Fagron genomics

NutriGen™ Literature review

6 September 2019

Table of Contents

Fagron BV
Lichtenauerlaan 182
3062 Rotterdam
The Netherlands

T +31 88 33 11 210
F +31 88 33 11 210

www.fagron.com

Introduction

Fagron NutriGen™ will be available in two different versions sold via different types of healthcare professionals, Fagron NutriGen™ and Fagron NutriGen™ Medical, offering different solutions based on the patient's genetic background and lifestyle.

The Fagron NutriGen™ literature review contains the most relevant scientific articles that support the genetic analysis performed in NutriGen. Some suggested APIs and DCIs are not directly associated with a genetic variation but have an important role in the metabolic pathways analyzed. For more detailed information regarding the analyzed genes and their relationship with APIs/DCIs for weight management, please be referred to Fagron NutriGen™ Medical Scientific validation document.

For more information regarding Fagron NutriGen™ Medical please be referred to the Fagron NutriGen™ Medical Introduction document.

For more information about basic genetics, genetic variations, analysis of genetic variations and the genotyping process, please be referred to Fagron Genomics Introduction document. All available materials can be found online on the concept page within the Innovation Platform.

Should you have any questions or remarks, please contact your local sales representative.

Fagron BV
Lichtenauerlaan 182
3062 Rotterdam
The Netherlands

T +31 88 33 11 210
F +31 88 33 11 210

www.fagron.com

1. Morphological genetics in overweight predisposition

1.1 Common genetic variants in the FTO gene are associated with substantial changes in BMI, hip circumference, and body weight.

Scuteri A, et al. *Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits*. PLoS Genet. 2007 Jul;3(7):e115.

The obesity epidemic is responsible for a substantial economic burden in developed countries and is a major risk factor for type 2 diabetes and cardiovascular disease. The disease is the result not only of several environmental risk factors, but also of genetic predisposition. To take advantage of recent advances in gene-mapping technology, we executed a genome-wide association scan to identify genetic variants associated with obesity-related quantitative traits in the genetically isolated population of Sardinia. Initial analysis suggested that several SNPs in the FTO and PFKP genes were associated with increased BMI, hip circumference, and weight. Within the FTO gene, rs9930506 showed the strongest association with BMI (p = 8.6 x10(-7)), hip circumference (p = 3.4 x 10(-8)), and weight (p = 9.1 x 10(-7)). In Sardinia, homozygotes for the rare "G" allele of this SNP (minor allele frequency = 0.46) were 1.3 BMI units heavier than homozygotes for the common "A" allele. Within the PFKP gene, rs6602024 showed very strong association with BMI (p = 4.9 x 10(-6)). Homozygotes for the rare "A" allele of this SNP (minor allele frequency = 0.12) were 1.8 BMI units heavier than homozygotes for the common "G" allele. To replicate our findings, we genotyped these two SNPs in the GenNet study. In European Americans (N = 1,496) and in Hispanic Americans (N = 839), we replicated significant association between rs9930506 in the FTO gene and BMI (p-value for meta-analysis of European American and Hispanic American follow-up samples, p = 0.001), weight (p = 0.001), and hip circumference (p = 0.0005). We did not replicate association between rs6602024 and obesity-related traits in the GenNet sample, although we found that in European Americans, Hispanic Americans, and African Americans, homozygotes for the rare "A" allele were, on average, 1.0-3.0 BMI units heavier than homozygotes for the more common "G" allele. In summary, we have completed a whole genome-association scan for three obesity-related quantitative traits and report that common genetic variants in the FTO gene are associated with substantial changes in BMI, hip circumference, and body weight. These changes could have a significant impact on the risk of obesity-related morbidity in the general population.

1.2 SH2B1 gene locus is significantly associated with complex obesity in a Caucasian population.

Beckers S, et al. *Replication of the SH2B1 rs7498665 Association with Obesity in a Belgian Study Population*. Obes Facts. 2011;4(6):473-7. doi: 10.1159/000335305.

Objective: SH2B1 has been identified as an interesting candidate gene for complex obesity through genome-wide association studies. Therefore, we set out to replicate the reported association with rs7498665 in our Belgian study population and to extend our study with an additional tagSNP for the SH2B1 gene region.
Methods: we genotyped both rs7498665 and rs7201929 in a population of 1,045 obese adults and 317 healthy lean individuals. Statistical analyses were performed to evaluate the role of these polymorphisms in the development of obesity.
Results: we found that the rs7498665 minor allele increases obesity risk by 26% (OR(age-sex adj) = 1.26, 95% CI 1.04-1.52, nominal p = 0.016). Logistic regression showed that the rs7201929 minor allele decreases obesity risk by 24% in the population investigated (OR(age-sex adj) = 0.76, 95% CI 0.61-0.94, nominal p = 0.011). Conditional analyses showed that both associations represent the same association signal (rs7498665 OR(adjusted for rs7201929) = 1.17, 95% CI 0.95-1.45, nominal P = 0.14; rs7201929 OR(adjusted for rs7498665) = 0.82, 95% CI 0.65-1.04, nominal p = 0.10).
Conclusion: with the current study we were able to replicate and confirm that the SH2B1 gene locus is significantly associated with complex obesity in a Caucasian population.

1.3 Common variants near MC4R are associated with fat mass, weight and risk of obesity.

Loos RJ, et al. *Common variants near MC4R are associated with fat mass, weight and risk of obesity*. Nat Genet. 2008 Jun;40(6):768-75. doi: 10.1038/ng.140.

To identify common variants influencing body mass index (BMI), we analyzed genome-wide association data from 16,876 individuals of European descent. After previously reported variants in FTO, the strongest association signal (rs17782313, P = 2.9 x 10(-6)) mapped 188 kb downstream of MC4R (melanocortin-4 receptor), mutations of which are the leading cause of monogenic severe childhood-onset obesity. We confirmed the BMI association in 60,352 adults (per-allele effect = 0.05 Z-score units; P = 2.8 x 10(-15)) and 5,988 children aged 7-11 (0.13 Z-score units; P = 1.5 x 10(-8)). In case-control analyses (n = 10,583), the odds for severe childhood obesity reached 1.30 (P = 8.0 x 10(-11)). Furthermore, we observed overtransmission of the risk allele to obese offspring in 660 families (P (pedigree disequilibrium test average; PDT-avg) = 2.4 x 10(-4)). The SNP location and patterns of phenotypic associations are consistent with effects mediated through altered MC4R function. Our findings establish that common variants near MC4R influence fat mass, weight and obesity risk at the population level and reinforce the need for large-scale data integration to identify variants influencing continuous biomedical traits.

1.4 Common genetic variation near MC4R is associated with waist circumference and insulin resistance.

Chambers JC, et al. *Common genetic variation near MC4R is associated with waist circumference and insulin resistance*. Nat Genet. 2008 Jun;40(6):716-8. doi: 10.1038/ng.156.

We carried out a genome-wide association study (318,237 SNPs) for insulin resistance and related phenotypes in 2,684 Indian Asians, with further testing in 11,955 individuals of Indian Asian or European ancestry. We found associations of rs12970134 near MC4R with waist circumference (P = 1.7 × 10−9) and, independently, with insulin resistance. Homozygotes for the risk allele of rs12970134 have ~2 cm increased waist circumference. Common genetic variation near MC4R is associated with risk of adiposity and insulin resistance.

1.5 LEPR rs1805094 C allele carriers showed nominally significant associations with change in BMI z categories

Almandil NB, et al. *Associations between the LEP -2548G/A Promoter and Baseline Weight and between LEPR Gln223Arg and Lys656Asn Variants and Change in BMI z Scores in Arab Children and Adolescents Treated with Risperidone*. Mol Neuropsychiatry. 2018 Oct;4(2):111-117. doi: 10.1159/000490463.

Data on baseline (antipsychotics-naïve) age, weight, and height, and change in these at 3 subsequent follow-up time points up to 313.6 days (95% CI 303.5-323.7) were collected from 181 risperidone-treated children and adolescents (mean age 12.58 years, SD 4.99, range 2.17-17.7) attending a pediatric neurology clinic in Saudi Arabia. Owing to differences in genotypic distributions in the subsamples, results are reported for the white Arab population (n = 144). Age- and gender-normed body mass index (BMI)-standardized z scores (BMI z) were calculated (LMSgrowth program). Linear regression was performed for baseline weight and BMI z, while change in BMI z was assessed using random effects ordered logistic regression. The following single nucleotide polymorphisms (SNPs) were analyzed: rs7799039 in the LEP promoter, rs1805094 (previously rs8179183), rs1137100 and rs1137101 in the LEPR, and rs1414334 in HTR2C. We found a nominally significant association between rs7799309 and baseline weight, adjusting for height, age, gender, and diagnosis (A/G, p = 0.035, β = -3.62 vs. G/G). The rs1137101 (G/G, p = 0.018, odds ratio [OR] = 4.13 vs. A/A) and rs1805094 C allele carriers (p = 0.019, OR = 0.51) showed nominally significant associations with change in BMI z categories. Our data support and replicate previous relevant associations for these variants (including with weight gain when on risperidone), whilst being the first report of such associations in patients of Arab ethnicity.

1.6 A significant association between LEPR SNP rs8179183 and overweight in Chinese Han adolescents has been found

Ren D, et al. *Association study between LEPR, MC4R polymorphisms and overweight/obesity in Chinese Han adolescents*. Gene. 2019 Apr 15; 692:54-59. doi: 10.1016/j.gene.2018.12.073.

Objective: obesity is one of the major health problems strongly influenced by lifestyle, genetic and environmental factors. Previous studies have reported many single-nucleotide polymorphisms (SNPs) are associated with obesity in different races. This study aimed to explore the genetic associations between LEPR, MC4R polymorphisms and overweight/obesity in Chinese Han adolescents.
Methods: 400 adolescents including 222 health controls and 178 overweight/obese adolescents were genotyped and their body compositions were also analyzed in this study.
Results: we found that allelic and genotypic frequencies of LEPR SNP rs8179183 were significantly different between controls and cases (allelic frequency $p < 0.001$; genotypic frequency $p = 0.004$). The difference was still significant (allelic frequency $p < 0.011$; genotypic frequency $p = 0.024$) after Bonferroni correction. Moreover, we found that rs8179183 was associated with serum triglyceride level after adjusting for age and body mass index (BMI) ($p = 0.037$).
Conclusion: in summary, our results found a significant association between LEPR SNP rs8179183 and overweight/obesity in Chinese Han adolescents. This study may provide a reference for future studies of obesity.

1.7 Adiponectin levels are associated with SNPs in two different regulatory regions

Hivert MF, et al. *Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study*. Diabetes. 2008 Dec;57(12):3353-9. doi: 10.2337/db08-0700.

Objective: variants in ADIPOQ have been inconsistently associated with adiponectin levels or diabetes. Using comprehensive linkage disequilibrium mapping, we genotyped single nucleotide polymorphisms (SNPs) in ADIPOQ to evaluate the association of common variants with adiponectin levels and risk of diabetes.
Research design and methods: participants in the Framingham Offspring Study (n = 2,543, 53% women) were measured for glycemic phenotypes and incident diabetes over 28 years of follow-up; adiponectin levels were quantified at exam 7. We genotyped 22 tag SNPs that captured common (minor allele frequency >0.05) variation at r(2) > 0.8 across ADIPOQ plus 20 kb 5' and 10 kb 3' of the gene. We used linear mixed effects models to test additive associations of each SNP with adiponectin levels and glycemic phenotypes. Hazard ratios (HRs) for incident diabetes were estimated using an adjusted Cox proportional hazards model.
Results: 2 promoter SNPs in strong linkage disequilibrium with each other (r(2) = 0.80) were associated with adiponectin levels (rs17300539; P(nominal) [P(n)] = 2.6 x 10(-8); P(empiric) [P(e)] = 0.0005 and rs822387; P(n) = 3.8 x 10(-5); P(e) = 0.001). A 3'-untranslated region (3'UTR) SNP (rs6773957) was associated with adiponectin levels (P(n) = 4.4 x 10(-4); P(e) = 0.005). A nonsynonymous coding SNP (rs17366743, Y111H) was confirmed to be associated with diabetes incidence (HR 1.94 [95% CI 1.16-3.25] for the minor C allele; P(n) = 0.01) and with higher mean fasting glucose over 28 years of follow-up (P(n) = 0.0004; P(e) = 0.004). No other significant associations were found with other adiposity and metabolic phenotypes.
Conclusions: adiponectin levels are associated with SNPs in two different regulatory regions (5' promoter and 3'UTR), whereas diabetes incidence and time-averaged fasting glucose are associated with a missense SNP of ADIPOQ.

1.8 ADIPOQ rs17300539 can be associated with obesity risk in Caucasian ethnicity.

Lu JF, et al. *Association of ADIPOQ polymorphisms with obesity risk: a meta-analysis*. Hum Immunol. 2014 Oct;75(10):1062-8. doi: 10.1016/j.humimm.2014.09.004.

Background: the association between ADIPOQ polymorphisms and the risk of obesity remains controversial. We perform a comprehensive meta-analysis to clarify the current understanding of this association.

Methods: we searched for relevant studies in PubMed, Embase and Cochrane library before February 2014. The strengths of the association between ADIPOQ polymorphisms and obesity risk were estimated by odds ratios (OR) with 95% confidence intervals (CI).
Results: eighteen case-control studies analyzing four SNPs (rs17300539, rs266729, rs1501299 and rs2241766) of ADIPOQ gene were eligible for the present meta-analysis. The pooling results showed that rs17300539 (2GG+GA vs. 2AA+GA: OR=0.78, 95%CI=0.69-0.89) and rs1501299 (2GG+GA vs. 2AA+GA: OR=0.89, 95%CI=0.80-0.98) were associated with obesity risk in Caucasian ethnicity. The rs266729 were associated with obesity risk in Asian ethnicity (2CC+CG vs. 2GG+GCG: OR=0.77, 95%CI=0.65-0.92). However, there were no associations between rs2241766 and the obesity risk (P>0.05). No publication bias was found among these studies (all P>0.05).
Conclusions: this study suggests that ADIPOQ rs17300539 and rs1501299 are associated with risk of obesity in Caucasian ethnicity, and the rs266729 is associated with obesity risk in Asian ethnicity. However, there is no association between rs2241766 and obesity risk.

1.9. Carriage of the ADIPOQ rs17300539 A allele confers protection from weight regain.

Goyenechea E, et al. *The - 11391 G/A polymorphism of the adiponectin gene promoter is associated with metabolic syndrome traits and the outcome of an energy-restricted diet in obese subjects*. Horm Metab Res. 2009 Jan;41(1):55-61. doi: 10.1055/s-0028-1087204.

Adiponectin is an adipose tissue-specific hormone that is commonly decreased in obese subjects. Furthermore, single-nucleotide polymorphisms (SNPs) of the adiponectin gene have been associated with metabolic phenotypes. The present study investigated whether the adiponectin gene promoter variant -11391 G/A (rs17300539) could predict the risk of developing traits characterizing the metabolic syndrome (MetS) and the impact of weight management. The -11391 G/A SNP was genotyped in 180 Spanish volunteers (BMI: 31.4+/-3.2 kg/m (2); age: 35+/-5 years). Clinical measurements were determined at baseline, following an 8-week low-calorie diet (LCD), and at 32 and 60 weeks. At baseline, the GG genotype was associated with higher HOMA-IR, insulin and triacylglyceride concentrations than other genotypes (p<0.05) and was also related with a higher risk of insulin resistance (OR: 2.437, p=0.025) and MetS clinical manifestations (OR: 3.236, p=0.003). Following the LCD, the increased risk in GG subjects compared with others disappeared (p>0.05). By 32 weeks after dietary therapy (n=84), GG carriers had recovered the risk of metabolic comorbidities (OR: 2.420, p=0.043). This risk was even more evident after 60 weeks (OR: 2.875, p=0.014). These data show an increased risk of insulin resistance and MetS complications in obese subjects of the -11391 GG genotype. The risk was markedly reduced during an energy-restricted diet but was not sustained. Carriage of the A allele therefore confers protection from weight regain, and the effect is particularly evident 32-60 weeks after the dietary intervention, when improvement in GG subjects had disappeared.

1.10 MC4R variants appear to contribute to body fat, body fat distribution, some metabolic traits and weight development during childhood

Kring SI, et al. *Common variants near MC4R in relation to body fat, body fat distribution, metabolic traits and energy expenditure*. Int J Obes (Lond). 2010 Jan;34(1):182-9. doi: 10.1038/ijo.2009.215.

Objective: common variants near melanocortin receptor 4 (MC4R) have been related to fatness and type 2 diabetes. We examined the associations of rs17782313 and rs17700633 in relation to body fat, body fat distribution, metabolic traits, weight development and energy expenditure.
Methods: obese young men (n = 753, BMI > or = 31.0 kg m(-2)) and a randomly selected group (n = 874) identified from a population of 174 800 men were re-examined in three surveys at mean ages 35, 46 and 49 years (S-35, S-46 and S-49). Measurements were available at upto eight times from birth to adulthood. Logistic regression analysis was used to assess odds ratio (OR) for the presence of the carrier allele for a given difference in phenotypic values.
Results: rs17782313 minor C-allele was associated with overall, abdominal and peripheral fatness (range of OR = 1.06-1.14 per z-score units) at all three surveys, although only consistently significant at S-35 and S-46. Rs17700633 minor A-allele was also associated with the fatness measures, but significantly so only at S-49 for

overall and abdominal fatness (range of OR = 1.03-1.15 per z-score units), and peripheral fatness (OR = 1.15-1.20 per z-score units). There were only few significant associations with metabolic traits. The rs17782313 C-allele and the rs17700633 A-allele were both associated with lower high-density lipoprotein cholesterol (range of OR = 0.64-0.84 per mol l(-1)), significantly at S-46. The rs17700633 A-allele was significantly associated with insulin (OR = 1.25 per 50 pmol l(-1)), leptin (OR = 1.42 per 10 ng microl(-1)) and insulin sensitivity (OR = 0.81 per model unit). The rs17782313 C-allele and the rs17700633 A-allele were both associated with BMI in childhood and adolescence (range of OR = 1.04-1.17 per z-score units), significant for the rs17782313 C-allele at the age of 13-19 years and for rs17700633 A-allele at age 7, 10, 13 and 19 years. No significant associations were found for energy expenditure.
Conclusion: near MC4R variants appear to contribute to body fat, body fat distribution, some metabolic traits, weight development during childhood, but not to energy expenditure.

1.11 SH2B *rs4788102* GG genotype is associated with low risk of obesity, and properly balance of leptin levels.

Jamshidi Y, et al. *The SH2B gene is associated with serum leptin and body fat in normal female twins. Obesity (Silver Spring)*. 2007 Jan;15(1):5-9.

Src-homology-2 (SH2)-B, a Janus tyrosine kinase 2-interacting protein, has been identified recently as a key regulator of leptin and insulin sensitivity, glucose homeostasis, and body weight in mice. The aim of this study was to determine whether single-nucleotide polymorphisms (SNPs) in the human SH2B gene are associated with these variables. A tagging SNP (tSNP), Ala484Thr (rs7498665), was selected to represent five common SNPs (minor allele frequency > 0.05) in perfect linkage disequilibrium in a 16-kb region encompassing the SH2B gene. The tSNP was genotyped in 2455 white female twins (mean age, 47.4 +/- 12.6 years) from the St. Thomas' United Kingdom Adult Twin Registry (Twins United Kingdom). Ala484Thr (minor allele frequency, 0.38) was associated with serum leptin, total fat, waist circumference, and body weight (P = 0.02 to 0.04). The coding SNP has no predicted effect on protein structure or function and is likely to be in linkage disequilibrium with an as-yet unidentified functional variant in the SH2B gene. Our results support a role for SH2-B in modulating the regulation of body weight and fat by leptin in this female population. If SH2-B signaling is attenuated in diet-induced obesity, it could become a target for drug-induced leptin sensitization.

2. Behavioral genetics in food intake

2.1 FTO rs9939609 polymorphism modifies the relationship between body mass index and affective symptoms through the life course

Koike S, et al. *Fat mass and obesity-associated (FTO) rs9939609 polymorphism modifies the relationship between body mass index and affective symptoms through the life course: a prospective birth cohort study*. Transl Psychiatry. 2018 Mar 13;8(1):62. doi: 10.1038/s41398-018-0110-1.

Although bi-directional relationships between high body mass index (BMI) and affective symptoms have been found, no study has investigated the relationships across the life course. There has also been little exploration of whether the fat mass and obesity-associated (FTO) rs9939609 single-nucleotide polymorphism (SNP) is associated with affective symptoms and/or modifies the relationship between BMI and affective symptoms. In the MRC National Survey of Health and Development (NSHD), 4556 participants had at least one measure of BMI and affective symptoms between ages 11 and 60-64 years. A structural equation modelling framework was used with the BMI trajectory fitted as latent variables representing BMI at 11, and adolescent (11-20 years), early adulthood (20-36 years) and midlife (36-53 years) change in BMI. Higher levels of adolescent emotional problems were associated with greater increases in adult BMI and greater increases in early adulthood BMI were associated with higher subsequent levels of affective symptoms in women. The rs9939609 risk variant (A allele) from 2469 participants with DNA genotyping at age 53 years showed mostly protective effect modification of these relationship. Increases in adolescent and early adulthood BMI were generally not associated with, or were

associated with lower levels, of affective symptoms in the FTO risk homozygote (AA) group, but positive associations were seen in the TT group. These results suggest bi-directional relationships between higher BMI and affective symptoms across the life course in women, and that the relationship could be ameliorated by rs9939609 risk variant.

2.2 The obesity risk variants of SH2B1 SNPs rs7498665 showed nominal evidence of association with increased snacking

Robiou-du-Pont S, et al. *Common variants near BDNF and SH2B1 show nominal evidence of association with snacking behavior in European populations*. J Mol Med (Berl). 2013 Sep;91(9):1109-15. doi: 10.1007/s00109-013-1027-z.

We investigated the effect of 24 obesity-predisposing single nucleotide polymorphisms (SNPs), separately and in combination, on snacking behavior in three European populations. The 24 SNPs were genotyped in 7,502 subjects (1,868 snackers and 5,634 non-snackers). We tested the hypothesis that obesity risk variants or a genetic risk score increases snacking using a logistic regression adjusted for sex, age, and body mass index. The obesity genetic risk score was not associated with snacking (odds ratio (OR) = 1.00 [0.98-1.02], P value = 0.48). The obesity risk variants of two SNPs (rs925946 and rs7498665) close to the BDNF and SH2B1 genes showed nominal evidence of association with increased snacking (OR = 1.09 [1.01-1.17], P value = 0.0348 and OR = 1.11 [1.04-1.19], P value = 0.00703, respectively) but did not survive Bonferroni corrections for multiple testing. The associations of rs925946 and rs7498665 obesity risk variants with increased BMI (β = 0.180 [0.022-0.339], P value = 0.0258 and β = 0.166 [0.019-0.313], P value = 0.0271, respectively) were slightly attenuated after adjusting for snacking (β = 0.151 [-0.006 to 0.309], P value = 0.0591 and β = 0.152 [0.006-0.297], P value = 0.0413). Our data suggest that genetic predisposition to obesity does not significantly contribute to snacking behavior. The nominal associations of rs925946 and rs7498665 obesity risk variants near the BDNF and SH2B1 genes with increased snacking deserve further investigation.

2.3 Common noncoding variation in the LEPR gene is associated with higher BMI and lower energy expenditure in Native Americans.

Traurig MT, et al. *Variants in the LEPR gene are nominally associated with higher BMI and lower 24-h energy expenditure in Pima Indians*. Obesity (Silver Spring). 2012 Dec;20(12):2426-30. doi: 10.1038/oby.2012.159.

Genome-wide association studies (GWASs) have been used to search for susceptibility genes for type 2 diabetes and obesity in the Pima Indians, a population with a high prevalence of both diseases. In these studies, a variant (rs2025804) in the LEPR gene was nominally associated with BMI in 1,082 subjects (P = 0.03 adjusted for age, sex, birth year, and family membership). Therefore the LEPR and leptin overlapping transcript (LEPROT) genes were selected for further sequencing and genotyping in larger population-based samples for association analyses with obesity-related phenotypes. Selected variants (n = 80) spanning these genes were genotyped in a sample of full-heritage Pima Indians (n = 2,842) and several common variants including rs2025804 were nominally associated with BMI (P = 0.05-0.003 adjusted for age, sex, birth year, and family membership). Four common tag variants associated with BMI in the full-heritage Pima Indian sample were genotyped in a second sample of mixed-heritage Native Americans (n = 2,969) and three of the variants showed nominal replication (P = 0.03-0.006 adjusted as above and additionally for Indian heritage). Combining both samples provided the strongest evidence for association (adjusted P = 0.0003-0.0001). A subset of these individuals (n = 403) had been metabolically characterized for predictors of obesity and the BMI risk alleles for the variants tagged by rs2025804 were also associated with lower 24-h energy expenditure (24hEE) as assessed in a human respiratory chamber (P = 0.0007 adjusted for age, sex, fat mass, fat-free mass, activity, and family membership). We conclude that common noncoding variation in the LEPR gene is associated with higher BMI and lower energy expenditure in Native Americans.

2.4 There is an association between the ADIPOQrs17300539 polymorphism and energy intake.

Kroll C, et al. *Associations of ADIPOQ and LEP Gene Variants with Energy Intake: A Systematic Review*. Nutrients. 2019 Mar 30;11(4). pii: E750. doi: 10.3390/nu11040750.

This systematic review aims to evaluate the association of adiponectin (ADIPOQ) and leptin (LEP) gene variants with energy intake. Cross-sectional, cohort, and case¯control studies that reported an association of leptin and/or adiponectin gene variants with energy intake were included in this review. Human studies without any age restrictions were considered eligible. Detailed individual search strategies were developed for each of the following bibliographic databases: Cochrane, Latin American and Caribbean Center on Health Sciences Information (LILACS), PubMed/MEDLINE, Scopus, and Web of Science. Risk of bias assessment was adapted from the Downs and Black scale and was used to evaluate the methodology of the included studies. Seven studies with a pooled population of 2343 subjects were included. The LEP and ADIPOQ gene variants studied were LEP-rs2167270 (k = 1), LEP-rs7799039 (k = 5), ADIPOQ-rs2241766 (k = 2), ADIPOQ-rs17300539 (k = 1), and ADIPOQ marker D3S1262 (k = 1). Two of the seven studies reviewed demonstrated a positive association between the LEP-rs7799039 polymorphism and energy intake. Two other studies-one involving a marker of the ADIPOQ gene and one examining the ADIPOQ-rs17300539 polymorphism-also reported associations with energy intake. More research is needed to further elucidate the contributions of genetic variants to energy metabolism.

2.5. LEP polymorphisms are related to the variation in the feeling of fullness and may play a role in the regulation of food intake

Dougkas A, et al. *The impact of obesity-related SNP on appetite and energy intake*. Br J Nutr. 2013 Sep 28;110(6):1151-6. doi: 10.1017/S0007114513000147.

An increasing number of studies have reported a heritable component for the regulation of energy intake and eating behavior, although the individual polymorphisms and their 'effect size' are not fully elucidated. The aim of the present study was to examine the relationship between specific SNP and appetite responses and energy intake in overweight men. In a randomized cross-over trial, forty overweight men (age 32 (sd 09) years; BMI 27 (sd 2) kg/m2) attended four sessions 1 week apart and received three isoenergetic and isovolumetric servings of dairy snacks or water (control) in random order. Appetite ratings were determined using visual analogue scales and energy intake at an ad libitum lunch was assessed 90 min after the dairy snacks. Individuals were genotyped for SNP in the fat mass and obesity-associated (FTO), leptin (LEP), leptin receptor (LEPR) genes and a variant near the melanocortin-4 receptor (MC4R) locus. The postprandial fullness rating over the full experiment following intake of the different snacks was 17·2 % (P= 0·026) lower in A carriers compared with TT homozygotes for rs9939609 (FTO, dominant) and 18·6 % (P= 0·020) lower in G carriers compared with AA homozygotes for rs7799039 (LEP, dominant). These observations indicate that FTO and LEP polymorphisms are related to the variation in the feeling of fullness and may play a role in the regulation of food intake. Further studies are required to confirm these initial observations and investigate the 'penetrance' of these genotypes in additional population subgroups.

2.6. COMT Val158Met (rs4680) is associated with higher blood pressure and higher prevalence of hypertension in Japanese men, and energy intake may interact with this effect.

Htun NC, et al. *Association of the catechol-O-methyl transferase gene Val158Met polymorphism with blood pressure and prevalence of hypertension: interaction with dietary energy intake*. Am J Hypertens. 2011 Sep;24(9):1022-6. doi: 10.1038/ajh.2011.93.

Background: previous studies of a functional variant of the catechol-O-methyl transferase (COMT) gene, Val158Met, have provided inconsistent results with regard to blood pressure or hypertension. We examined the effect of this variant, the considering environmental factors of daily salt and energy intakes.
Methods: a total of 735 Japanese men (mean age, 47 years) were recruited from two separate occupational cohorts from Kanagawa and Kyoto prefectures. Participants were genotyped for the presence of COMT

Val158Met (rs4680, G/A). Daily salt and energy intakes were evaluated by the food frequency questionnaire (FFQ).
Results: Met/Met carriers had higher adjusted systolic blood pressure (SBP) (+4.79 mm Hg, P < 0.001) and diastolic blood pressure (DBP) (+2.33 mm Hg, P = 0.001) than Met/Val or Val/Val carriers. There was a significant association between being a Met/Met carrier and having a higher prevalence of hypertension (odds ratio = 2.448, 95% confidence interval = 1.426-4.205, P = 0.001). When salt and energy intakes were dichotomized, the effect of Val158Met on hypertension was observed only in the high-energy intake group and was equivalent between low- and high-salt groups.
Conclusion: the Met allele of COMT Val158Met is associated with higher blood pressure and higher prevalence of hypertension in Japanese men, and energy intake may interact with this effect.

