

Polymorphism rs3123554 in CNR2 Reveals Gender-Specific Effects on Body Weight and Affects Loss of Body Weight and Cerebral Insulin Action

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Objective: The cannabinoid-receptor system is involved in the regulation of food intake. Here, we test whether single nucleotide polymorphisms (SNPs) in *CNR2*, encoding the cannabinoid-receptor 2, are associated with weight in a cross-sectional cohort. Furthermore, we wanted to investigate if the identified hits influence weight loss during lifestyle intervention; and study a potential involvement of cerebral insulin action.

Methods: 2006 subjects at increased risk for type 2 diabetes mellitus were genotyped for 5 tagging SNPs in the *CNR2* locus. All subjects underwent a 75-g OGTT. 345 subjects participated in a lifestyle intervention (Tübingen Lifestyle Intervention Programme). Cerebrocortical insulin sensitivity was measured by magnetoencephalography after intranasal insulin application in 43 subjects.

Results: In the cross-sectional cohort, the minor allele of rs3123554 was associated with lower BMI ($P_{\text{add}} = 0.01$, $P_{\text{rec}} = 0.004$), and this was attributable to its effect in women only. Interestingly, during lifestyle intervention, carriers of the same allele lost less body weight ($P_{\text{add}} = 0.03$, $P_{\text{rec}} = 0.008$). Moreover, carriers of this minor allele showed lower cerebral insulin sensitivity ($P_{\text{rec}} = 0.0402$).

Conclusions: The minor allele of rs3123554 is associated cross-sectionally with lower body weight, whereas during intervention the same allele led to less reduction of body weight. Reduced cerebral insulin sensitivity in carriers of this allele might contribute to these disadvantageous effects during lifestyle intervention.

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Introduction

In Western countries, the prevalence of obesity with related diseases such as type 2 diabetes (T2DM) is still growing and has reached epidemic proportions. Not only environmental, but also genetic factors contribute to the development of obesity and T2DM (1,2). In lifestyle interventions designed to prevent T2DM, the loss of body weight is one main target (3). Because the environment and the genetic background are interacting rather than acting independently, in a considerable number of participants, the lifestyle interventions seem to be ineffective and especially body weight change is very

heterogeneous. In this context, single nucleotide polymorphisms (SNPs) influencing the outcome of such prevention programs have been identified (4,5).

The cannabinoid receptor system consists of two receptors (CB1 and CB2). CB1 is mainly expressed in the brain; its role in eating behavior is well established and has already led to therapeutical approaches in the treatment of obesity (6). In contrast, CB2 has long been referred to as the peripheral cannabinoid receptor isoform that is mainly expressed in cells of the immune system. Interestingly,

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there is now evidence of CB2 expression in several different cells of the brain (7-10); however, the physiological role of central CB2 receptors could not be entirely elucidated yet.

The cerebral insulin signal represents a feed-back loop on body weight regulation (11). Measurements of cerebrocortical activity by magnetoencephalography (MEG) revealed that obesity correlates with cerebral insulin resistance (12). Furthermore, we were able to show, that high insulin sensitivity in the brain ameliorates loss of body weight during lifestyle intervention with a most pronounced decrease of the metabolic unfavorable visceral fat (13).

In this study, we tested whether 5 tagging SNPs in the *CNR2* gene, encoding for CB2, are associated with weight and body adiposity in a cross-sectional study population. We furthermore wanted to investigate whether the hits from the cross-sectional study might lead to significant differences in body weight and visceral fat reduction during a lifestyle intervention, and if so whether cerebral insulin signaling might account for these differences.

