Research Highlights

Highlights from the latest articles in pharmacogenomics

Tamoxifen treatment outcome may be determined by host genotype

Evaluation of: Rokavec M, Schroth W, Amaral SM *et al.*: A polymorphism in the *TC21* promoter associates with an unfavorable tamoxifen treatment outcome in breast cancer. *Cancer Res.* 68(23), 9799–9808 (2008).

– MJ Higgins & V Stearns

Although the majority of estrogen receptor (ER)-positive tumors respond to treatment with the selective ER modulator tamoxifen, resistance to the drug is a clinically relevant problem. Resistance to therapy may be intrinsic or acquired. One possible cause of intrinsic resistance to tamoxifen is a genetic polymorphism in the enzyme pathway involved in metabolizing the drug to its more active metabolite, endoxifen [1]. The presence of alleles which code for reduced or inactive CYP2D6 enzymes have been shown to correlate with a less favorable recurrence-free time (RFT) and disease-free survival [2–4]. Potential mechanisms of intrinsic or acquired resistance may include altered receptor expression [5], and altered crosstalk between ERs and growth factor signaling, including the Ras superfamily of proteins [6]. *TC21* is a member of this superfamily. Although *TC21* mutations are rare in breast cancer cell lines, *TC21* protein overexpression is not infrequent. *TC21* oncogenic signals are mediated via the PI3K/Akt, NF-κB and Ral signaling pathways. The *TC21* -582C>T promoter SNP has a minor allele frequency of 34% in Europeans. The investigators hypothesized that the *TC21*-582C>T polymorphism may alter protein expression and outcomes of tamoxifen-treated women [7].

Microarray analyses of breast tumors consistently demonstrated higher *TC21* mRNA and *TC21* protein expression in

ER-negative compared with ER-positive tumors and cell lines. *TC21* expression appeared to be repressed by ER. *TC21* expression in ER-positive tumors was found to be higher in carriers of the *TC21*-582C>T polymorphism, an observation which suggests that this repression is allele dependent. The investigators studied whether *TC21* protein expression and, in particular, the *TC21*-582C>T polymorphism could potentially predict the treatment effect of tamoxifen. In a group of patients treated with a single agent adjuvant tamoxifen, patients with strong cytoplasmic *TC21* expression in their tumors ($n = 45$) or the presence of the -582T allele ($n = 206$), had increased recurrence rates (hazard ratio [HR]: 3.06; 95% CI: 1.16–18.05; HR: 1.79; 95% CI: 1.08–3.00, respectively). A significant association between *TC21* expression and overall survival was not seen in either patients who received tamoxifen treatment or those who did not.

Using the patient collection previously described by Schroth *et al.* when reporting on adjuvant tamoxifen breast cancer treatment outcome relative to the patients' *CYP2D6* and *CYP2D19* genotypes [4], a combined analysis of *CYP2D6* and *TC21* genotype status was performed. Patients with a mutated genotype in either *TC21* or *CYP2D6* had a less favorable RFT when compared with patients with both wild-type genotypes but had a more favorable RFT when compared with patients with who had both genes mutated.

These preliminary data are provocative but will need to be confirmed in larger, prospective clinical studies. The conclusion that the *TC21*-582C>T polymorphism is a potential classifier of tamoxifen treatment outcome is in agreement with current observations of ER and growth factor signaling [8–10]. It is likely that in the

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future, analyses of both host and tumor factors will be used to select personalized treatment choices.

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Host and tumor characteristics determine response to tamoxifen in hormone receptor-positive breast cancer

Evaluation of: Goetz M, Suman V, Couch F *et al.*: Cytochrome P450 and homeobox13/interleukin-17B receptor: combining inherited and tumor gene markers for prediction of tamoxifen resistance. *Clin. Cancer Res.* 14(18), 5864–5868 (2008).

– MJ Higgins & V Stearns

The selective estrogen receptor (ER) modulator, tamoxifen, has been used for over 30 years to treat and, more recently, to prevent breast cancer. Unfortunately, even among patients with ER-positive tumors, the response achieved with tamoxifen treatment is variable. Efficacy and safety of anticancer therapies such as tamoxifen may depend not only on tumor, but also on treatment and host characteristics.

