Evaluation of gene-obesity interaction effects on cholesterol levels: A genetic predisposition score on HDL-cholesterol is modified by obesity

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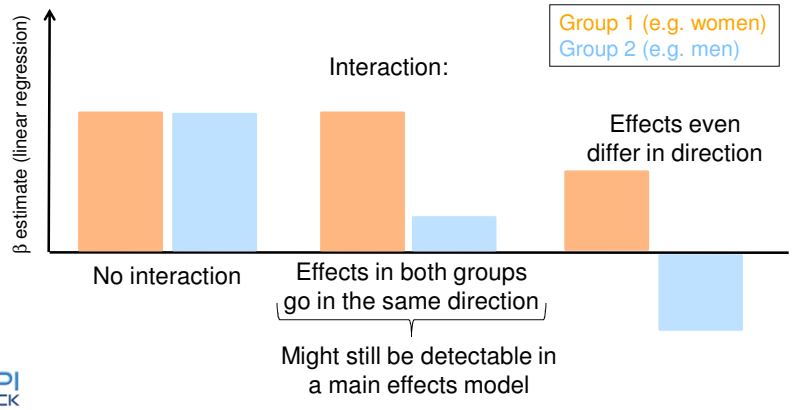
Background – Gene-environment interaction

- Definition "**Statistical interaction**" in general:

Departure from a pure main effects model

Example: interaction between one continuous and one dichotomous variable on a continous linear outcome



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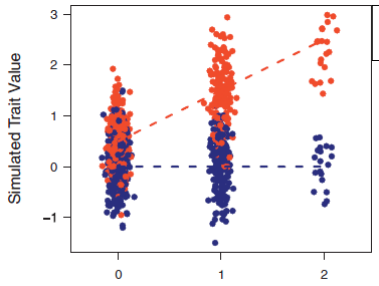
Background – Gene-environment interaction

- Definition "**Gene-Environment interaction**":

Interaction between a genetic variant (a SNP or SNP-Score) and an environmental parameter on a phenotype (disease risk or continuous phenotype). The environmental parameter can also be (partly) genetically determined, e.g. obesity.

Example: SNP*Group interaction effect on a continous trait:



Group 1: no effect of the genotype on the trait
Group 2: Additive genetic effect of the genotype on the trait

Davies et al, 2009, Genetic Epidemiology

Two different study scenarios possible:

- Hypothesis-driven candidate gene studies (genetic variant already known)
- Genome-wide GxE interaction analysis → also possible to find interaction effects that wouldn't have been found with main effect models

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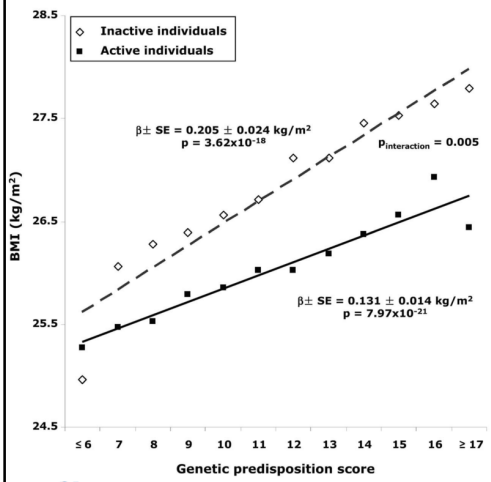
Background – Gene-environment interaction

Example: SNP-Score*Physical activity interaction effect on BMI:

SNP-Score: counting the numbers of trait increasing alleles



Advantage:

- Bigger effect size for the score compared to SNPs
- Compared to GWAs: Significance level does not have to bee too strict

→ Higher power

Disadvantage:

- Restricted to already known genetic variants
- Differing interaction effects will not be detected

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Li et al, 2010, Plos Medicine

Background – Genetics of lipids

- Lipids are affected by:
  - Genetics
  - Diet
  - Obesity
  - Physical activity, etc…
- 95 susceptibility loci have been identified for total cholesterol (TC), LDL cholesterol, HDL cholesterol and triglycerides*
- They explain ~10-12% of phenotypic variance (25-30% of genetic variance)
- Some part of missing heritability can possibly be explained by so far not identified gene-environment interaction effects

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* Teslovich et al, 2010, Nature

Introduction and aim

Studies on gene-environment interaction (GxE) on lipids:

- Accumulating evidence from single candidate gene association studies: genetic effects on lipids are modified by obesity and/or factors involved in obesity, primarily by physical activity, diet or other genes
- One Genome-wide meta-analysis (n~50,000) for GxE (gender, BMI, waist-hip-ratio (WHR), alcohol consumption and smoking) identified one significant SNP-interaction effect with WHR on TC (p-value interaction term: 4.79×10^{-9}).

