Silvia Funke
Dr. sc. hum.

Genetic influence on the risk of colorectal cancer and the survival after chemo- and/or radiotherapy

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Doktormutter: Frau Prof. Dr. sc. hum. Jenny Chang-Claude

It is widely accepted that the interplay of genetic predisposition and environmental factors contribute to the development of the majority of colorectal malignancies. Thus, genetic variation leading to altered generation or detoxification of potential carcinogens may influence individual susceptibility to colorectal cancer. Genetic variants related to either low phase II metabolic activity of glutathione-S-transferases (GSTs) or to high levels of reactive oxygen species (ROS), both leading to prolonged exposure to carcinogens and oxidative stress, might increase the risk of colorectal cancer. Enzymes participating in oxidative stress mechanisms include ROS neutralizing GSTs, catalase (CAT), and manganese superoxide dismutase (MnSOD) and ROS generating myeloperoxidase (MPO), and endothelial nitric oxide synthase (eNOS).
Genetic factors may also account for individual differences in treatment outcome once colorectal cancer has occurred. Prolonged exposure to chemotherapeutic agents due to genetic variation in *GST* genes might increase chemotherapeutic benefit. Since chemotherapeutic drugs and radiation therapy exert part of their anticarcinogenic activity via the generation of oxidative stress, genetic variants related to high levels of ROS might also increase treatment benefit after chemotherapy and/or radiotherapy. The present study presents an explorative epidemiological investigation of how genetic polymorphisms in *GST* genes (*GSTP1* Ile^{105}Val, *GSTT1* CNV, *GSTM1* CNV) and in other genes related to oxidative stress mechanisms (*CAT* C262T, *MnSOD* Val^{9}Ala, *MPO* G463A, *eNOS* Glu^{298}Asp) influence both the risk of colorectal cancer taking effect modification by environmental modifiers into account and the survival of colorectal cancer patients who received chemotherapy and/or radiotherapy.
In this thesis, data of a population-based case-control study (DACHS) recruiting patients with primary invasive colorectal cancer were used to assess the association of genetic polymorphisms and colorectal cancer risk and possible effect modification by smoking or alcohol consumption. Comprehensive information on sociodemographic factors, lifestyle habits, and nutrition was available. To assess the association between genetic polymorphisms and survival, colorectal cancer cases of the DACHS study and a further cohort study (ESTHER II) were used. Detailed information on colorectal cancer treatment and clinical course of the disease was collected. Genotyping was performed with fluorescence based melting curve analysis, relative quantification method based on multiplex real time PCR, PCR followed by gel electrophoresis, and sequencing analysis (Pyrosequencing^{TM}). The final sample size consisted of 632 cases and 606 controls (DACHS study) for the association analyses and of 1083 cases (DACHS and ESTHER II studies) for the survival analyses. Conditional logistic regression adjusted for potential confounders calculating odds ratios (OR) and 95% confidence intervals (CI) was used to assess the effect of genetic variants on colorectal cancer risk. Statistical interaction between smoking or alcohol consumption and

genetic polymorphisms was evaluated by means of the likelihood ratio test. The effect of genetic variation on survival after chemotherapy and/or radiotherapy was assessed by Kaplan-Meier method and log-rank test. To take potential confounders into account Cox proportional hazards regression was further applied, calculating multivariate adjusted hazard ratios (HR) and corresponding 95% CIs.

None of the genetic polymorphisms under investigation were associated with colorectal cancer risk. Furthermore, no effect modification by smoking status (never/ever) was observed. Assessing the association between colorectal cancer risk and *MnSOD* Val^{9}Ala polymorphism revealed statistically significant heterogeneity by pack-years of smoking. Among smokers who had smoked 1-10 pack-years, carriers of high ROS generating *MnSOD* alanine allele showed an increased risk (OR: 5.99, 95% CI: 2.50-14.38). In contrast, among stronger smokers *MnSOD* alanine allele was associated with a decreased risk. Effect modification by alcohol was observed for the association between genetic polymorphisms in *GSTP1*, *GSTM1* or *MPO* and colorectal cancer risk. For carriers of low activity *GSTP1* valine allele, risk increase was restricted to individuals with an ethanol consumption of more than 20.3 g/day. Furthermore, the increased risk for the *GSTM1* null genotype was only found among individuals with high consumption of alcohol. Moreover, carriers of low ROS generating *MPO* A allele were associated with a decreased risk of colorectal cancer only among individuals with high ethanol intake.

In the survival analysis none of the genetic polymorphisms were found to be prognostic factors independent of type of therapy. Among colorectal cancer patients who received chemotherapy, *GSTM1* genotype was associated with survival. Carriers of at least one copy of the *GSTM1* gene showed a reduced hazard of death (HR: 0.48, 95% CI: 0.25-0.94 for carriers of one copy compared to carriers of two *GSTM1* copies). Furthermore, a reduced hazard of death was observed for carriers of low activity *GSTP1* valine allele among colon cancer patients who received oxaliplatin treatment (HR: 0.41, 95% CI: 0.17-0.99). Among rectal cancer patients who received radiotherapy, carriers of low ROS producing *eNOS* asparagine allele showed an increased hazard of death (HR: 2.10, 95% CI: 1.01-4.38). In addition, for rectal cancer patients carrying low ROS generating *MPO* A allele a reduced hazard of death was observed (HR: 0.44, 95% CI: 0.21-0.93).

Our data provided suggestive evidence of an interaction between smoking and *MnSOD* genotype with respect to colorectal cancer risk. We also found suggestive evidence of effect modification by alcohol consumption on the association between *GSTP1*, *GSTM1* or *MPO* genotype and colorectal cancer risk. However, the observed findings were based on small sample sizes and were not consistent among different categories of smoking or alcohol consumption. Since previous studies on this topic, mainly performed with respect to other cancer sites, are scarce and sometimes contradictory, findings of the present study require further elucidation in large study populations for colorectal cancer.

Data of the survival analyses indicate an association between the numbers of *GSTM1* copies, corresponding drug metabolizing activity and survival after chemotherapeutic treatment. This points to the importance of discrimination between the numbers of *GSTM1* copies in further studies instead of comparing the null genotype to the non-null genotype as performed usually. Furthermore, our data confirm prior suggestions that low activity *GSTP1* valine allele may prolong survival after oxaliplatin therapy in colon cancer patients. This study also firstly examined the association of genetic polymorphisms in genes related to oxidative stress and survival of rectal cancer patients who received radiotherapy and found survival benefit for carriers of high ROS generating *eNOS* glutamine allele but for carriers of low ROS generating *MPO* A allele. Further large pharmacogenetic studies that allow comparison of different treatment regimens between colon and rectal cancer patients with adequate power would be required for a more reliable estimation of the observed effects.