

**Network: Obesity and Related Disorders****Project: Variation in Candidate Genes for Obesity: Relative and Attributable Risk with Obesity Related Diseases and Association with Quantitative Traits of the Metabolic Syndrome****Heiner Boeing/Andreas Pfeiffer/Joachim Spranger - German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke - boeing@mail.dife.de/afhp@mail.dife.de/spranger@mail.dife.de****Introduction**

The pathogenesis of obesity and related disorders are within the focus of the project group since many years and some relevant pathogenic mechanisms with potential therapeutic implications and disease associations have been elucidated in the past such as the role of specific cytokines as predictors of type 2 diabetes [1,2]. The aim of this subproject is to prospectively investigate the relation of genetic variations for obesity related phenotypes with risk of diabetes mellitus, cardiovascular diseases, stroke, and hypertension. In addition, the role of quantitative traits which are assumed to be functionally relevant in this context, will be further evaluated in a cross-sectional analysis of a second, independent study population. Particular emphasis will be given to estimate the impact of the SNPs with and without control for anthropometric parameters such as Body-Mass-Index (BMI), Waist-Hip-Ratio (WHR) and body composition. The human study populations will also be used to clarify the impact of polymorphism, which were identified to be related to obesity in genome-wide animal experiments or by other approaches within the pipeline of NeuroNet.

Depending on the number of genes and SNPs, haplotype analyses will be conducted in order to understand and/or simplify the genetic information. Traditional epidemiological approaches such as multiple and logistic regression will be used to establish the association between genotype and phenotype/disease information. Major focus of the statistical evaluation with respect to incidence is directed to relative as well as attributable risk. The pathway information associated with the candidate genes will be used to develop disease models that allow considering their specific potential function in the pathogenesis of disease.

**Project Status****Study populations**

Over the past 10 years the Department of Epidemiology established the EPIC-Potsdam study which is part of a large European cohort study that includes more than 520,000 participants from 10 European countries allowing a prospective evaluation of the role of diet in conjunction with biomarker and genetic information on risk of cancer and non-cancer diseases. The local EPIC study population includes 27.548 study participants from whom 26.444 gave blood and are therefore eligible for genetic studies [3]. The study is currently processing the data from the 6 year follow up. In the past the return rate of questionnaires to report about new chronic diseases exceeded 96 %. Each reported disease of interest is verified by the treating physician or hospital. Currently we estimate that about 800 new cases of medically verified diabetes mellitus, 150 of myocardial infarction, 170 of stroke, and 360 of hypertension are available for this study. Past and upcoming publications of national and international EPIC-data make reference to obesity as risk factor for diseases (diabetes mellitus, cardiovascular diseases, stroke, and hypertension as well as specific cancer types). In addition, international and local EPIC activities refer to obesity as endpoint and a joint project between the involved Departments could give proof of successful evaluation of genetic effects with the identification of the effect modification of obesity in respect to one IL6 polymorphism and risk of diabetes mellitus [4].

An important potential of this prospective study is the availability of information on many lifestyle factors including

diet, collected at baseline and updated during follow up. This information can be used for extended genotype-phenotype analyses. The collected information includes variables on socioeconomic status, physical activity, reproductive history, medication, smoking, alcohol consumption, dietary intake, and medical history. Direct measurements include anthropometry and blood pressure. The anthropometric measurements comprised weight, height, waist and hip circumferences, and 4 skin fold thickness measurements by caliper for estimating body composition.

The prospective data collection will be amended by a local well phenotyped cross-sectional study population, MesyBePo, established by the Department of Clinical Nutrition. Interviews elicit detailed information on social, family and medical history. We also ask for habitual leisure time and physical activity in the preceding year. Information on usual dietary intake is obtained using a semi-quantitative food frequency questionnaire. Further direct characterization includes anthropometry (including various skin folds), standard laboratory (cholesterol, triglyzerides, fasting glucose and insulin), 3 hour oral glucose tolerance test (OGTT), intima-media-thickness. Standardized measurements of blood pressure are obtained. Various additional procedures are performed in subcohorts such as euglycemic-hyperinsulinemic clamp and indirect calorimetry.

**Genotyping**

Genotyping will be performed as a medium-throughput approach 'in house' using either TaqMan allelic discrimination or Single Nucleotide Primer Elongation technology (SnuPE). Both techniques are well established. Given that much lower costs can be achieved by high-throughput approaches of other partners within the network, a collaboration with respect to genotyping is intended. Ring trials will be performed when genes are analysed (with different methods) in different labs. This is of utmost importance to ensure comparable results between labs.

**Statistical modelling**

We will use the nested case-control approach with about twice the number of randomly selected controls (n=2850) to estimate the relative risk in connection with genetic variation. Relative risk estimates for each endpoint will be obtained from logistic regression models which also include confounders such as age, sex, education, smoking and alcohol drinking. A particular focus will be given to the factor obesity that has been measured as BMI, waist and hip circumferences and body composition (by skin-fold thickness measurements). It will particularly be investigated whether obesity modifies the risk. This research strategy requires more complex statistical models which include interaction terms. However, due to the limited number of cases a formal gene-environment interaction analysis will be hard to be conducted. Currently, a meta-analysis with respect to a polymorphism within the IL-6 gene is performed in collaboration with another group of the NeuroNet and various international partners. Only such meta-analyses will have sufficient power to address gene-environment interactions.