Effect of rs6448771 on total cholesterol (TC) by waist-to-hip ratio (WHR) tertiles:

SNP effect on TC by WHR tertiles	Beta	CI-95 lower	CI-95 upper
WHR <0.84375	-0.031	-0.069	0.008
0.84375< WHR <0.92891	-0.011	-0.053	0.031
WHR >0.92891	0.083	0.036	0.129

Would not have been detected in a main effects model

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Surakka et al, 2011, Plos Genetics

Introduction and aim

- Aim: Show potential obesity-modifying effects on genetic predisposition on lipids (TC, HDL, LDL)
- We use:
  - "G": Weighted genetic risk scores based on known lipid-genes
  - "E": Obesity parameters:
    "overall" obesity: BMI
    "central" obesity: WHR, waist

→ We look at interaction effects between two continous variables

→ Secondary aim: How can we display/report these effects without using arbitrary categorizations & still be understandable for clinicians/geneticists?

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Available data

The studies: KORA F3 & KORA F4

- Population-based studies
- Genome-wide data and lipids available from:
  - KORA F4: n = 1405
  - KORA F3: n = 1524

The genotypes

- Affy 500 K chip (KORA F3), Affy 6.0 (KORA F4)
- IMPUTE imputed genotypes → **SNP-dosages** ranging from [0;2], which is in accordance with an additive model (meaning that the statistical effect of two "risk" alleles is assumed to be twice as high as one "risk" allele)

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SNP selection

95 lipid loci from Teslovich et. al, 2010, Nature

Biological, clinical and population relevance of 95 loci for blood lipids

SNP selection

Table 1 | Meta-analysis of plasma lipid concentrations in >100,000 individuals of European descent.

Locus	Chr	Lead SNP	Lead trait	Other traits	P
LDLRAP1	1	rs12027135	TC	LDL	4×10^{-11}
PABPC4	1	rs4660293	HDL		4×10^{-10}
PCSK9	1	rs2479409	LDL	TC	2×10^{-28}
ANGPTL3	1	rs2131925	TG	TC, LDL	9×10^{-43}
EVI5	1	rs7515577	TC		3×10^{-8}
SORT1	1	rs629301	LDL	TC	1×10^{-170}
ZNF648	1	rs1689800	HDL		3×10^{-10}
MOSC1	1	rs2642442	TC	LDL	6×10^{-13}
GALNT2	1	rs4846914	HDL	TG	4×10^{-21}
IRF2BP2	1	rs514230	TC	LDL	5×10^{-14}
APOB	2	rs1367117	LDL	TC	4×10^{-114}
		rs1042034	TG	HDL	1×10^{-45}

All SNPs from these loci, which are associated with Total cholesterol (TC), LDL- or HDL- cholesterol

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106 SNPs

HDL: 46 SNPs

LDL: 37 SNPs

TC: 51 SNPs

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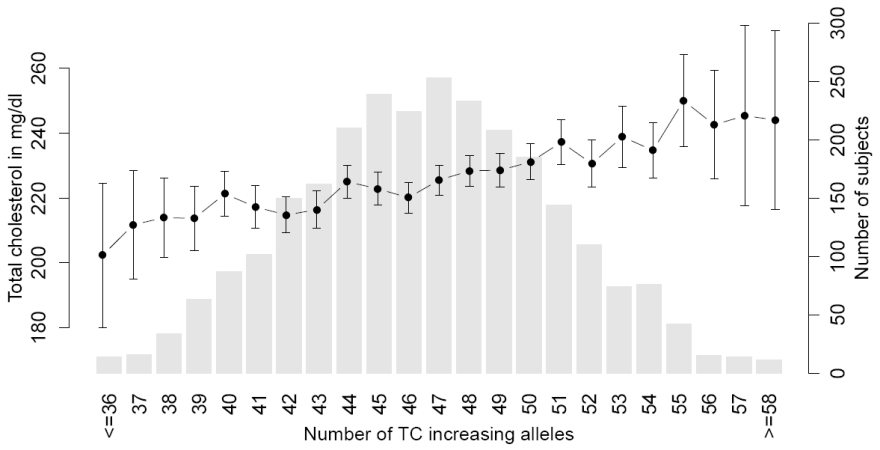
Association of SNP-scores with TC