2.7 Polymorphisms in the MC4R gene do not have a role in regulating food intake and preference for specific food items.

Hasselbalch AL, et al. *A variant in the fat mass and obesity-associated gene (FTO) and variants near the melanocortin-4 receptor gene (MC4R) do not influence dietary intake*. J Nutr. 2010 Apr;140(4):831-4. doi: 10.3945/jn.109.114439.

We investigated the role of the fat mass and obesity associated gene (FTO) and variants near the melanocortin-4 receptor gene (MC4R) in modulating habitual intake of total energy and macronutrients, glycemic index, glycemic load, dietary energy density, and energy from 20 food groups in adults. In a population-based sample of 756 healthy adult twin pairs, we studied associations between FTO rs9939609, near-MC4R rs12970134, rs17700633, and rs17782313 single nucleotide polymorphisms (SNP) and habitual dietary intake. Habitual dietary intake was assessed by a 247-question FFQ. Nontransformed variables and variables transformed by natural logarithm were analyzed by linear regression and dichotomized variables were analyzed by logistic regression. FTO SNP rs9939609 was not associated with habitual dietary intake. For the near-MC4R SNP rs12970134 and rs17700633, we found significant positive associations with intake of energy from whole grains (P >or= 0.04). These associations did not remain significant after controlling for multiple testing. The outcome of this study indicates that polymorphisms in the FTO gene and near the MC4R gene do not have a role in regulating food intake and preference for specific food items.

2.8 A polymorphism in TAS2R38 is associated with differences in ingestive behavior.

Dotson CD, et al. *Variation in the gene TAS2R38 is associated with the eating behavior disinhibition in Old Order Amish women*. Appetite. 2010 Feb;54(1):93-9. doi: 10.1016/j.appet.2009.09.011.

Insensitivity to the bitter-tasting compound 6-n-propylthiouracil (PROP) has been proposed as a marker for individual differences in taste perception that influence food preference and intake. The principal genetic determinants of phenotypic variation in PROP taste sensitivity are alleles of the TAS2R38 gene, which encodes a chemosensory receptor sensitive to thiourea compounds including PROP and phenylthiocarbamide. Members of the TAS2R family are expressed in the gustatory system, where they function as bitter taste receptors, and throughout the gut, where their physiological roles in prandial, gut-derived hormone release are beginning to be elucidated. To better understand the relationship between TAS2R function and ingestive behaviors, we asked if TAS2R38 variants are associated with one or more of three eating behaviors: restraint, disinhibition, and hunger. We genotyped a single nucleotide polymorphism (SNP) located within the TAS2R38 gene, rs1726866 (T785C, Val262Ala) in 729 nondiabetic individuals (381 females, 348 males) within the Amish Family Diabetes Study. Eating behaviors were assessed using the Three-Factor Eating Questionnaire. An association analysis between rs1726866 and these three traits revealed a significant association of the PROP-insensitive "T" allele with increased disinhibition (p=0.03). Because eating behaviors differ substantially between males and females, we subsequently performed sex-stratified analyses, which revealed a strong association in females (p=0.0002) but not in males. Analyses with other SNPs in close proximity to rs1726866 suggest that this locus is principally responsible for the association. Therefore, our results indicate that a polymorphism in TAS2R38 is associated with differences in ingestive behavior.

2.9 DRD2 rs1800497 and rs6277 are associated with increased compulsory eating desire.

Davis C, et al. *Binge eating disorder and the dopamine D2 receptor: genotypes and sub-phenotypes*. Prog Neuropsychopharmacol Biol Psychiatry. 2012 Aug 7;38(2):328-35. doi: 10.1016/j.pnpbp.2012.05.002.

Objective: while the study of binge eating disorder (BED) has burgeoned in the past decade, an understanding of its neurobiological underpinnings is still in the early stages. Previous research suggests that BED may be an overeating syndrome characterized by a hyper-responsiveness to reward, and a strong dopamine signaling in the neuro-circuitry that regulates pleasure and appetitive behaviors. We investigated the D2 receptors genes (DRD2/ANKK1) and their relation to the BED phenotype and four sub-phenotypes of BED that reflect an enhanced response to positive food stimuli.
Methods: in a sample of 230 obese adults with and without BED, we genotyped five functional markers of the D2 receptor: rs1800497, rs1799732, rs2283265, rs12364283, and rs6277, and assessed binge eating, emotional eating, hedonic eating, and food craving from dimensionally-scored, self-report questionnaires.
Results: compared to weight-matched controls, BED was significantly related to the rs1800497 and rs6277 genotypes that reflect enhanced dopamine neurotransmission. BED participants were also less likely to carry the minor T allele of rs2283265. The same markers related to the sub-phenotypes of BED with rs1800497 showing the strongest effects in the predicted direction.
Conclusions: this study supports the view that BED may be a condition that has its causal origins in a hypersensitivity to reward - a predisposition that is likely to foster overeating in our current environment with abundant availability of highly palatable and calorically-dense processed foods.

2.10 APOB rs515135 TT genotype is associated with increase circulating LDL levels and risk of coronary artery disease.

Vrablík M, et al. *Impact of variants within seven candidate genes on statin treatment efficacy*. Physiol Res. 2012;61(6):609-17.

Statins are the most commonly used drugs in patients with dyslipidemia. Among the patients, a significant inter-individual variability with supposed strong genetic background in statin treatment efficacy has been observed. Genome wide screenings detected variants within the CELSR2/PSRC1/SORT1, CILP2/PBX4, APOB, APOE/C1/C4, HMGCoA reductase, LDL receptor and PCSK9 genes that are among the candidates potentially modifying response to statins. Ten variants (SNPs) within these genes (rs599838, rs646776, rs16996148, rs693, rs515135, rs4420638, rs12654264, rs6511720, rs6235, rs11206510) were analyzed in 895 (46 % men, average age 60.3+/-13.1 years) patients with dyslipidemia treated with equipotent doses of statins (~90 % on simvastatin or atorvastatin, doses 10 or 20 mg) and selected 672 normolipidemic controls (40 % men, average age 46.5 years). Lipid parameters were available prior to the treatment and after 12 weeks of therapy. Statin treatment resulted in a significant decrease of both total cholesterol (7.00+/-1.53-->5.15+/-1.17 mmol/l, P<0.0001) and triglycerides (2.03+/-1.01-->1.65+/-1.23 mmol/l, P<0.0005). Rs599838 variant was not detected in first analyzed 284 patients. After adjustment for multiple testing, there was no significant association between individual SNPs and statin treatment efficacy. Only the rs4420638 (APOE/C1/C4 gene cluster) G allele carriers seem to show more profitable change of HDL cholesterol (P=0.007 without and P=0.06 after adjustment). Results demonstrated that, although associated with plasma TC and LDL cholesterol per se, variants within the CELSR2/PSRC1/SORT1, CILP2/PBX4, APOB, APOE/C1/C4, HMGCoA reductase, LDL receptor and PCSK9 genes do not modify therapeutic response to statins.

2.11 HNF1A rs2650000 is associated with an alteration of LDL cholesterol plasma levels.

Hindorff LA, et al. *Potential etiologic and functional implications of genome-wide association loci for human diseases and traits*. Proc Natl Acad Sci U S A. 2009 Jun 9;106(23):9362-7. doi: 10.1073/pnas.0903103106.

We have developed an online catalog of SNP-trait associations from published genome-wide association studies for use in investigating genomic characteristics of trait/disease-associated SNPs (TASs). Reported TASs were

common [median risk allele frequency 36%, interquartile range (IQR) 21%-53%] and were associated with modest effect sizes [median odds ratio (OR) 1.33, IQR 1.20-1.61]. Among 20 genomic annotation sets, reported TASs were significantly overrepresented only in nonsynonymous sites [OR = 3.9 (2.2-7.0), p = 3.5 x 10(-7)] and 5kb-promoter regions [OR = 2.3 (1.5-3.6), p = 3 x 10(-4)] compared to SNPs randomly selected from genotyping arrays. Although 88% of TASs were intronic (45%) or intergenic (43%), TASs were not overrepresented in introns and were significantly depleted in intergenic regions [OR = 0.44 (0.34-0.58), p = 2.0 x 10(-9)]. Only slightly more TASs than expected by chance were predicted to be in regions under positive selection [OR = 1.3 (0.8-2.1), p = 0.2]. This new online resource, together with bioinformatic predictions of the underlying functionality at trait/disease-associated loci, is well-suited to guide future investigations of the role of common variants in complex disease etiology.

2.12 rs7903146 TT genotype is associated with decreased levels of HDL cholesterol.

Sanghera DK, et al. *TCF7L2 polymorphisms are associated with type 2 diabetes in Khatri Sikhs from North India: genetic variation affects lipid levels*. Ann Hum Genet. 2008 Jul;72(Pt 4):499-509. doi: 10.1111/j.1469-1809.2008.00443.x.

Recently, the transcription factor-7-like 2 (TCF7L2) gene has been identified as the most important type 2 diabetes mellitus (T2DM) susceptibility gene. Common intronic polymorphisms in this gene have been found to be strongly associated with T2DM susceptibility showing marked reproducibility in multiple populations. The purpose of this study was to confirm the reported association of six TCF7L2 variants in a Khatri Sikh diabetic sample from North India. We genotyped six-associated SNPs in a case-control sample consisting of 556 T2DM cases and 537 controls. We also examined the impact of these variants on body mass index (BMI), waist to hip ratio (WHR), fasting insulin, and glucose and lipid levels. We report replication of association of four of the six SNPs with T2DM in this Khatri Sikh sample [rs7903146, (p = 0.010); rs11196205, (p = 0.011); rs10885409, (p = 0.002) and rs4918789, (p = 0.029)], under a dominant model conferring odds ratios (ORs) of 1.39, 1.44, 1.57 and 1.36, respectively. Haplotype analysis provided further evidence of association by showing a significant difference between cases and controls as revealed by the global omnibus test (chi(2)= 19.36; p = 0.0036). Multiple linear regression analysis also revealed the risk allele carriers of three of four significant SNPs (rs7903146, rs11196205, rs10885409) to be significantly associated with increased fasting total cholesterol (p value = 0.019, 0.025, 0.006) and LDL cholesterol levels (p value = 0.021, 0.018, 0.005), respectively. Our findings confirm that the TCF7L2 gene is a major risk factor for development of T2DM in Khatri Sikhs. It also provides new information about the significant impact of TCF7L2 gene variants on plasma cholesterol levels that appear to be independent of BMI.

2.13 NMB rs1051168 TT genotype is associated with increased levels of disinhibition and susceptibility to hunger and increased body weight.

Bouchard L, et al. *Neuromedin beta: a strong candidate gene linking eating behaviors and susceptibility to obesity*. Am J Clin Nutr. 2004 Dec;80(6):1478-86.

Background: obesity is frequently associated with eating disorders, and evidence indicates that both conditions are influenced by genetic factors. However, little is known about the genes influencing eating behaviors.
Objective: the objective was to identify genes associated with eating behaviors.
Design: three eating behaviors were assessed in 660 adults from the Quebec Family Study with the use of the Three-Factor Eating Questionnaire. A genome-wide scan was conducted with a total of 471 genetic markers spanning the 22 autosomes to identify quantitative trait loci for eating behaviors. Body composition and macronutrient and energy intakes were also measured.
Results: hour quantitative trait loci were identified for disinhibition and susceptibility to hunger. Of these, the best evidence of linkage was found between a locus on chromosome 15q24-q25 and disinhibition (P <0.0058) and susceptibility to hunger (P <0.0001). After fine-mapping, the peak linkage was found between markers D15S206 and D15S201 surrounding the neuromedin beta (NMB) gene. A missense mutation (p.P73T) located within the NMB gene showed significant associations with eating behaviors and obesity phenotypes. The T73T homozygotes were 2 times as likely to exhibit high levels of disinhibition (odds ratio: 1.8; 95% CI: 1.07, 2.89;

P=0.03) and susceptibility to hunger (odds ratio: 1.9; 95% CI: 1.15, 3.06; P=0.01) as were the P73 allele carriers. Six-year follow-up data showed that the amount of body fat gain over time in T73T subjects was >2 times that than in P73P homozygotes (3.6 compared with 1.5 kg; P <0.05).
Conclusion: the results suggest that NMB is a very strong candidate gene of eating behaviors and predisposition to obesity.

3. Efficacy of exercise

3.1. A physically active lifestyle can have an impact on the genetic susceptibility to obesity induced by FTO rs1121980.

Vimaleswaran KS, et al. *Physical activity attenuates the BMI increasing influence of genetic variation in the FTO gene*. Am J Clin Nutr. 2009 Aug;90(2):425-8. doi: 10.3945/ajcn.2009.27652.

Background: intronic variation in the FTO (fat mass and obesity-associated) gene has been unequivocally associated with increased body mass index (BMI; in kg/m(2)) and the risk of obesity in populations of different ethnicity.
Objective: we examined whether this robust genetic predisposition to obesity can be attenuated by being more physically active.
Design: the FTO variant rs1121980 was genotyped in 20,374 participants (39-79 y of age) from the European Prospective Investigation into Cancer and Nutrition-Norfolk Study, an ethnically homogeneous population-based cohort. Physical activity (PA) was assessed with a validated self-reported questionnaire. The interaction between rs1121980 and PA on BMI and waist circumference (WC) was examined by including the interaction term in mixed-effect models.
Results: we confirmed that the risk (T) allele of rs1121980 was significantly associated with BMI (0.31-unit increase per allele; P < 0.001) and WC (0.77-cm increase per allele; P < 0.001). The PA level attenuated the effect of rs1121980 on BMI and WC; ie, whereas in active individuals the risk allele increased BMI by 0.25 per allele, the increase in BMI was significantly (P for interaction = 0.004) more pronounced (76%) in inactive individuals (0.44 per risk allele). We observed similar effects for WC (P for interaction = 0.02): the risk allele increased WC by 1.04 cm per allele in inactive individuals but by only 0.64 cm in active individuals.
Conclusions: our results showed that PA attenuates the effect of the FTO rs1121980 genotype on BMI and WC. This observation has important public health implications because we showed that a genetic susceptibility to obesity induced by FTO variation can be overcome, at least in part, by adopting a physically active lifestyle.

3.2 Physical training-induced alterations in body composition and plasma leptin may be influenced by a genetic variation of leptin promoter

Huuskonen A, et al. *Genetic variations of leptin and leptin receptor are associated with body composition changes in response to physical training*. Cell Biochem Funct. 2010 Jun;28(4):306-12. doi: 10.1002/cbf.1658.

Leptin regulates body weight, metabolism, and tissue adaptations to environmental stressors. We examined the association of single nucleotide polymorphism (SNP) of leptin promoter G-2548A (rs7799039) and leptin receptor Gln223Arg (rs1137101) with body composition, plasma leptin levels, and peak oxygen uptake (VO(2)peak) in response to 8 weeks of physical training in 48 male military conscripts. AA homozygotes of leptin promoter SNP-2548 showed higher body fat and BMI values than G allele carriers. Acute exercise decreased leptin levels in G allele carriers, but increased in AA homozygotes. Physical training significantly decreased BMI values and also a tendency for decreased plasma leptin levels was observed in all subjects. In G allele carriers, BMI loss was mainly due to decreased fat mass, whereas in AA homozygotes due to loss of fat-free mass. Training increased VO(2)peak in all subjects with most prominent effects in G allele carriers. Regarding leptin receptor SNP, there were no statistically significant differences in BMI values between the genotype groups at baseline or after physical training. Our results suggest that physical training-induced alterations in body composition and plasma leptin may be influenced by a genetic variation of leptin promoter but not of leptin receptor.

3.3 Effects of common variants in the LPL, LIPC, and CETP genes on HDL-C levels are modified by physical activity

Ahmad T, et al. *Physical activity modifies the effect of LPL, LIPC, and CETP polymorphisms on HDL-C levels and the risk of myocardial infarction in women of European ancestry*. Circ Cardiovasc Genet. 2011 Feb;4(1):74-80. doi: 10.1161/CIRCGENETICS.110.957290.

Background: recent genome-wide association studies have identified common variants associated with high-density lipoprotein cholesterol (HDL-C). Whether these associations are modified by physical activity, which increases HDL-C levels and reduces the risk of cardiovascular disease, is uncertain.
Methods and results: in a prospective cohort study of 22 939 apparently healthy US women of European ancestry, we selected 58 single nucleotide polymorphisms (SNPs) in 9 genes that demonstrated genome-wide association (P<5×10(-8)) with HDL-C levels and sought evidence of effect modification according to levels of physical activity. Physical activity modified the effects on HDL-C of 7 SNPs at 3 loci, and the strongest evidence of effect was observed for rs10096633 at lipoprotein lipase (LPL), rs1800588 at hepatic lipase (LIPC), and rs1532624 at cholesteryl ester transfer protein (CETP) (each P-interaction<0.05). The per-minor-allele increase in HDL-C for rs1800588 at LIPC and rs1532624 at CETP was greater in active than inactive women, whereas the reverse was observed for rs10096633 at LPL. Minor-allele carrier status at the LPL SNP was associated with a reduced risk of myocardial infarction in active (hazard ratio, 0.51; 95% confidence interval 0.30-0.86) but not among inactive women (hazard ratio 1.13; 95% confidence interval 0.79 to 1.61; P-interaction=0.007). By contrast, carrier status at the CETP SNP was associated with a reduced risk of myocardial infarction regardless of activity level (hazard ratio, 0.72; 95% confidence interval, 0.57 to 0.92; P-interaction=0.71). No association between LIPC SNP carrier status and myocardial infarction risk was noted.
Conclusions: the effects of common variants in the LPL, LIPC, and CETP genes on HDL-C levels are modified by physical activity. For a common variant in LPL, the impact on myocardial infarction varied by activity level, whereas the effects of a common variant in CETP on myocardial infarction risk did not.

3.4 Effect of endothelin 1 genotype on blood pressure is dependent on physical activity or fitness levels

Rankinen T, et al. *Effect of endothelin 1 genotype on blood pressure is dependent on physical activity or fitness levels*. Hypertension. 2007 Dec;50(6):1120-5.

Contributions of the DNA sequence variation at the endothelin 1 locus to the risk of hypertension and to endurance training-induced changes in blood pressure were investigated in the Aerobics Center Longitudinal Study and the Health, Risk Factors, Exercise Training and Genetics Family Study cohorts. We identified 586 normotensive control subjects and 607 incident hypertensive case subjects from the Aerobics Center Longitudinal Study cohort (all whites) who were normotensive and healthy at their first clinic visit. The case subjects were diagnosed with hypertension during an average follow-up of 9.5 years, whereas the control subjects remained normotensive. The allele and genotype frequencies of 5 endothelin 1 haplotype tagging single nucleotide polymorphisms did not differ significantly between the case and control subjects. However, we observed a significant (P=0.0025) interaction between the endothelin 1 rs5370 (G/T; Lys198Asn) genotype and cardiorespiratory fitness level on the risk of hypertension: among low-fit subjects, the rs5370 minor allele (T; 198Asn) was associated with higher risk of hypertension (odds ratio: 1.95; 95% CI: 1.36 to 2.81; P=0.0003), whereas the risk did not differ among genotypes in high-fit subjects. In the white Health, Risk Factors, Exercise Training and Genetics subjects (N=480), the rs5370 T allele was associated with blunted systolic blood pressure (P=0.0046) and pulse pressure (P=0.0016) responses to a 20-week endurance training program. The Lys198Asn variant of the endothelin 1 locus is associated with blood pressure phenotypes in whites. However, the expression of the genotype effect is modulated by physical activity or cardiorespiratory fitness level. Our study provides an illustrative example of how physical activity and fitness level modifies the associations between a candidate gene and outcome phenotype.

3.5 PPARD rs2016520 polymorphism is associated with increased levels of HDL (good cholesterol) with endurance exercise.

Ahmetov II, et al. *The combined impact of metabolic gene polymorphisms on elite endurance athlete status and related phenotypes*. Hum Genet. 2009 Dec;126(6):751-61. doi: 10.1007/s00439-009-0728-4.

Endurance performance is a complex phenotype subject to the influence of both environmental and genetic factors. Although the last decade has seen a variety of specific genetic factors proposed, many in metabolic pathways, each is likely to make a limited contribution to an 'elite' phenotype: it seems more likely that such status depends on the simultaneous presence of multiple such variants. The aim of the study was to investigate individually and in combination the association of common metabolic gene polymorphisms with endurance athlete status, the proportion of slow-twitch muscle fibers and maximal oxygen consumption. A total of 1,423 Russian athletes and 1,132 controls were genotyped for 15 gene polymorphisms, of which most were previously reported to be associated with athlete status or related intermediate phenotypes. Muscle fiber composition of m. vastus lateralis in 45 healthy men was determined by immunohistochemistry. Maximal oxygen consumption of 50 male rowers of national competitive standard was determined during an incremental test to exhaustion on a rowing ergometer. Ten 'endurance alleles' (NFATC4 Gly160, PPARA rs4253778 G, PPARD rs2016520 C, PPARGC1A Gly482, PPARGC1B 203Pro, PPP3R1 promoter 5I, TFAM 12Thr, UCP2 55Val, UCP3 rs1800849 T and VEGFA rs2010963 C) were first identified showing discrete associations with elite endurance athlete status. Next, to assess the combined impact of all 10 gene polymorphisms, all athletes were classified according to the number of 'endurance' alleles they possessed. The proportion of subjects with a high (≥9) number of 'endurance' alleles was greater in the best endurance athletes compared with controls (85.7 vs. 37.8%, P = 7.6 × 10(-6)). The number of 'endurance' alleles was shown to be positively correlated (r = 0.50; P = 4.0 × 10(-4)) with the proportion of fatigue-resistant slow-twitch fibers, and with maximal oxygen consumption (r = 0.46; P = 7.0 × 10(-4)). These data suggest that the likelihood of becoming an elite endurance athlete depends on the carriage of a high number of endurance-related alleles.

3.6 MATK rs12104221 GG genotype is associated with a better result in weight loss, reduced BMI and waist circumference, when conducting a daily physical activity.

Comuzzie AG, et al. *Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population*. PLoS One. 2012;7(12):e51954. doi: 10.1371/journal.pone.0051954.

Genetic variants responsible for susceptibility to obesity and its comorbidities among Hispanic children have not been identified. The VIVA LA FAMILIA Study was designed to genetically map childhood obesity and associated biological processes in the Hispanic population. A genome-wide association study (GWAS) entailed genotyping 1.1 million single nucleotide polymorphisms (SNPs) using the Illumina Infinium technology in 815 children. Measured genotype analysis was performed between genetic markers and obesity-related traits i.e., anthropometry, body composition, growth, metabolites, hormones, inflammation, diet, energy expenditure, substrate utilization and physical activity. Identified genome-wide significant loci: 1) corroborated genes implicated in other studies (MTNR1B, ZNF259/APOA5, XPA/FOXE1 (TTF-2), DARC, CCR3, ABO); 2) localized novel genes in plausible biological pathways (PCSK2, ARHGAP11A, CHRNA3); and 3) revealed novel genes with unknown function in obesity pathogenesis (MATK, COL4A1). Salient findings include a nonsynonymous SNP (rs1056513) in INADL (p = 1.2E-07) for weight; an intronic variant in MTNR1B associated with fasting glucose (p = 3.7E-08); variants in the APOA5-ZNF259 region associated with triglycerides (p = 2.5-4.8E-08); an intronic variant in PCSK2 associated with total antioxidants (p = 7.6E-08); a block of 23 SNPs in XPA/FOXE1 (TTF-2) associated with serum TSH (p = 5.5E-08 to 1.0E-09); a nonsynonymous SNP (p = 1.3E-21), an intronic SNP (p = 3.6E-13) in DARC identified for MCP-1; an intronic variant in ARHGAP11A associated with sleep duration (p = 5.0E-08); and, after adjusting for body weight, variants in MATK for total energy expenditure (p = 2.7E-08) and in CHRNA3 for sleeping energy expenditure (p = 6.0E-08). Unprecedented phenotyping and high-density SNP genotyping enabled localization of novel genetic loci associated with the pathophysiology of childhood obesity.

4. Diet type

4.1. Metabolic improvement of FTO rs9939609 polymorphism carriers is related with diet type.

De Luis DA, et al. *Role of rs9939609 FTO gene variant in weight loss, insulin resistance and metabolic parameters after a high monounsaturated vs a high polyunsaturated fat hypocaloric diet*. Nutr Hosp. 2015 Jul 1;32(1):175-81. doi: 10.3305/nh.2015.32.1.9169.

Introduction: common polymorphisms (rs9939609) of the fat mass and obesity associated gene (FTO) have been linked to obesity.
Objectives: our aim was to investigate the role of this polymorphism on insulin resistance, metabolic changes and weight loss secondary to a high monounsaturated fat vs a high polyunsaturated fat hypocaloric diets.
Material and Methods: a sample of 233 obese subjects was enrolled in a prospective way. In the basal visit, patients were randomly allocated during 3 months to; Diet M (high monounsaturated fat hypocaloric diet) or Diet P (high polyunsaturated fat hypocaloric diet).
Results: after treatment with two diets and in both genotypes, weight, fat mass and waist circumference decreased. Lower levels of body mass index (BMI), weight and fat mass were detected after Diet P in A allele carriers than TT genotype subjects. With the diet type P and in both genotypes (TT and AT + AA), total cholesterol levels (-15.3 + 35.1 mg/dl vs -11.6 + 32.1 mg/dl: $p > 0.05$) and LDL cholesterol levels (-11.5 + 34.1 mg/dl vs -8.5 + 30.1 mg/dl: $p > 0.05$) decreased. In A allele carriers a significant decreased was detected in insulin levels (-2.8 + 2.1 UI/L vs -1.3 + 8.0 UI/L: $p < 0.05$) and HOMA index (-1.0 + 1.3 vs -0.2 + 2.1: $p > 0.05$), too. With the diet M and in both genotype groups, leptin levels (-8.0 + 17.1 ng/ml vs -4.9 + 18.7 ng/ml: $p > 0.05$) decreased.
Conclusion: metabolic improvement secondary to weight loss was better in A carriers with a high polyunsaturated fat hypocaloric diet.

4.2. LEPR rs1805134 polymorphism could be involved in the development of morbid obesity.

Rojano-Rodriguez ME, et al. *Leptin receptor gene polymorphisms and morbid obesity in Mexican patients*. Hereditas. 2016 Feb 22;153:2. doi: 10.1186/s41065-016-0006-0

Background: human obesity is due to a complex interaction among environmental, behavioural, developmental and genetic factors, including the interaction of leptin (LEP) and leptin receptor (LEPR). Several LEPR mutations and polymorphisms have been described in patients with early onset severe obesity and hyperphagic eating behavior; however, some contradictory findings have also been reported. In the present study we explored the association of six LEPR gene polymorphisms in patients with morbid obesity.
Findings: 28 patients with morbid obesity and 56 non-obese Mexican Mestizo individuals were included. Typing of rs1137100, rs1137101, rs1805134, Ser492Thr, rs1805094 and rs1805096 LEPR polymorphisms was performed by PCR and allele specific hybridization. The LEPR Ser492Thr polymorphism was monomorphic with the presence of only the Ser492Thr-G allele. Allele C and genotype T/C for rs1805134 polymorphism were associated with susceptibility to morbid obesity ($p = 0.02$ and $p = 0.03$, respectively). No association was observed with any haplotype. Linkage disequilibrium (LD) showed that five polymorphisms (rs1137100, rs1137101, rs1805134, rs1805094 and rs1805096) were in absolute ($D' = 1$) but none in perfect ($r2 = 1$) LD.
Conclusions: our results suggest that rs1805134 polymorphism could be involved in the development of morbid obesity, whilst none of the alleles of the LEPR gene, rs1137100, rs1137101, rs1805094 and rs1805096 were associated as risk factors. However, more studies are necessary to confirm or reject this hypothesis.

4.3 Fat intake on diet influences plasma HDL-C according to LIPC genotype

Nettleton JA, et al. *Associations between HDL-cholesterol and polymorphisms in hepatic lipase and lipoprotein lipase genes are modified by dietary fat intake in African American and White adults*. Atherosclerosis. 2007 Oct;194(2):e131-40.

Polymorphisms in genes involved in HDL-cholesterol (HDL-C) metabolism influence plasma HDL-C concentrations. We examined whether dietary fat intake modified relations between HDL-C and polymorphisms in hepatic lipase (LIPC-514C-->T), cholesteryl ester transfer protein (CETP TaqIB), and lipoprotein lipase (LPL S447X) genes. Diet (food frequency questionnaire), plasma lipids, and LIPC, CETP, and LPL genotypes were assessed in approximately 12,000 White and African American adults. In both races and all genotypes studied, minor allele homozygotes had highest HDL-C concentrations compared to the other genotypes (P<0.001). However, main effects were modified by usual dietary fat intake. In African Americans - women somewhat more strongly than men -LIPC TT homozygotes with fat intake >or=33.2% of energy had approximately 3-4 mg/dL higher HDL-C concentrations than CC and CT genotypes. In contrast, when fat intake was <33.2% of energy, TT homozygotes had HDL-C concentrations approximately 3.5mg/dL greater than those with the CC genotype but not different from those with the CT genotype (P(interaction)=0.013). In Whites, LPLGG homozygotes had greatest HDL-C at lower total, saturated, and monounsaturated fat intakes but lowest HDL-C at higher intakes of these fats (P(interaction)<or=0.002). Dietary fat did not modify associations between CETP and HDL-C. In conclusion, these data show that plasma HDL-C differs according to LIPC, LPL, and CETP genotypes. In the case of LIPC and LPL, data suggest dietary fat modifies these relations.