Relative risk analysis, based on multivariate logistic regression, will be extended towards the analysis of attributable risk, factor-specific and population-based. Attributable risk analysis utilizes estimates of relative risk and

information on the prevalence of the SNP in the population (or cases) and estimates the percentage of incidence which is linked with the SNP. The statistical modelling will also investigate whether a SNP improve the predictability of a disease.

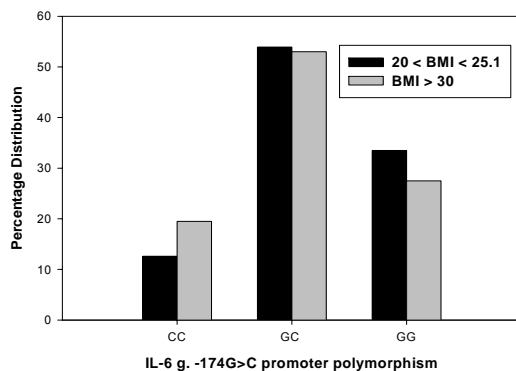
The relationship of specific genotype information with quantitative traits such as BMI, fasting and 2-h plasma glucose and insulin, and HOMA index will be investigated in the MesyBePo study population. Adjustment for additional covariates such as age, sex, physical activity, smoking and alcohol drinking will be performed using General Linear Model procedures. Emphasis will be given to potential interactions between anthropometric variables and the genotypes on the respective trait.

New statistical approaches will be pursued in the area of genomic data. One approach is the construction of haplotypes within and across genes of the same chromosome. Here we will particularly interact with Dr Rohde from GEM-Berlin. A further topic will be the biological hierarchy of genes and SNPs which might help to construct appropriate statistical models.

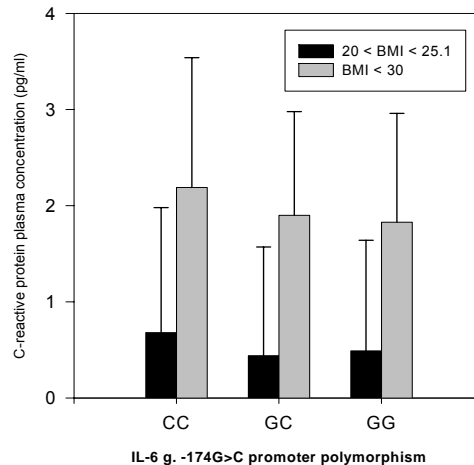
**First results**

A interleukin-6 (IL-6) g. -174G>C promoter polymorphism has recently been associated with indices of overweight [5]. Homozygous subjects were observed to have reduced energy expenditure, suggesting that lower IL-6 gene transcription, caused by the IL-6 g. -174G>C promoter polymorphism may be associated with obesity. For 334 normal weight (20<BMI<25) and 334 obese (BMI>30) subjects matched by age and sex originating from the population-based EPIC-Potsdam Study recalled weight change from age 25 to study enrolment was determined, the IL-6 g. -174G>C promoter polymorphism was defined and plasma concentrations of IL-6 and C-reactive protein (CRP) were measured.

The IL-6 g. -174G>C promoter polymorphism was significantly associated with obesity ( $\chi^2=7,34$ ,  $p=0.026$ ). Odds ratios for subjects with GC and CC genotypes for obesity were 1.19 [95% CI: 0.84-1.68;  $p=0.323$ ] and 1.91 [95% CI: 1.19 – 3.08;  $p=0.007$ ], respectively. Recalled weight change from age 25 years to study enrolment differed



**Fig 1:** Distribution of the IL-6 g. -174G>C in normal weight and obese subjects.



**Fig 2:** CRP levels adjusted for smoking and prevalence of diabetes mellitus type 2, myocardial infarction, and stroke according to the genotypes of the IL-6 g. -174G>C SNP.

significantly according to genotype ( $p=0.044$ ) and was most pronounced in subjects with the CC genotype suggesting the IL-6 g. -174G>C promoter polymorphism to be a susceptibility or modifying locus for common obesity and weight gain

**Outlook**

Currently, genetic variations of four candidate genes are under investigation. On medium-term, genome-wide linkage studies in animal and humans within the NeuroNet pipeline have suggested to provide specific genes/polymorphisms, which will be further evaluated within the here established study populations.

Lit.: 1. Spranger, J., Kroke, A., Möhlig, M., Hoffmann, K., Bergmann, M., Ristow, M., Boeing, H., Pfeiffer, A.F.H. (2003) Inflammatory cytokines and the risk to develop type 2 diabetes. Results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. *Diabetes* 52: 812-817. 2. Spranger, J., Kroke, A., Möhlig, M., Bergmann, M.M., Ristow, M., Boeing, H., Pfeiffer, A.F. (2003) Adiponectin independently protects against type 2 diabetes mellitus. *Lancet* 361: 226-228. 3. Boeing, H., Wahrendorf, J., Becker, N. (1999) EPIC-Germany - a source for studies into diet and risk of chronic diseases. *Ann Nutr Metab* 43:195-204. 4. Möhlig, M., Boeing, H., Spranger, J., Osterhoff, M., Kroke, A., Fisher, E., Bergmann, M.M., Ristow, M., Hoffmann, K., Pfeiffer, A.F.H. (2003). BMI and C-174G Interleukin-6 Promoter Polymorphism interact in predicting Type 2 diabetes. 5. Klipstein-Grobusch K., Möhlig M., Spranger J., Hoffmann K, Rodrigues FUS., Sharma AM, Klaus S, Pfeiffer AFH, Boeing H. The interleukin-6 g. -174G>C promoter polymorphism is associated with obesity in the EPIC-Potsdam study (*Obes Res*, in press).