Re: Polymorphisms Associated With Circulating Sex Hormone Levels in Postmenopausal Women

The Journal recently published a study analyzing the role of sequence variation of genes involved in steroid hormone metabolism in the serum sex hormone levels of postmenopausal women (1). This study concluded that CYP19 and SBGH polymorphisms contribute to the variability in circulating sex hormones in postmenopausal women, although CYP19 or SHBG polymorphisms are only marginally related to breast cancer susceptibility. However, after comparing results from a study we recently published (2) with those of Dunning et al. (1), we think that because of the complexity of the genetic control of estrogen production in pre- and postmenopausal women, a multilocus rather than a marker-by-marker statistical analysis may be best suited for the study of the genetic component of complex traits such as hormone levels or breast cancer risk (3). Therefore, we would like to suggest further analysis of the data presented by Dunning et al.

Using a pharmacogenetics approach, we are studying the role of different loci looking at the effect of gonadotropin releasing hormone agonist (GnRHa) and other hormones during controlled ovarian stimulation (COS) in premenopausal women (2). Hormone-related cancers and assisted reproduction techniques are routine GnRHa indications. GnRHa administration suppresses the estrogen production in the ovary via the biochemical ablation of the hypothalamus–hypophysis–ovary axis (pituitary suppression). Pituitary suppression represents a unique pharmacogenetics model to study the function and regulation of the CYP19 aromatase, because only a small proportion of women reach partial estrogen synthesis suppression after GnRHa treatment (4,5). To study the association of different genetic polymorphisms with pituitary suppression caused by GnRHa treatment, 213 premenopausal women were treated with GnRHa tryptorelin, as previously reported (2). Patients were grouped according to degree of pituitary suppression and genotyped for several loci (2). Table 1 summarizes the results of our study using four single-nucleotide polymorphism (SNP) markers located at FSHR, CYP19, ESR1, and ESR2. Twenty-four percent of the patients responded only partially to GnRHa treatment. In addition, our results suggest that the rs10046 marker of the CYP19 locus is associated with pituitary suppression in premenopausal women (Pallele positivity test for risk allele c < .001). These results are also consistent with quantitative analysis that showed a higher mean number of days to reach pituitary suppression in women who carry the cc genotype than the tt genotype at the rs10046 locus (Kruskall–

| Parameter                              | Partially suppressed (estradiol >40 pmol/L) | Fully suppressed (estradiol <40 pmol/L) | P  
|----------------------------------------|---------------------------------------------|----------------------------------------|------
| No. of patients                        | 51                                          | 162                                    |      
| Age, y                                 | 32.2 ± 2.7                                  | 32.9 ± 2.5                             | .12† 
| Cause of sterility, male/tubaric/both/unknown | 32/12/70                                   | 96/61/72                               | .76‡ 
| Time of treatment with tryptorelin, days | 29.8 ± 11                                  | 20.4 ± 8.3                             | <.001† 
| FSHR Ser680Asn genotypes, Asn/Asn, Ser/Asn, Ser/Ser§ | 17, 19, 6                                   | 36, 59, 29                             | .101 (allele freq. difference) 
| CYP19 rs10046 genotypes, CC/CT/TT¶     | 15/30/6                                     | 34/69/59                               | <.001 (allele positivity) 
| ESR1 g.938C>T genotypes, TT/TC/CC§     | 13/20/9                                     | 45/66/23                               | .509 (allele freq. difference) 
| ESR2 39A>G genotypes, AA/AG/GG§        | 11/21/10                                    | 22/64/38                               | .207 (homozygous) 

*Mean value ± standard deviation.
†Kruskall–Wallis test.
§One hundred and sixty-six genotypes available for FSHR, ESR1, and ESR2 genes.
¶Analysis was performed using the online resource at http://ihg.gsf.de and includes the following: deviation from Hardy–Weinberg equilibrium, allele frequency differences test, heterozygous test, homozygous test, allele positivity test, and Armitage’s trend test. Because of the multiple testing, Bonferroni’s correction has been applied. All statistical tests were two-sided.
∫Two hundred and thirteen genotypes available for the CYP19 gene.
Finally, following a conservative multilocus analysis (2), a genetic interaction between CYP19, ESR1, and ESR2 loci and pituitary suppression was detected ($P = .0199$), suggesting that pituitary suppression could be a polygenic trait.

In accordance with Dunning et al., our data support the finding that genetic variation in CYP19 contributes to variance in circulating estrogen levels. However, despite an almost identical distribution of genotype/allele frequencies at the rs10046 locus in English and Spanish populations ($P_{	ext{genotype distribution}} = .55$), serum estrogen levels after GnRHa suppression in our study were radically different from those obtained by Dunning et al. (1).

