Insulin gene polymorphism and premature male pattern baldness in the general population

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ABSTRACT

Insulin is found in hair follicles and may play a role in the regulation of androgen metabolism and the hair growth cycle, which are relevant to the loss of scalp hair known as male pattern baldness. An excess of dihydrotestosterone on balding scalp indicates that the condition is androgen dependent. Premature male pattern baldness may be the male phenotype of familial polycystic ovary syndrome, a condition characterized by high levels of androgens and insulin that has been linked to insulin gene polymorphism. Therefore, we studied possible associations between relevant insulin gene polymorphisms and premature male pattern baldness in the general community. We examined the distribution of three dimorphic restriction fragment length polymorphisms: HphI, PstI and FokI in cases consisting of 56 men aged 18–30 years with significant baldness, and in 107 control men aged 50 years or more with no indication of baldness. No significant differences between cases and controls in allele, genotype or haplotype frequencies were identified. We conclude that, in the general population, the insulin gene is not associated with premature male pattern baldness.

INTRODUCTION

Male pattern baldness (MPB) is defined as the genetically predisposed loss of scalp hair which follows a defined pattern and is androgen dependent [1,2]. The mode of inheritance of MPB is often cited as autosomal dominant [3], but a polygenic aetiology appears more likely [4,5]. The genetic defect(s) underlying this condition are unknown, although clues are provided by the excess production of dihydrotestosterone on balding scalp [6]. Dihydrotestosterone is formed from the reduction of testosterone by the action of the enzyme 5α-reductase. The genes encoding the two forms of 5α-reductase were for some time suspected of involvement in MPB, but we have recently shown that common variants of these genes do not appear to be associated with this disorder [5].

Polycystic ovary syndrome (PCOS) is a familial endocrine disorder affecting as many as 10% of females of reproductive age. It is characterized by hyperandrogenism and hyperinsulinaemia. Peripheral insulin resistance appears to be the main cause of the hyperinsulinaemia, and PCOS patients have a 7-fold increased risk of Type II (non-insulin-dependent) diabetes [7]. In males from families with PCOS, an increase in the prevalence of premature MPB (significant balding before 30 years of age) in an autosomal dominant pattern of inheritance [8,9] has led investigators to suggest that premature MPB is the male phenotype of the disease [9,10]. The genetic disorder(s) underlying PCOS may therefore provide clues for MPB. Recently, linkage and association of familial PCOS with the insulin gene (INS) regulatory variable number of tandem repeats (VNTR) was reported [7].

Apart from their association in PCOS [11–13], androgen and insulin pathways appear to be closely linked in both women and men [14]. There is a strong association between circulating levels of insulin and sex hormone-binding globulin, and possibly between insulin and...
testosterone in normal, obese and diabetic men [14–20]. Furthermore, insulin found in hair follicles appears to play a role in the control of the growth cycle of the hair [21].

In view of its linkage with PCOS and potentially important effects on androgen metabolism generally, the INS gene is a good candidate for MBP. Importantly, the INS polymorphism linked to PCOS is thought to have functional effects on INS gene expression [22]. Therefore, we performed a population-based case-control association study of the INS gene in premature MBP using three dimorphic restriction fragment length polymorphisms (RFLPs), one of which is in strong allelic association with the VNTR [7].

METHODS

Sampling

The details of recruitment and the characteristics of participants have been reported previously [5]. Approximately 3000 Caucasian individuals were recruited as part of the Victorian Family Heart Study. This is a general population survey of coronary risk factors and consists of approximately 800 families, comprising a mother, father and at least one natural offspring. Baldness survey questionnaires were sent to all males (approximately one half of the sample number) asking them to detail their degree of baldness, if any, in terms of the Hamilton baldness scale [2,23]. The survey described all categories of the scale, and provided diagrams to assist in the self-assessment. Validation of this self-reported questionnaire approach has been reported previously [5].

A total of 1073 men returned the completed baldness questionnaires. This group contained 529 sons aged 18 to 30 years and 544 fathers aged 50 years and above. Cosmetically significant baldness of type III or greater [23] was reported by 58 (11.0%) sons aged 18 to 30 years and 544 fathers aged 50 years and above. These individuals were defined as cases and DNA was available for analysis in 56 of these subjects. A total of 114 fathers aged 50 and above (21.0%) reported no baldness (Hamilton baldness score of 1). Two individuals were identified as fathers of young balding men used in the case group, and were therefore excluded from the study. DNA was available from 107 of the remaining individuals who comprised the control group for our genetic analyses.