4.4 Two copies of the Thr allele in the FABP2 is associated with significantly increased sensitivity to saturated fats.

Solà R, et al. *Soluble fibre (Plantago ovata husk) reduces plasma low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, oxidised LDL and systolic blood pressure in hypercholesterolaemic patients: A randomised trial*. Atherosclerosis. 2010 Aug;211(2):630-7. doi: 10.1016/j.atherosclerosis.2010.03.010.

Objective: the objective was to evaluate whether the soluble fiber Plantago ovata (Po)-husk improves cardiovascular disease (CVD) risk biomarkers including low-density lipoprotein cholesterol (LDL-C).
Methods: in a multi-centered, double-blind, placebo-controlled, parallel, randomized trial conducted in primary care-clinics in Spain, France and Holland, mild-moderate hypercholesterolemic patients (age range: 43-67 years) received 14 g/d of Po-husk (n=126) or placebo (microcrystalline-cellulose 14 g/d; n=128) in a low saturated fat diet for 8 weeks. Subsequently, if LDL-C remained > or = 3.35 mmol/L [130 mg/dL], participants proceeded with the fiber plus simvastatin (20mg/d) for further 8 weeks. Lipid profile, blood pressure (BP), insulin, oxidized LDL and some gene polymorphisms involved in CVD risk were measured.
Results: relative to placebo, Po-husk reduced plasma LDL-C by -6% (P<0.0002), total cholesterol (TC) by -6%, triglycerides (TG) by -21.6%, apolipoprotein (Apo) B-100 by -6.7%, oxidized LDL by a mean of -6.82 U/L (95%CI: 3.15-10.48), insulin by -4.68 pmol/L (95%CI: 0.68-8.67) and systolic BP by -4.0mm Hg (95%CI; 1.2-6.7) (P<0.05). The TG-lowering effect in the Po-husk group was magnified by variants in plasminogen-activator-inhibitor (PAI-1; rs1799768) and fatty acid-binding protein (FABP-2; rs1799883) genes. At 16 weeks, the intra-group action of simvastatin (20mg/d) added to Po-husk or placebo was a similar LDL-C reduction.
Conclusions: Po-husk, apart from lowering LDL-C, also reduced TG, TG related to certain gene variants, TC, Apo B-100, oxLDL, insulin-resistance and systolic BP in mild-moderate hypercholesterolemic individuals. Thus, the target patients to receive Po-husk would be those who present a cluster of various CVD risk factors, such as metabolic syndrome.

4.5 Overweight and obese individuals carrying the AMY1-AMY2 rs11185098 genotype associated with higher amylase activity may have greater loss of adiposity during low-calorie diet interventions.

Heianza Y, et al. *Starch Digestion-Related Amylase Genetic Variant Affects 2-Year Changes in Adiposity in Response to Weight-Loss Diets: The POUNDS Lost Trial*. Diabetes. 2017 Sep;66(9):2416-2423. doi: 10.2337/db16-1482.

Salivary and pancreatic amylases (encoded by AMY1 and AMY2 genes, respectively) are responsible for digesting starchy foods. AMY1 and AMY2 show copy number variations that affect differences in amylase amount and activity, and AMY1 copies have been associated with adiposity. We investigated whether genetic variants determining amylase gene copies are associated with 2-year changes in adiposity among 692 overweight and obese individuals who were randomly assigned to diets varying in macronutrient content. We found that changes in body weight (BW) and waist circumference (WC) were significantly different according to the AMY1-AMY2 rs11185098 genotype. Individuals carrying the A allele (indicating higher amylase amount and activity) showed a greater reduction in BW and WC at 6, 12, 18, and 24 months than those without the A allele ($P < 0.05$ for all). The association was stronger for long-term changes compared with short-term changes of these outcomes. The genetic effects on these outcomes did not significantly differ across diet groups. In conclusion, the genetic variant determining starch metabolism influences the response to weight-loss dietary intervention. Overweight and obese individuals carrying the AMY1-AMY2 rs11185098 genotype associated with higher amylase activity may have greater loss of adiposity during low-calorie diet interventions.

4.6 Individuals with the TCF7L2 rs7903146 risk genotype had significantly greater decreases in fat-free mass and fat mass after 10 week of consumption of a reduced-calorie low-fat diet

Mattei J, et al. *TCF7L2 genetic variants modulate the effect of dietary fat intake on changes in body composition during a weight-loss intervention*. Am J Clin Nutr. 2012 Nov;96(5):1129-36. doi: 10.3945/ajcn.112.038125.

Background: TCF7L2 gene variants have been associated with increased risk of type 2 diabetes and higher adiposity. Observational studies and short-term trials have suggested that macronutrients may modify these effects. However, to our knowledge, this has yet to be verified in long-term interventions.
OBJECTIVE: in a long-term intervention setting, we investigated the effects of TCF7L2 polymorphisms rs7903146 and rs12255372 and dietary total fat on changes in body composition and subsequent glycemic control.
Design: data were analyzed for 591 participants in the Preventing Overweight Using Novel Dietary Strategies (Pounds Lost) trial, which is a 2-y weight-loss randomized clinical trial of diets that differed in macronutrient proportions. Adjusted means for changes in body composition at 6 and 24 mo were obtained for gene main effects and interactions with a low-fat diet (20% from energy) compared with a high-fat diet (40% from energy). Interactions with protein and carbohydrate intakes were also tested. Predicted changes in glycemic control from changes in adiposity were determined by genotype and diet type.
Results: significant interactions were observed for rs12255372 TT (risk genotype) and fat intake for changes in BMI, total fat mass, and trunk fat mass (all $P/q < 0.05$) at 6 mo, with nonsignificant larger decreases for TT carriers on a low-fat diet. No significant associations were observed at 24 mo or for other macronutrients. Changes in body composition for TT carriers predicted reductions in plasma glucose and insulin only on the low-fat diet.
Conclusions: individuals with the TCF7L2 rs12255372 risk genotype may reduce body adiposity by consuming a diet lower in total fat. These reductions may induce better glycemic control for such individuals predisposed to type 2 diabetes. The Pounds Lost trial was registered at clinicaltrials.gov as NCT00072995.

4.7 ADRB2 rs1042714 GG genotype is associated with high response to lose weight with calories restricted diet.

Ruiz JR, et al. *Role of β_2-adrenergic receptor polymorphisms on body weight and body composition response to energy restriction in obese women: preliminary results*. Obesity (Silver Spring). 2011 Jan;19(1):212-5. doi: 10.1038/oby.2010.130.

We investigated the role of common β2-adrenergic receptor (ADRB2) rs1042714 (Gln27Glu) and rs1042713 (Arg16Gly) polymorphisms on body weight and body composition response to 12-week energy-restricted diet in women. The study comprised 78 Spanish obese (BMI: 34.0 ± 2.8 kg/m^2) women (age: 36.7 ± 7 years). We measured (before and after the dietary intervention) weight and height, and BMI calculated. Moreover, body fat mass and lean mass (LM) were measured by dual energy X-ray absorptiometry. We observed an interaction effect between the Gln27Glu polymorphism and diet-induced changes on body weight (P = 0.006), BMI (P = 0.004), and LM (P = 0.001). Women carrying the Glu allele had a greater reduction in body weight than non-Glu allele carriers (9.5 ± 2.9 vs. 7.0 ± 3.5%, respectively, P = 0.002). Moreover, women with the Glu allele lost more LM than the Gln27Gln group (5.9 ± 2.7 vs. 4.0 ± 2.7%, respectively, P = 0.001). We did not find any significant interaction effect between the Arg16Gly polymorphism and diet-induced changes on the outcome variables (all P > 0.1). The results suggest that the ADRB2 Gln27Glu polymorphism has a modulating effect on diet-induced changes on body weight and body composition, and should be considered in future obesity treatments. These findings should be taken as preliminary and be replicated in further energy restriction studies with larger sample sizes.

4.8 GHSR rs490683 CC genotype is associated with high response to lose weight with low fat diet.

Matzko ME, et al. *Association of ghrelin receptor promoter polymorphisms with weight loss following Roux-en-Y gastric bypass surgery*. Obes Surg. 2012 May;22(5):783-90. doi: 10.1007/s11695-012-0631-2.

Background: ghrelin plays a role in appetite and has been hypothesized to play a role in the mechanism of Roux-en-Y gastric bypass (RYGB) surgery. Single nucleotide polymorphisms (SNPs) in the promoter region of its receptor gene (growth hormone secretagogue receptor type 1a--GHSR) have also been associated with weight loss outcomes following long-term dietary intervention in adults with impaired glucose tolerance. Our objectives were to evaluate changes in serum ghrelin levels and determine the effect of GHSR promoter polymorphisms on post-RYGB surgery weight loss.
Methods: preoperative and 6-month postoperative serum ghrelin levels were measured in 37 patients with extreme obesity undergoing RYGB surgery. Total ghrelin was also measured in liver tissue collected intraoperatively. Association analysis between genotypes for SNPs rs9819506 and rs490683 in the promoter region of the GHSR gene and weight loss outcomes in the 30 months following surgery was performed in over 650 RYGB patients.
Results: serum ghrelin levels increased after RYGB surgery. Weight loss trajectories were significantly different using an additive model for both ghrelin SNPs, with patients homozygous for the rs490683 CC genotype exhibiting the most weight loss. Weight loss trajectories were also different using a dominant model. The rs490683 risk allele demonstrated decreased promoter activity in vitro.
Conclusions: the role of increased ghrelin levels in weight loss outcomes following RYGB surgery may be influenced by variation in the GHSR gene.

4.9 ACSL5 rs2419621(T) is associated with more rapid diet-induced weight loss.

Teng AC, et al. *Functional characterization of a promoter polymorphism that drives ACSL5 gene expression in skeletal muscle and associates with diet-induced weight loss*. FASEB J. 2009 Jun;23(6):1705-9. doi: 10.1096/fj.08-120998.

Diet-induced weight loss is affected by a wide range of factors, including genetic variation. Identifying functional polymorphisms will help to elucidate mechanisms that account for variation in dietary metabolism. Previously, we reported a strong association between a common single nucleotide polymorphism (SNP) rs2419621 (C>T) in the

promoter of acyl-CoA synthetase long chain 5 (ACSL5), rapid weight loss in obese Caucasian females, and elevated ACSL5 mRNA levels in skeletal muscle biopsies. Here, we showed by electrophoretic mobility shift assay (EMSA) that the T allele creates a functional cis-regulatory E-box element (CANNTG) that is recognized by the myogenic regulatory factor MyoD. The T allele promoted MyoD-dependent activation of a 1089-base pair ACSL5 promoter fragment in nonmuscle CV1 cells. Differentiation of skeletal myoblasts significantly elevated expression of the ACSL5 promoter. The T allele sustained promoter activity 48 h after differentiation, whereas the C allele showed a significant decline. These results reveal a mechanism for elevated transcription of ACSL5 in skeletal muscle of carriers of the rs2419621(T) allele, associated with more rapid diet-induced weight loss. Natural selection favoring promoter polymorphisms that reduced expression of catabolic genes in skeletal muscle likely accounts for the resistance of obese individuals to dietary intervention.

4.10 rs2289487 PLIN1 CC genotype is associated with high response to lose weight with calories restricted diet.

Soenen S, et al. *Relationship between perilipin gene polymorphisms and body weight and body composition during weight loss and weight maintenance*. Physiol Behav. 2009 Mar 23;96(4-5):723-8.
Background: genetic variation in the perilipin (PLIN) gene may play a role in the etiology and treatment of obesity.
Objective: to examine different polymorphisms in the PLIN gene in relation to body-weight regulation.
Methods: 118 subjects followed a 6 wk VLCD, followed by 1 year weight maintenance. Body-weight (BW), body composition, leptin concentration, and polymorphisms of the PLIN gene: PLIN1:rs2289487, PLIN4:rs894160, PLIN6:rs1052700, PLIN5:rs2304795 and PLIN7:rs 2304796 were determined.
Results: BW loss during VLCD was 7.0+/-3.1 kg ($p<0.05$), and BW regain was 3.7+/-1.4 kg ($p<0.05$), including changes in body mass index (BMI), waist-circumference, body-composition and leptin concentrations ($p<0.05$). Linkage disequilibria were observed between PLIN1 and PLIN4: D' >0.9, r2=0.72; PLIN5 and PLIN7: D' >0.9, r2=0.85. In men, body weight, BMI, waist circumference, body fat, leptin concentrations were significantly lower for the haplotype of PLIN1 (C-alleles) and PLIN4 (A-alleles). In women weight loss and loss of fat mass were larger for the haplotype of PLIN1 (C-alleles) and PLIN4 (A-alleles). For PLIN6 genotypes body weight and body fat were lower for homozygotes of the minor allele (T/T) in the men; in the women leptin concentrations were lower. The haplotype of PLIN5 and PLIN7 consisting of A/G and G/G of PLIN5 and A/A of PLIN7 showed a reduction in FM: 5.9+/-0.6 kg vs 3.1+/-0.4 kg, % body fat: 5.5+/-0.6% vs 2.2+/-0.2%, and leptin: 20.5+/-10.8 ng/ml vs 12.9+/-6.7 ng/ml over time in the women ($p<0.05$).
Conclusion: since the haplotype of the minor alleles PLIN1-4, PLIN5-7 and PLIN6, was related to body-weight regulation at a lower level of body-weight in the men as well in the women we conclude that the PLIN1-4, 6, and 5-7 locus appears as a genetic influencer of obesity risk in humans.

4.11 GAL rs694066 AA genotype is associated with high response to lose weight with carbohydrates restricted diet.

Ruaño G, et al. *Physiogenomic analysis of weight loss induced by dietary carbohydrate restriction*. Nutr Metab (Lond). 2006 May 15;3:20.

Background: diets that restrict carbohydrate (CHO) have proven to be a successful dietary treatment of obesity for many people, but the degree of weight loss varies across individuals. The extent to which genetic factors associate with the magnitude of weight loss induced by CHO restriction is unknown. We examined associations among polymorphisms in candidate genes and weight loss in order to understand the physiological factors influencing body weight responses to CHO restriction.
Methods: we screened for genetic associations with weight loss in 86 healthy adults who were instructed to restrict CHO to a level that induced a small level of ketosis (CHO approximately 10% of total energy). A total of 27 single nucleotide polymorphisms (SNPs) were selected from 15 candidate genes involved in fat digestion/metabolism, intracellular glucose metabolism, lipoprotein remodeling, and appetite regulation. Multiple linear regression was used to rank the SNPs according to probability of association, and the most significant associations were analyzed in greater detail.

Results: mean weight loss was 6.4 kg. SNPs in the gastric lipase (LIPF), hepatic glycogen synthase (GYS2), cholesteryl ester transfer protein (CETP) and galanin (GAL) genes were significantly associated with weight loss. **Conclusion:** a strong association between weight loss induced by dietary CHO restriction and variability in genes regulating fat digestion, hepatic glucose metabolism, intravascular lipoprotein remodeling, and appetite were detected. These discoveries could provide clues to important physiologic adaptations underlying the body mass response to CHO restriction.

4.12 LIPF rs814628 AA genotype is associated with high response to lose weight with carbohidrates restricted diet.

AbuMweis SS, et al. *Triacylglycerol-Lowering Effect of Docosahexaenoic Acid Is Not Influenced by Single-Nucleotide Polymorphisms Involved in Lipid Metabolism in Humans*. Lipids. 2018 Sep;53(9):897-908. doi: 10.1002/lipd.12096.

The triacylglycerol (TAG)-lowering effects of long-chain n-3 fatty acids, and in particular docosahexaenoic acid (DHA), are well documented, although these effects manifest large interindividual variability. The objective of this secondary analysis is to investigate whether common single-nucleotide polymorphisms (SNP) in genes involved in DHA synthesis and TAG metabolism are associated with the responsiveness of blood lipids, lipoprotein, and apolipoprotein concentration to dietary treatment by DHA supplied in high-oleic canola oil (HOCO). In a randomized, crossover-controlled feeding trial, 129 subjects with metabolic syndrome received high-oleic canola oil (HOCO) and high-oleic canola oil supplemented with DHA (HOCO-DHA), each for 4 weeks. During the HOCO-DHA phase, the intake of DHA ranged from 1 to 2.5 g/day. The subjects were genotyped for apolipoprotein E (APOE) isoforms, and SNP including FADS1-rs174561, FADS2-rs174583, ELOVL2-rs953413, ELOVL5-rs2397142, CETP-rs5882, SCD1-rs2234970, PPARA-rs6008259, and LIPF-rs814628 were selected as important genes controlling fatty acid metabolism. Overall, consumption of HOCO-DHA oil reduced blood concentrations of TAG by 24% compared to HOCO oil. The reduction in TAG was independent of genetic variations in the studied genes. Similarly, no treatment-by-gene interactions were evident in the response to other lipids, lipoproteins, or apolipoproteins to DHA supplementation. Nevertheless, a lower interindividual variation in the TAG response to DHA supplementation compared to other studies was observed in this analysis. The TAG-lowering effect of a supplemental body-weight-based dose of DHA was not influenced by genetic variations in APOE, FADS1, FADS2, ELOVL2, ELOVL5, CETP, SCD1, PPARA, and LIPF.

4.13 AGTR2 rs5950584 TT genotype is associated with high response to lose weight with carbohydrates restricted diet.

Seip RL, et al. *Physiogenomic comparison of human fat loss in response to diets restrictive of carbohydrate or fat*. Nutr Metab (Lond). 2008 Feb 6;5:4. doi: 10.1186/1743-7075-5-4.

Background: genetic factors that predict responses to diet may ultimately be used to individualize dietary recommendations. We used physiogenomics to explore associations among polymorphisms in candidate genes and changes in relative body fat (Delta%BF) to low fat and low carbohydrate diets.
Methods: we assessed Delta%BF using dual energy X-ray absorptiometry (DXA) in 93 healthy adults who consumed a low carbohydrate diet (carbohydrate ~12% total energy) (LC diet) and in 70, a low fat diet (fat ~25% total energy) (LF diet). Fifty-three single nucleotide polymorphisms (SNPs) selected from 28 candidate genes involved in food intake, energy homeostasis, and adipocyte regulation were ranked according to probability of association with the change in %BF using multiple linear regression.
Results: dieting reduced %BF by 3.0 +/- 2.6% (absolute units) for LC and 1.9 +/- 1.6% for LF ($p < 0.01$). SNPs in nine genes were significantly associated with Delta%BF, with four significant after correction for multiple statistical testing: rs322695 near the retinoic acid receptor beta (RARB) ($p < 0.005$), rs2838549 in the hepatic phosphofructokinase (PFKL), and rs3100722 in the histamine N-methyl transferase (HNMT) genes (both $p < 0.041$) due to LF; and the rs5950584 SNP in the angiotensin receptor Type II (AGTR2) gene due to LC ($p < 0.021$).
Conclusion: fat loss under LC and LF diet regimes appears to have distinct mechanisms, with PFKL and HNMT and RARB involved in fat restriction; and AGTR2 involved in carbohydrate restriction. These discoveries could

provide clues to important physiologic mechanisms underlying the Delta%BF to low carbohydrate and low fat diets.

4.14 PPARG rs1801282 GG genotype is associated with high response to lose weight with calories restricted diet.

Delahanty LM, et al. *Genetic predictors of weight loss and weight regain after intensive lifestyle modification, metformin treatment, or standard care in the Diabetes Prevention Program*. Diabetes Care. 2012 Feb;35(2):363-6. doi: 10.2337/dc11-1328.

Objective: we tested genetic associations with weight loss and weight regain in the Diabetes Prevention Program, a randomized controlled trial of weight loss-inducing interventions (lifestyle and metformin) versus placebo.
Research design and methods: sixteen obesity-predisposing single nucleotide polymorphisms (SNPs) were tested for association with short-term (baseline to 6 months) and long-term (baseline to 2 years) weight loss and weight regain (6 months to study end).
Results: irrespective of treatment, the Ala12 allele at PPARG associated with short- and long-term weight loss (-0.63 and -0.93 kg/allele, P ≤ 0.005, respectively). Gene-treatment interactions were observed for short-term (LYPLAL1 rs2605100, P(lifestyle*SNP) = 0.032; GNPDA2 rs10938397, P(lifestyle*SNP) = 0.016; MTCH2 rs10838738, P(lifestyle*SNP) = 0.022) and long-term (NEGR1 rs2815752, P(metformin*SNP) = 0.028; FTO rs9939609, P(lifestyle*SNP) = 0.044) weight loss. Three of 16 SNPs were associated with weight regain (NEGR1 rs2815752, BDNF rs6265, PPARG rs1801282), irrespective of treatment. TMEM18 rs6548238 and KTCD15 rs29941 showed treatment-specific effects (P(lifestyle*SNP) < 0.05).
Conclusions: genetic information may help identify people who require additional support to maintain reduced weight after clinical intervention.

4.15 AMY1 rs4244372 AA genotype is associated with decreased capacity to digest starch.

Sorkin R, et al. *Genetic variation in the AMY1 gene is associated with dietary carbohydrate and starch intake in a young adult population*. The FASEB Journal 2017 31:1_supplement, 299.5-299.5

Background: genetic variation contributes to individual differences in energy and nutrient intake and may be a contributing factor to the selection of certain foods and macronutrient preference. Salivary amylase is encoded by the AMY1 gene, which displays high copy number variation (CNV) and plays an important role in starch digestion. Research has found that AMY1 CNV is positively correlated with both salivary amylase concentration and activity and higher AMY1 copy numbers have been found in populations with high starch diets. Individual single nucleotide polymorphisms (SNPs) near the amylase genes have been found to correlate with AMY1 copy number within populations. The objective of this study was to determine whether common variants in the AMY1 gene are associated with habitual dietary intake.
Methods: fasting blood samples were drawn from a total of 1,600 ethno-culturally diverse participants aged 20–29 years from the Toronto Nutrigenomics and Health Study for genotyping of 9 SNPs associated with AMY1 copy number: rs4244372 (T/A), rs11577390 (C/T), rs1566154 (A/G), rs10881197(G/C), rs2132957 (A/G), rs11185098(G/A), rs1999478(C/A), rs1330403(A/G) and rs6696797(A/G). Dietary intake was assessed using a one-month, 196-item Toronto-modified Willett food frequency questionnaire. An analysis of covariance adjusted for age, sex, ethnicity, BMI, and physical activity was used to determine the association between AMY1 genotypes and dietary intake.
Results: among Caucasians (n = 620), carriers of the minor allele in rs10881197 had a significantly lower energy intake (1838 vs 2089 kcal/day, p = 0.005). Carriers of the minor allele in rs11185098 had significantly higher total carbohydrate (CHO) and starch intakes (273 vs 266 g CHO/day, p =0.03 and 122 vs 117g starch/day, p= 0.02 respectively). Among East Asians (n = 465), carriers of the minor allele in rs1999478 had significantly higher total energy and sugar intakes (1985 vs 1828 kcal/day, p = 0.02 and 113 vs 105 g sugar/day, p = 0.03 respectively). No other associations were observed between AMY1 variants and carbohydrate or starch intake.

Conclusion: polymorphisms in the AMY1 gene are associated with dietary intake patterns. Identifying the genetic determinants of dietary intake and food preference may enhance our understanding of excessive caloric intake and obesity, a common risk factor of many chronic conditions and provide further evidence to support the use of personalized nutrition recommendations.

4.16 PPARG rs1801282GG genotype is associated with poor response to lose weight with low fat diet.

Garaulet M, et al. *PPARγ Pro12Ala interacts with fat intake for obesity and weight loss in a behavioural treatment based on the Mediterranean diet*. Mol Nutr Food Res. 2011 Dec;55(12):1771-9. doi: 10.1002/mnfr.201100437.

Scope: the goal of this study was to examine whether the Pro12Ala polymorphism of peroxisome proliferator-activated receptor γ (PPARγ) is associated with insulin resistance, obesity and weight loss and to analyze potential interactions between fat intake and PPARγ polymorphism in a Spanish overweight/obese population.
Materials and methods: we recruited 1465 subjects enrolled in a behavioural treatment program for obesity based on a Mediterranean diet, which included the following: dietary treatment, physical activity, nutritional education and behavioral techniques. A significant association was found between PPARγ2 Pro12Ala genotype and plasma insulin concentration and homeostasis model assessment insulin resistance. Subjects with the Ala12 genotype had lower insulin levels than those with the Pro12Pro genotype. We detected a gene-diet interaction between the PPARγ Pro12Ala polymorphism and MUFA for BMI and body fat. Furthermore, we detected an interaction between the PPARγ Pro12Ala polymorphism and fat intake for total weight loss ($p<0.001$). When total fat intake was high, Ala12-carriers exhibited a significantly lower percentage of total weight loss than major-allele-carriers ($p=0.037$).
Conclusion: data are consistent with previous results showing a protective role for the Ala12 allele against insulin resistance and replicate an earlier study that detected an interaction between dietary MUFA and PPARγ2 for BMI. Our detection of a gene-diet interaction between PPARγ Pro12Ala and fat intake for weight loss may explain previous discrepancies among different studies.

4.17 CETP rs5883 and GYS2 rs2306179 CC genotype is associated with poor response to lose weight with carbohydrate restricted diet.

Ruaño G, et al. *Physiogenomic analysis of weight loss induced by dietary carbohydrate restriction*. Nutr Metab (Lond). 2006; 3: 20. doi: 10.1186/1743-7075-3-20

Background: diets that restrict carbohydrate (CHO) have proven to be a successful dietary treatment of obesity for many people, but the degree of weight loss varies across individuals. The extent to which genetic factors associate with the magnitude of weight loss induced by CHO restriction is unknown. We examined associations among polymorphisms in candidate genes and weight loss in order to understand the physiological factors influencing body weight responses to CHO restriction.
Methods: we screened for genetic associations with weight loss in 86 healthy adults who were instructed to restrict CHO to a level that induced a small level of ketosis (CHO ~10% of total energy). A total of 27 single nucleotide polymorphisms (SNPs) were selected from 15 candidate genes involved in fat digestion/metabolism, intracellular glucose metabolism, lipoprotein remodeling, and appetite regulation. Multiple linear regression was used to rank the SNPs according to probability of association, and the most significant associations were analyzed in greater detail.
Results: mean weight loss was 6.4 kg. SNPs in the gastric lipase (LIPF), hepatic glycogen synthase (GYS2), cholesteryl ester transfer protein (CETP) and galanin (GAL) genes were significantly associated with weight loss.
Conclusion: a strong association between weight loss induced by dietary CHO restriction and variability in genes regulating fat digestion, hepatic glucose metabolism, intravascular lipoprotein remodeling, and appetite were detected. These discoveries could provide clues to important physiologic adaptations underlying the body mass response to CHO restriction.

5. Insulin resistance

5.1. Adiponectin -11426A > G polymorphism could contribute to type II diabetes mellitus risk

Chu H, et al. *AdipoQ polymorphisms are associated with type 2 diabetes mellitus: a meta-analysis study*. Diabetes Metab Res Rev. 2013 Oct;29(7):532-45. doi: 10.1002/dmrr.2424.

Objective: adiponectin (AdipoQ) plays an important role in the pathogenesis of diabetes mellitus and is considered as an important candidate gene for type 2 diabetes mellitus (T2DM). So far, there have been many studies to investigate the association between the adiponectin polymorphisms and T2DM risk. However, the results are conflicting. To derive a more precise estimation, we performed a meta-analysis to assess the association between five AdipoQ polymorphisms [-11426A > G (rs16861194), -11391G > A (rs17300539), -11377C > G (rs266729), +45T > G (rs2241766) and +276G > T (rs1501299)], and T2DM risk.
Methods: the fixed and random-effects model should be used to assess the summary odds ratios (ORs) of each study. ORs with 95% confidence intervals (CIs) were used to evaluate the strength of association. On the basis of the included criteria, we selected 39 papers, among which eight for -11426A > G, 14 for -11391G > A, 21 for -11377C > G, 28 for +45 T > G and 24 for +276G > T. Sensitivity analyses were conducted to assess the stability of the results. Both Begg's funnel plots and Egger's test are commonly used to evaluate publication bias.
Results: overall, we found that individuals with the -11426G allele had a 0.15-fold significantly increased T2DM risk (additive model: 1.15, 1.04-1.27, 0.222). In the stratified analyses, we found that the -11426A > G, -11391G > A and -11377C > G polymorphisms could increase T2DM risk in European populations in the additive model. For Asian populations, we found that the -11377C > G polymorphism also could elevate T2DM risk.
Conclusions: our results suggested that the adiponectin -11426A > G polymorphism could contribute to the T2DM risk.

5.2. The adiponectin gene variants and haplotype contribute to the genetic risk towards the development of type 2 diabetes in the south Indian population.

Ramya K, et al. *Genetic association of ADIPOQ gene variants with type 2 diabetes, obesity and serum adiponectin levels in south Indian population*. Gene. 2013 Dec 15;532(2):253-62. doi: 10.1016/j.gene.2013.09.012.

Objective: to investigate the genetic association of eight variants of the adiponectin gene with type 2 diabetes mellitus (T2DM), obesity and serum adiponectin level in the south Indian population.
Methods: the study comprised of 1100 normal glucose tolerant (NGT) and 1100 type 2 diabetic, unrelated subjects randomly selected from the Chennai Urban Rural Epidemiology Study (CURES), in southern India. Fasting serum adiponectin levels were measured by radioimmunoassay. The variants were screened by polymerase chain reaction-restriction fragment length polymorphism. Linkage disequilibrium was estimated from the estimates of haplotype frequencies.
Results: of the 8 variants, four SNPs namely, +276 G/T (rs1501299), -4522 C/T (rs822393), -11365 C/G (rs266729), and +712 G/A (rs3774261) were significantly associated with T2DM in our study population. The -3971 A/G (rs822396) and -11391 G/A (rs17300539) SNPs' association with T2DM diabetes was mediated through obesity (where the association with type 2 diabetes was lost after adjusting for BMI). There was an independent association of +276 G/T (rs1501299) and -3971 A/G (rs822396) SNPs with generalized obesity and +349 A/G (rs2241767) with central obesity. Four SNPs, -3971 A/G (rs822396), +276 G/T (rs1501299), -4522 C/T (rs822393) and Y111H T/C (rs17366743) were significantly associated with hypoadiponectinemia. The haplotypes GCCATGAAT and AGCGTGGGT conferred lower risk of T2DM in this south Indian population.
Conclusion: the adiponectin gene variants and haplotype contribute to the genetic risk towards the development of type 2 diabetes, obesity and hypoadiponectinemia in the south Indian population.