In their study of postmenopausal women, those carrying the rs10046 locus t allele had high serum estradiol, whereas in our study of premenopausal women, those carrying the c allele had high levels of serum estradiol after pituitary suppression ($P < .001$). A simple explanation for this difference would be that the same marker is tracking different polygenes in different populations. Alternatively, if the rs10046 marker is tracking independent CYP19 functional alleles in tissue-specific promoters that are activated differentially before and after menopause, the same marker could yield different results in pre- and postmenopausal women (6,7). To test the differential promoter hypothesis, patient stratification (pre- and postmenopausal) would be required to study the association of the CYP19 allele with breast cancer risk. This analysis should be easily addressed by Dunning et al., given the extraordinary breast cancer cohort obtained available to these researchers (1). Finally, the existence of genetic interactions between CYP19, ESR1, and ESR2 loci detected in pituitary suppression outcome underscores the necessity of a multilocus, rather than a marker-by-marker, analysis to explore the role of genetic polymorphisms in hormone levels and cancer risk.

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REFERENCES

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Table 1. CYP 19 SNPs rs10046 genotype distributions in pre- and postmenopausal breast cancer case patients and control subjects and breast cancer risk.*

<table>
<thead>
<tr>
<th>Menopausal status</th>
<th>SNP rs10046 genotype</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tt</td>
<td>tc</td>
</tr>
<tr>
<td>Premenopausal women, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case patients (n = 1407)</td>
<td>408</td>
<td>674</td>
</tr>
<tr>
<td>Control subjects (n = 487)</td>
<td>151</td>
<td>254</td>
</tr>
<tr>
<td>OR (95% CIs)</td>
<td>1.00 (0.83 to 1.21)</td>
<td>1.07 (0.92 to 1.24)</td>
</tr>
<tr>
<td>Postmenopausal women, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case patients (n = 902)</td>
<td>242</td>
<td>454</td>
</tr>
<tr>
<td>Control subjects (n = 3127)</td>
<td>895</td>
<td>1530</td>
</tr>
<tr>
<td>OR (95% CIs)</td>
<td>1.00 (0.87 to 1.15)</td>
<td>1.10 (0.99 to 1.22)</td>
</tr>
</tbody>
</table>

*Patients were drawn from the Anglican Breast Cancer Study, and matched control subjects, were recruited through the European Prospective Investigation of Cancer study (1). Odds ratios (ORs) and confidence intervals (CIs) were calculated using unconditional logistic regression; all statistical tests were two-sided (1). SNP = single nucleotide polymorphism.

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RESPONSE

De Castro et al. have studied the response of 213 premenopausal women to gonadotrophin releasing hormone agonist (GnRHa) in relation to genotype at several single nucleotide polymorphisms (SNPs) in hormone signaling and metabolism genes. For the CYP19 3’untranslated region (UTR) t–c SNP (rs10046), they report an association between the c allele and circulating estradiol levels opposite to the one we recently reported in 1975 postmenopausal women (1), although they do not present the mean values observed in each genotype class for comparison. They argue that the opposing results may be due to SNP rs10046 acting as a marker for functional alleles in the multiple CYP19 gene promoters and that these promoters may be activated differently in pre- and postmenopausal women.

From our data we calculated that we would expect a 5–10% relative increase in the risk of breast cancer due to an...
increase in mean circulating estradiol level from 15.0 pmol/L (cc genotype) to 17.1 pmol/L (tt genotype). In our breast cancer case–control study (N = 2635 case patients and 3630 control subjects), we observed an odds ratio of 1.07 (95% confidence interval = 0.96 to 1.19) (1) for risk of breast cancer in women with the cc genotype versus those with the tt genotype. To investigate the suggestion of De Castro et al. that there should be different odds ratios in pre- and postmenopausal women, we determined the menopausal status for 2309 case patients and 3614 control subjects (Table 1). The genotype distribution between pre- and postmenopausal case patients ($\chi^2 = 1.6$, $P = .45$, 2 degrees of freedom) is similar. The estimated odds ratios for the two groups are also similar and are both consistent with the predicted 5–10% relative increase from the effect of circulating hormone levels (1) (although they are also consistent with no association between genotypes and breast cancer risk).

Thus, we do not have any evidence for a differential effect of SNP rs10046 in risk of pre- versus postmenopausal breast cancer. Moreover, Haiman et al. (2) demonstrate that multiple promoter exons of CYP19 fall into four different linkage disequilibrium blocks, with recombination occurring rarely between each block. Hence, rs10046, which is in block 4, is unlikely to be a good marker for promoter variants in blocks 1, 2, and 3, as proposed by De Castro et al. In addition, Kristensen et al. (3) reported that the c allele of rs10046 is associated with reduced CYP19 RNA levels, which would lead in turn to low CYP19 levels and activity. Therefore, the 3’UTR SNP c allele appears to be a likely cause of the differences observed in circulating estradiol levels in postmenopausal women in our study. The association of the rs10046 SNP allele with circulating estradiol levels reported by De Castro et al. may reflect a response to GnRHa treatment, rather than natural variation in estradiol levels.

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