Tamoxifen is biotransformed to the potent anti-estrogen, endoxifen, almost exclusively via the cytochrome P450 (CYP) 2D6 isoform [1]. Although prospective data are lacking, the balance of evidence available at this time suggests that SNPs in the *CYP2D6* gene, in particular the

presence of two null alleles, predicts for reduced tamoxifen metabolism and possibly a poorer outcome than that expected in patients with a wild-type genotype [2]. Women with normal wild-type *CYP2D6* who take concomitant drugs that strongly inhibit CYP2D6 have reductions in their serum endoxifen concentrations similar to those seen in women with two null alleles (poor metabolizers) [1,3].

Using microarray analysis of tumors from women with ER-positive breast cancers, Ma *et al.* have previously described the homeobox13 (*HOXB13*)/interleukin-17B receptor (*IL17BR*) gene ratio as an independent predictor of treatment outcome [4]. In both hormone-sensitive cell lines and follow-up studies in clinical databases, *HOXB1* expression has been shown to be a marker of reduced sensitivity to tamoxifen and the *HOXB13:IL17BR* ratio as both a prognostic marker and a biomarker predictive of tamoxifen benefit [5–8].

The study investigators hypothesized that because *CYP2D6* genotype and *HOXB13:IL17BR* represent biologically independent markers of tamoxifen

resistance, a combined index may provide a better indicator of tamoxifen treatment benefit. Using the original study population that provided earlier findings on *CYP2D6* and *HOXB13:IL17BR* [5,9,10], the combined effect of *CYP2D6* and *HOXB13:IL17BR* on the outcomes of disease-free survival (DFS) and overall survival (OS) in 110 women with lymph node-negative breast cancer treated with adjuvant tamoxifen monotherapy was retrospectively evaluated [11]. Information on the concomitant use of CYP2D6 inhibitors was incorporated in this study.

A risk factor (*CYP2D6:HOXB13:IL17BR*) representing the four categories of combining CYP2D6 metabolism (extensive or decreased) and *HOXB13:IL17BR* (low or high) was created. The combined *CYP2D6:HOXB13:IL17BR* risk factor was significantly associated with DFS (log-rank $p = 0.004$) and ($p = 0.009$). Relative to women with extensive CYP2D6 metabolism and low *HOXB13:IL17BR*, those with either decreased metabolism or a high *HOXB13:IL17BR* ratio had a significantly worse OS (adjusted

hazard ratio [HR]: 2.41; 95% CI: 1.08–05.37; $p = 0.031$) whereas women with both decreased metabolism and high *HOXB13:IL17BR* ratio had the shortest survival (HR: 3.15; 95% CI: 1.17–18.52; $p = 0.024$).

The findings suggest that an index composed of host (*CYP2D6*) and tumor (*HOXB13:IL17BR*) gene variation could be used to identify patients with varying degrees of tamoxifen resistance. It is anticipated that such personalized recommendations will be used in future to guide therapeutic choices for breast cancer patients.

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Genetic determinants of heavy smoking

Evaluation of: Stevens V, Bierut L, Talbot J *et al.*: Nicotinic receptor gene variants influence susceptibility to heavy smoking. *Cancer Epidemiol. Biomarkers Prev.* 17(12), 3517–3525 (2008).

– EC Johnstone & MR Munafò

Since the heritability of smoking behavior was first established, research into genetic influences of aspects of this behavior, including ability to quit, has focused on two areas: the neurobiological basis of reward, mainly via dopaminergic pathways, and the rate of nicotine metabolism. Emerging evidence from this study [1], as well as preceding genome-wide association studies (GWAS), strongly supports a role for genetic variation in certain nicotinic receptors in moderating the smoking habit. In particular, the *CHRNA5–CHRNA3–CHRNA4* gene cluster has been associated with amount smoked [2], nicotine dependence

(defined by Fagerström Test for Nicotine Dependence [FTND] score) [3], and early age of initiation of smoking [4]. Importantly, SNPs within the same cluster have also been implicated in risk of lung cancer [5–7], but there remains some debate as to whether this genetic effect is an indirect effect operating via heaviness of smoking, or is a direct effect independent of smoking quantity.

