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Evaluation of the dissertation thesis '**Genetic profile of genes involved in cell cycle control and the risk of sporadic colorectal cancer in the Czech Republic**' by **Veronika Polakova**

Dear Prof. RNDr. Zadrazil,

The dissertation thesis '**Genetic profile of genes involved in cell cycle control and the risk of sporadic colorectal cancer in the Czech Republic**' by **Veronika Polakova** takes up the question of the 'common disease-common variant' hypothesis in colorectal cancer (CRC), which is a serious public health problem in the Czech Republic, where both the incidence of and the mortality due to CRC are among the highest worldwide. As for other common diseases, risk factors for CRC are thought to involve both a genetic and an environmental component. While high-penetrance mutations are connected to the rare CRC syndromes and explain only a small proportion of all CRC cases, a combination of low penetrance mutations, in the form of genetic polymorphisms, and together with environmental factors, are thought to explain the etiology of the major proportion of CRC.

In this thesis, Veronika Polakova has used a candidate gene approach and selected polymorphisms in genes related to cell cycle control and to closely linked DNA repair, a well justified decision, as deregulation of both cell cycle and DNA repair pathways play an important role in carcinogenesis. By analyzing haplotypes in the studied genes and gene-gene interactions, she also takes up the question of the effect of several polymorphisms both within a gene and between two genes. The polymorphisms selection is scientifically solid and it was based on the principle of the tagging single nucleotide polymorphism (SNP) approach for *TP53*, covering all the common genetic variation in the gene region, where most of the somatic mutations occur. For other cell cycle genes (*CDKN1A*, *CDKN2A* and *CCND1*) common SNPs were selected. For the DNA repair genes, SNPs were selected based on previous studies of the research group about the functional effects of the SNPs on DNA repair capacities and DNA and chromosomal damage. Additionally, one SNP, Glu185Gln, in the *NBN* DNA repair gene, was genotyped, because its frequency is relatively high in the Czech population as is the incidence of Nijmegen breakage syndrome (NBS), a syndrome characterized by marked susceptibility to cancer.

The study population is relatively large, including 614 hospital-based CRC cases and 614 age and gender matched, colonoscopically negative, controls, ensuring disease-free individuals as controls. In one study, an additional

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control group consisting of healthy blood donors was used to present a more population-based population.

While none of the studied polymorphisms were independently associated with CRC, Veronika Polakova observed a haplotype effect in the *TP53* gene, with the most common haplotypes to increase the risk of CRC and several rare haplotypes to decrease the risk of CRC. Additionally, she observed a gene-gene interaction between two DNA repair genes, with individuals simultaneously homozygous for the variant alleles of the *APE1* Asn148Glu and *OGG1* Ser326Cys SNPs at an increased risk of CRC.

In conclusion, the thesis of Veronika Polakova shows, that a study design taking into account several polymorphisms in a gene or several genes with a signaling pathway is able to discover genetic effects which would have been missed if only single SNPs had been studied.

By these words I recommend the acceptance of the thesis by Veronika Polakova.

Sincerely yours,

Asta Försti

Attachment: Comments to the thesis of Veronika Polakova 'Genetic profile of genes involved in cell cycle control and the risk of sporadic colorectal cancer in the Czech Republic'

Comments to the thesis of Veronika Polakova '**Genetic profile of genes involved in cell cycle control and the risk of sporadic colorectal cancer in the Czech Republic**'

What would you regard as the main outcome of your thesis?

You mention only shortly the International HapMap Project and the recent whole genome association studies (GWASs) in your thesis. What have these two projects given to the research of susceptibility to and/or clinical outcome of common diseases? How would you evaluate your SNP selection and your results in relation to the data available in HapMap and in the GWASs?

In your paper on cell cycle genes (publication 1) you created haplotypes for the *TP53* gene and analyzed their association with CRC. The two most common haplotypes increased the risk of CRC and several rare haplotypes decreased the risk of CRC. As you mention in the footnote of table 3, each individual carries two haplotypes (i.e. a diplotype). Did you also do the analyses for the diplotypes, which would be a more relevant way to correlate the effect of haplotypes on CRC risk? If yes, what was the outcome?

Your results both on the *TP53* gene and the DNA repair genes show, that a combination of SNPs (in haplotypes or in the form of gene-gene interaction) may show some effects, although you may not observe any individual SNP effects. Can you, please, comment the gene-gene interaction analysis between the *TP53* haplotypes and the DNA repair genes? Was the stratification made only for carrier status (carriers with one or two of the risk haplotypes having the same weight)? Why did you do the *TP53* gene-gene interaction analysis only between haplotypes and SNPs which already individually showed an affect and not between the *TP53* haplotypes (or diplotypes) and the other cell cycle genes or with other DNA repair genes?