demonstration of the influence of the chromosome 20p11 locus on the transcription of the PAX1 gene requires further investigation.

For the HDAC9 locus, the statistical power calculations showed that we had >80% power to detect 5 SNPs (rs13230142, rs12056282, rs56853, rs2249817 and rs10247184) associated with AGA, whereas other 3 SNPs (rs3852255, rs10252945 and rs17350355) had low power (≤70%), with SNP rs17350355 having the lowest power (36%). For those SNPs that had low power to detect an association, we could not confirm that these SNPs lacked an association with AGA because we might lack the power to detect a true association. Larger sample sizes will help improve the power and ensure the correct conclusion with respect to whether these SNP are associated with AGA. For those SNPs that had >80% power to detect an association, we confirmed that these SNPs are not associated with AGA; however, we do not deny that HDAC9 may be a risk factor for AGA because there could be different functional variants between Chinese and Caucasian populations or different LD patterns between the markers and hidden functional variants. For the AR/EDA2R locus, our genotype data showed that the three SNPs (rs1385699, rs2497911 and rs5919393) of the AR/EDA2R locus, which show significant association with AGA in European populations, were allelic and genotypic effects using Plink 1.07 software (Harvard, Boston, MA, USA). The power was calculated using the power Fisher exact test in SPSS (SPSS version 13.0). In total, 16 SNPs were genotyped in 445 cases and 546 controls. Conserva- tion was estimated from the genotypes of the SNPs on chromosome 20 using the Armitage trend test. The five SNPs that showed the highest statistical power were subsequently selected for replication (rs2180439, rs1998076, rs1131491, rs6137444 and rs201571) (Table 4). These five AGA susceptibility SNPs identified in the Chinese population were associated with one genetic locus (20p11). The SNP rs2180439 showed a stronger association (OR = 1.92, P = 1.84 × 10^{-4}) than the other four SNPs. Furthermore, controlling for the genetic effect of the SNP rs2180439 by using conditional logistic regression analysis also abolished the association of the other four SNPs (P > 0.05, Table 3). These findings indicate that the other four SNPs are correlated with the SNP rs2180439 and that the association of the other four SNPs is mainly driven by the SNP rs2180439. The difference in the distribution of the rs2180439 genotype between the AGA cases and controls was significant (P = 1.29 × 10^{-30}) (Table 4).

Acknowledgments
We thank all study participants and all volunteers who so willingly participated in this study, thus making this study possible.
### Table 2. Case-control association analysis for SNPs within AR/EDA2R, HDAC9 and 20p11 in 445 Han Chinese patients with AGA and 546 controls.