Using GWAS to identify genomic regions of association has the advantage of being unbiased, as no *a priori* hypothesis is required, but follow-up fine-mapping studies are necessary to refine the results and identify the causal SNP and likely mechanism of effect. This paper identifies a group of eight SNPs, frequently inherited together, which are strongly associated with heavy smoking, and suggest one SNP in the *CHRNA5* subunit (rs16969968) as the most likely candidate for causal SNP [1]. This variant has been shown to reduce the response of the receptor to nicotine, perhaps thereby

diminishing the rewarding effects per cigarette and increasing the number of cigarettes smoked. Future work in this area is needed to fully elucidate these mechanistic effects and open possible avenues of exploitation for novel smoking cessation therapies. It would also be interesting to see if these SNPs also affect the success of quit-smoking attempts with existing agents, in a randomized trial setting.

As with all association studies, the importance of the phenotype should not be underestimated. Subtle differences in phenotype definition between studies are one likely source of nonreplication, a common problem in association studies. Stevens and colleagues address this issue by validating their definitions of heavy (at least 30 cigarettes per day for 5 years) and light (less than 5 cigarettes per day for at least 1 year) smoking, derived from questionnaire responses, against lifetime FTND score, albeit in a separate cohort. Further research is necessary to pinpoint the relative effect sizes of the genetic (nicotine activation and

metabolism) and the environmental contribution to smoking quantity, before we can hope to translate these findings into real clinical benefit.

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Genetic determinants of smoking cessation

Evaluation of: Omidvar M, Stolk L, Uitterlinden AG *et al.*: The effect of catechol-O-methyltransferase Met/Val polymorphism on smoking cessation: retrospective and prospective analyses in a cohort study. *Pharmacogenet. Genomics* 19(1), 45–51 (2009).

– MR Munafò & EC Johnstone

An endemic difficulty in the search for genetic variants associated with complex behavioral phenotypes is the lack of robust replication [1]: initially promising findings are frequently followed by failures to replicate, or opposite findings. Of the various candidate genes that have been investigated in the context of cigarette smoking phenotypes, catechol-O-methyltransferase (*COMT*) has emerged as one of the more promising, with a particular focus on the Val^{108/158}Met polymorphism. This is a plausible candidate given the role of the *COMT* enzyme in dopamine metabolism, in particular in the prefrontal cortex, and the functional consequences of the Met allele, which confers reduced enzyme activity relative to the Val allele.

A major contributor to the inconsistent replication of the genetic association studies is the small sample size, and consequent lack of statistical power, which characterizes many of these studies. Omidvar and colleagues [2] report data from over 5000 individuals, which is a

considerable strength. The combination of retrospective and prospective analyses also lends weight to their findings. Their data suggest that, in elderly smokers, a higher likelihood of cessation is associated with Val/Val homozygote status. The authors argue that this may be owing to the higher levels of dopamine activity in Met allele carriers, with the result that these individuals are more likely to miss the extrinsic stimulation from nicotine when they stop smoking.

However, the data reported by Omidvar and colleagues also suggest potential complexity in the role of *COMT* in smoking cessation, given previous findings in treatment-seeking smokers using nicotine replacement therapy to support their cessation attempt. In these studies, the presence of the Val allele has generally been associated with increased likelihood of relapse to smoking [3–5]. This suggests that the effects of *COMT* on smoking cessation may differ as a function of whether the cessation attempt is aided or unaided, and in particular whether nicotine replacement therapies are used. Clearly further research, ideally in large prospective cohorts, is needed to investigate this possibility.

More generally, this study illustrates that apparently minor differences in phenotype definition, or the environmental context within which the phenotype is assessed, may have an impact on the strength and even direction of possible associations. It is an empirical question

whether this heterogeneity between studies represents true (and therefore informative) heterogeneity, or whether it simply reflects the presence of false-positive findings. Given the history of the search for genetic determinants of behavioral and psychiatric traits, a certain degree of healthy skepticism and caution is always appropriate.

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