Variance explained: 10-11%

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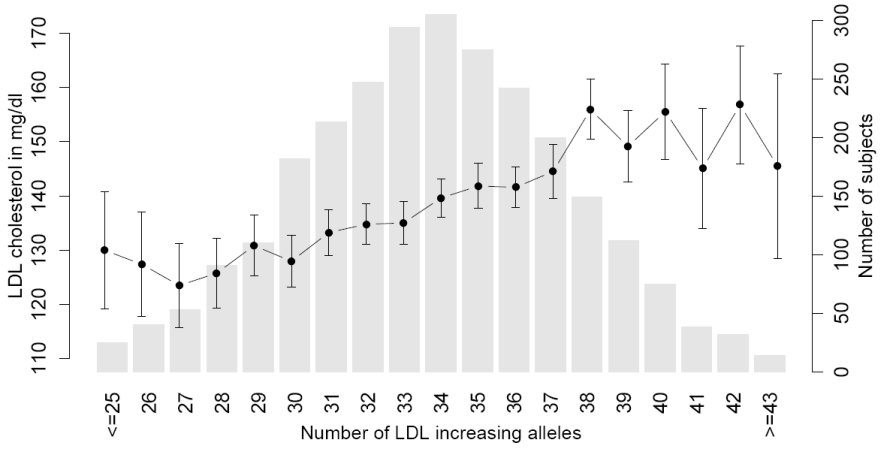
Association of SNP-scores with LDL



Variance explained: ~9%

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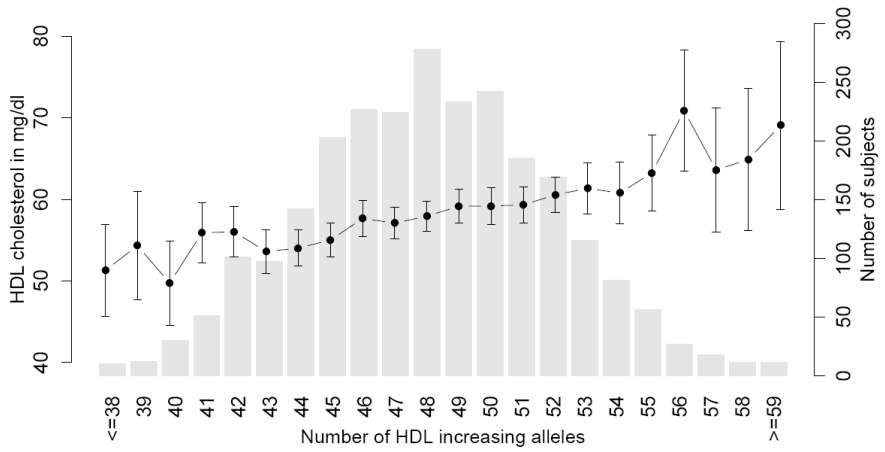
Association of SNP-scores with HDL



Variance explained: 8-10%

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Calculation of SNP score

Exemplified on HDL-C:

For each SNP of the 46 SNPs in the HDL-C SNP-score:

Linear regression in KORA F4:

$$HDL - C = \alpha + \beta_{KORA\,F4_i} * SNPdosage_{KORA\,F4_i} + \epsilon$$

Example:

Gene	SNP	β estimate from F4
rs3764261	CETP	4.21
rs7241918	LIPG	-1.73
rs10808546	TRIB1	0.10

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→ Reference allele was changed in KORA F3

→ All effect estimates add positively to the weighted SNP-score.

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The HDL-SNP-Score is then calculated as:

$$\sum_{i=1}^{46} |\beta_{KORA\,F4_i}| * SNPdosage_{KORA\,F3_i}$$

HDL-Score =
4.21*rs3764261-dosage +
1.73*rs7241918-dosage +
0.10 *rs10808546-dosage +
.....

TC-SNP-Score LDL-SNP-Score HDL-SNP-Score

Results SNP-score*Obesity parameter on HDL

Linear regression of (SNP-score*Obesity parameter) on HDL:

- Each model is additionally adjusted for age and sex
- Variables are centralized to their mean*:
  - Mean HDL-SNP-Score = 41.47
  - Mean BMI = 27.97
  - Mean WHR = 0.89
  - Mean waist in cm = 96.13

Outcome Variable	Explaining variables	With interaction term* β	se	p-value
TC	BMI	-1.0838	0.0895	3.95×10^{-32}
	HDL SNP-Score	0.9782	0.1001	7.89×10^{-22}
	BMI*HDL SNP-Score	-0.0590	0.0215	**0.0062**
	WHR	-72.0625	6.9551	2.98×10^{-24}
	HDL SNP-Score	0.9823	0.1016	1.95×10^{-21}
	WHR*HDL SNP-Score	-3.3780	1.1622	**0.0037**
	Waist	-0.4649	0.0362	9.58×10^{-36}
	HDL SNP-Score	0.9966	0.0995	8.18×10^{-23}
	Waist*HDL SNP-Score	-0.0234	0.0073	**0.0014**