5.3. Gene polymorphisms in ADIPOQ (rs1501299 and rs17300539), LepR (rs1137101 and rs1045895), IRS2 (rs1805092), GRB14 (rs10195252 and rs3923113) and PPARG (rs1801282) have been associated with overweight and obesity in uncontrolled T2DM.

Kasim NB, et al. *Genetic polymorphisms associated with overweight and obesity in uncontrolled Type 2 diabetes mellitus*. Biomark Med. 2016;10(4):403-15. doi: 10.2217/bmm-2015-0037.

Generally, obese and overweight individuals display higher free fatty acid levels, which stimulate insulin resistance. The combination of overweight or obesity with insulin resistance can trigger Type 2 diabetes mellitus (T2DM) and are primary contributing factors to the development of uncontrolled T2DM. Genetic polymorphisms also play an important role as they can impact a population's susceptibility to becoming overweight or obese and developing related chronic complications, such as uncontrolled T2DM. This review specifically examines the genetic polymorphisms associated with overweight and obesity in patients with uncontrolled T2DM. Particularly, gene polymorphisms in ADIPOQ (rs1501299 and rs17300539), LepR (rs1137101 and rs1045895), IRS2 (rs1805092), GRB14 (rs10195252 and rs3923113) and PPARG (rs1801282) have been associated with overweight and obesity in uncontrolled T2DM.

5.4 PPARG rs1801282 (Pro12Ala) is associated with the risk of type 2 diabetes

Kilpeläinen TO, et al. *SNPs in PPARG associate with type 2 diabetes and interact with physical activity*. Med Sci Sports Exerc. 2008 Jan;40(1):25-33.

Purpose: to study the associations of seven single-nucleotide polymorphisms (SNPs) in the peroxisome proliferator-activated receptor gamma (PPARG) gene with the conversion from impaired glucose tolerance (IGT) to type 2 diabetes (T2D), and the interactions of the SNPs with physical activity (PA).
Methods: overweight individuals with IGT who participated in the Finnish Diabetes Prevention Study (DPS) (N = 479) were followed, on average, 4.2 yr. PA was assessed yearly with a 12-month validated questionnaire.
Results: in Cox regression analyses, the rare alleles of rs17036314 and rs1801282 (Pro12Ala) predicted conversion to T2D (P = 0.038 and 0.037, respectively), but only rs17036314 predicted T2D after adjustment for baseline fasting glucose (P = 0.030). The change in the total amount of PA, stratified by median, modified the association of rs17036314 and rs1801282 with the risk of T2D during the intervention (P = 0.002 and 0.031, respectively, for interaction between PA change and genotype); an increase in PA seemed to remove the effect of the risk alleles. The distinct rs1152003 polymorphism interacted with the study group on the conversion to T2D (P = 0.027) and tended to increase the risk of T2D in the intervention group (P = 0.050). No interaction between rs1152003 and the change in PA was found.
Conclusions: the rs17036314, rs1801282 (Pro12Ala), and rs1152003 were associated with the risk of T2D in the DPS. Increased PA seemed to decrease the effect of the risk alleles of rs17036314 and rs1801282 on the conversion to T2D. The effect of rs1152003 was modified by other lifestyle changes or the lifestyle intervention as a whole.

5.5 Common variants in the TCF7L2 gene increase risk of type 2 diabetes

Lyssenko V, et al. *Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes*. J Clin Invest. 2007 Aug;117(8):2155-63.

Genetic variants in the gene encoding for transcription factor-7-like 2 (TCF7L2) have been associated with type 2 diabetes (T2D) and impaired beta cell function, but the mechanisms have remained unknown. We therefore studied prospectively the ability of common variants in TCF7L2 to predict future T2D and explored the mechanisms by which they would do this. Scandinavian subjects followed for up to 22 years were genotyped for 3 SNPs (rs7903146, rs12255372, and rs10885406) in TCF7L2, and a subset of them underwent extensive metabolic studies. Expression of TCF7L2 was related to genotype and metabolic parameters in human islets. The CT/TT genotypes of SNP rs7903146 strongly predicted future T2D in 2 independent cohorts (Swedish and Finnish). The risk T allele was associated with impaired insulin secretion, incretin effects, and enhanced rate of hepatic glucose production. TCF7L2 expression in human islets was increased 5-fold in T2D, particularly in

carriers of the TT genotype. Overexpression of TCF7L2 in human islets reduced glucose-stimulated insulin secretion. In conclusion, the increased risk of T2D conferred by variants in TCF7L2 involves the enteroinsular axis, enhanced expression of the gene in islets, and impaired insulin secretion.

5.6. Polymorphisms in the GHSR promoter may modify changes in body weight during long-term lifestyle intervention and affect ghrelin receptor signaling through modulation of GHSR gene expression.

Mager U, et al. *Variations in the ghrelin receptor gene associate with obesity and glucose metabolism in individuals with impaired glucose tolerance*. PLoS One. 2008 Aug 13;3(8):e2941. doi: 10.1371/journal.pone.0002941.

Background: ghrelin may influence the development of obesity through its role in the control of energy balance, food intake, and regulation of body weight. The effects of ghrelin are mediated via the growth hormone secretagogue receptor (GHSR).
Methodology: we genotyped 7 single nucleotide polymorphisms (SNPs) in the GHSR gene and assessed the association between those SNPs and obesity and type 2 diabetes-related phenotypes from 507 middle-aged overweight persons with impaired glucose tolerance participating in the Finnish Diabetes Prevention Study (DPS). Additionally, we performed in silico screening of the 5'-regulatory region of GHSR and evaluated SNPs disrupting putative transcription factor (TF) binding sites in vitro with gelshift assays to determine differences in protein binding between different alleles of SNPs. Rs9819506 in the promoter region of GHSR was associated with body weight ($p = 0.036$); persons with rs9819506-AA genotype having the lowest body weight. Individuals with rs490683-CC genotype displayed highest weight loss in the whole study population ($p = 0.032$). The false discovery rate for these results was <10%. Rs490683 and rs509035 were associated with several measures of glucose and insulin metabolism during the follow-up. Rs490683 may be a functional SNP, since gelshift experiments showed differential protein binding between the alleles, with higher binding to the G-allele. Rs490683-C may disrupt a putative binding site for the TF nuclear factor 1 (NF-1), thus rs4906863-GG genotype where the NF-1 site is intact may lead to a higher GHSR gene expression.
Conclusion: polymorphisms in the GHSR promoter may modify changes in body weight during long-term lifestyle intervention and affect ghrelin receptor signaling through modulation of GHSR gene expression.

5.7 Central obesity may modify the associations between PLIN variations and diabetes risk in women.

Qi L, et al. *Common variations in perilipin gene, central obesity, and risk of type 2 diabetes in US women*. Obesity (Silver Spring). 2008 May;16(5):1061-5. doi: 10.1038/oby.2008.26.

Objective: the variations in perilipin gene (PLIN) were previously associated with obesity and insulin sensitivity. We examined whether PLIN variability was associated with diabetes risk and obesity status modified such associations.
Methods: we conducted a nested case-control study of 431 incident cases of type 2 diabetes and 791 healthy control women from the Nurses' Health Study. Obesity was defined by BMI or waist circumference (central obesity).
Results: in the sample of all participants, PLIN variations were not significantly associated with the incidence of diabetes. The central obesity status (by National Cholesterol Education Program Adult Treatment Panel III definition of waist circumference>35 inches) significantly interacted with PLIN polymorphisms in relation to diabetes risk (P for interaction=0.027, 0.009, and 0.02 for rs2289487, rs8179043, and rs894160, respectively). In nonobese (central) women, carriers of rs2289487, rs8179043, and rs894160 had significantly greater risk of type 2 diabetes, adjusting for diabetes risk factors (odds ratio (OR)=1.52, 1.03-2.25; 1.54, 1.07-2.23; and 1.57, 1.09-2.27, respectively). Haplotypes possessing the three polymorphisms were also significantly associated with diabetes risk (global test, P=0.01). As compared with the most common haplotype 111, haplotype 222 and 211 (1 codes the common and 2 codes the minor alleles) were associated with 44% (OR=1.44, 95% confidence interval (CI) 1.09-1.91; P=0.01) and 70% (OR=1.70, 95% CI 1.04-2.77; P=0.03) greater risk, respectively. The PLIN variations were not significantly associated with the disease risk among women with central obesity.
Discussion: our data indicate that central obesity may modify the associations between PLIN variations and diabetes risk in women.

5.8 FTO rs1121980 might be important in the progression of insulin resistance in Japanese subjects

Shimaoka I, et al. *Association of gene polymorphism of the fat-mass and obesity-associated gene with insulin resistance in Japanese*. Hypertens Res. 2010 Mar;33(3):214-8. doi: 10.1038/hr.2009.215.

It was reported that gene polymorphisms in the fat-mass and obesity-associated gene (FTO) were associated with obesity and diabetes in several genome-wide association studies. A recent report indicated that FTO-knockout mice exhibited phenotypes of skinny body shape and normal metabolic profiles. Thus, FTO could be important in metabolic disorders. The aim of this study was to clarify the role of single nucleotide polymorphisms (SNPs) in FTO in metabolic disorders such as hypertension, obesity, diabetes, dyslipidemia, insulin resistance and metabolic syndrome in the Japanese general population using data from a cohort study in Hokkaido, namely the Tanno-Sobetsu study. Written informed consent for the genetic analysis was obtained from each subject participating in the study. A total of 1514 subjects were genotyped by TaqMan PCR methods for three SNPs, rs9939609, rs1121980 and rs1558902, in FTO. Association analyses between the SNPs and metabolic parameters were performed. Although two SNPs, rs9939609 and rs1558902, were not significantly associated with hypertension, obesity, metabolic syndrome or any metabolic parameters, additive and recessive models of rs1121980 were strongly associated with plasma immunoreactive insulin (IRI) level and homeostasis model assessment insulin resistance (HOMA-IR), even after adjusting for confounding factors such as age, gender and body mass index. A haplotype of three SNPs was also significantly associated with IRI and HOMA-IR. One SNP, rs1121980, and a haplotype of three SNPs in FTO that contains this SNP, might be important in the progression of insulin resistance in Japanese subjects.

5.9 A genetic effect of FTO rs9939609 on fasting insulin levels was observed

Tan S, et al. *Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS).* BMC Med Genet. 2010 Jan 21;11:12. doi: 10.1186/1471-2350-11-12.

Background: the polycystic ovary syndrome (PCOS), a common endocrine disorder in women of child-bearing age, mainly characterized by chronic anovulation and hyperandrogenism, is often associated with insulin resistance (IR) and obesity. Its etiology and the role of IR and obesity in PCOS are not fully understood. We examined the influence of validated genetic variants conferring susceptibility to obesity and/or type 2 diabetes mellitus (T2DM) on metabolic and PCOS-specific traits in patients with PCOS.
Methods: we conducted an association study in 386 patients with PCOS (defined by the Rotterdam-criteria) using single nucleotide polymorphisms (SNPs) in or in proximity to the fat mass and obesity associated gene (FTO), insulin-induced gene-2 (INSIG2), transcription factor 7-like 2 gene (TCF7L2) and melanocortin 4 receptor gene (MC4R). To compare the effect of FTO obesity risk alleles on BMI in patients with PCOS to unselected females of the same age range we genotyped 1,971 females from the population-based KORA-S4 study (Kooperative Gesundheitsforschung im Raum Augsburg, Survey 4).
Results: the FTO risk allele was associated with IR traits and measures of increased body weight. In addition, the TCF7L2 SNP was associated with body weight traits. For the SNPs in the vicinity of INSIG2 and MC4R and for the other examined phenotypes there was no evidence for an association. In PCOS the observed per risk allele effect of FTO intron 1 SNP rs9939609 on BMI was +1.56 kg/m2, whereas it was +0.46 kg/m2 in females of the same age range from the general population as shown previously.
Conclusion: the stronger effect on body weight of the FTO SNP in PCOS might well have implications for the etiology of the disease.

5.10 The increased risk of type II diabetes conferred by variants in TCF7L2 involves the enteroinsular axis, enhanced expression of the gene in islets, and impaired insulin secretion

Lyssenko V, et al. *Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes*. J Clin Invest. 2007 Aug;117(8):2155-63.

Genetic variants in the gene encoding for transcription factor-7-like 2 (TCF7L2) have been associated with type 2 diabetes (T2D) and impaired beta cell function, but the mechanisms have remained unknown. We therefore studied prospectively the ability of common variants in TCF7L2 to predict future T2D and explored the mechanisms by which they would do this. Scandinavian subjects followed for up to 22 years were genotyped for 3 SNPs (rs7903146, rs12255372, and rs10885406) in TCF7L2, and a subset of them underwent extensive metabolic studies. Expression of TCF7L2 was related to genotype and metabolic parameters in human islets. The CT/TT genotypes of SNP rs7903146 strongly predicted future T2D in 2 independent cohorts (Swedish and Finnish). The risk T allele was associated with impaired insulin secretion, incretin effects, and enhanced rate of hepatic glucose production. TCF7L2 expression in human islets was increased 5-fold in T2D, particularly in carriers of the TT genotype. Overexpression of TCF7L2 in human islets reduced glucose-stimulated insulin secretion. In conclusion, the increased risk of T2D conferred by variants in TCF7L2 involves the enteroinsular axis, enhanced expression of the gene in islets, and impaired insulin secretion.

5.11 There was observed a strong association between the distal susceptibility CDKN2A/CDKN2B variant rs10811661 and type 2 diabetes

Duesing K, et al. *Strong association of common variants in the CDKN2A/CDKN2B region with type 2 diabetes in French Europids*. Diabetologia. 2008 May;51(5):821-6. doi: 10.1007/s00125-008-0973-4.

Hypothesis: genome-wide association studies (GWASs) recently identified common variants in the CDKN2A/CDKN2B region on chromosome 9p as being strongly associated with type 2 diabetes. Since these association signals were not picked up by the French-Canadian GWAS, we sought to replicate these findings in the French Europid population and to further characterize the susceptibility variants at this novel locus.
Methods: we genotyped 20 single nucleotide polymorphisms (SNPs) spanning the CDKN2A/CDKN2B locus in our type 2 diabetes case-control cohort. The association between CDKN2A/CDKN2B SNPs and quantitative metabolic traits was also examined in the normoglycemic participants comprising the control cohort.
Results: we report replication of the strong association of rs10811661 with type 2 diabetes found in the GWASs ($P= 3.8 X 10(-7)$; OR 1.43 [95% CI 1.24-1.64]). The other CDKN2A/CDKN2B susceptibility variant, rs564398, did not attain statistical significance ($p = 0.053$; OR 1.11 [95% CI 1.00-1.24]) in the present study. We also obtained several additional nominal association signals ($p < 0.05$) at the CDKN2A/CDKN2B locus; however, only the rs3218018 result ($p = 0.002$) survived Bonferroni correction for multiple testing (adjusted $p = 0.04$).
Conclusions: our comprehensive association study of common variation spanning the CDKN2A/CDKN2B locus confirms the strong association between the distal susceptibility variant rs10811661 and type 2 diabetes in the French population. Further genetic and functional studies are required to identify the etiological variants at this locus and determine the cellular and physiological mechanisms by which they act to modulate type 2 diabetes susceptibility.

5.12 CDKAL1 rs7756992 AA genotype is associated with average population risk for diabetes type II.

Horikawa Y, et al. *SNP rs7756992 in CDKAL1 has been associated with type 2 diabetes in Han Chinese individuals from Hong Kong*. J Clin Endocrinol Metab. 2008 Aug;93(8):3136-41. doi: 10.1210/jc.2008-0452.

Background: in Europeans and populations of European origin, several groups have recently identified novel type 2 diabetes susceptibility genes, including FTO, SLC30A8, HHEX, CDKAL1, CDKN2B, and IGF2BP2, none of which were in the list of functional candidates.
Objective and design: The aim of this study was to replicate in a Japanese population previously identified associations of single nucleotide polymorphisms (SNPs) within 10 candidate loci with type 2 diabetes using a relatively large sample size: 1921 subjects with type 2 diabetes and 1622 normal controls.

Results: A total of 15 SNPs were genotyped. Eight SNPs in five loci were found to be associated with type 2 diabetes: rs3802177 [odds ratio (OR) = 1.16 (95% confidence interval (CI) 1.05-1.27); P = 4.5 x 10(-3)] in SLC30A8; rs1111875 [OR = 1.27 (95% CI 1.14-1.40); P = 1.4 x 10(-5)] and rs7923837 [OR = 1.27 (95% CI 1.13-1.43); P = 1.0 x 10(-4)] in HHEX; rs10811661 [OR = 1.27 (95% CI 1.15-1.40); P = 1.9 x 10(-6)] in CDKN2B; rs4402960 [OR = 1.23 (95% CI 1.11-1.36); P = 8.1 x 10(-5)] and rs1470579 [OR = 1.18 (95% CI 1.07-1.31); P = 8.3 x 10(-4)] in IGF2BP2; and rs7754840 [OR = 1.28 (95% CI 1.17-1.41); P = 4.5 x 10(-7)] and rs7756992 [OR = 1.27 (95% CI 1.15-1.40); P = 9.8 x 10(-7)] in CDKAL1. The first and second strongest associations were found at variants in CDKAL1 and CDKN2B, both of which are involved in the regenerative capacity of pancreatic beta-cells.
Conclusion: Some of these variants represent common type 2 diabetes-susceptibility genes in both Japanese and Europeans.

5.13 TCF7L2 rs7901695 is associated with type-2 diabetes

Mayans S, et al. *TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden*. Eur J Hum Genet. 2007 Mar;15(3):342-6.

A recent study found association of one microsatellite and five single nucleotide polymorphisms (SNPs) in intron 3 of the TCF7L2 gene with type 2 diabetes (T2D) in the Icelandic, Danish and American populations. The aim of the present study was to investigate if those SNPs were associated to T2D in two (family- and population-based) cohorts from northern Sweden. We genotyped four of the associated SNPs in a case-control cohort consisting of 872 T2D cases and 857 controls matched with respect to age, sex and geographical origin and in a sample of 59 extended families (148 affected and 83 unaffected individuals). Here, we report replication of association between T2D and three SNPs in the case-control (rs7901695, P=0.003; rs7901346, P=0.00002; and rs12255372, P=0.000004) and two SNPs in the family-based (rs7901695, P=0.01 and rs7901346, P=0.04) samples from northern Sweden. This replication strengthens the evidence for involvement of TCF7L2 in T2D.

5.14 Common genetic variation in KCNQ1 is associated with insulin secretion upon oral glucose load in a German population at increased risk of type 2 diabetes.

Müssig K, et al. *Association of type 2 diabetes candidate polymorphisms in KCNQ1 with incretin and insulin secretion.* Diabetes. 2009 Jul;58(7):1715-20. doi: 10.2337/db08-1589.

Objective: KCNQ1 gene polymorphisms are associated with type 2 diabetes. This linkage appears to be mediated by altered beta-cell function. In an attempt to study underlying mechanisms, we examined the effect of four KCNQ1 single nucleotide polymorphisms (SNPs) on insulin secretion upon different stimuli.
Methods: we genotyped 1,578 nondiabetic subjects at increased risk of type 2 diabetes for rs151290, rs2237892, rs2237895, and rs2237897. All participants underwent an oral glucose tolerance test (OGTT); glucagon-like peptide (GLP)-1 and gastric inhibitory peptide secretion was measured in 170 participants. In 519 participants, a hyperinsulinemic-euglycemic clamp was performed, in 314 participants an intravenous glucose tolerance test (IVGTT), and in 102 subjects a hyperglycemic clamp combined with GLP-1 and arginine stimuli.
Results: rs151290 was nominally associated with 30-min C-peptide levels during OGTT, first-phase insulin secretion, and insulinogenic index after adjustment in the dominant model (all P < or = 0.01). rs2237892, rs2237895, and rs2237897 were nominally associated with OGTT-derived insulin secretion indexes (all P < 0.05). No SNPs were associated with beta-cell function during intravenous glucose or GLP-1 administration. However, rs151290 was associated with glucose-stimulated gastric inhibitory polypeptide and GLP-1 increase after adjustment in the dominant model (P = 0.0042 and P = 0.0198, respectively). No associations were detected between the other SNPs and basal or stimulated incretin levels (all P > or = 0.05).
Conclusions: common genetic variation in KCNQ1 is associated with insulin secretion upon oral glucose load in a German population at increased risk of type 2 diabetes. The discrepancy between orally and intravenously administered glucose seems to be explained not by altered incretin signaling but most likely by changes in incretin secretion.

5.15 CDKN2A, CDKN2B rs2383208 GG genotype is associated with average population risk for diabetes type II.

Wang Y, et al. *Association of six single nucleotide polymorphisms with gestational diabetes mellitus in a Chinese population*. PLoS One. 2011;6(11):e26953. doi: 10.1371/journal.pone.0026953

Background: to investigate whether the candidate genes that confer susceptibility to type 2 diabetes mellitus are also correlated with gestational diabetes mellitus (GDM) in pregnant Chinese women.
Methodology: in this study, 1764 unrelated pregnant women were recruited, of which 725 women had GDM and 1039 served as controls. Six single nucleotide polymorphisms (rs7754840 in CDKAL1, rs391300 in SRR, rs2383208 in CDKN2A/2B, rs4402960 in IGF2BP2, rs10830963 in MTNR1B, rs4607517 in GCK) were genotyped using TaqMan allelic discrimination assays. The genotype and allele distributions of each SNP between the GDM cases and controls and the combined effects of alleles for the risk of developing GDM were analyzed. We found that the rs4402960, rs2383208 and rs391300 were statistically associated with GDM (OR=1.207, 95%CI=1.029-1.417, p=0.021; OR=1.242, 95%CI=1.077-1.432, p=0.003; OR=1.202, 95%CI=1.020-1.416, P=0.028, respectively). In addition, the effect was greater under a recessive model in rs391300 (OR=1.820, 95%CI=1.226-2.701, p=0.003). Meanwhile, the joint effect of these three loci indicated an additive effect of multiple alleles on the risk of developing GDM with an OR of 1.196 per allele (p=1.08×10(-4)). We also found that the risk alleles of rs2383208 (b=-0.085, p=0.003), rs4402960 (b=-0.057, p=0.046) and rs10830963 (b=-0.096, p=0.001) were associated with HOMA-B, while rs7754840 was associated with decrease in insulin AUC during a 100 g OGTT given at the time of GDM diagnosis (b=-0.080, p=0.007).
Conclusions: several risk alleles of type 2 diabetes were associated with GDM in pregnant Chinese women. The effects of these SNPs on GDM might be through the impairment of beta cell function and these risk loci contributed additively to the disease.

5.16 GHSR rs490683 GG genotype is associated increased plasmatic glucose levels.

Mager U, et al. *Variations in the ghrelin receptor gene associate with obesity and glucose metabolism in individuals with impaired glucose tolerance*. PLoS One. 2008 Aug 13;3(8):e2941. doi: 10.1371/journal.pone.0002941.

Background: ghrelin may influence the development of obesity through its role in the control of energy balance, food intake, and regulation of body weight. The effects of ghrelin are mediated via the growth hormone secretagogue receptor (GHSR).
Methodology/principal findings: we genotyped 7 single nucleotide polymorphisms (SNPs) in the GHSR gene and assessed the association between those SNPs and obesity and type 2 diabetes-related phenotypes from 507 middle-aged overweight persons with impaired glucose tolerance participating in the Finnish Diabetes Prevention Study (DPS). Additionally, we performed in silico screening of the 5'-regulatory region of GHSR and evaluated SNPs disrupting putative transcription factor (TF) binding sites in vitro with gelshift assays to determine differences in protein binding between different alleles of SNPs. Rs9819506 in the promoter region of GHSR was associated with body weight (p = 0.036); persons with rs9819506-AA genotype having the lowest body weight. Individuals with rs490683-CC genotype displayed highest weight loss in the whole study population (p = 0.032). The false discovery rate for these results was <10%. Rs490683 and rs509035 were associated with several measures of glucose and insulin metabolism during the follow-up. Rs490683 may be a functional SNP, since gelshift experiments showed differential protein binding between the alleles, with higher binding to the G-allele. Rs490683-C may disrupt a putative binding site for the TF nuclear factor 1 (NF-1), thus rs4906863-GG genotype where the NF-1 site is intact may lead to a higher GHSR gene expression.
Conclusion/significance: polymorphisms in the GHSR promoter may modify changes in body weight during long-term lifestyle intervention and affect ghrelin receptor signalling through modulation of GHSR gene expression.

6. Fat response

6.1. A significant association between the -11391G>A SNP (rs17300539) and obesity-related traits and the potential to moderate such effects using dietary modification has been observed.

Warodomwichit D, et al. *ADIPOQ polymorphisms, monounsaturated fatty acids, and obesity risk: the GOLDN study*. Obesity (Silver Spring). 2009 Mar;17(3):510-7. doi: 10.1038/oby.2008.583.

Serum adiponectin levels have been positively associated with insulin sensitivity and are decreased in type 2 diabetes (T2D) and obesity. Genetic and environmental factors influence serum adiponectin and may contribute to risk of metabolic syndrome and T2D. Therefore, we investigated the effect of ADIPOQ single-nucleotide polymorphisms (SNPs), -11377C>G and -11391G>A, on metabolic-related traits, and their modulation by dietary fat in white Americans. Data were collected from 1,083 subjects participating in the Genetics of Lipid Lowering Drugs and Diet Network study. Mean serum adiponectin concentration was higher for carriers of the -11391A allele (P = 0.001) but lower for the -11377G allele carriers (P = 0.017). Moreover, we found a significant association with obesity traits for the -11391G>A SNP. Carriers of the -11391A allele had significantly lower weight (P = 0.029), BMI (P = 0.019), waist (P = 0.003), and hip circumferences (P = 0.004) compared to noncarriers. Interestingly, the associations of the -11391G>A with BMI and obesity risk were modified by monounsaturated fatty acids (MUFAs) intake (P-interaction = 0.021 and 0.034 for BMI and obesity risk, respectively). In subjects with MUFA intake above the median (> or =13% of energy intake), -11391A carriers had lower BMI (27.1 kg/m(2) for GA+AA vs. 29.1 kg/m(2) for GG, P = 0.002) and decreased obesity risk (odds ratio for -11391A = 0.52, 95% confidence interval (CI); 0.28-0.96; P = 0.031). However, we did not detect genotype-related differences for BMI or obesity in subjects with MUFA intake <13%. Our findings support a significant association between the -11391G>A SNPs and obesity-related traits and the potential to moderate such effects using dietary modification.

6.2 APOA5 polymorphims is associated with significantly less weight gain on a high fat diet.

Corella D, et al. *APOA5 gene variation modulates the effects of dietary fat intake on body mass index and obesity risk in the Framingham Heart Study*. J Mol Med (Berl). 2007 Feb;85(2):119-28.

Diet is an important environmental factor interacting with our genes to modulate the likelihood of developing lipid disorders and, consequently, cardiovascular disease risk. Our objective was to study whether dietary intake modulates the association between APOA5 gene variation and body weight in a large population-based study. Specifically, we have examined the interaction between the APOA5-1131T>C and 56C>G (S19W) polymorphisms and the macronutrient intake (total fat, carbohydrate, and protein) in their relation to the body mass index (BMI) and obesity risk in 1,073 men and 1,207 women participating in the Framingham Offspring Study. We found a consistent and statistically significant interaction between the -1131T>C single-nucleotide polymorphism (SNP; but not the 56C>G) and total fat intake for BMI. This interaction was dose-dependent, and no statistically significant heterogeneity by gender was detected. In subjects homozygous for the -1131T major allele, BMI increased as total fat intake increased. Conversely, this increase was not present in carriers of the -1131C minor allele. Accordingly, we found significant interactions in determining obesity and overweight risks. APOA5-1131C minor allele carriers had a lower obesity risk (OR, 0.61, 95%; CI, 0.39-0.98; P = 0.032) and overweight risk (OR, 0.63, 95%; CI, 0.41-0.96; P = 0.031) compared with TT subjects in the high fat intake group (>or=30% of energy) but not when fat intake was low (OR, 1.16, 95%; CI, 0.77-1.74; P = 0.47 and OR = 1.15, 95%; CI, 0.77-1.71; P = 0.48) for obesity and overweight, respectively). When specific fatty acid groups were analyzed, monounsaturated fatty acids showed the highest statistical significance for these interactions. In conclusion, the APOA5-1131T>C SNP, which is present in approximately 13% of this population, modulates the effect of fat intake on BMI and obesity risk in both men and women.

6.3 PPARγ polymorphism interact with dietary intake of fat in determination of plasma lipid concentrations

Alsaleh A, et al. *PPARγ2 gene Pro12Ala and PPARα gene Leu162Val single nucleotide polymorphisms interact with dietary intake of fat in determination of plasma lipid concentrations*. J Nutrigenet Nutrigenomics. 2011;4(6):354-66. doi: 10.1159/000336362.