| SNP       | Samplea | locus       | Positionb | European | Chinese Han |
|-----------|---------|-------------|-----------|----------|-------------|
|           | Cases   | Controls    | A/B Allele | MAF (Case/Control) Risk Allele | Allelic P | OR(95%CI) | Risk Allele | Allelic OR(95%CI) | Power(%) |
| rs6137444 | 445     | 546         | C/T       | 0.269/0.391 | 2.20 x 10⁻¹⁰ 1.74(1.37-2.21) | C/T       | 0.272/0.401 | 34/174/237 | 96/246/204 | T       | 1.68 x 10⁻⁷ 1.79(1.49-2.17) | 98       |
| rs2180439 | 444     | 540         | C/T       | 0.293/0.452 | 2.67 x 10⁻⁸ 1.82(1.45-2.30) | C/T       | 0.271/0.417 | 29/183/232 | 108/234/198 | T       | 1.84 x 10⁻⁷ 1.92(1.59-2.33) | 99       |
| rs1998076 | 445     | 540         | A/G       | 0.292/0.448 | 7.73 x 10⁻⁹ 1.91(1.50-2.41) | A/G       | 0.270/0.411 | 29/182/234 | 102/240/198 | G       | 5.28 x 10⁻⁷ 1.89(1.56-2.27) | 99       |
| rs201571  | 443     | 540         | C/T       | 0.298/0.444 | 1.21 x 10⁻¹⁰ 1.72(1.36-2.17) | T/C       | 0.428/0.324 | 77/225/141 | 72/210/264 | T       | 2.10 x 10⁻⁷ 1.56(1.30-1.87) | 97       |
| rs6113491 | 445     | 540         | A/C       | 0.641/0.488 | 1.13 x 10⁻⁸ 1.66(1.33-2.08) | A/C       | 0.245/0.313 | 81/216/148 | 66/210/270 | A       | 1.92(1.59-2.27) | 98       |
| rs3852255 | 446     | 540         | T/C       | 0.045/0.030 | 1.45 x 10⁻⁶ 1.54(0.87-2.72) | T/C       | 0.136/0.149 | 10/101/334 | 6/144/396 | C       | 6.59 x 10⁻¹ 1.06(0.82-1.37) | 70       |
| rs3203142 | 445     | 534         | A/G       | 0.030/0.013 | 3.10 x 10⁻² 2.37(1.05-5.37) | A/G       | 0.117/0.118 | 8/88/349 | 6/114/414 | G       | 9.39 x 10⁻¹ 1.01(0.77-1.33) | 90       |
| rs12056282| 443     | 540         | C/T       | 0.043/0.022 | 2.90 x 10⁻¹³ 1.98(1.05-3.75) | C/T       | 0.135/0.144 | 11/98/334 | 6/144/390 | T       | 5.67 x 10⁻¹ 1.08(0.83-1.39) | 95       |
| rs756853  | 442     | 534         | G/A       | 0.496/0.380 | 4.65 x 10⁻¹ 1.61(1.30-1.98) | A/G       | 0.397/0.410 | 68/215/159 | 90/258/186 | G       | 5.59 x 10⁻¹ 1.05(0.88-1.27) | 92       |
| rs2249817 | 445     | 540         | C/T       | 0.475/0.411 | 8.90 x 10⁻¹ 1.59(1.29-1.95) | A/G       | 0.300/0.267 | 39/189/217 | 42/204/294 | A       | 1.02 x 10⁻¹ 1.18(0.97-1.44) | 82       |
| rs10247184| 445     | 540         | C/T       | 0.002/0.002 | 3.34 x 10⁻¹ 1.61(1.30-1.98) | T/C       | 0.123/0.137 | 9/91/345 | 18/114/414 | T       | 3.28 x 10⁻¹ 1.14(0.88-1.49) | 90       |
| rs10252945| 441     | 528         | A/C       | 0.286/0.256 | 1.97 x 10⁻¹ 1.17(0.92-1.47) | A/C       | 0.231/0.222 | 19/166/256 | 36/162/330 | A       | 6.11 x 10⁻¹ 1.06(0.85-1.31) | 52       |
| rs1730355 | 444     | 540         | G/A       | 0.441/0.354 | 5.81 x 10⁻¹ 1.44(1.17-1.78) | A/G       | 0.389/0.412 | 68/209/167 | 102/246/198 | G       | 2.87 x 10⁻¹ 1.10(0.92-1.32) | 36       |
| rs1385699 | 445     | 546         | AC/EDAR2R | 0.113/0.292 | 1.60 x 10⁻³ 3.23(1.86-5.59) | T/T       | 1.00/1.000 | 445 | 546 – | NA | NA | 90       |
| rs5919393 | 445     | 546         | AR/EDA2R  | 0.064/0.218 | 6.00 x 10⁻⁷ 5.82(2.73-12.41) | T/T       | 1.00/1.000 | 445 | 546 – | NA | NA | 90       |
| rs2497911 | 445     | 546         | AR/EDA2R  | 0.038/0.283 | 2.30 x 10⁻⁶ 1.44(1.17-1.78) | C/C       | 1.00/1.000 | 445 | 546 – | NA | NA | 100      |

**Note:**

a, Total samples (445 cases and 546 controls), some samples failed to genotype in the experiment.

b, In bp. NCBI build 36.3.

c, Minor allele.

d, MAF = Minor allele frequency.

e, Numbers shown correspond to the following genotypes: AA/AB/BB (A = minor allele; B = major allele).