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* the marginal effects and p-values of both interaction variables are the effects/p-values of variable 1 (e.g. BMI) at the mean value of variable 2 (e.g. HDL SNP-score) and vice versa

Results SNP-score*Obesity parameter on HDL

Linear regression analysis results for HDL-C SNP-score on HDL-C (age-adjusted), stratified by obesity using <u>different categorizations</u>:

Categorization of obesity by		Linear regression results from HDL-C SNP-Score on HDL-C n	β	se	p-value
BMI	≥30	400	0.6207	0.1666	2.24×10^{-04}
	<30	999	1.0566	0.1270	3.05×10^{-16}
WHR:	Men: >1; Women: >0.85	436	0.5932	0.1630	3.09×10^{-04}
	Men: ≤1; Women: ≤0.85	966	1.0822	0.1319	7.76×10^{-16}
Waist:	Men: >102 cm; Women: >88 cm	724	0.7183	0.1281	3.55×10^{-08}
	Men: ≤102 cm; Women: ≤88 cm	678	1.1958	0.1594	2.09×10^{-13}
BMI & WHR & waist obesity condition fulfilled		198	0.4561	0.2081	0.0296
At least one condition not fulfilled		1208	1.0251	0.1156	2.79×10^{-18}

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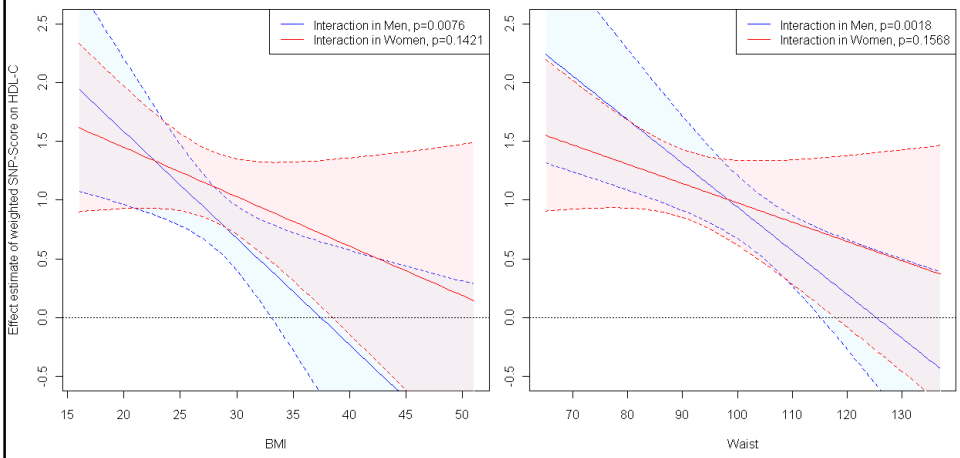
Graphical presentation of interaction effects



- The effect of the SNP-score on HDL diminishes for increasing level of obesity
- This modifying effect seems to be stronger in men
  - It is <u>not</u> triggered by specific single SNPs (highest associated interaction effect present for *APOB*-SNP, p(BMI-interaction)=0.018)

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R-function can be found at: http://www.i-med.ac.at/genepi

Summary

- A modifying effect of obesity on lipids can only be seen for HDL (primarily in men)
- The effect of the SNP-score on HDL diminishes for increasing level of obesity (~ twice as high for obese than for non-obese)
- Inclusion of SNP-obesity interaction effect on HDL additionally explains ~3% of phenotypic variance

→ reduces the missing heritability problem

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Summary

What we have learned (from a statistical, methodological view):

→ SNP-Scores are appropriate tools for the detection of gene-environment interaction effects, if there is not enough power for single SNP*Environment interaction effects

→ Weighting necessary, using independent, but similar populations!

→ Limitation:
- No „new“ loci can be found
- One has to assume that interaction effects in all SNPs point in the same direction and accumulate in the SNP-Score

→ Interaction effects between continuous variables can be presented and interpreted easily without arbitrary dichotomizations (although still useful sometimes, if cutpoints are clinically relevant)

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References:

Lamina C, Forer L, Schönherr S, Kollerits B, Ried JS, Gieger C, Peters A, Wichmann HE, Kronenberg F.: Evaluation of gene-obesity interaction effects on cholesterol levels: a genetic predisposition score on HDL-cholesterol is modified by obesity. Atherosclerosis. 2012 Dec;225(2):363-9

Lamina C, Sturm G, Kollerits B, Kronenberg F: Visualizing interaction effects: a proposal for presentation and interpretation. J Clin Epidemiol. 2012 Aug;65(8):855-62.