Background: the peroxisome proliferator-activated receptors (PPARs) are transcriptional regulators of lipid metabolism, activated by unsaturated fatty acids. We investigated independent and interactive effects of PPARγ2 gene PPARG Pro12Ala (rs1801282) andPPARαgene PPARA Leu162Val (rs1800206) genotypes with dietary intake of fatty acids on concentrations of plasma lipids in subjects of whom 47.5% had metabolic syndrome.
Methods: the RISCK study is a parallel design, randomised controlled trial. Plasma lipids were quantified at baseline after a 4-week high saturated fatty acids diet and after three parallel 24-week interventions with reference (high saturated fatty acids), high monounsaturated fatty acids and low-fat diets. Single nucleotide polymorphisms were genotyped in 466 subjects.
Results: at baseline, the PPARG Ala12allele was associated with increased plasma total cholesterol (n = 378; p = 0.04), LDL cholesterol (p = 0.05) and apoB (p =0.05) after adjustment for age, gender and ethnicity. At baseline, PPARA Leu162Val × PPARG Pro12Ala genotype interaction did not significantly influence plasma lipid concentrations. After dietary intervention, gene-gene interaction significantly influenced LDL cholesterol (p =0.0002) and small dense LDL as a proportion of LDL (p = 0.005) after adjustments.
Conclusions: interaction between PPARG Pro12Ala and PPARA Leu162Val genotypes may influence plasma LDL cholesterol concentration and the proportion as small dense LDL after a high monounsaturated fatty acids diet.

6.4 Intake levels of dietary long-chain polyunsaturated fatty acids modify the association between genetic variation in FADS and LDL cholesterol

Hellstrand S, et al. *Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C.* J Lipid Res. 2012 Jun;53(6):1183-9. doi: 10.1194/jlr.P023721.

Polymorphisms of the FA desaturase (FADS) gene cluster have been associated with LDL, HDL, and triglyceride concentrations. Because FADS converts α-linolenic acid (ALA) and linoleic acid into PUFAs, we investigated the interaction between different PUFA intakes and the FADS polymorphism rs174547 (T>C) on fasting blood lipid and lipoprotein concentrations. We included 4,635 individuals (60% females, 45-68 years) from the Swedish population-based Malmö Diet and Cancer cohort. Dietary intakes were assessed by a modified diet history method including 7-day registration of cooked meals. The C-allele of rs174547 was associated with lower LDL concentration (P = 0.03). We observed significant interaction between rs174547 and long-chain ω-3 PUFA intakes on LDL (P = 0.01); the C-allele was only associated with lower LDL among individuals in the lowest tertile of long-chain ω-3 PUFA intakes (P < 0.001). In addition, significant interaction was observed between rs174547 and the ratio of ALA and linoleic FA intakes on HDL (P = 0.03). However, no significant associations between the C-allele and HDL were detected within the intake tertiles of the ratio. Our findings suggest that dietary intake levels of different PUFAs modify the associated effect of genetic variation in FADS on LDL and HDL.

6.5 APOA II polymorphism may influence the saturated fatty acid intake required to prevent dyslipidemia in the type 2 diabetic population

Noorshahi N, et al. *APOA II genotypes frequency and their interaction with saturated fatty acids consumption on lipid profile of patients with type 2 diabetes*. Clin Nutr. 2016 Aug;35(4):907-11. doi: 10.1016/j.clnu.2015.06.008.

Background: several studies have suggested that APOA II-265T/C polymorphism affect lipid profile. The aim of this study was to investigate the effect of -265T/C APOA II polymorphism and saturated fatty acids (SFA) intake interaction on lipid profile in diabetic population who are at risk for lipid disorders.
Methods: in this cross-sectional study, 697 type 2 diabetic patients participated. Food consumption data were collected using validated semi-quantitative FFQ during the last year. Realtime-PCR was used to determine APOA II-265T/C genotypes. The interaction between the genotypes and SFA intake with lipid profile was tested using analysis of covariance (ANCOVA).
Results: according to APOA II-265T/C (rs5082) genotype distribution results, CC genotype with a frequency of 12.9% and TC with that of 47.7% showed the lowest and highest frequency in our population, respectively. CC genotype subjects had significantly lower total cholesterol, triglyceride, Cholesterol/HDL-c ratio and non-HDL cholesterol than T allele carriers ($p = 0.009$, $p = 0.02$, $p = 0.02$ and $p = 0.002$, respectively). The interaction between genotype and SFA intake contributed to significant higher levels of LDL-c and LDL/HDL in CCs ($p = 0.05$ and $p = 0.01$), suggesting vulnerability of these individuals to high intake of SFA in the diet.
Conclusion: APOA II polymorphism may influence the saturated fatty acid intake required to prevent dyslipidemia in the type 2 diabetic population.

7. Flavours preference

7.1 Subjects with RS713598 polymorphism had a higher bitter taste perception threshold

Perna S, et al. *Association of the bitter taste receptor gene TAS2R38 (polymorphism RS713598) with sensory responsiveness, food preferences, biochemical parameters and body-composition markers. A cross-sectional study in Italy*. Int J Food Sci Nutr. 2018 Mar;69(2):245-252. doi: 10.1080/09637486.2017.1353954.

This study examined the relationship between TAS2R38 gene polymorphism (RS713598), G/G, C/G or C/C genotype, and sensory responsiveness, food preferences, biochemical parameters and body composition in a cross-sectional study in 118 adults (24 men and 94 women). The frequencies of C/C, G/G and C/G were respectively 20.3%, 29.7% and 50.0%. As regards taste responsiveness, subjects with G-allele had a higher perception threshold than the C/C genotype for 6-n-propyl-2-thiouracil (PROP) ($p < .05$), and caffeine ($p < .05$). The G-alleles had higher preferences for beer (OR: 6.25; $p < .05$), but lower for butter (OR: 0.64; $p < .05$) and cured meat (OR: 0.55; $p < .05$). Biochemical parameters and body composition markers did not differ between genotypes. Subjects with RS713598 polymorphism had a higher bitter taste perception threshold and higher or lower preferences for selected nutrient/energy dense foods, such as beer, butter and cured meat.

7.2 rs1726866 polymorphism has influence on TAS2R bitter taste receptor family.

Kim UK, et al. *Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide*. Science. 2003 Feb 21;299(5610):1221-5.

The ability to taste the substance phenylthiocarbamide (PTC) has been widely used for genetic and anthropological studies, but genetic studies have produced conflicting results and demonstrated complex inheritance for this trait. We have identified a small region on chromosome 7q that shows strong linkage disequilibrium between single-nucleotide polymorphism (SNP) markers and PTC taste sensitivity in unrelated subjects. This region contains a single gene that encodes a member of the TAS2R bitter taste receptor family. We identified three coding SNPs giving rise to five haplotypes in this gene worldwide. These haplotypes

completely explain the bimodal distribution of PTC taste sensitivity, thus accounting for the inheritance of the classically defined taste insensitivity and for 55 to 85% of the variance in PTC sensitivity. Distinct phenotypes were associated with specific haplotypes, which demonstrates that this gene has a direct influence on PTC taste sensitivity and that sequence variants at different sites interact with each other within the encoded gene product.

7.3 Allelic polymorphism within the TAS1R3 promoter is associated with human taste sensitivity to sucrose

Fushan AA, et al. *Allelic polymorphism within the TAS1R3 promoter is associated with human taste sensitivity to sucrose*. Curr Biol. 2009 Aug 11;19(15):1288-93. doi: 10.1016/j.cub.2009.06.015.

Human sweet taste perception is mediated by the heterodimeric G protein-coupled receptor encoded by the TAS1R2 and TAS1R3 genes. Variation in these genes has been characterized, but the functional consequences of such variation for sweet perception are unknown. We found that two C/T single-nucleotide polymorphisms (SNPs) located at positions -1572 (rs307355) and -1266 (rs35744813) upstream of the TAS1R3 coding sequence strongly correlate with human taste sensitivity to sucrose and explain 16% of population variability in perception. By using a luciferase reporter assay, we demonstrated that the T allele of each SNP results in reduced promoter activity in comparison to the C alleles, consistent with the phenotype observed in humans carrying T alleles. We also found that the distal region of the TAS1R3 promoter harbors a composite cis-acting element that has a strong silencing effect on promoter activity. We conclude that the rs307355 and rs35744813 SNPs affect gene transcription by altering the function of this regulatory element. A worldwide population survey reveals that the T alleles of rs307355 and rs35744813 occur at lowest frequencies in European populations. We propose that inherited differences in TAS1R3 transcription account for a substantial fraction of worldwide differences in human sweet taste perception.

7.4. A genetic variation in GLUT2 is associated with habitual consumption of sugars, suggesting an underlying glucose-sensing mechanism that regulates food intake.

Eny KM, et al. *Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations*. Physiol Genomics. 2008 May 13;33(3):355-60. doi: 10.1152/physiolgenomics.00148.2007.

Glucose sensing in the brain has been proposed to be involved in regulating food intake, but the mechanism is not known. Glucose transporter type 2 (GLUT2)-null mice fail to control their food intake in response to glucose, suggesting a potential role for this transporter as a glucose sensor in the brain. Here we show that individuals with a genetic variation in GLUT2 (Thr110Ile) have a higher daily intake of sugars in two distinct populations. In the first population, compared with individuals with the Thr/Thr genotype, carriers of the Ile allele had a significantly higher intake of sugars as assessed from 3-day food records administered on two separate visits (visit 1: 112 +/- 9 vs. 86 +/- 4 g/day, P = 0.01; visit 2: 111 +/- 8 vs. 82 +/- 4 g/day, P = 0.003), demonstrating within-population reproducibility. In a second population, carriers of the Ile allele also reported consuming a significantly greater intake of sugars (131 +/- 5 vs. 115 +/- 3 g/day, P = 0.007) over a 1-mo period as measured from a food frequency questionnaire. GLUT2 genotypes were not associated with fat, protein, or alcohol intake in either population. These observations were consistent across older and younger adults as well as among subjects with early Type 2 diabetes and healthy individuals. Taken together, our findings show that a genetic variation in GLUT2 is associated with habitual consumption of sugars, suggesting an underlying glucose-sensing mechanism that regulates food intake.

7.5 ACE rs699 TT genotype is associated with average salt sensibility.

Imaizumi T, et al. *Association of interactions between dietary salt consumption and hypertension-susceptibility genetic polymorphisms with blood pressure among Japanese male workers*. Clin Exp Nephrol. 2017 Jun; 21(3):457-464. doi: 10.1007/s10157-016-1315-3.

Background: blood pressure is influenced by hereditary factors and dietary habits. The objective of this study was to examine the effect of dietary salt consumption and single-nucleotide polymorphisms (SNPs) on blood pressure (BP).
Methods: this was a cross-sectional analysis of 2728 male participants who participated in a health examination in 2009. Average dietary salt consumption was estimated using electronically collected meal purchase data from cafeteria. A multivariate analysis, adjusting for clinically relevant factors, was conducted to examine whether the effect on BP of salt consumption, SNPs, and interaction between salt consumption and each SNP. This study examined the SNPs AGT rs699 (Met235Thr), ADD1 rs4961 (Gly460Trp), NPPA rs5063 (Val32Met), GPX1 rs1050450 (Pro198Leu), and AGTR1 rs5186 (A1166C) in relation to hypertension and salt sensitivity.
Results: BP was not significantly associated with SNPs or salt consumption. The interaction between salt consumption and SNPs with systolic BP showed a significant association in NPPA rs5063 (Val32Met) (P = 0.023) and a marginal trend toward significance in rs4961 and rs1050450 (P = 0.060 and 0.067, respectively).
Conclusion: the effect of salt consumption on BP differed by genotype. Dietary salt consumption and genetic variation can predict a high risk of hypertension.

8. Alchool and caffeine metabolism

8.1 The rs671(A) allele of the ALDH2 gene encodes a form of aldehyde dehydrogenase 2 protein that is defective at metabolizing alcohol.

Yokoyama M, et al. *Hangover susceptibility in relation to aldehyde dehydrogenase-2 genotype, alcohol flushing, and mean corpuscular volume in Japanese workers*. Alcohol Clin Exp Res. 2005 Jul;29(7):1165-71.

Background: a study of Asian-American students suggested a positive association between inactive ALDH2*2 and susceptibility to hangover. A biomarker for moderate-to-heavy drinking in persons with inactive aldehyde dehydrogenase-2 (ALDH2) is increased mean corpuscular volume (MCV).
Methods: associations between hangover and ALDH2 genotype, alcohol flushing, and MCV were examined for 251 Japanese workers (139 men, 112 women).
Results: inactive ALDH2*1/2*2 heterozygotes drank less alcohol than active ALDH2*1/2*1 homozygotes ($p < 0.0001$), but the frequency of hangover did not significantly differ between the two groups for either gender. The amount of drinking reported to lead to hangover was significantly less for male and female ALDH2*1/2*2 heterozygotes than for their ALDH2*1/2*1 homozygous counterparts ($p < 0.005$). The proportion of men who had hangover three times or more during the past year increased significantly with increased daily alcohol consumption in men with the ALDH2*1/2*2 genotype ($p = 0.0002$) but not in those with the ALDH2*1/2*1 genotype. For men who usually consumed <44 g of ethanol/day, the median amount of drinking before hangover was significantly lower for ALDH2*1/2*2 men than for ALDH2*1/2*1 men reporting the same level of consumption. Hangover occurred with consistently high frequency among ALDH2*1/2*1 men, regardless of their daily consumption. Similar findings were observed in a comparison of men who never flushed and those who reported current or former flushing, a surrogate marker of inactive ALDH2. Assessment of hangover risk by quartiles of MCV showed that men with MCV of > or =96 had a significantly higher risk of hangover than did men with MCV of <91 (odds ratio = 5.56; 95% confidence interval = 1.69-18.25).
Conclusions: inactive heterozygous ALDH2, alcohol flushing, and increased MCV were positively associated with hangover susceptibility in Japanese workers, suggesting that acetaldehyde is etiologically linked to the development of hangover.

8.2 rs762551, also known as -164A>C or -163C>A, is a SNP encoding the CYP1A2*1F allele, codifyingCYP1A2 is an enzyme responsible for the metabolism of caffeine and some drugs.

Wang L, et al. *Association between common CYP1A2 polymorphisms and theophylline metabolism in non-smoking healthy volunteers*. Basic Clin Pharmacol Toxicol. 2013 Apr;112(4):257-63. doi: 10.1111/bcpt.12038.

This study was designed to investigate the impact of cytochrome P450 (CYP) 1A2 polymorphisms on theophylline metabolism in a non-smoking healthy male Chinese population. Four polymorphisms CYP1A2 1C (G-3860A), G-3113A, CYP1A2 1F (C-163A) and CYP1A2 1B (C-5347T) were screened in 238 unrelated male volunteers. Then, a single oral 200-mg dose of theophylline was administered to 37 volunteers, who were selected from 238 volunteers based on the CYP1A2 genotype. CYP1A2 activities were evaluated by plasma 1,7-dimethylxanthine/caffeine ratios (17X/137X) after administration of 100-mg caffeine. The plasma concentrations of theophylline, 17X and 137X were determined by high-performance liquid chromatography. The activity of CYP1A2 was lower in volunteers with the -3113 AA genotype compared with those with the -3113 AG genotype (0.35 ± 0.04 versus 0.48 ± 0.07, p = 0.016) or the -3113 GG genotype (0.35 ± 0.04 versus 0.58 ± 0.22, p = 0.037). CYP1A2 1F polymorphisms were associated with increased CYP1A2 activity in volunteers with -3860G/-3113G/5347C homozygosity (0.66 ± 0.24 versus 0.46 ± 0.05, p = 0.034). However, theophylline metabolism showed no difference among volunteers carrying different haplotype pairs. CYP1A2 genetic polymorphisms influenced CYP1A2 enzyme activity as measured by caffeine, but CYP1A2 gene polymorphisms appeared to have limited influence on theophylline metabolism in our study.

8.3 rs2470893 influences habitual caffeine consumption.

Josse AR, et al. *Associations between polymorphisms in the AHR and CYP1A1-CYP1A2 gene regions and habitual caffeine consumption*. Am J Clin Nutr. 2012 Sep;96(3):665-71. doi: 10.3945/ajcn.112.038794.

Background: recent genome-wide association studies (GWASs) from populations of European descent identified single nucleotide polymorphisms (SNPs) in aryl-hydrocarbon receptor (AHR) and cytochrome P450 1A1 and 1A2 (CYP1A1-CYP1A2) genes that are associated with habitual caffeine and coffee consumption.
Objective: we examined whether these SNPs (AHR: rs6968865 and rs4410790; CYP1A1-CYP1A2: rs2472297 and rs2470893) and 6 additional tag SNPs in the AHR gene were associated with habitual caffeine consumption in a Costa Rican population.
Design: subjects were from a case-control study of gene-diet interactions and myocardial infarction. Subjects with hypertension or missing information on smoking, caffeine intake, or genotype were excluded. Subjects were genotyped by using polymerase chain reaction with mass spectrometry-based detection, and caffeine intake was assessed by using a validated food-frequency questionnaire.
Results: compared with subjects who consumed <100 mg caffeine/d, subjects who consumed >400 mg caffeine/d were more likely to be carriers of the T, C, or T allele for rs6968865, rs4410790, and rs2472297, respectively. The corresponding ORs and 95% CIs were 1.41 (1.03, 1.93), 1.41 (1.04, 1.92), and 1.55 (1.01, 2.36). Multivariate-adjusted ORs (95% CIs) for rs6968865 were 1.44 (1.03, 2.00) for all subjects, 1.75 (1.16, 2.65) for nonsmokers, 1.15 (0.58, 2.30) for current smokers, 2.42 (1.45, 4.04) for subjects >57 y old, and 1.00 (0.65, 1.56) for subjects ≤57 y old. A similar effect modification was observed for rs4410790 but not for rs2472297.
Conclusion: our findings show that previous associations between SNPs in AHR and CYP1A1-CYP1A2 and caffeine and coffee consumption from GWASs in European populations are also observed in an ethnically distinct Costa Rican population, but age and smoking are important effect modifiers.

8.4 CYP1A2 polymorphism are associated with higher coffee consumption.

Cornelis MC, et al. *Genome-wide meta-analysis identifies six novel loci associated with habitual coffee consumption*. Mol Psychiatry. 2015 May;20(5):647-656. doi: 10.1038/mp.2014.107.

Coffee, a major dietary source of caffeine, is among the most widely consumed beverages in the world and has received considerable attention regarding health risks and benefits. We conducted a genome-wide (GW) meta-analysis of predominately regular-type coffee consumption (cups per day) among up to 91,462 coffee consumers of European ancestry with top single-nucleotide polymorphisms (SNPs) followed-up in ~30 062 and 7964 coffee consumers of European and African-American ancestry, respectively. Studies from both stages were combined in a trans-ethnic meta-analysis. Confirmed loci were examined for putative functional and biological relevance. Eight loci, including six novel loci, met GW significance (log10Bayes factor (BF)>5.64) with per-allele effect sizes of 0.03-0.14 cups per day. Six are located in or near genes potentially involved in pharmacokinetics (ABCG2, AHR, POR and CYP1A2) and pharmacodynamics (BDNF and SLC6A4) of caffeine. Two map to GCKR and MLXIPL genes related to metabolic traits but lacking known roles in coffee consumption. Enhancer and promoter histone marks populate the regions of many confirmed loci and several potential regulatory SNPs are highly correlated with the lead SNP of each. SNP alleles near GCKR, MLXIPL, BDNF and CYP1A2 that were associated with higher coffee consumption have previously been associated with smoking initiation, higher adiposity and fasting insulin and glucose but lower blood pressure and favorable lipid, inflammatory and liver enzyme profiles (P<5 × 10(-8)).Our genetic findings among European and African-American adults reinforce the role of caffeine in mediating habitual coffee consumption and may point to molecular mechanisms underlying inter-individual variability in pharmacological and health effects of coffee.

9. Cholesterol and Triglycerides

9.1 APOA5 rs662799 polymorphism is associated with normal levels of HDL.

Boes E, et al. *Genetic-epidemiological evidence on genes associated with HDL cholesterol levels: a systematic in-depth review*. Exp Gerontol. 2009 Mar;44(3):136-60. doi: 10.1016/j.exger.2008.11.003.

High-density lipoprotein (HDL) particles exhibit multiple antiatherogenic effects. They are key players in the reverse cholesterol transport which shuttles cholesterol from peripheral cells (e.g. macrophages) to the liver or other tissues. This complex process is thought to represent the basis for the antiatherogenic properties of HDL particles. The amount of cholesterol transported in HDL particles is measured as HDL cholesterol (HDLC) and is inversely correlated with the risk for coronary artery disease: an increase of 1mg/dL of HDLC levels is associated with a 2% and 3% decrease of the risk for coronary artery disease in men and women, respectively. Genetically determined conditions with high HDLC levels (e.g. familial hyperalphalipoproteinemia) often coexist with longevity, and higher HDLC levels were found among healthy elderly individuals. HDLC levels are under considerable genetic control with heritability estimates of up to 80%. The identification and characterization of genetic variants associated with HDLC concentrations can provide new insights into the background of longevity. This review provides an extended overview on the current genetic-epidemiological evidence from association studies on genes involved in HDLC metabolism. It provides a path through the jungle of association studies which are sometimes confusing due to the varying and sometimes erroneous names of genetic variants, positions and directions of associations. Furthermore, it reviews the recent findings from genome-wide association studies which have identified new genes influencing HDLC levels. The yet identified genes together explain only a small amount of less than 10% of the HDLC variance, which leaves an enormous room for further yet to be identified genetic variants. This might be accomplished by large population-based genome-wide meta-analyses and by deep-sequencing approaches on the identified genes. The resulting findings will probably result in a re-drawing and extension of the involved metabolic pathways of HDLC metabolism.

9.2 The minor allele of ABCG8 rs6544713 SNP was associated with increased LDL cholesterol.

Deloukas P, et al. *Large-scale association analysis identifies new risk loci for coronary artery disease*. Nat Genet. 2013 Jan;45(1):25-33. doi: 10.1038/ng.2480.

Coronary artery disease (CAD) is the commonest cause of death. Here, we report an association analysis in 63,746 CAD cases and 130,681 controls identifying 15 loci reaching genome-wide significance, taking the number of susceptibility loci for CAD to 46, and a further 104 independent variants ($r(2) < 0.2$) strongly associated with CAD at a 5% false discovery rate (FDR). Together, these variants explain approximately 10.6% of CAD heritability. Of the 46 genome-wide significant lead SNPs, 12 show a significant association with a lipid trait, and 5 show a significant association with blood pressure, but none is significantly associated with diabetes. Network analysis with 233 candidate genes (loci at 10% FDR) generated 5 interaction networks comprising 85% of these putative genes involved in CAD. The four most significant pathways mapping to these networks are linked to lipid metabolism and inflammation, underscoring the causal role of these activities in the genetic etiology of CAD. Our study provides insights into the genetic basis of CAD and identifies key biological pathways.

9.3 CELSR2 rs12740374 GG genotype is not associated with increased LDL cholesterol levels.

Sandhu MS, et al. *LDL-cholesterol concentrations: a genome-wide association study*. Lancet. 2008 Feb 9;371(9611):483-91. doi: 10.1016/S0140-6736(08)60208-1.

Background: LDL cholesterol has a causal role in the development of cardiovascular disease. Improved understanding of the biological mechanisms that underlie the metabolism and regulation of LDL cholesterol might help to identify novel therapeutic targets. We therefore did a genome-wide association study of LDL-cholesterol concentrations.
Methods: we used genome-wide association data from up to 11,685 participants with measures of circulating LDL-cholesterol concentrations across five studies, including data for 293 461 autosomal single nucleotide polymorphisms (SNPs) with a minor allele frequency of 5% or more that passed our quality control criteria. We also used data from a second genome-wide array in up to 4337 participants from three of these five studies, with data for 290,140 SNPs. We did replication studies in two independent populations consisting of up to 4979 participants. Statistical approaches, including meta-analysis and linkage disequilibrium plots, were used to refine association signals; we analysed pooled data from all seven populations to determine the effect of each SNP on variations in circulating LDL-cholesterol concentrations.
Findings: in our initial scan, we found two SNPs (rs599839 [p=1.7x10(-15)] and rs4970834 [p=3.0x10(-11)]) that showed genome-wide statistical association with LDL cholesterol at chromosomal locus 1p13.3. The second genome screen found a third statistically associated SNP at the same locus (rs646776 [p=4.3x10(-9)]). Meta-analysis of data from all studies showed an association of SNPs rs599839 (combined p=1.2x10(-33)) and rs646776 (p=4.8x10(-20)) with LDL-cholesterol concentrations. SNPs rs599839 and rs646776 both explained around 1% of the variation in circulating LDL-cholesterol concentrations and were associated with about 15% of an SD change in LDL cholesterol per allele, assuming an SD of 1 mmol/L.
Interpretation: we found evidence for a novel locus for LDL cholesterol on chromosome 1p13.3. These results potentially provide insight into the biological mechanisms that underlie the regulation of LDL cholesterol and might help in the discovery of novel therapeutic targets for cardiovascular disease.

9.4 HMGCR rs3846663 CC genotype is not associated with increased LDL cholesterol levels.

Lowe JK, et al. *Genome-wide association studies in an isolated founder population from the Pacific Island of Kosrae*. PLoS Genet. 2009 Feb;5(2):e1000365. doi: 10.1371/journal.pgen.1000365.

It has been argued that the limited genetic diversity and reduced allelic heterogeneity observed in isolated founder populations facilitates discovery of loci contributing to both Mendelian and complex disease. A strong founder effect, severe isolation, and substantial inbreeding have dramatically reduced genetic diversity in natives from the island of Kosrae, Federated States of Micronesia, who exhibit a high prevalence of obesity and other

metabolic disorders. We hypothesized that genetic drift and possibly natural selection on Kosrae might have increased the frequency of previously rare genetic variants with relatively large effects, making these alleles readily detectable in genome-wide association analysis. However, mapping in large, inbred cohorts introduces analytic challenges, as extensive relatedness between subjects violates the assumptions of independence upon which traditional association test statistics are based. We performed genome-wide association analysis for 15 quantitative traits in 2,906 members of the Kosrae population, using novel approaches to manage the extreme relatedness in the sample. As positive controls, we observe association to known loci for plasma cholesterol, triglycerides, and C-reactive protein and to a compelling candidate loci for thyroid stimulating hormone and fasting plasma glucose. We show that our study is well powered to detect common alleles explaining >/=5% phenotypic variance. However, no such large effects were observed with genome-wide significance, arguing that even in such a severely inbred population, common alleles typically have modest effects. Finally, we show that a majority of common variants discovered in Caucasians have indistinguishable effect sizes on Kosrae, despite the major differences in population genetics and environment.

9.5 A common variant at the LDLR gene locus affects LDL-C levels.

Linsel-Nitschke P, et al. *Lifelong reduction of LDL-cholesterol related to a common variant in the LDL-receptor gene decreases the risk of coronary artery disease--a Mendelian Randomisation study*. PLoS One. 2008 Aug 20;3(8):e2986. doi: 10.1371/journal.pone.0002986.

Background: rare mutations of the low-density lipoprotein receptor gene (LDLR) cause familial hypercholesterolemia, which increases the risk for coronary artery disease (CAD). Less is known about the implications of common genetic variation in the LDLR gene regarding the variability of cholesterol levels and risk of CAD.
Methods: imputed genotype data at the LDLR locus on 1 644 individuals of a population-based sample were explored for association with LDL-C level. Replication of association with LDL-C level was sought for the most significant single nucleotide polymorphism (SNP) within the LDLR gene in three European samples comprising 6 642 adults and 533 children. Association of this SNP with CAD was examined in six case-control studies involving more than 15 000 individuals.
Findings: each copy of the minor T allele of SNP rs2228671 within LDLR (frequency 11%) was related to a decrease of LDL-C levels by 0.19 mmol/L (95% confidence interval (CI) [0.13-0.24] mmol/L, p = 1.5x10(-10)). This association with LDL-C was uniformly found in children, men, and women of all samples studied. In parallel, the T allele of rs2228671 was associated with a significantly lower risk of CAD (Odds Ratio per copy of the T allele: 0.82, 95% CI [0.76-0.89], p = 2.1x10(-7)). Adjustment for LDL-C levels by logistic regression or Mendelian Randomization models abolished the significant association between rs2228671 with CAD completely, indicating a functional link between the genetic variant at the LDLR gene locus, change in LDL-C and risk of CAD.
Conclusion: a common variant at the LDLR gene locus affects LDL-C levels and, thereby, the risk for CAD.

9.6 The oxLDL levels increasing variant rs676210 associates with CVD events in patients undergoing coronary angiography.

Mäkelä KM, et al. *Association of the novel single-nucleotide polymorphism which increases oxidized low-density lipoprotein levels with cerebrovascular disease events*. Atherosclerosis. 2014 May;234(1):214-7. doi: 10.1016/j.atherosclerosis.2014.03.002.

Background: patients with genetic background for high circulating oxidized low-density lipoprotein (oxLDL) levels might be at an increased risk of cerebrovascular disease (CVD).
Methods: the association of oxLDL-variant rs676210 with CVD events was studied in patients undergoing coronary angiography (study A; N = 2913 [271 cases]). We sought to replicate the results in a large genome-wide association study meta-analysis of ischaemic stroke (study B; N = 3548 cases, 5972 controls).
Results: in study A, the prevalence of hypertension, diabetes and >50% carotid stenosis as well as the levels of LDL cholesterol differed significantly between cases and controls. In a logistic regression model adjusted for the significant covariates, rs676210 associated with CVD events (p = 0.030; odds ratio = 1.29 [95% confidence interval 1.03–1.63] for risk allele G). In study B, rs676210 did not associate with the history of ischaemic stroke.

Conclusions: The oxLDL levels increasing variant rs676210 associates with CVD events in patients undergoing coronary angiography.