Analysis of RFLPs of the INS gene

DNA was extracted from leucocytes using standard phenol/chloroform techniques. Approximately 50 ng of DNA from each individual was used in polymerase chain reactions (PCRs). The HphI RFLP, located 573 bp distal to the regulatory VNTR and −23 bp from the start site of the INS gene, was detected by amplification of DNA using primers ins04 (5′-TCCAGGACAGGCTGCA-T-CAG-3′) and ins05 (5′-AGCAATGGGGCTTG-GCTCA-3′) [24]. DNA was added to a mix containing 0.5 μM of each primer, 1 × PCR buffer (Perkin–Elmer Applied Biosystems, Norwalk, CT, U.S.A.), 250 μM dNTP (Perkin–Elmer Applied Biosystems), 1.5 mM MgCl$_2$ (Perkin–Elmer Applied Biosystems) and 1 unit AmpliTag Gold DNA polymerase (Perkin–Elmer Applied Biosystems), to give a total reaction volume of 20 μl. The thermal conditions required for the reaction were 95 °C for 10 min (for activation of the AmpliTag Gold enzyme), followed by 35 cycles of 95 °C for 1 min, 64 °C for 1 min and 72 °C for 1 min, followed by a final extension time of 72 °C for 10 min. Products were then digested by the addition of 5 units of HphI restriction endonuclease (New England Biolabs, Beverly, MA, U.S.A.) in the presence of 1 × buffer ‘4’ (New England Biolabs) at 37 °C for 1 h. Digested products were then electrophoresed through a 2% agarose gel (Type II-A, Sigma, St. Louis, MO, U.S.A.) containing ethidium bromide. DNA was visualized by placing the gel on an UV transilluminator.

The PsI RFLP located +1127 bp from the INS start site was detected in a similar manner. Primers ins01 (5′-GAAGGAGGTGGAAGCATGT-3′) and ins02 (5′-GCTGGTTCAGGCTTTA-3′) were used to amplify DNA [24]. Again, approximately 50 ng of DNA was added to a reaction mixture as described above. Thermal conditions required for the reaction were 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, followed by a final extension of 72 °C for 10 min. PCR products were digested with 5 units of PsI (Boehringer Mannheim, Indianapolis, IN, U.S.A.) in the presence of 1 × buffer ‘H’ (Boehringer Mannheim) at 37 °C for 1 h. Similarly, the FokI RFLP located +1428 bp from the INS start site was detected by amplification of DNA with primers ins13 (5′-TAAAGCCCTTGAACCAGC-3′) and DS02 (5′-CAGCCAGGCTCCTCCCTCCAAC-3′) [24]. Thermal conditions required were 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 62 °C for 1 min and 72 °C for 1 min, followed by a final extension of 72 °C for 10 min. PCR products were digested with 5 units of FokI (Boehringer Mannheim) in the presence of 1 × buffer ‘M’ (Boehringer Mannheim) at 37 °C for 1 h. Digested products were electrophoresed and visualized as above.

RESULTS

Lack of association of the INS RFLPs with baldness

Table 1 summarizes the distribution of the resulting alleles after digestion of PCR products by HphI, PsI and
Table 1  Distribution of INS RFLP alleles in young bald men (cases) and older non-bald men (controls)

<table>
<thead>
<tr>
<th></th>
<th>HphI RFLP alleles</th>
<th>PstI RFLP alleles</th>
<th>FokI RFLP alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Cases</td>
<td>34 (30.4)</td>
<td>78 (69.6)</td>
<td>84 (75.0)</td>
</tr>
<tr>
<td>Controls</td>
<td>63 (29.4)</td>
<td>151 (70.6)</td>
<td>156 (72.9)</td>
</tr>
</tbody>
</table>

Table 2  Distribution of genotypes for each INS RFLP analysed in young bald men (cases) and older non-bald men (controls)