f, the threshold for statistical significance was P<3.13 x 10⁻⁷. doi:10.1371/journal.pone.0071771.t002
Table 3. Association and conditional logistic analysis for rs2180439, rs6137444, rs1998076, rs201571 and rs6113491 in 445 cases and 546 controls.

| SNP       | CHR | Position* | Risk Allele | Risk Allele Frequency | OR(95%CI) | Condition rs2180439 |
|-----------|-----|-----------|-------------|-----------------------|-----------|---------------------|
| rs2180439 | 20  | 21801100  | T           | 0.729                 | 1.84×10⁻¹⁵| 1.92(1.59–2.33)     |
| rs6137444 | 20  | 21733659  | T           | 0.728                 | 1.68×10⁻¹⁵| 1.79(1.49–2.17)     |
| rs1998076 | 20  | 21828045  | G           | 0.73                  | 5.28×10⁻¹⁵| 1.89(1.56–2.27)     |
| rs201571  | 20  | 21961514  | T           | 0.428                 | 2.10×10⁻¹⁵| 1.56(1.30–1.87)     |
| rs6113491 | 20  | 22005415  | A           | 0.425                 | 2.82×10⁻⁷ | 1.62(1.35–1.95)     |

Note:

* In bp. NCBI build 36.3.

doi:10.1371/journal.pone.0071771.t003

Table 4. Genotypic effects analysis for rs2180439 in the AGA patients and controls.

| Genotype | Cases (n = 445) | Controls (n = 546) | OR (95%CI) | P |
|----------|-----------------|--------------------|------------|---|
| CC       | 29(6.53%)       | 108(20%)           | Reference  | 1.29×10⁻¹⁰|
| TC       | 183(41.22%)     | 234(43.33%)        | 2.91(1.85–4.58) | 6	×10⁻² |
| TT       | 232(52.25%)     | 198(36.67%)        | 4.36(2.78–6.85) | 6	×10⁻² |

doi:10.1371/journal.pone.0071771.t004

Author Contributions

Performed the experiments: BL, JZ, GC, FSZ, YTD. Analyzed the data: XBZ, YL, XDZ. Contributed reagents/materials/analysis tools: HYT, YJS, HC, SY, XFT. Wrote the paper: LDS, BL, YL. Participated with aspects of study design and interpretation of the data: XHF, TW, Y. Dong, ZWZ, JPG, DWD. Conceived this study and obtained financial support: CJY, XJZ. Designed the experiments: CJY, XJZ, BL, XBZ, YL.

References

1. Hamilton JB (1951) Patterned loss of hair in man; types and incidence. Ann N Y Acad Sci 53: 708–728.
2. Norwood OT (1975) Male pattern baldness: classification and incidence. South Med J 68: 1359–1365.
3. Wang TL, Zhou C, Shen YW, Wang XY, Ding XL, et al. (2010) Prevalence of androgenetic alopecia in China: a community-based study in six cities. Br J Dermatol 162: 843–847.
4. Xu F, Sheng YY, Mu ZL, Lou W, Zhou J, et al. (2009) Prevalence and types of androgenetic alopecia in Shanghai, China: a community-based study. Br J Dermatol 160: 629–32.
5. Prodi DA, Pirastu N, Maninchedda G, Sassa A, Picciau A, et al. (2008) EDA2R is associated with androgenetic alopecia. J Invest Dermatol 128: 2268–2270.
6. Hillmer AM, Hanneken S, Ritzmann S, Becker T, Freudenberg J, et al. (2005) Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia. Am J Hum Genet 77: 140–148.
7. Cobb JE, Zaloumis SG, Scurrah KJ, Harrap SB, Ellis JA (2010) Evidence for two independent functional variants for androgenetic alopecia around the androgen receptor gene. Exp Dermatol 19:1026–8.
8. Broschmidt FF, Hanneken S, Hanneken S, Hellmann S, et al. (2011) Susceptibility variants on chromosome 7p21.1 suggest HDAC9 as a new candidate gene for male-pattern baldness. Br J Dermatol 165: 1293–1302.