9.7 Polymorphisms on CETP gene have been associated with HDL levels.

Papp AC, et al. *Cholesteryl Ester Transfer Protein (CETP) polymorphisms affect mRNA splicing, HDL levels, and sex-dependent cardiovascular risk*. PLoS One. 2012;7(3):e31930. doi: 10.1371/journal.pone.0031930.

Polymorphisms in and around the Cholesteryl Ester Transfer Protein (CETP) gene have been associated with HDL levels, risk for coronary artery disease (CAD), and response to therapy. The mechanism of action of these polymorphisms has yet to be defined. We used mRNA allelic expression and splice isoform measurements in human liver tissues to identify the genetic variants affecting CETP levels. Allelic CETP mRNA expression ratios in 56 human livers were strongly associated with several variants 2.5-7 kb upstream of the transcription start site (e.g., rs247616 p = 6.4 × 10(-5), allele frequency 33%). In addition, a common alternatively spliced CETP isoform lacking exon 9 (Δ9), has been shown to prevent CETP secretion in a dominant-negative manner. The Δ 9 expression ranged from 10 to 48% of total CETP mRNA in 94 livers. Increased formation of this isoform was exclusively associated with an exon 9 polymorphism rs5883-C>T (p = 6.8 × 10(-10)) and intron 8 polymorphism rs9930761-T>C (5.6 × 10(-8)) (in high linkage disequilibrium with allele frequencies 6-7%). rs9930761 changes a key splicing branch point nucleotide in intron 8, while rs5883 alters an exonic splicing enhancer sequence in exon 9.The effect of these polymorphisms was evaluated in two clinical studies. In the Whitehall II study of 4745 subjects, both rs247616 and rs5883T/rs9930761C were independently associated with increased HDL-C levels in males with similar effect size (rs247616 p = 9.6 × 10(-28) and rs5883 p = 8.6 × 10(-10), adjusted for rs247616). In an independent multiethnic US cohort of hypertensive subjects with CAD (INVEST-GENE), rs5883T/rs9930761C alone were significantly associated with increased incidence of MI, stroke, and all-cause mortality in males (rs5883: OR 2.36 (CI 1.29-4.30), p = 0.005, n = 866). These variants did not reach significance in females in either study. Similar to earlier results linking low CETP activity with poor outcomes in males, our results suggest genetic, sex-dependent CETP splicing effects on cardiovascular risk by a mechanism independent of circulating HDL-C levels.

9.8 CDKN2A/B rs10811661 CC genotype is not associated with increased levels of triglycerides.

Saxena R, et al. Gen*ome-wide association analysis identifies loci for type 2 diabetes and triglyceride level*s. Science. 2007 Jun 1;316(5829):1331-6.

New strategies for prevention and treatment of type 2 diabetes (T2D) require improved insight into disease etiology. We analyzed 386,731 common single-nucleotide polymorphisms (SNPs) in 1464 patients with T2D and 1467 matched controls, each characterized for measures of glucose metabolism, lipids, obesity, and blood pressure. With collaborators (FUSION and WTCCC/UKT2D), we identified and confirmed three loci associated with T2D-in a noncoding region near CDKN2A and CDKN2B, in an intron of IGF2BP2, and an intron of CDKAL1- and replicated associations near HHEX and in SLC30A8 found by a recent whole-genome association study. We identified and confirmed association of a SNP in an intron of glucokinase regulatory protein (GCKR) with serum triglycerides. The discovery of associated variants in unsuspected genes and outside coding regions illustrates the ability of genome-wide association studies to provide potentially important clues to the pathogenesis of common diseases.

9.9 KCTD10 rs10850219 GG genotype is associated with decreased HDL cholesterol levels when the diet contains high levels of carbohydrates.

Demirkan A, et al. *Insight in genome-wide association of metabolite quantitative traits by exome sequence analyses*. PLoS Genet. 2015 Jan 8;11(1):e1004835. doi: 10.1371/journal.pgen.1004835.

Metabolite quantitative traits carry great promise for epidemiological studies, and their genetic background has been addressed using Genome-Wide Association Studies (GWAS). Thus far, the role of less common variants

has not been exhaustively studied. Here, we set out a GWAS for metabolite quantitative traits in serum, followed by exome sequence analysis to zoom in on putative causal variants in the associated genes. 1H Nuclear Magnetic Resonance (1H-NMR) spectroscopy experiments yielded successful quantification of 42 unique metabolites in 2,482 individuals from The Erasmus Rucphen Family (ERF) study. Heritability of metabolites were estimated by SOLAR. GWAS was performed by linear mixed models, using HapMap imputations. Based on physical vicinity and pathway analyses, candidate genes were screened for coding region variation using exome sequence data. Heritability estimates for metabolites ranged between 10% and 52%. GWAS replicated three known loci in the metabolome wide significance: CPS1 with glycine (P-value =1.27×10-32), PRODH with proline (P-value =1.11×10-19), SLC16A9 with carnitine level (P-value =4.81×10-14) and uncovered a novel association between DMGDH and dimethyl-glycine (P-value =1.65×10-19) level. In addition, we found three novel, suggestively significant loci: TNP1 with pyruvate (P-value =1.26×10-8), KCNJ16 with 3-hydroxybutyrate (P-value =1.65×10-8) and 2p12 locus with valine (P-value =3.49×10-8). Exome sequence analysis identified potentially causal coding and regulatory variants located in the genes CPS1, KCNJ2 and PRODH, and revealed allelic heterogeneity for CPS1 and PRODH. Combined GWAS and exome analyses of metabolites detected by high-resolution 1H-NMR is a robust approach to uncover metabolite quantitative trait loci (mQTL), and the likely causative variants in these loci. It is anticipated that insight in the genetics of intermediate phenotypes will provide additional insight into the genetics of complex traits.

9.10 PPAR-Y rs1801282 GG genotype is associated with increased levels of LDL cholesterol.

Bego T, et al. *Association of PPARG and LPIN1 gene polymorphisms with metabolic syndrome and type 2 diabetes*. Med Glas (Zenica). 2011 Feb;8(1):76-83.

Aim: lipin 1 is a recently discovered multifunctional protein involved in the metabolism of lipids, while PPARgamma is involved in adipocyte differentiation, and regulation of lipid metabolism. Up to now, LPIN1 and PPARG gene polymorphisms have been associated with type 2 diabetes, metabolic syndrome, and central obesity. In this study, we hypothesized that genetic variants within LPIN1 and PPARG genes were associated with traits of metabolic syndrome. Correlation between biochemical parameters (including but not limited to, glucose, HbA1c, insulin levels, HDL and LDL cholesterol, triglycerides, serum proteins, liver enzymes) and frequency of polymorphisms in LPIN1 (rs11693809 and rs2716610) and PPARG gene (rs10865710, rs3856806 and rs1801282), was tested in this study.
Methods: the study included 70 patients diagnosed with metabolic syndrome and type 2 diabetes. Two polymorphisms of LPIN1 gene (rs11693809 and rs2716610), and three polymorphisms of PPARG gene (rs10865710, rs385806 and rs1801282) were analyzed by real time PCR and conventional PCR-RFLP methods
Results: our analysis revealed correlation between insulin levels and rs11693809 LPIN1 polymorphism in diabetic patients. Also the results of this study showed an association of rs10865710 and rs385806 polymorphism of PPARG with HDL cholesterol and LDL plus total cholesterol levels, respectively.
Conclusion: these data reflect an association of analyzed PPARG and LPIN1 gene polymorphisms with values of insulin, HDL, LDL and total cholesterol which indicates an important role of these genes in lipid metabolism and pathogenesis of type 2 diabetes and metabolic syndrome.

10. Hypertension

10.1 ACE 2350A allele is associated with a significantly reduced hypertension risk

Wenquan Niu, et al. *Review: association between angiotensin converting enzyme G2350A polymorphism and hypertension risk: a meta-analysis*. J Renin Angiotensin Aldosterone Syst. 2011 Mar;12(1):8-14. doi: 10.1177/1470320310375859.

Background: an exonic polymorphism G2350A (rs4343) in angiotensin converting enzyme (protein: ACE; gene: ACE) was shown to exert the most significant influence on plasma ACE levels. We therefore performed a meta-analysis to investigate association of ACE G2350A polymorphism with hypertension.

Methods: published case-control studies in English were identified. A total of four studies with 1699 cases and 1274 controls were identified. A random-effects model was performed irrespective of the between-study heterogeneity. Study quality was assessed in duplicate.
Results: compared with 2350G, the ACE 2350A allele conferred a protective effect on hypertension (odds ratio (OR) = 0.81; 95% confidence interval (CI), 0.56-1.18; p = .28). Similarly, comparisons of 2350AA and 2350GA with 2350GG generated a nonsignificant reduced risk, respectively. Under the dominant model, the ACE 2350A allele conferred a reduced hypertension risk and such associations were divergent between Han Chinese and Muslims from the Arab Gulf and Pakistan. Under the recessive model, this protective effect was totally reversed (OR = 1.01; 95% CI, 0.77-1.33; p = .94). Subgroup analyses indicated a significant protective effect of ACE 2350A compared with 2350G among Muslims from the Arab Gulf and Pakistan (OR = 0.55; 95% CI, 0.42-0.71; p < .00001). No publication biases were observed.
Conclusions: Our results demonstrate that the ACE 2350A allele is associated with a significantly reduced hypertension risk among Muslims from the Arab Gulf and Pakistan, yet an elevated risk among Han Chinese.

10.2 ADRB2 rs1042714 CC genotype is not associated with increased risk of hypertension.

Gjesing AP, et al. *No consistent effect of ADRB2 haplotypes on obesity, hypertension and quantitative traits of body fatness and blood pressure among 6,514 adult Danes*. PLoS One. 2009 Sep 25;4(9):e7206. doi: 10.1371/journal.pone.0007206.

Background: evidence regarding the association of variation within ADRB2, the gene encoding the beta-adrenergic receptor 2 (ADRB2) with obesity and hypertension is exceedingly ambiguous. Despite negative reports, functional impacts of individual genetic variants have been reported. Also, functional haplotypes as well as haplotype combinations affecting expression levels in vivo of ADRB2 mRNA and protein as well as receptor sensitivity have been reported. The aim of the present study was therefore to evaluate if variations within ADRB2 as haplotypes or as haplotype combinations confer an increased prevalence of obesity and hypertension among adults.
Methodology/principal findings: we genotyped five variants required to capture common variation in a region including the ADRB2 locus in a population-based study of 6,514 unrelated, middle-aged Danes. Phases of the genotypes were estimated in silico. The variations were then investigated for their combined association with obesity, hypertension and related quantitative traits. The present study did not find consistent evidence for an association of ADRB2 variants with either obesity or hypertension when variations were analysed in a case-control study. The same lack of impact was also seen in the quantitative trait analyses, apart from nominal differences on waist-to-hip ratio and systolic blood pressure between specific haplotype combinations.
Conclusions: in a population-based sample of 6,514 Danes we found no consistent associations between five common variants which tag the ADRB2 locus and prevalence of obesity or hypertension neither when analysed as individual haplotypes nor as haplotype pairs.

10.3 ADD1 rs4961 GG genotype is not associated with increased risk of hypertension.

Li Y, et al. *Cardiovascular risk in relation to alpha-adducin Gly460Trp polymorphism and systolic pressure: a prospective population study*. Hypertension. 2005 Sep;46(3):527-32.

Preliminary evidence from 1 case-control study suggested that in hypertensive patients, the alpha-adducin 460Trp allele might be associated with a 2-fold higher risk of coronary heart disease. In a prospective population study, we investigated whether the alpha-adducin Gly460Trp polymorphism predicted mortality and morbidity. From August 1985 until July 2003, we randomly recruited 2235 Belgian residents. We obtained information on vital status (until July 1, 2004) and the incidence of events via registries and repeat examinations (median 3). In Cox regression, before and after adjustment for other risk factors, we found strong interaction between systolic blood pressure at baseline, analyzed as a continuous variable, and the alpha-adducin polymorphism in relation to total (P=0.01) and cardiovascular mortality (P=0.007) and all cardiovascular (P=0.003), cardiac (P=0.001), and coronary events (P=0.03). The hazard ratio for total mortality associated with the Trp allele relative to GlyGly homozygosity was 2.30 (95% confidence interval, 1.12 to 4.72; P=0.02) in patients with stage-2 systolic hypertension (> or =160 mm Hg) and 0.88 (0.61 to 1.26; P=0.48) in the other participants. For all cardiovascular

complications, these estimates were 2.94 (1.28 to 6.74; P=0.01) and 0.83 (0.58 to 1.20; P=0.32), respectively. For all cardiovascular events, the positive predictive value and the attributable risk associated with the Trp allele in patients with stage-2 systolic hypertension were 76.9% and 44.3%, respectively. In conclusion, the alpha-adducin Gly460Trp polymorphism, in combination with systolic blood pressure, is a strong predictor of cardiovascular mortality and morbidity.

10.4 AGTR1 rs5186 is associated with increased hypertension risk.

Bonnardeaux A, et al. *Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension*. Hypertension. 1994 Jul;24(1):63-9.

We conducted the present study to determine whether the angiotensin II type I receptor (AT1) gene might be implicated in human essential hypertension by using case-control and linkage studies. The entire coding and 3' untranslated regions of the AT1 receptor gene (2.2 kb) were amplified by polymerase chain reaction and submitted to single-strand conformation polymorphism in 60 hypertensive subjects with a familial susceptibility. We identified five polymorphisms (T573-->C, A1062-->G, A1166-->C, G1517-->T, and A1878-->G). However, no mutations that alter the encoded amino acid sequence were detected. A case-control study performed on white hypertensive (n = 206; blood pressure, 168 +/- 16/103 +/- 9 mm Hg) and normotensive (n = 298; blood pressure, 122 +/- 10/75 +/- 9 mm Hg) subjects using three of five polymorphisms showed a significant increase in allelic frequency of C1166 in hypertensive subjects (0.36 versus 0.28 for normotensive subjects, chi 2 = 6.8, P < .01). Frequencies for the alleles of the other two polymorphisms (T573-->C, A1878-->G) were similar in both groups. We performed a linkage study using the affected sib pair method and a highly polymorphic marker of the AT1 receptor gene. There was no evidence for linkage in 267 sib pairs analyzed from 138 pedigrees. These findings would be compatible with a common variant of the AT1 receptor imparting a small effect on blood pressure; further studies will be needed to address this possibility.

10.5 RYR2 rs2820037 AT genotype, rs6997709 GG genotype, BCAT1 rs7961152 AA genotype and chr13:67461239 (GRCh38.p12) rs1937506 AA genotype are associated with an increased risk for high blood pressure.

Wellcome Trust Case Control Consortium. *Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls*. Nature. 2007 Jun 7;447(7145):661-78.

There is increasing evidence that genome-wide association (GWA) studies represent a powerful approach to the identification of genes involved in common human diseases. We describe a joint GWA study (using the Affymetrix GeneChip 500K Mapping Array Set) undertaken in the British population, which has examined approximately 2,000 individuals for each of 7 major diseases and a shared set of approximately 3,000 controls. Case-control comparisons identified 24 independent association signals at P < 5 x 10(-7): 1 in bipolar disorder, 1 in coronary artery disease, 9 in Crohn's disease, 3 in rheumatoid arthritis, 7 in type 1 diabetes and 3 in type 2 diabetes. On the basis of prior findings and replication studies thus-far completed, almost all of these signals reflect genuine susceptibility effects. We observed association at many previously identified loci, and found compelling evidence that some loci confer risk for more than one of the diseases studied. Across all diseases, we identified a large number of further signals (including 58 loci with single-point P values between 10(-5) and 5 x 10(-7)) likely to yield additional susceptibility loci. The importance of appropriately large samples was confirmed by the modest effect sizes observed at most loci identified. This study thus represents a thorough validation of the GWA approach. It has also demonstrated that careful use of a shared control group represents a safe and effective approach to GWA analyses of multiple disease phenotypes; has generated a genome-wide genotype database for future studies of common diseases in the British population; and shown that, provided individuals with non-European ancestry are excluded, the extent of population stratification in the British population is generally modest. Our findings offer new avenues for exploring the pathophysiology of these important disorders. We anticipate that our data, results and software, which will be widely available to other investigators, will provide a powerful resource for human genetics research.

10.6 AGTR2 rs5950584 TT genotype is associated with increased blood pressure.

Zhang Y, et al. *Angiotensin II type 2 receptor gene polymorphisms and essential hypertension*. Acta Pharmacol Sin. 2003 Nov;24(11):1089-93.

Aim: to identify the genetic variants of angiotensin II type 2 receptor (AT2R) gene in a Chinese population and to determine whether the AT2R gene polymorphisms are associated with essential hypertension (EH).
Methods: the detection of single nucleotide polymorphisms (SNPs) was performed in 19 subjects by a direct DNA sequencing. Two hundred fifty patients with EH and 250 normotensive controls were included in the study to assess the contribution of polymorphism of AT2R gene to hypertension.
Results: we identified 9 SNPs in the promoter, intron, exons and 3' untranslated region (3'UTR) of AT2R gene; among them 5 SNPs were novel molecular variants. A case-control study using a most frequent SNP (1334T/C) in the promoter region, showed a significant increase in allele frequency of C1334 in male hypertensive subjects (17.5 % vs 10.3 % for normotensive subjects, P<0.05).
Conclusion: the catalogue of SNPs of AT2R gene in Chinese population showed ethnic difference in DNA sequence variation. A polymorphism in the promoter region (1334T/C) of AT2R gene might be involved in the development of hypertension in Chinese population.

10.7 FGF5 rs1902859 CC genotype is associated with hypertension and increased risk of cardiovascular disease.

Takeuchi F, et al. *Interethnic analyses of blood pressure loci in populations of East Asian and European descent*. Nat Commun. 2018 Nov 28;9(1):5052. doi: 10.1038/s41467-018-07345-0.

Blood pressure (BP) is a major risk factor for cardiovascular disease and more than 200 genetic loci associated with BP are known. Here, we perform a multi-stage genome-wide association study for BP (max N = 289,038) principally in East Asians and meta-analysis in East Asians and Europeans. We report 19 new genetic loci and ancestry-specific BP variants, conforming to a common ancestry-specific variant association model. At 10 unique loci, distinct non-rare ancestry-specific variants colocalize within the same linkage disequilibrium block despite the significantly discordant effects for the proxy shared variants between the ethnic groups. The genome-wide transethnic correlation of causal-variant effect-sizes is 0.898 and 0.851 for systolic and diastolic BP, respectively. Some of the ancestry-specific association signals are also influenced by a selective sweep. Our results provide new evidence for the role of common ancestry-specific variants and natural selection in ethnic differences in complex traits such as BP.

11. Chron’s disease

11.1 There is a reported association of NOD2 rs2066847 with susceptibility to Crohn's disease.

Hugot JP, et al. *Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease*. Nature. 2001 May 31;411(6837):599-603.

Crohn's disease and ulcerative colitis, the two main types of chronic inflammatory bowel disease, are multifactorial conditions of unknown etiology. A susceptibility locus for Crohn's disease has been mapped to chromosome 16. Here we have used a positional-cloning strategy, based on linkage analysis followed by linkage disequilibrium mapping, to identify three independent associations for Crohn's disease: a frameshift variant and two missense variants of NOD2, encoding a member of the Apaf-1/Ced-4 superfamily of apoptosis regulators that is expressed in monocytes. These NOD2 variants alter the structure of either the leucine-rich repeat domain of the protein or the adjacent region. NOD2 activates nuclear factor NF-kB; this activating function is regulated by the carboxy-terminal leucine-rich repeat domain, which has an inhibitory role and also acts as an intracellular receptor for components of microbial pathogens. These observations suggest that the NOD2 gene product confers susceptibility to Crohn's disease by altering the recognition of these components and/or by over-activating NF-kB in monocytes, thus documenting a molecular model for the pathogenic mechanism of Crohn's disease that can now be further investigated.

11.2 rs12994997 AA genotype is associated with increased risk of Crohn disease, and inflammatory bowel disease.

Jimmy Z Liu, et al*. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations*. Nat Genet. 2015 Sep; 47(9): 979–986.

Ulcerative colitis and Crohn's disease are the two main forms of inflammatory bowel disease (IBD). Here, we report the first trans-ethnic association study of IBD, with genome-wide or Immunochip genotype data from an extended cohort of 86,640 European individuals and Immunochip data from 9,846 individuals of East-Asian, Indian or Iranian descent. We implicate 38 loci in IBD risk for the first time. For the majority of IBD risk loci, the direction and magnitude of effect is consistent in European and non-European cohorts. Nevertheless, we observe genetic heterogeneity between divergent populations at several established risk loci driven by a combination of differences in allele frequencies (NOD2), effect sizes (TNFSF15, ATG16L1) or a combination of both (IL23R, IRGM). Our results provide biological insights into the pathogenesis of IBD, and demonstrate the utility of trans-ethnic association studies for mapping complex disease loci and understanding genetic architecture across diverse populations.

11.3 In several European populations, the minor NOD2 allele, rs2076756(G), is associated with increased risk for Crohn's disease.

Franke A, et al. Sy*stematic association mapping identifies NELL1 as a novel IBD disease gene*. PLoS One. 2007 Aug 8;2(8):e691.

Crohn disease (CD), a sub-entity of inflammatory bowel disease (IBD), is a complex polygenic disorder. Although recent studies have successfully identified CD-associated genetic variants, these susceptibility loci explain only a fraction of the heritability of the disease. Here, we report on a multi-stage genome-wide scan of 393 German CD cases and 399 controls. Among the 116,161 single-nucleotide polymorphisms tested, an association with the known CD susceptibility gene NOD2, the 5q31 haplotype, and the recently reported CD locus at 5p13.1 was confirmed. In addition, SNP rs1793004 in the gene encoding nel-like 1 precursor (NELL1, chromosome 11p15.1) showed a consistent disease-association in independent German population- and family-based samples (942 cases, 1082 controls, 375 trios). Subsequent fine mapping and replication in an independent sample of 454 French/Canadian CD trios supported the authenticity of the NELL1 association. Further confirmation in a large German ulcerative colitis (UC) sample indicated that NELL1 is a ubiquitous IBD susceptibility locus (combined p<10(-6); OR = 1.66, 95% CI: 1.30-2.11). The novel 5p13.1 locus was also replicated in the French/Canadian sample and in an independent UK CD patient panel (453 cases, 521 controls, combined p<10(-6) for SNP rs1992660). Several associations were replicated in at least one independent sample, point to an involvement of ITGB6 (upstream), GRM8 (downstream), OR5V1 (downstream), PPP3R2 (downstream), NM_152575 (upstream) and HNF4G (intron).

11.4 IL23R rs11209026 is associated with inflammatory bowel disease.

Weersma RK, et al. *ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in the Netherlands*. Am J Gastroenterol. 2008 Mar;103(3):621-7. Epub 2007 Nov 28.

Background: inflammatory bowel disease (IBD)--Crohn's disease (CD) and ulcerative colitis (UC)--and celiac disease are intestinal inflammatory disorders with a complex genetic background. Recently, two novel genes were found to be associated with IBD susceptibility. One, an uncommon coding variant (rs11209026) in the gene

encoding for the interleukin-23 receptor (IL23R), conferred strong protection against CD. The other, rs2241880 in the autophagy-related 16-like 1 gene (ATG16L1), was associated with CD. We performed a case-control study for the association of IBD with IL23R and ATG16L1 in a Dutch cohort. We also looked at the association of IL23R and ATG16L1 with celiac disease.
Methods: five hundred eighteen Dutch white IBD patients (311 CD and 207 UC, including 176 trios of patients with both parents), 508 celiac disease patients, and 893 healthy controls were studied for association with the rs11209026 (IL23R) and rs2241880 (ATG16L1) single nucleotide polymorphisms (SNP).
Results: The rs11209026 SNP in IL23R had a protective effect for IBD in the case-control analysis (odds ratio [OR] 0.19, 95% confidence interval [CI] 0.10-0.37, P= 6.6E-09). Both CD (OR 0.14, CI 0.06-0.37, P= 3.9E-07) and UC (OR 0.33, CI 0.15-0.73, P= 1.4E-03) were associated with IL23R. For ATG16L1, the rs2241880 SNP was associated with CD susceptibility (OR 1.36, CI 1.12-1.66, P= 0.0017). The population-attributable risk of carrying allele G is 0.24 and is 0.19 for homozygosity for allele G in CD. No association was found between IL23R or ATG16L1 and celiac disease.
Conclusions: we confirmed the association of IL23R and ATG16L1 with CD susceptibility and also the association of IL23R with UC. We found IL23R and ATG16L1 were not associated with celiac disease susceptibility.

12. Gout (uric acid)

12.1 SLC16A9 rs734553, ABCG2 rs2231142, SLC22A11 rs17300741 and SLC2A9 rs4698014 can influence uric acid concentrations.

Kolz M, et al. *Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations*. PLoS Genet. 2009 Jun;5(6):e1000504. doi: 10.1371/journal.pgen.1000504.

Elevated serum uric acid levels cause gout and are a risk factor for cardiovascular disease and diabetes. To investigate the polygenetic basis of serum uric acid levels, we conducted a meta-analysis of genome-wide association scans from 14 studies totalling 28,141 participants of European descent, resulting in identification of 954 SNPs distributed across nine loci that exceeded the threshold of genome-wide significance, five of which are novel. Overall, the common variants associated with serum uric acid levels fall in the following nine regions: SLC2A9 (p = 5.2x10(-201)), ABCG2 (p = 3.1x10(-26)), SLC17A1 (p = 3.0x10(-14)), SLC22A11 (p = 6.7x10(-14)), SLC22A12 (p = 2.0x10(-9)), SLC16A9 (p = 1.1x10(-8)), GCKR (p = 1.4x10(-9)), LRRC16A (p = 8.5x10(-9)), and near PDZK1 (p = 2.7x10(-9)). Identified variants were analyzed for gender differences. We found that the minor allele for rs734553 in SLC2A9 has greater influence in lowering uric acid levels in women and the minor allele of rs2231142 in ABCG2 elevates uric acid levels more strongly in men compared to women. To further characterize the identified variants, we analyzed their association with a panel of metabolites. rs12356193 within SLC16A9 was associated with DL-carnitine (p = 4.0x10(-26)) and propionyl-L-carnitine (p = 5.0x10(-8)) concentrations, which in turn were associated with serum UA levels (p = 1.4x10(-57) and p = 8.1x10(-54), respectively), forming a triangle between SNP, metabolites, and UA levels. Taken together, these associations highlight additional pathways that are important in the regulation of serum uric acid levels and point toward novel potential targets for pharmacological intervention to prevent or treat hyperuricemia. In addition, these findings strongly support the hypothesis that transport proteins are key in regulating serum uric acid levels.

13. Risk of gallstones

13.1 The UGT1A1 Gilbert syndrome variant rs6742078 is associated with gallstone disease in men.

Buch S, et al. *Loci from a genome-wide analysis of bilirubin levels are associated with gallstone risk and composition*. Gastroenterology. 2010 Dec;139(6):1942-1951.e2. doi: 10.1053/j.gastro.2010.09.003. E

Background: genome-wide association studies have mapped loci that are associated with serum levels of bilirubin. Bilirubin is a major component of gallstones so we investigated whether these variants predict gallstone bilirubin content and overall risk for gallstones.
Methods: loci that were identified in a meta-analysis to attain a genome-wide significance level of a P value less than 1.0×10(-7) (UGT1A1, SLCO1B1, LST-3TM12, SLCO1A2) were analyzed in 1018 individuals with known gallstone composition. Gallstone risk was analyzed in 2606 German choleystecomized individuals and 1121 controls and was replicated in 210 cases and 496 controls from South America.
Results: by using the presence of bilirubin as a phenotype, variants rs6742078 (UGT1A1; P = .003), rs4149056 (SLCO1B1; P = .003), and rs4149000 (SLCO1A2; P = .015) were associated with gallstone composition. In regression analyses, only UGT1A1 and SLCO1B1 were independently retained in the model. UGT1A1 (rs6742078; P = .018) was associated with overall gallstone risk. In a sex-stratified analysis, only male carriers of rs6742078 had an increased risk for gallstone disease (P = 2.1×10(-7); odds ratio(recessive), 2.34; P(women) = .47). The sex-specific association of rs6742078 was confirmed in samples from South America (P(men) = .046; odds ratio(recessive), 2.19; P(women) = .96).
Conclusions: the UGT1A1 Gilbert syndrome variant rs6742078 is associated with gallstone disease in men; further studies are required regarding the sex-specific physiology of bilirubin and bile acid metabolism. Variants of ABCG8 and UGT1A1 are the 2 major risk factors for overall gallstone disease, they contribute a population attributable risk of 21.2% among men.

14. Intolerances

14.1 MCM6 rs4988235 and rs182549 are two SNPs that is associated with the primary haplotype associated with hypolactasia, more commonly known as lactose intolerance in European Caucasian populations.

Enattah NS, et al. *Identification of a variant associated with adult-type hypolactasia*. Nat Genet. 2002 Feb;30(2):233-7. Epub 2002 Jan 14.

Adult-type hypolactasia, also known as lactase non-persistence (lactose intolerance), is a common autosomal recessive condition resulting from the physiological decline in activity of the lactase-phlorizin hydrolase (LPH) in intestinal cells after weaning. LPH hydrolyzes lactose into glucose and galactose. Sequence analyses of the coding and promoter regions of LCT, the gene encoding LPH, has revealed no DNA variations correlating with lactase non-persistence. An associated haplotype spanning LCT, as well as a distinct difference in the transcript levels of 'non-persistence' and 'persistence' alleles in heterozygotes, suggest that a cis-acting element contributes to the lactase non-persistence phenotype. Using linkage disequilibrium (LD) and haplotype analysis of nine extended Finnish families, we restricted the locus to a 47-kb interval on 2q21. Sequence analysis of the complete region and subsequent association analyses revealed that a DNA variant, C/T-13910, roughly 14 kb upstream from the LCT locus, completely associates with biochemically verified lactase non-persistence in Finnish families and a sample set of 236 individuals from four different populations. A second variant, G/A-

22018, 8 kb telomeric to C/T-13910, is also associated with the trait in 229 of 236 cases. Prevalence of the C/T-13910 variant in 1,047 DNA samples is consistent with the reported prevalence of adult-type hypolactasia in four different populations. That the variant (C/T-13910) occurs in distantly related populations indicates that it is very old.