<table>
<thead>
<tr>
<th></th>
<th>HphI RFLP genotypes</th>
<th>PstI RFLP genotypes</th>
<th>FokI RFLP genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
</tr>
<tr>
<td>Cases</td>
<td>8 (14.3)</td>
<td>18 (32.1)</td>
<td>30 (53.6)</td>
</tr>
<tr>
<td>Controls</td>
<td>7 (6.5)</td>
<td>49 (45.8)</td>
<td>51 (47.7)</td>
</tr>
</tbody>
</table>

FokI. No significant differences were found between cases and controls for either allele of each RFLP (HphI: \( \chi^2 = 0.029, df = 1, P = 0.86 \); PstI: \( \chi^2 = 0.168, df = 1, P = 0.68 \); FokI: \( \chi^2 = 0.099, df = 1, P = 0.75 \)). The possible individual genotypes for each RFLP (i.e. AA, AB or BB) were also compared in cases and controls. The distribution of genotypes is shown in Table 2. Again, no significant differences in genotypes were detected between groups (HphI: \( \chi^2 = 4.33, df = 2, P = 0.11 \); PstI: \( \chi^2 = 4.89, df = 2, P = 0.087 \); FokI: \( \chi^2 = 3.26, df = 2, P = 0.20 \)). Finally, we analysed haplotypes of the three INS RFLPs in cases and controls. A total of 27 different haplotypes resulting from combinations of all alleles of the three RFLPs was possible. No significant differences in haplotype distributions were demonstrated between the groups (\( \chi^2 = 10.26, df = 26, P = 0.997 \)) (results not shown).

**DISCUSSION**

We have shown that the INS gene does not appear to play a role in the aetiology of premature MPB in the general population. Our primary interest was in a polymorphism of functional relevance to INS gene expression. The INS VNTR, located within the promoter region of the INS gene, is thought to regulate insulin expression [22] and it is this region which has been linked to PCOS. In our study we utilized the HphI RFLP which is located 573 bp 3’ to the VNTR. Our marker has been shown to be in very close allelic association with the INS VNTR and also in strong association with PCOS [7]. Nevertheless, our analysis showed that the distribution of alleles and genotypes relevant to the HphI RFLP was indistinguishable statistically between subjects with premature MPB and controls. Additionally, we analysed two RFLPs 3’ to the INS gene (FokI and PstI) [24] to investigate the possibility of functional mutations elsewhere in the INS gene. The FokI and PstI RFLPs were in strong allelic association but were not associated with MPB in our population.

Our interest in the INS gene arose because of reported genetic linkage with PCOS. PCOS is reportedly one of the most common female endocrine disorders [12], and may be expressed in males as premature MPB [9,10]. The negative findings in this study might be explained if, in our population, PCOS is not common, premature MPB is not a male phenotype of PCOS or the INS gene is not linked with PCOS.

The prevalence of PCOS in the Australian population has not been reported but the generally accepted figure is 5–10% of women of reproductive age [12]. In comparison, the overall prevalence of premature MPB in our participants was 11%. In males, the phenotypic expression of PCOS related to hair growth includes premature baldness and excessive hairiness. In first-degree male relatives of female cases of PCOS, 19.7% had early baldness and/or excessive hairiness, compared with 6.5% of first-degree male relatives of controls (\( P < 0.01 \)) [9]. However, when premature balding is separated from excessive hairiness, the percentage of first-degree male relatives of PCOS patients was only 11.1%. It is possible that the earlier appearance of MPB in first-degree relatives is secondary to high androgen levels per se rather than sharing a common pathophysiology related to the INS gene.

Further independent confirmation of linkage between INS VNTR and PCOS has not yet been reported.
Interestingly, the LOD score achieved by Waterworth et al. [7] was borderline (maximum LOD score = 3.25) [25] and was observed in only 9 of 17 families.

It should be noted that there are several limitations to this type of association study, such as the possibility of population stratification between the case and control groups, which we have discussed previously [5]. It is unlikely, however, that such limitations are masking an association of MPB to INS, since population stratification is normally associated with a type I, or false-positive, error. In addition, we have surveyed three separate RFLPs of the INS gene and all produce a similar result.

We conclude that the gene encoding insulin does not appear to be associated with premature MPB in the general population. This does not necessarily indicate that MPB and PCOS are not in some way associated. However, given the link between the androgen and insulin pathways, and the role of insulin in hair growth cycle regulation, other genes involved in the insulin pathway may be worthy of investigation.

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REFERENCES

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