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Investigations into the role of Glutathione-*S*-transferase (GST) and Cytochrome-P450-monooxygenase (CYP) polymorphisms as risk factors for early onset lung cancer

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Lung cancer is considered the result of complex interactions between environmental and genetic factors. There is a growing realization that there are individual differences in the bioactivation and detoxification of carcinogens such as tobacco carcinogens, and that these individual differences in susceptibility may be the result of genetic polymorphisms in enzymes involved in the metabolism of carcinogens (e.g. Cytochrome-P450-monooxygenase (CYP450), Glutathione-*S*-transferase (GSTs)). A particularly strong genetic component may exist in cases with early onset lung cancer.

A case-control study was carried out to identify genetic polymorphisms in CYP450 and GST that modify the risk of developing lung cancer in young individuals (under the age of 51). 638 patients lung cancer, aged 50 and younger, were included in the current study from two existing German studies: a) the LUCY study and b) young cases from a case-control study of lung cancer carried out in Heidelberg. 1300 control individuals, matched by age and sex, were selected from the population based KORA study.

Overall, 18 SNPs in the genes *CYP1A1, CYP1B1, CYP2A13, CYP3A4, CYP3A5* and *GSTP1* as well as two deletion polymorphisms in *GSTM1* and *GSTT1* were selected for analysis. Genotyping of the *CYP1A1*, *CYP1B1*, *CYP2A13* and *GSTP1* polymorphisms was carried out using MALDI-TOF (matrix assisted laser desorption/ionization time-of-flight) mass spectrometry. Genotyping of the *CYP3A4* rs2740574*, CYP3A5* rs776746 and *CYP1B1* rs1056836 polymorphisms was carried out using fluorescence-based melting curve analysis methods on either the LightTyper or the LightCycler 480. A new high-throughput real-time multiplex genotyping PCR assay on the LightCycler 480 was established for determining

GSTM1 and *GSTT1* copy number variants. This method was shown to be reproducible and reliable assay suitable for future large scale epidemiological and clinical studies.

Multivariate conditional logistic regression analysis was applied to assess the odds ratios (ORs) and their 95% confidence intervals (CI). A generalized linear model (GLM) framework, adjusted for age, gender and smoking, was used to test for haplotype-trait association and to calculate ORs and 95% CIs.

No significant association between any of the analysed polymorphisms and lung cancer risk overall was found. No significant associations were found in a haplotype analysis. However, among women a significantly elevated risk of early onset lung cancer overall was observed for carriers of the variant allele of the *CYP1B1 SNP* rs1056836 (Leu432Val) (OR 1.97; 95% CI 1.32-2.94, p=0.0009, significant after Bonferroni correction for 40 tests). Also, an increased risk of early onset small cell lung cancer was observed in the group of women who carry the variant allele of the *CYP2A13* tagging SNP rs1709084 (OR 1.64; 95% CI 1.00-2.70, p=0.0515, not significant after Bonferroni correction for 40 tests). The effect of these polymorphisms was shown to be modified by smoking. The 3-way interactions between gender, smoking and polymorphism were statistically significant for both polymorphisms.

Among GST polymorphisms, the tagging SNP *GSTP1* rs947895 was shown to be associated with a decreased risk of lung cancer in men (CA+AA vs. CC OR 0.76, 95% CI 0.58-1.00, p=0.0463). The functional SNP *GSTP1* rs1695 (Ile105Val), which is highly linked with rs947895, was also shown to be non-significantly associated with a decreased risk of early onset lung cancer in women. The present study is the first study on lung cancer where a new method for distinguishing between *GSTM1* and *GSTT1* heterozygous (1/0) and homozygous (1/1) genotypes was applied. However, the expected gene-dose effect for these deletion polymorphisms was not observed. The results of this study suggest that *GSTT1* deletion is associated with an increased risk of lung cancer in heavy smoking women (≥21 PY) (1/0+0/0 vs. 1/1 OR 2.33, 95%CI 1.31-4.14, p=0.0040). These effects were not confirmed by interaction tests and were not significant after Bonferroni correction for multiple testing. Therefore, the results have to be interpreted with caution.

The reported study is the biggest study on early onset lung cancer to date. Early onset lung cancer cases, due to the high prevalence of women, are an ideal study group to investigate gender specific differences in lung caner. Indeed, the present study observed gender specific effects of *CYP1B1*, *CYP2A13*, *GSTP1* and *GSTT1* polymorphisms. Previous studies investigating the role of CYP450 and GST polymorphisms in lung cancer risk often showed conflicting results. However, given the involvement of CYP450s and GSTs in the

metabolism of both tobacco carcinogen and oestrogens, the observed gender specific effects are biological plausible.

To summarize, the findings suggest a modifying effect of gender on the effect of *CYP1B1*, *CYP2A13*, *GSTP1* and *GSTT1* polymorphisms on the risk of early onset lung cancer. To confirm these gender-specific effects on the risk of lung cancer further larger studies on early onset lung cancer have to be conducted.