14.2 rs4713586, rs7775228 and rs2395182 are three HLA region SNPs used to tag the DQ2.2 haplotype in gs221. rs2187668 tags the tightly linked DQB1*0201 allele, which in turn is linked to DQA1*0501; together, these are known as the DQ2.5 haplotype. This is the most common haplotype associated with celiac disease in Europeans. rs4639334(A) is said to tag (i.e. identify the presence of) the HLA-DQ7 haplotype.

Monsuur AJ, et al. *Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms*. PLoS One. 2008 May 28;3(5):e2270. doi: 10.1371/journal.pone.0002270.

Background: the HLA genes, located in the MHC region on chromosome 6p21.3, play an important role in many autoimmune disorders, such as celiac disease (CD), type 1 diabetes (T1D), rheumatoid arthritis, multiple sclerosis, psoriasis and others. Known HLA variants that confer risk to CD, for example, include DQA1*05/DQB1*02 (DQ2.5) and DQA1*03/DQB1*0302 (DQ8). To diagnose the majority of CD patients and to study disease susceptibility and progression, typing these strongly associated HLA risk factors is of utmost importance. However, current genotyping methods for HLA risk factors involve many reactions, and are complicated and expensive. We sought a simple experimental approach using tagging SNPs that predict the CD-associated HLA risk factors.
Methodology: our tagging approach exploits linkage disequilibrium between single nucleotide polymorphism (SNPs) and the CD-associated HLA risk factors DQ2.5 and DQ8 that indicate direct risk, and DQA1*0201/DQB1*0202 (DQ2.2) and DQA1*0505/DQB1*0301 (DQ7) that attribute to the risk of DQ2.5 to CD. To evaluate the predictive power of this approach, we performed an empirical comparison of the predicted DQ types, based on these six tag SNPs, with those executed with current validated laboratory typing methods of the HLA-DQA1 and -DQB1 genes in three large cohorts. The results were validated in three European celiac populations.
Conclusion: using this method, only six SNPs were needed to predict the risk types carried by >95% of CD patients. We determined that for this tagging approach the sensitivity was >0.991, specificity >0.996 and the predictive value >0.948. Our results show that this tag SNP method is very accurate and provides an excellent basis for population screening for CD. This method is broadly applicable in European populations.

14.3 rs1800546 is linked to approximately 65% of hereditary fructosuria (also known as fructose intolerance or HFI) cases in those of European ancestry. rs76917243 usually considered the second most common ALDOB gene mutation after the most common one (rs1800546).

Cross NC, et al. *Catalytic deficiency of human aldolase B in hereditary fructose intolerance caused by a common missense mutation*. Cell. 1988 Jun 17;53(6):881-5.

Hereditary fructose intolerance (HFI) is a human autosomal recessive disease caused by a deficiency of aldolase B that results in an inability to metabolize fructose and related sugars. We report here the first identification of a molecular lesion in the aldolase B gene of an affected individual whose defective protein has previously been characterized. The mutation is a G----C transversion in exon 5 that creates a new recognition site for the restriction enzyme Ahall and results in an amino acid substitution (Ala----Pro) at position 149 of the protein within a region critical for substrate binding. Utilizing this novel restriction site and the polymerase chain

reaction, the patient was shown to be homozygous for the mutation. Three other HFI patients from pedigrees unrelated to this individual were found to have the same mutation: two were homozygous and one was heterozygous. We suggest that this genetic lesion is a prevailing cause of hereditary fructose intolerance.

15. Anemia

15.1 Patients with deficiencies in water-soluble B vitamins folate, vitamin B6, and vitamin B12 can develop anemia. rs4654748 polymorphisms are associated with lower vitamin B6 levels and rs602662 polymorphisms are associated with lower vitamin B12 levels.

Tanaka T, et al. *Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations*. Am J Hum Genet. 2009 Apr;84(4):477-82.

The B vitamins are components of one-carbon metabolism (OCM) that contribute to DNA synthesis and methylation. Homocysteine, a by-product of OCM, has been associated with coronary heart disease, stroke and neurological disease. To investigate genetic factors that affect circulating vitamin B6, vitamin B12, folate and homocysteine, a genome-wide association analysis was conducted in the InCHIANTI (N = 1175), SardiNIA (N = 1115), and BLSA (N = 640) studies. The top loci were replicated in an independent sample of 687 participants in the Progetto Nutrizione study. Polymorphisms in the ALPL gene (rs4654748, p = 8.30 × 10−18) were associated with vitamin B6 and FUT2 (rs6022662, p = 2.83 × 10−20) with vitamin B12 serum levels. The association of MTHFR, a gene consistently associated with homocysteine, was confirmed in this meta-analysis. The ALPL gene likely influences the catabolism of vitamin B6 while FUT2 interferes with absorption of vitamin B12. These findings highlight mechanisms that affect vitamin B6, vitamin B12 and homocysteine serum levels.

15.2 rs1801133 is a SNP that is relatively common that encodes a variant in the MTHFR gene, encoding an enzyme involved in folate metabolism.

Ganz AB, et al. *Genetic impairments in folate enzymes increase dependence on dietary choline for phosphatidylcholine production at the expense of betaine synthesis*. FASEB J. 2016 Oct;30(10):3321-3333.

Although single nucleotide polymorphisms (SNPs) in folate-mediated pathways predict susceptibility to choline deficiency during severe choline deprivation, it is unknown if effects persist at recommended intakes. Thus, we used stable isotope liquid chromatography-mass spectrometry (LC-MS) methodology to examine the impact of candidate SNPs on choline metabolism in a long-term, randomized, controlled feeding trial among pregnant, lactating, and nonpregnant (NP) women consuming 480 or 930 mg/d choline (22% as choline-d9, with d9 indicating a deuterated trimethyl amine group) and meeting folate-intake recommendations. Variants impairing folate metabolism, methylenetetrahydrofolate reductase (MTHFR) rs1801133, methionine synthase (MTR) rs1805087 [wild-type (WT)], MTR reductase (MTRR) rs1801394, and methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase-formyltetrahydrofolate synthetase (MTHFD1) rs2236225, influenced choline dynamics, frequently through interactions with reproductive state and choline intake, with fewer genotypic alterations observed among pregnant women. Women with these variants partitioned more dietary choline toward phosphatidylcholine (PC) biosynthesis via the cytidine diphosphate (CDP)-choline pathway at the expense of betaine synthesis even when use of betaine as a methyl donor was increased. Choline intakes of 930 mg/d restored partitioning of dietary choline between betaine and CDP-PC among NP (MTHFR rs1801133 and MTR rs1805087 WT) and lactating (MTHFD1 rs2236225) women with risk genotypes. Overall, our findings indicate that loss-of-function variants in folate-metabolizing enzymes strain cellular PC production, possibly via

impaired folate-dependent phosphatidylethanolamine-N-methyltransferase (PEMT)-PC synthesis and suggest that women with these risk genotypes may benefit from choline intakes exceeding current recommendations.

15.3 TF rs8177253 CC genotype is associated with average levels of transferrin levels.

Li J, et al. *Genome-wide admixture and association study of serum iron, ferritin, transferrin saturation and total iron binding capacity in African Americans*. Hum Mol Genet. 2015 Jan 15;24(2):572-81. doi:10.1093/hmg/ddu454.

Iron is an essential component of many important proteins and enzymes, including hemoglobin, which is responsible for carrying oxygen to the cells. African Americans (AAs) have a greater prevalence of iron deficiency compared with European Americans. We conducted genome-wide admixture-mapping and association studies for serum iron, serum ferritin, transferrin saturation (SAT) and total iron binding capacity (TIBC) in 2347 AAs participating in the Jackson Heart Study (JHS). Follow-up replication analyses for JHS iron-trait associated SNPs were conducted in 329 AA participants in the Healthy Aging in Neighborhoods of Diversity across the Life Span study (HANDLS). Higher estimated proportions of global African ancestry were significantly associated with lower levels of iron (P = 2.4 × 10(-5)), SAT (P = 0.0019) and TIBC (P = 0.042). We observed significant associations (P < 5 × 10(-8)) between serum TIBC levels and two independent SNPs around TF on chromosome 3, the first report of a genome-wide significant second independent signal in this region, and SNPs near two novel genes: HDGFL1 on chromosome 6 and MAF on chromosome 16. We also observed significant associations between ferritin levels and SNPs near GAB3 on chromosome X. We replicated our two independent associations at TF and our association at GAB3 in HANDLS. Our study provides evidence for both shared and unique genetic risk factors that are associated with iron-related measures in AAs. The top two variants in TF explain 11.2% of the total variation in TIBC levels in AAs after accounting for age, gender, body mass index and background ancestry.

16. Supplementation

16.1 The rs3811647 and rs4820268 variants in the TFR2 gene are implicated in the physiological regulation of serum iron levels.

Pichler I, et al. *Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels*. Hum Mol Genet. 2011 Mar 15;20(6):1232-40

The genetic determinants of variation in iron status are actively sought, but remain incompletely understood. Meta-analysis of two genome-wide association (GWA) studies and replication in three independent cohorts was performed to identify genetic loci associated in the general population with serum levels of iron and markers of iron status, including transferrin, ferritin, soluble transferrin receptor (sTfR) and sTfR-ferritin index. We identified and replicated a novel association of a common variant in the type-2 transferrin receptor (TFR2) gene with iron levels, with effect sizes highly consistent across samples. In addition, we identified and replicated an association between the HFE locus and ferritin and confirmed previously reported associations with the TF, TMPRSS6 and HFE genes. The five replicated variants were tested for association with expression levels of the corresponding genes in a publicly available data set of human liver samples, and nominally statistically significant expression differences by genotype were observed for all genes, although only rs3811647 in the TF gene survived the Bonferroni correction for multiple testing. In addition, we measured for the first time the effects of the common variant in TMPRSS6, rs4820268, on hepcidin mRNA in peripheral blood (n = 83 individuals) and on hepcidin levels in urine (n = 529) and observed an association in the same direction, though only borderline significant.

These functional findings require confirmation in further studies with larger sample sizes, but they suggest that common variants in TMPRSS6 could modify the hepcidin-iron feedback loop in clinically unaffected individuals, thus making them more susceptible to imbalances of iron homeostasis.

16.2 CASR rs1801725 GG genotype is associated with average plasma levels of Calcium.

Kapur K, et al. *Genome-wide meta-analysis for serum calcium identifies significantly associated SNPs near the calcium-sensing receptor (CASR) gene*. PLoS Genet. 2010 Jul 22;6(7):e1001035. doi: 10.1371/journal.pgen.1001035.

Calcium has a pivotal role in biological functions, and serum calcium levels have been associated with numerous disorders of bone and mineral metabolism, as well as with cardiovascular mortality. Here we report results from a genome-wide association study of serum calcium, integrating data from four independent cohorts including a total of 12,865 individuals of European and Indian Asian descent. Our meta-analysis shows that serum calcium is associated with SNPs in or near the calcium-sensing receptor (CASR) gene on 3q13. The top hit with a p-value of 6.3 x 10(-37) is rs1801725, a missense variant, explaining 1.26% of the variance in serum calcium. This SNP had the strongest association in individuals of European descent, while for individuals of Indian Asian descent the top hit was rs17251221 (p = 1.1 x 10(-21)), a SNP in strong linkage disequilibrium with rs1801725. The strongest locus in CASR was shown to replicate in an independent Icelandic cohort of 4,126 individuals (p = 1.02 x 10(-4)). This genome-wide meta-analysis shows that common CASR variants modulate serum calcium levels in the adult general population, which confirms previous results in some candidate gene studies of the CASR locus. This study highlights the key role of CASR in calcium regulation.

16.3 rs17251221 GG genotype is associated with calcium-sensing receptor gene (CASR), associated with higher serum calcium levels. The G allele of rs17251221 was also associated with higher serum magnesium levels.

O'Seaghdha CM, et al. *Common variants in the calcium-sensing receptor gene are associated with total serum calcium levels*. Hum Mol Genet. 2010 Nov 1;19(21):4296-303. doi: 10.1093/hmg/ddq342.

Serum calcium levels are tightly regulated. We performed genome-wide association studies (GWAS) in population-based studies participating in the CHARGE Consortium to uncover common genetic variations associated with total serum calcium levels. GWAS of serum calcium concentrations was performed in 20 611 individuals of European ancestry for ~2.5 million genotyped and imputed single-nucleotide polymorphisms (SNPs). The SNP with the lowest P-value was rs17251221 (P = 2.4 * 10(-22), minor allele frequency 14%) in the calcium-sensing receptor gene (CASR). This lead SNP was associated with higher serum calcium levels [0.06 mg/dl (0.015 mmol/l) per copy of the minor G allele] and accounted for 0.54% of the variance in serum calcium concentrations. The identification of variation in CASR that influences serum calcium concentration confirms the results of earlier candidate gene studies. The G allele of rs17251221 was also associated with higher serum magnesium levels (P = 1.2 * 10(-3)), lower serum phosphate levels (P = 2.8 * 10(-7)) and lower bone mineral density at the lumbar spine (P = 0.038), but not the femoral neck. No additional genomic loci contained SNPs associated at genome-wide significance (P < 5 * 10(-8)). These associations resemble clinical characteristics of patients with familial hypocalciuric hypercalcemia, an autosomal-dominant disease arising from rare inactivating mutations in the CASR gene. We conclude that common genetic variation in the CASR gene is associated with similar but milder features in the general population.

16.4 rs1570669 GG genotype is associated with correct assimilation and metabolization of calcium. rs1550532 CC and rs780094 TT genotype is associated with increased levels of calcium. rs7481584 AA genotype is associated decreased calcium levels

O'Seaghdha CM, et al. *Meta-analysis of genome-wide association studies identifies six new Loci for serum calcium concentrations*. PLoS Genet. 2013;9(9):e1003796. doi: 10.1371/journal.pgen.1003796.

Calcium is vital to the normal functioning of multiple organ systems and its serum concentration is tightly regulated. Apart from CASR, the genes associated with serum calcium are largely unknown. We conducted a genome-wide association meta-analysis of 39,400 individuals from 17 population-based cohorts and investigated the 14 most strongly associated loci in ≤ 21,679 additional individuals. Seven loci (six new regions) in association with serum calcium were identified and replicated. Rs1570669 near CYP24A1 (P = 9.1E-12), rs10491003 upstream of GATA3 (P = 4.8E-09) and rs7481584 in CARS (P = 1.2E-10) implicate regions involved in Mendelian calcemic disorders: Rs1550532 in DGKD (P = 8.2E-11), also associated with bone density, and rs7336933 near DGKH/KIAA0564 (P = 9.1E-10) are near genes that encode distinct isoforms of diacylglycerol kinase. Rs780094 is in GCKR. We characterized the expression of these genes in gut, kidney, and bone, and demonstrate modulation of gene expression in bone in response to dietary calcium in mice. Our results shed new light on the genetics of calcium homeostasis.

16.5 rs10766197 AA genotype is associated with average vitamin D levels and calcium absorption.

Zhang Z, et al. *An analysis of the association between the vitamin D pathway and serum 25-hydroxyvitamin D levels in a healthy Chinese population*. J Bone Miner Res. 2013 Aug;28(8):1784-92. doi: 10.1002/jbmr.1926.

Vitamin D deficiency has been recognized as a major public health issue worldwide. Recent studies have indicated that genetic factors might play an important role in determining serum 25-hydroxyvitamin D [25(OH)D] levels in Caucasians and African Americans. However, the genes that contribute to the variation in serum 25(OH)D levels in Chinese are unknown. In this study, we screened 15 key genes within the vitamin D metabolic pathway using 96 single-nucleotide polymorphism (SNP) markers in a group of 2897 unrelated healthy Chinese subjects. Significant confounding factors that may influence the variability in serum 25(OH)D levels were used as covariates for association analyses. An association test for quantitative traits was performed to evaluate the association between candidate genes and serum 25(OH)D levels. In the present study, variants and/or haplotypes in GC, CYP2R1, and DHCR7/NADSYN1 were identified as being associated with 25(OH)D levels. Participants with three or four risk alleles of the two variants (GC-rs4588 and CYP2R1-rs10766197) had an increased chance of presenting with a 25(OH)D concentration lower than 20 ng/mL (odds ratio 2.121, 95% confidence interval 1.586-2.836, p = 6.1 × 10(-8)) compared with those lacking the risk alleles. Each additional copy of a risk allele was significantly associated with a 0.12-fold decrease in the log-25(OH)D concentration (p = 3.7 × 10(-12)). Haplotype TGA of GC rs705117-rs2282679-rs1491710, haplotype GAGTAC of GC rs842999-rs705120-rs222040-rs4588-rs7041-rs10488854, haplotype CA of GC rs1155563-rs222029, and haplotype AAGA of CYP2R1 rs7936142-rs12794714-rs2060793-rs16930609 were genetic risk factors toward a lower 25(OH)D concentration. In contrast, haplotype TGGGCCC of DHCR7/NADSYN1 rs1790349-rs7122671-rs1790329-rs11606033-rs2276360-rs1629220-rs2282618 were genetic protective factors. The results suggest that the GC, CYP2R1, and DHCR7/NADSYN1 genes might contribute to variability in the serum 25(OH)D levels in a healthy Chinese population in Shanghai. These markers could be used as tools in Mendelian randomization analyses of vitamin D, and they could potentially be drug targets in the Chinese population in Shanghai.

16.6 HFE rs1800562 represents a SNP that accounts for ~85% of all cases of hemochromatosis.

Valenti L, et al. *The mitochondrial superoxide dismutase A16V polymorphism in the cardiomyopathy associated with hereditary haemochromatosis*. J Med Genet. 2004 Dec;41(12):946-50.
The A16V mitochondrial targeting sequence polymorphism influences the antioxidant activity of MnSOD, an enzyme involved in neutralising iron induced oxidative stress. Patients with hereditary haemochromatosis develop parenchymal iron overload, which may lead to cirrhosis, diabetes, hypogonadism, and heart disease. The objective of this study was to determine in patients with haemochromatosis whether the presence of the Val MnSOD allele, associated with reduced enzymatic activity, affects tissue damage, and in particular heart disease, as MnSOD knockout mice develop lethal cardiomyopathy. We studied 217 consecutive unrelated probands with haemochromatosis, and 212 healthy controls. MnSOD polymorphism was evaluated by restriction analysis. The frequency distribution of the polymorphism did not differ between patients and controls. Patients carrying the Val allele had higher prevalence of cardiomyopathy (A/A 4%, A/V 11%, V/V 30%, p = 0.0006) but not of cirrhosis, diabetes, or hypogonadism, independently of age, sex, alcohol misuse, diabetes, and iron overload (odds ratio 10.1 for V/V, p = 0.006). The frequency of the Val allele was higher in patients with cardiomyopathy (0.67 v 0.45, p = 0.003). The association was significant in both C282Y+/+ (p = 0.02), and in non-C282Y+/+ patients (p = 0.003), and for both dilated (p = 0.01) and non-dilated stage (p = 0.04) cardiomyopathy, but not for ischaemic heart disease. In patients with hereditary haemochromatosis, the MnSOD genotype affects the risk of cardiomyopathy related to iron overload and possibly to other known and unknown risk factors and could represent an iron toxicity modifier gene.

16.7 MUC1 rs4072037 CC genotype is associated with lower serum magnesium levels. ATP2B1 rs7965584 AA genotype, TRPM6 rs11144134 CC genotype, DCDC5 rs3925584 AA, SHROOM3 rs13146355 AA genotype are associated with normal metabolization of magnesium.

Meyer TE, et al. *Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels*. PLoS Genet. 2010 Aug 5;6(8). pii: e1001045. doi: 10.1371/journal.pgen.1001045.

Magnesium, potassium, and sodium, cations commonly measured in serum, are involved in many physiological processes including energy metabolism, nerve and muscle function, signal transduction, and fluid and blood pressure regulation. To evaluate the contribution of common genetic variation to normal physiologic variation in serum concentrations of these cations, we conducted genome-wide association studies of serum magnesium, potassium, and sodium concentrations using approximately 2.5 million genotyped and imputed common single nucleotide polymorphisms (SNPs) in 15,366 participants of European descent from the international CHARGE Consortium. Study-specific results were combined using fixed-effects inverse-variance alysis. SNPs demonstrating genome-wide significant ($p<5 \times 10(-8)$) or suggestive associations ($p<4 \times 10(-7)$) were evaluated for replication in an additional 8,463 subjects of European descent. The association of common variants at six genomic regions (in or near MUC1, ATP2B1, DCDC5, TRPM6, SHROOM3, and MDS1) with serum magnesium levels was genome-wide significant when meta-analyzed with the replication dataset. All initially significant SNPs from the CHARGE Consortium showed nominal association with clinically defined hypomagnesemia, two showed association with kidney function, two with bone mineral density, and one of these also associated with fasting glucose levels. Common variants in CNNM2, a magnesium transporter studied only in model systems to date, as well as in CNNM3 and CNNM4, were also associated with magnesium concentrations in this study. We observed no associations with serum sodium or potassium levels exceeding $p<4 \times 10(-7)$. Follow-up studies of newly implicated genomic loci may provide additional insights into the regulation and homeostasis of human serum magnesium levels.

16.8 ACE rs4343 AA genotype is associated with salt sensitivity, leading to a high increase in high blood pressure risk.

Gu D, et al. *Genetic variants in the renin-angiotensin-aldosterone system and salt sensitivity of blood pressure*. J Hypertens. 2010 Jun;28(6):1210-20.

Objective: to examine the association between renin-angiotensin-aldosterone system (RAAS) genes and salt sensitivity of blood pressure (BP).
Methods: a 7-day low-sodium dietary intervention followed by a 7-day high-sodium dietary intervention was conducted among 1906 participants living in a rural region of north China where habitual sodium intake is high. BP measurements were obtained at baseline and following each intervention using a random-zero sphygmomanometer.
Results: DBP and mean arterial pressure responses increased with the number of rs4524238 A alleles in the angiotensin II receptor type 1 gene. For example, mean DBP responses (95% confidence interval) among those with genotypes G/G, G/A, and A/A were -2.53 (-2.89 to -2.18), -3.49 (-4.13 to -2.86), and -5.78 (-9.51 to -2.06) mmHg, respectively, following the low-sodium intervention (P=0.0008). Carriers of the rare A allele of rs5479 in the hydroxysteroid (11-beta) dehydrogenase 2 gene had decreased DBP responses to low sodium (P=0.00004). Those with the C/A and C/C genotypes had DBP responses of -0.70 (-6.62 to 5.22) and -2.71 (-4.88 to -0.54) mmHg, respectively. X chromosome renin-binding protein gene markers rs1557501 and rs2269372 were associated with SBP response to low sodium in men (P=0.00004 and 0.0001, respectively). SBP responses (95% confidence interval) were -6.13 (-6.68 to -5.58) versus -4.07 (-4.88 to -3.26) and -6.04 (-6.57 to -5.52) versus -3.94 (-4.90 to -2.99) mmHg among men with major versus those with minor alleles of rs1557501 and rs2269372, respectively. Haplotype analyses of these genes supported our single-marker findings.
Conclusion: we identified renin-angiotensin-aldosterone system variants that were predictive of salt sensitivity in a Han population with habitually high-sodium intake.

16.9 AGA rs1395479 CC genotype and SLC39A11 rs891684 AA genotype are associated with lower serum selenium levels.

Gong J, et al. *Genome-wide association study of serum selenium concentrations*. Nutrients. 2013 May 21;5(5):1706-18. doi: 10.3390/nu5051706.

Selenium is an essential trace element and circulating selenium concentrations have been associated with a wide range of diseases. Candidate gene studies suggest that circulating selenium concentrations may be impacted by genetic variation; however, no study has comprehensively investigated this hypothesis. Therefore, we conducted a two-stage genome-wide association study to identify genetic variants associated with serum selenium concentrations in 1203 European descents from two cohorts: the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening and the Women's Health Initiative (WHI). We tested association between 2,474,333 single nucleotide polymorphisms (SNPs) and serum selenium concentrations using linear regression models. In the first stage (PLCO) 41 SNPs clustered in 15 regions had $p < 1 \times 10(-5)$. None of these 41 SNPs reached the significant threshold ($p = 0.05/15$ regions $= 0.003$) in the second stage (WHI). Three SNPs had $p < 0.05$ in the second stage (rs1395479 and rs1506807 in 4q34.3/AGA-NEIL3; and rs891684 in 17q24.3/SLC39A11) and had p between $2.62 \times 10(-7)$ and $4.04 \times 10(-7)$ in the combined analysis (PLCO + WHI). Additional studies are needed to replicate these findings. Identification of genetic variation that impacts selenium concentrations may contribute to a better understanding of which genes regulate circulating selenium concentrations.

17. Vitamin requirements

17.1 rs12934922 (R267S) and rs7501331 (A379V) double mutant have a reduced catalytic activity of beta-carotene by 57%. Female volunteers carrying the T variant of rs7501331 (379V) had a 32% lower ability to convert Beta-carotene, and those carrying at least one T in both SNPs show a 69% lower ability to convert Beta-carotene into retinyl esters.

Leung WC, et al. *Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15'-monoxygenase alter beta-carotene metabolism in female volunteers*. FASEB J. 2009 Apr;23(4):1041-53. doi: 10.1096/fj.08-121962.

The key enzyme responsible for beta-carotene conversion into retinal is beta-carotene 15,15'-monoxygenase (BCMO1). Since it has been reported that the conversion of beta-carotene into vitamin A is highly variable in up to 45% of healthy individuals, we hypothesized that genetic polymorphisms in the BCMO1 gene could contribute to the occurrence of the poor converter phenotype. Here we describe the screening of the total open reading frame of the BCMO1 coding region that led to the identification of two common nonsynonymous single nucleotide polymorphisms (R267S: rs12934922; A379V: rs7501331) with variant allele frequencies of 42 and 24%, respectively. In vitro biochemical characterization of the recombinant 267S + 379V double mutant revealed a reduced catalytic activity of BCMO1 by 57% (P<0.001). Assessment of the responsiveness to a pharmacological dose of beta-carotene in female volunteers confirmed that carriers of both the 379V and 267S + 379V variant alleles had a reduced ability to convert beta-carotene, as indicated through reduced retinyl palmitate:beta-carotene ratios in the triglyceride-rich lipoprotein fraction [-32% (P=0.005) and -69% (P=0.001), respectively] and increased fasting beta-carotene concentrations [+160% (P=0.025) and +240% (P=0.041), respectively]. Our data show that there is genetic variability in beta-carotene metabolism and may provide an explanation for the molecular basis of the poor converter phenotype within the population.

17.2 There is an association between the rs1279683 SLC23A2 gene variation and plasma vitamin C levels.

Zanon-Moreno V, et al. *Association between a SLC23A2 gene variation, plasma vitamin C levels, and risk of glaucoma in a Mediterranean population*. Mol Vis. 2011;17:2997-3004.

Purpose: several dietary factors have been associated with glaucoma. Among them, dietary antioxidant intake (i.e., vitamin C and vitamin A) in association with glaucoma has been analyzed, but with mixed results. Genetic factors may play a role in modulating the effect of dietary antioxidant intake on glaucoma; however, nutrigenetic studies in this field are scarce. Our aim was to study the association between selected polymorphisms in key proteins related to vitamin C and vitamin A concentrations and primary open-angle glaucoma (POAG).
Methods: we performed a case-control study matched for age, sex, and bodyweight. We recruited 300 subjects (150 POAG cases and 150 controls) from a Mediterranean population and determined the plasma concentrations of vitamin C and vitamin A for each subject. We selected the following single-nucleotide polymorphisms (SNPs) in genes related to vitamin A and vitamin C concentrations: rs176990 and rs190910 in the retinol-binding protein 1 (RBP1) gene; and rs10063949 and rs1279683 in the Na^+-dependent L-ascorbic acid transporters 1 and 2, respectively (encoded by the SLC23A1 and SLC23A2 genes).
Results: we found a statistically significant association between the rs1279386 (A>G) SNP in SLC23A2 and POAG risk. In the crude analysis, homozygous subjects for the G allele (GG subjects) had higher risk of POAG than other genotypes (OR: 1.67; 95% CI: 1.03-2.71). This association remained statistically significant (p=0.010) after multivariate adjustment for potential confounders. We also found that POAG patients had lower plasma vitamin C concentrations than control subjects (9.9±1.7 µg/ml versus 11.7±1.8 µg/ml, p<0.001). Moreover, we consistently detected a significant association between the rs1279386 SNP in SLC23A2 and plasma vitamin C concentrations: GG subjects had significantly lower plasma vitamin C concentrations than the other genotypes (9.0±1.4 µg/ml versus 10.5±1.6 µg/ml, p<0.001 in POAG cases and 10.9±1.6 µg/ml versus 12.1±1.8 µg/ml, p<0.001 in controls). The rs10063949 SNP in SLC23A1 was not associated with either plasma vitamin C concentrations or POAG risk. Similarly, SNPs in RBP1 were not associated with vitamin A concentrations or POAG risk.

Conclusions: the rs1279683 SNP in SLC23A2 was significantly associated with lower plasma concentrations of vitamin C and with higher risk of POAG in GG subjects.

17.3 rs33972313 SNP at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C).

Timpson NJ, et al. *Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): evidence from 5 independent studies with >15,000 participants*. Am J Clin Nutr. 2010 Aug;92(2):375-82. doi: 10.3945/ajcn.2010.29438.

Background: L-ascorbic acid is an essential part of the human diet and has been associated with a wide range of chronic complex diseases, including cardiovascular outcomes. To date, there are no confirmed genetic correlates of circulating concentrations of L-ascorbic acid.
Objective: we aimed to confirm the existence of an association between common variation at the SLC23A1 gene locus and circulating concentrations of L-ascorbic acid.
Design: we used a 2-stage design, which included a discovery cohort (the British Women's Heart and Health Study), a series of follow-up cohorts, and meta-analysis (totaling 15,087 participants) to assess the relation between variation at SLC23A1 and circulating concentrations of L-ascorbic acid.
Results: in the discovery cohort, variation at rs33972313 was associated with a reduction in circulating concentrations of L-ascorbic acid (-4.15 micromol/L; 95% CI: -0.49, -7.81 micromol/L; P = 0.03 reduction per minor allele). Pooled analysis of the relation between rs33972313 and circulating L-ascorbic acid across all studies confirmed this and showed that each additional rare allele was associated with a reduction in circulating concentrations of L-ascorbic acid of -5.98 micromol/L (95% CI: -8.23, -3.73 micromol/L; P = 2.0 x 10(-7) per minor allele).
Conclusions: a genetic variant (rs33972313) in the SLC23A1 vitamin C active transporter locus was identified that is reliably associated with circulating concentrations of L-ascorbic acid in the general population. This finding has implications more generally for the epidemiologic investigation of relations between circulating L-ascorbic acid and health outcomes.

17.4 rs2282679, located in the group-specific component (vitamin D binding protein) GC gene on chromosome 4p12, has been linked to vitamin D serum concentrations. rs12785878 has been linked by several studies to vitamin D serum concentrations. rs10741657(G) is associated with lower vitamin D and potential vitamin D insufficiency.

Wang TJ, et al. *Common genetic determinants of vitamin D insufficiency: a genome-wide association study*. Lancet. Volume 376, Issue 9736, 17–23 July 2010, 180-188.

Background: vitamin D is crucial for maintenance of musculoskeletal health, and might also have a role in extraskeletal tissues. Determinants of circulating 25-hydroxyvitamin D concentrations include sun exposure and diet, but high heritability suggests that genetic factors could also play a part. We aimed to identify common genetic variants affecting vitamin D concentrations and risk of insufficiency.
Methods: we undertook a genome-wide association study of 25-hydroxyvitamin D concentrations in 33 996 individuals of European descent from 15 cohorts. Five epidemiological cohorts were designated as discovery cohorts (n=16 125), five as in-silico replication cohorts (n=9367), and five as de-novo replication cohorts (n=8504). 25-hydroxyvitamin D concentrations were measured by radioimmunoassay, chemiluminescent assay, ELISA, or mass spectrometry. Vitamin D insufficiency was defined as concentrations lower than 75 nmol/L or 50 nmol/L. We combined results of genome-wide analyses across cohorts using Z-score-weighted meta-analysis. Genotype scores were constructed for confirmed variants.

Findings: variants at three loci reached genome-wide significance in discovery cohorts for association with 25-hydroxyvitamin D concentrations, and were confirmed in replication cohorts: 4p12 (overall p=1·9×10 −109 for rs2282679, in GC); 11q12 (p=2·1×10 −27 for rs12785878, near DHCR7); and 11p15 (p=3·3×10 −20 for rs10741657, near CYP2R1). Variants at an additional locus (20q13, CYP24A1) were genome-wide significant in the pooled sample (p=6·0×10 −10 for rs6013897). Participants with a genotype score (combining the three confirmed variants) in the highest quartile were at increased risk of having 25-hydroxyvitamin D concentrations lower than 75 nmol/L (OR 2·47, 95% CI 2·20–2·78, p=2·3×10 −48) or lower than 50 nmol/L (1·92, 1·70–2·16, p=1·0×10 −26) compared with those in the lowest quartile.
Interpretation: variants near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status. Genetic variation at these loci identifies individuals who have substantially raised risk of vitamin D insufficiency.

17.5 Levels of 25-hydroxyvitamin D in familial longevity: the Leiden Longevity Study concluded that familial longevity was associated with a lower vitamin D levels and a lower frequency of the allelic variation rs2060793 in the CYP2R1 gene.

Noordam R, et al. *Levels of 25-hydroxyvitamin D in familial longevity: the Leiden Longevity Study*. CMAJ. 2012 Dec 11;184(18):E963-8. doi: 10.1503/cmaj.120233.

Background: low levels of 25(OH) vitamin D are associated with various age-related diseases and mortality, but causality has not been determined. We investigated vitamin D levels in the offspring of nonagenarians who had at least one nonagenarian sibling; these offspring have a lower prevalence of age-related diseases and a higher propensity to reach old age compared with their partners.
Methods: we assessed anthropometric characteristics, 25(OH) vitamin D levels, parathyroid hormone levels, dietary vitamin D intake and single nucleotide polymorphisms (SNPs) associated with vitamin D levels. We included offspring (n = 1038) of nonagenarians who had at least one nonagenarian sibling, and the offsprings' partners (n = 461; controls) from the Leiden Longevity Study. We included age, sex, body mass index, month during which blood sampling was performed, dietary and supplemental vitamin D intake, and creatinine levels as possible confounding factors.
Results: the offspring had significantly lower levels of vitamin D (64.3 nmol/L) compared with controls (68.4 nmol/L; p = 0.002), independent of possible confounding factors. There was no difference in the levels of parathyroid hormone between groups. Compared with controls, the offspring had a lower frequency of a genetic variant in the CYP2R1 gene (rs2060793) (p = 0.04). The difference in vitamin D levels between offspring and controls persisted over the 2 most prevalent genotypes of this SNP.
Interpretation: compared with controls, the offspring of nonagenarians who had at least one nonagenarian sibling had a reduced frequency of a common variant in the CYP2R1 gene, which predisposes people to high vitamin D levels; they also had lower levels of vitamin D that persisted over the 2 most prevalent genotypes. These results cast doubt on the causal nature of previously reported associations between low levels of vitamin D and age-related diseases and mortality.

17.6 There are reported associations between common variants in GC and DHCR7/NADSYN1 rs3829251 and vitamin D concentration in Chinese Hans.

Lu L, et al. *Associations between common variants in GC and DHCR7/NADSYN1 and vitamin D concentration in Chinese Hans*. Hum Genet. 2012 Mar;131(3):505-12. doi: 10.1007/s00439-011-1099-1.

Recent studies have identified common variants in or near GC, CYP2R1 and NADSYN1/DHCR7 to be associated with 25-hydroxyvitamin D [25(OH)D] levels in European populations. We aimed to examine whether these variants also influence 25(OH)D levels in Chinese. Seven common variants were successfully genotyped and tested for associations with plasma 25(OH)D levels in a population-based cohort of 3,210 Chinese Hans from Beijing and Shanghai. Six common variants at GC (rs4588, rs7041, rs2282679 and rs1155563) and NADSYN1/DHCR7 (rs3829251 and rs1790349) loci were all significantly associated with lower plasma 25(OH)D levels ($-0.036 \leq \beta \leq -0.076$ per risk-allele, $P \leq 5.7 \times 10(-5)$), while CYP2R1-rs2060793 showed a trend toward association with 25(OH)D levels in the Shanghai subpopulation ($P = 0.08$), but not in the Beijing subpopulation ($P = 0.82$). Haplotype-based association analyses of the four GC variants showed that only the haplotype that contained all risk-alleles (TACC) was significantly associated with lower plasma 25(OH)D levels ($\beta = -0.085$, $P = 2.3 \times 10(-9)$), while the haplotype containing the risk-alleles of rs4588 and rs2282679 (TATC) was marginally associated with lower 25(OH)D levels ($\beta = -0.054$, $P = 0.0562$) when compared with GCTA haplotype carrying the four protective alleles. Most notably, conditional analyses showed that only GC-rs4588 and GC-rs2282679 (r (2) = 0.97) remained significantly associated with 25(OH)D concentrations ($P \leq 1.9 \times 10(-5)$) after adjusting for the other two SNPs in GC. In conclusion, GC and NADSYN1/DHCR7 loci individually and collectively contribute to variation in plasma vitamin D levels in Chinese Hans.

17.7 rs12272004 is associated with alpha-tocopherol levels.

Ferrucci L, et al. *Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study*. Am J Hum Genet. 2009 Feb;84(2):123-33. doi: 10.1016/j.ajhg.2008.12.019.

Low plasma levels of carotenoids and tocopherols are associated with increased risk of chronic disease and disability. Because dietary intake of these lipid-soluble antioxidant vitamins is only poorly correlated with plasma levels, we hypothesized that circulating carotenoids (vitamin A-related compounds) and tocopherols (vitamin E-related compounds) are affected by common genetic variation. By conducting a genome-wide association study in a sample of Italians (n = 1190), we identified novel common variants associated with circulating carotenoid levels and known lipid variants associated with alpha-tocopherol levels. Effects were replicated in the Women's Health and Aging Study (n = 615) and in the alpha-Tocopherol, beta-Carotene Cancer Prevention (ATBC) study (n = 2136). In meta-analyses including all three studies, the G allele at rs6564851, near the beta-carotene 15,15'-monooxygenase 1 (BCMO1) gene, was associated with higher beta-carotene ($p = 1.6 \times 10(-24)$) and alpha-carotene ($p = 0.0001$) levels and lower lycopene (0.003), zeaxanthin ($p = 1.3 \times 10(-5)$), and lutein ($p = 7.3 \times 10(-15)$) levels, with effect sizes ranging from 0.10-0.28 SDs per allele. Interestingly, this genetic variant had no significant effect on plasma retinol ($p > 0.05$). The SNP rs12272004, in linkage disequilibrium with the S19W variant in the APOA5 gene, was associated with alpha-tocopherol (meta-analysis $p = 7.8 \times 10(-10)$) levels, and this association was substantially weaker when we adjusted for triglyceride levels ($p = 0.002$). Our findings might shed light on the controversial relationship between lipid-soluble antioxidant nutrients and human health.

17.8 The rs964184 variant allele, associated with increased alpha-tocopherol, has also been associated with decreased circulating HDL cholesterol and increased triglyceride concentrations

Jacqueline M, et al. *Genome-wide association study identifies common variants associated with circulating vitamin E levels*. Hum Mol Genet. 2011 Oct 1; 20(19): 3876–3883.

In genome-wide association studies (GWAS) of common genetic variants associated with circulating alpha- and gamma-tocopherol concentrations in two adult cohorts comprising 5006 men of European descent, we observed three loci associated with alpha-tocopherol levels, two novel single-nucleotide polymorphisms (SNPs), rs2108622 on 19pter-p13.11 (P= 1.7 × 10−8) and rs11057830 on 12q24.31 (P= 2.0 × 10−8) and confirmed a previously reported locus marked by rs964184 on 11q23.3 (P= 2.7 × 10−10). The three SNPs have been reported to be associated with lipid metabolism and/or regulation. We replicated these findings in a combined meta-analysis with two independent samples, P= 7.8 × 10−12 (rs964184 on 11q23.3 near BUD13, ZNF259 and APOA1/C3/A4/A5), P= 1.4 × 10−10 (rs2108622 on 19pter-p13.11 near CYP4F2) and P= 8.2 × 10−9 (rs11057830 on 12q24.31 near SCARB1). Combined, these SNPs explain 1.7% of the residual variance in log alpha-tocopherol levels. In one of the two male GWAS cohorts (n= 992), no SNPs were significantly associated with gamma-tocopherol concentrations after including data from the replication sample for 71 independent SNPs with P< 1 × 10−4 identified.

17.9 The rs6994076 polymorphism was significantly associated with plasma vitamin E.

Zanon-Moreno V, et al. *Effects of polymorphisms in vitamin E-, vitamin C-, and glutathione peroxidase-related genes on serum biomarkers and associations with glaucoma*. Mol Vis. 2013;19:231-42. Epub 2013 Feb 3.

Purpose: to study the association of selected polymorphism in genes related to vitamin E, vitamin C, and glutathione peroxidase with these biomarkers and primary open-angle glaucoma (POAG) risk.
Methods: a case-control study matched for age, sex, and bodyweight was undertaken. Two hundred fifty POAG cases and 250 controls were recruited from a Mediterranean population. Plasma concentrations of vitamin C, vitamin E, and glutathione peroxidase (GPx) activity were measured. We analyzed the polymorphisms rs1279683 in the Na(+)-dependent L-ascorbic acid transporter 2 (SLC23A2) gene, rs6994076 in the tocopherol alpha transfer protein (TTPA) gene, rs737723 in the tocopherol-associated protein (SEC14L2/TAP) gene, and rs757228 in the glutathione peroxidase 4 (GPX4) gene. We also analyzed expression of the SLC23A2 gene in a subsample.
Results: we found a novel association between the rs737723 polymorphism and POAG risk. Homozygous subjects for the C allele had a higher POAG risk than carriers of the ancestral G allele (adjusted odds ratio 1.73, 95% confidence interval 1.13-2.65, p=0.011). This association remained statistically significant after adjustment for multiple comparisons. We also confirmed the association between the rs1279683 polymorphism and a higher POAG risk in GG homozygous subjects and detected statistically significant differences in SLC23A2 gene expression between POAG cases and controls, even after adjustment for multiple testing. We observed a nominally significant (p<0.05) gene-gene interaction between the SEC14L2/TAP and SLC23A2 polymorphisms in determining POAG risk, increasing POAG risk in those subjects who had both risk genotypes at the same time (p<0.01). This increase was statistically significant even after adjustment for multiple comparisons. We did not detect any association with POAG risk for the rs6994076 or rs757228 polymorphisms. We also found that POAG patients had statistically significant (after correction for multiple testing) lower plasma vitamin E (p<0.001) and vitamin C (p<0.001) concentrations than control subjects. However, we detected a higher plasma GPx activity in POAG cases than in controls (p<0.001). The rs6994076 and rs1279683 polymorphisms were significantly

(p<0.001) associated with plasma vitamin E and vitamin C, respectively. However, the rs757228 polymorphism in the GPX4 gene was not associated with plasma GPx activity.
Conclusions: we have described a novel association between the rs737723 polymorphism (SEC14L2/TAP) and higher POAG risk and confirmed the association between rs1279683 (SLC23A2) and POAG. Our results also suggested a gene-gene interaction between both polymorphisms that increases POAG risk.

18. Detox ability

18.1 GPx1P1 rs1050450 TT genotype is associated with lower capacity of detoxifying hydrogen peroxide.

Tang TS, et al. *Association between the rs1050450 glutathione peroxidase-1 (C > T) gene variant and peripheral neuropathy in two independent samples of subjects with diabetes mellitus*. Nutr Metab Cardiovasc Dis. 2012 May;22(5):417-25. doi: 10.1016/j.numecd.2010.08.001.

Glutathione peroxidase-1 (GPx-1) is an endogenous anti-oxidant enzyme. The T allele of the GPx-1 rs1050450 (C > T) gene variant is associated with reduced enzyme activity. Our aim was to examine the association between this gene variant and peripheral neuropathy in two cross-sectional samples of subjects with diabetes: (i) 773 Caucasian subjects were genotyped from the UCL Diabetes and Cardiovascular disease Study (UDACS) and (ii) 382 Caucasian subjects from the Ealing Diabetes Study (EDS). Peripheral neuropathy status (and oxidised-LDL [Ox-LDL:LDL] and plasma Total Ant-ioxidant Status [TAOS] in UDACS), were analysed in relation to genotype. We observed that: (i) In UDACS, the odds ratio (OR) for peripheral neuropathy in the T allele carriers compared to the CC genotype was 1.61 [1.10-2.28], p = 0.01. This remained significant after adjustment for other risk factors. Ox-LDL:LDL ratio was significantly elevated in T allele carriers (CC vs. CT/TT: 16.3 ± 2.4 v 18.0 ± 2.9 U/mmol LDL, p = 0.02). (ii) In EDS, the OR for peripheral neuropathy in the T allele carriers compared to the CC genotype was 1.95 [1.11-3.42], p = 0.02. This remained significant after adjustment for other risk factors. In conclusion, we observed a significant association between the T allele and peripheral neuropathy and LDL oxidation. This is the first paper to examine the rs1050450 variant in two samples of Caucasian subjects with diabetes. Prospective analysis of the gene variant is required in diabetic and healthy cohorts with measured plasma markers of oxidative stress to investigate the described association further.

18.2 GG genotype is associated with reduced GSTP1 activity GG genotype is associated with reduced GSTP1 activity.

Moyer AM, et al. *Glutathione s-transferase p1: gene sequence variation and functional genomic studies*. Cancer Res. 2008 Jun 15;68(12):4791-801. doi: 10.1158/0008-5472.CAN-07-6724.

Glutathione S-transferase P1 (GSTP1) is of importance for cancer research because of its role in detoxifying carcinogens, activating antineoplastic prodrugs, metabolizing chemotherapeutic agents, and its involvement in cell cycle and apoptosis regulation. Two common GSTP1 genetic polymorphisms have been studied extensively. However, the full range of GSTP1 genetic variation has not been systematically characterized in the absence of disease pathology. We set out to identify common GSTP1 polymorphisms in four ethnic groups, followed by functional genomic studies. All exons, splice junctions, and the 5'-flanking region of GSTP1 were resequenced using 60 DNA samples each from four ethnic groups. The 35 single-nucleotide polymorphisms (SNP) identified included six nonsynonymous SNPs and 17 previously unreported polymorphisms. GSTP1 variant allozymes were then expressed in COS-1 cells, and five displayed significantly altered levels of enzyme activity. One decreased to 22% of the wild-type (WT) activity. Four variant allozymes had K(m) values that differed significantly from that of the WT, and five showed altered levels of immunoreactive protein compared with WT,

with a significant correlation (r = 0.79, P < 0.007) between levels of immunoreactive protein and enzyme activity in these samples. In the Mexican American population, five linked SNPs were significantly associated with GSTP1 mRNA expression, one of which was found by electrophoretic mobility shift assay to alter protein binding. These studies have identified functionally significant genetic variation, in addition to the two frequently studied GSTP1 nonsynonymous SNPs, that may influence GSTP1's contribution to carcinogen and drug metabolism, and possibly disease pathogenesis and/or drug response.

18.3 SULT1A1*1/2 rs9282861 GG genotype is associated with reduced SULT1A activity.

Hebbring SJ, et al. *Sulfotransferase gene copy number variation: pharmacogenetics and function*. Cytogenet Genome Res. 2008;123(1-4):205-10. doi: 10.1159/000184710.

Pharmacogenetics is the study of the role of inheritance in variation to drug response. Drug response phenotypes can vary from adverse drug reactions at one end of the spectrum to equally serious lack of the desired effect of drug therapy at the other. Many of the current important examples of pharmacogenetics involve inherited variation in drug metabolism. Sulfate conjugation catalyzed by cytosolic sulfotransferase (SULT) enzymes, particularly SULT1A1, is a major pathway for drug metabolism in humans. Pharmacogenetic studies of SULT1A1 began over a quarter of a century ago and have advanced from biochemical genetic experiments to include cDNA and gene cloning, gene resequencing, and functional studies of the effects of single nucleotide polymorphisms (SNPs). SNP genotyping, in turn, led to the discovery of functionally important copy number variations (CNVs) in the SULT1A1 gene. This review will briefly describe the evolution of our understanding of SULT1A1 pharmacogenetics and CNV, as well as challenges involved in utilizing both SNP and CNV data in an attempt to predict SULT1A1 function. SULT1A1 represents one example of the potential importance of CNV for the evolving disciplines of pharmacogenetics and pharmacogenomics.

18.4 SOD2 rs4880 is associated with SOD2 increased/decreased activity.

Bastaki M, et al. *Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans*. Pharmacogenet Genomics. 2006 Apr;16(4):279-86.

Objectives: this study examined the association between genetic polymorphisms and enzyme activity for antioxidant enzymes that share a common detoxification pathway: manganese superoxide dismutase (MnSOD), glutathione peroxidase-1 (GPX1) and catalase.
Methods: MnSOD, GPX1, and catalase activities were measured in isolated erythrocytes of 231 healthy, non-smoking student volunteers (55% women, ages 17-21, majority Asian or Caucasian). DNA from blood clots was genotyped by Taqman PCR (C47T : MnSOD and C593T : GPX1) and standard PCR (-262C>T : catalase). Associations between genotype and enzyme activity were analyzed by multiple linear regression, adjusted for baseline factors including gender and ethnicity.
Results: minor allele frequencies ranged from 13% for catalase (T) to 18% for GPX1 (T), and 33% for MnSOD(C) with significant variation between ethnicities. Median GPX1 activity was 13.2 U/g Hb with a six-fold difference between lowest and highest levels. Catalase activity ranged eight-fold (median: 86.3 k/g Hb), while median MnSOD activity was 2.8 U/mg Hb with a 56-fold range of values. MnSOD enzyme activity was 15% higher in females than males (95%CI : -1%, 32%), and 33% higher in CT or TT individuals (C47T) versus CC individuals (95%CI : 7-59%). On average, catalase activity was 18.1 k/g Hb lower for TT subjects (-262C>T) versus CC subjects (95% CI: -32.3, -4.0). All enzyme activities were correlated (r=0.3-0.4, P<0.001).
Conclusions: interindividual variability of antioxidant enzyme activity in healthy young adults was partially explained by significant associations with three known genetic polymorphisms and was further modified by

gender and ethnicity. A substantial component of this variability may be attributable to differences in diet, environmental exposures, and additional genetic factors.

18.5 COMT rs4680 AA genotype is associated with reduced COMT enzyme activity, leading to reduced neurotransmitters and catechol estrogens breakdown, high levels of dopamine and lower estrogen metabolism (increased estradiol levels).

Stein DJ, et al. *Warriors versus worriers: the role of COMT gene variants.* CNS Spectr. 2006 Oct;11(10):745-8.

Behavioral phenotypes are generally complex, reflecting the action of multiple different genes. Nevertheless, there is growing evidence that key gene variants can alter activity within specific neuronal circuits and, therefore, influence particular cognitive-affective phenomena. One example is the catechol-O-methyltransferase (COMT) gene, which has a common variant at codon 158. Those with valine (Val158) alleles have increased greater COMT activity and lower prefrontal extracellular dopamine compared with those with the methionine (Met158) substitution. Val158 alleles may be associated with an advantage in the processing of aversive stimuli (warrior strategy), while Met158 alleles may be associated with an advantage in memory and attention tasks (worrier strategy). Under conditions of increased dopamine release (eg, stress), individuals with Val158 alleles may have improved dopaminergic transmission and better performance, while individuals with Met158 alleles may have less efficient neurotransmission and worse performance. Some evidence suggests that Val158 alleles are associated with schizophrenia, while Met158 alleles are associated with anxiety.

18.6 CYP1A1 rs4646903 CC genotype is associated with an accelerated CYP1A1 enzyme activity, decreased xenobiotic metabolism capacity, increased accumulation of carcinogenic products and cellular DNA damage.

Wright CM, et al. *Genetic association study of CYP1A1 polymorphisms identifies risk haplotypes in nonsmall cell lung cancer*. Eur Respir J. 2010 Jan;35(1):152-9. doi: 10.1183/09031936.00120808.

Lung cancer remains a leading cause of disease globally, with smoking being the largest single cause. Phase I enzymes, including cytochrome P(450), family 1, subfamily A, polypeptide 1 (CYP1A1), are involved in the activation of carcinogens, such as polycyclic aromatic hydrocarbons, to reactive intermediates that are capable of binding covalently to DNA to form DNA adducts, potentially initiating the carcinogenic process. The aim of the present study was to investigate the association of CYP1A1 gene polymorphisms and haplotypes with lung cancer risk. A case-control study was carried out on 1,040 nonsmall cell lung cancer (NSCLC) cases and 784 controls to investigate three CYP1A1 variants, CYP1A1*2A (rs4646903; thymidine to cytosine substitution at nucleotide 3801 (3801T>C)), CYP1A1*2C (rs1048943; 2455A>G; substitution of isoleucine 462 with valine (exon 7)) and CYP1A1*4 (rs1799814; 2453C>A; substitution of threonine 461 with asparagine (exon 7)) using PCR restriction fragment length polymorphism methods. The CYP1A1*2A and CYP1A1*2C variants were significantly over-represented in NSCLC cases compared with controls, whereas the CYP1A1*4 variant was under-represented. CYP1A1 haplotypes (in allele order CYP1A1*4, CYP1A1*2C, CYP1A1*2A) CGC and CGT were associated with an increased risk of lung cancer, whereas AAT was associated with decreased lung cancer risk in this population. The present study has identified risk haplotypes for CYP1A1 in NSCLC and confirmed that CYP1A1 polymorphisms are a minor risk factor for NSCLC.

18.7 CYP1B1 rs1056836 GG genotype is associated with increased CYP1B1 enzyme activity, related with higher ROS levels and higher cellular damage.

Gold LS, et al. *Associations of common variants in genes involved in metabolism and response to exogenous chemicals with risk of multiple myeloma*. Cancer Epidemiol. 2009 Oct;33(3-4):276-80. doi: 10.1016/j.canep.2009.08.005.

Background: we examined risk of multiple myeloma (MM) associated with variants in genes involved in metabolism and response to exogenous chemicals [cytochrome P450 enzymes (CYP1B1, CYP2C9), epoxide hydrolase (EPHX1), paraoxonase 1 (PON1), arylhydrocarbon hydroxylase receptor (AHR), and NAD(P)H:quinone oxidoreductase (NQO1)].
Methods: this study included 279 MM cases and 782 controls in a pooled analysis of two population-based case-control studies. One common variant from each candidate gene was genotyped using DNA from blood or buccal cells. We estimated risk of MM associated with each genotype, controlling for race, gender, study site, and age, using odds ratios (OR) and 95% confidence intervals (CI).
Results: evaluations of the CYP1B1 V432L variant (rs1056836) suggested increased risk of MM among persons with the CG and GG genotypes compared to the CC genotype [OR (95% CI)=1.4 (1.0-2.0)]. Similar results were seen in analyses stratified by race and gender. We did not find any associations between MM and the CYP2C9, EPHX1, NQO1, or PON1 genes.
Conclusions: CYP1B1 activates chemicals such as polycyclic aromatic hydrocarbons and dioxins to create oxidized, reactive intermediates, and higher gene activity has been shown for the G allele. We conducted the largest analysis to date on MM and these genetic variants and our results provide preliminary evidence that variation in CYP1B1 may influence susceptibility to MM.

18.8 NQO1 rs1800566 GG genotype is associated with a normal NQO1 enzyme activity.

Zai CC, et al. *Oxidative stress in tardive dyskinesia: genetic association study and meta-analysis of NADPH quinine oxidoreductase 1 (NQO1) and Superoxide dismutase 2 (SOD2, MnSOD) genes*. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Feb 1;34(1):50-6. doi: 10.1016/j.pnpbp.2009.09.020.

Introduction: tardive dyskinesia (TD) is a potentially irreversible side effect of antipsychotic medication treatment that occurs in approximately 25% of chronically treated schizophrenia patients. Oxidative stress has been one of the proposed mechanisms influencing TD risk. Pae et al. (2004) originally reported a significant association between TD and the NADPH quinine oxidoreductase 1 (NQO1) gene Pro187Ser (C609T, rs1800566) polymorphism in Korean schizophrenia patients; however, subsequent studies have not consistently replicated these findings. Similarly, Hori et al. (2000) reported an association between TD and the Manganese superoxide dismutase SOD2 (MnSOD) gene Ala9Val (rs4880) polymorphism in a Japanese sample, but most research groups failed to replicate their positive findings.
Aims: we investigated the role of the NQO1 polymorphism Pro187Ser and SOD2 (Ala9Val) in a group of well-characterized schizophrenia patients (N=223) assessed for TD. We also performed a meta-analysis of all the previously published TD studies, including data from our sample, on these polymorphisms, Pro187Ser (N=5 studies) and Ala9Val (N=9 studies).
Results: we did not observe a significant association of the Pro187Ser or Ala9Val polymorphism with TD occurrence or AIMS scores in our Caucasian and African American samples when analyzed independently. Meta-analysis did not reveal a significant association of the Pro187Ser/Ala9Val alleles or genotypes with TD occurrence.

Conclusions: neither the NQO1 Pro187Ser nor the SOD2 Ala9Val appear to play a major role in TD risk, although additional polymorphisms should be tested before the role of NQO1 and SOD2 in TD can be completely excluded.

LEGAL DISCLAIMER: Fagron Genomics, S.L.U carries out genetic tests upon request by healthcare professionals, in relation to biological samples from patients obtained by the healthcare professional. Our tests do not replace a medical consultation, nor do they make up a diagnostic or treatment, nor should they be interpreted this way. Only healthcare professionals can interpret the results of said tests, based on their knowledge of the clinical records of the patients and other relevant factors and, under their responsibility, give a diagnostic or prescribe treatment to the patient. We decline all responsibility derived from the use and interpretation of the results of our tests by the solicitant healthcare professional. Fagron Genomics, S.L.U expressly reserves any legal actions in case of an innapropiate, negligent or incorrect use or interpretation of the results of our tests. It is the responsibility of the healthcare professional who requests a test to guarantee to the patient the appropriate genetic advice as foreseen by Law 14/2007, of 3rd July, of biomedical research. As Fagron Genomics, S.L.U does not have access to the personal identifiable information about the patient from whom the sample comes, it is the responsibility of the requesting healthcare professional to comply with the applicable data protection Laws and regulations.

Fagron BV
Lichtenauerlaan 182
3062 Rotterdam
The Netherlands

T +31 88 33 11 210
F +31 88 33 11 210

www.fagron.com