Methods

Human subjects and experimental design

Two thousand and six subjects at increased risk for T2DM (nondiabetic individuals with family history of type 2 diabetes or diagnosis of impaired fasting glycaemia) from the TUEF study were genotyped for five tagging SNPs (rs2501392, rs2229579, rs3123554, rs9424398, rs4625225) in the *CNR2* gene. Anthropometric parameters were assessed and all subjects underwent a 75-g OGTT. Because associations between gene variants and body weight could affect different aspects of body composition, we decided to include percentage of body fat as one measure for adiposity and waist-to-hip ratio as an estimate of body fat distribution. Three hundred and forty five subjects also took part in lifestyle intervention aimed to reduce body weight by decreased energy intake and moderate physical activity (Tübingen Lifestyle Intervention Programme, TULIP). Because of most comprehensive datasets we analyzed the baseline and 9-month visit data in this study. To assess cerebral insulin action, a subset of 43 participants was studied by magnetencephalography (MEG) and application of intranasal insulin. Informed written consent was obtained from all participants, and the ethics committee of the medical faculty of the University Tübingen approved the protocol.

Selection of tagging SNPs

Based on publicly available phase III data of the International HapMap Project derived from the Central European (CEU) population (release #28 August 2010, <http://hapmap.ncbi.nlm.nih.gov/index.html.en>), we screened *in silico* a genomic area on human chromosome 1 encompassing the complete *CNR2* gene as well as 5-kb each of its 5'- and 3'-flanking regions. The *CNR2* gene is flanked 45kb upstream by the *PNRC2* gene and 5 kb downstream by the *FUCA1* gene. No linkage blocks within the screened *CNR2* locus region were found to overlap with the gene *PNRC2*. However, there was a linkage block ranging from the 3'-UTR of the *CNR2* gene to about 1.5 kb upstream of the *FUCA1* gene.

Within the screened *CNR2* locus, 19 informative HapMap SNPs were present with Hardy-Weinberg *P* values and MAFs ≥ 0.05

(HapMap data). Among these SNPs, five SNPs were selected as tagging SNPs covering all the other common SNPs within the locus with an $r^2 > 0.8$ (100% coverage) based on Tagger analysis using Haploview software (<http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview>). The five tagging SNPs were rs2501392, rs4625225 and rs9424398 in intron 1, rs2229579 in exon 2 (3'-coding region) and rs3123554 in the 5' flanking region.

Genotyping

DNA from whole blood was isolated using a commercial DNA isolation kit (NucleoSpin, Macherey& Nagel, Düren, Germany) and genotyping was performed using TaqMan assays (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 7000 and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). The genotyping success rates were 99.8% for rs2229579, 99.1% for rs3123554, 99.9% for rs9424398, 99.7% for rs4625225, and 99.8% for rs2501392.

The genotypes were verified in 50 randomly selected subjects by bidirectional sequencing, and both methods gave 100% identical results. The 5 SNPs were in Hardy-Weinberg equilibrium ($P \geq 0.26$).

For replication, publicly available BMI data from European subjects of the GIANT consortium were interrogated (http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files). In lieu of *CNR2* SNPs that were not depicted on the GWAS arrays, proxy SNPs with $r^2 > 0.8$ were searched using SNAP freeware (<http://www.broadinstitute.org/mpg/snap/ldsearch.php>) and tested for association with BMI.

Oral glucose tolerance test (OGTT)

After an overnight fast for at least 10 h, all subjects underwent a 75-g OGTT. Venous blood samples were obtained at 0, 30, 60, 90, and 120 min for determination of plasma glucose, insulin, and C-peptide.

Peripheral insulin sensitivity was calculated from glucose and insulin values during the OGTT as proposed by Matsuda and DeFronzo (14).

Analytical procedures

Plasma glucose was determined during the OGTT using the glucose oxidase method (YSI, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were measured by commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany).

Bioelectrical impedance measurements

Body composition was measured by bioelectrical impedance analysis (BIA-101A, RJL Systems, Detroit, MI) and expressed as percent body fat.

Lifestyle intervention

The Tübingen Lifestyle Intervention Programme (TULIP) was described earlier (15). Individuals were asked to perform at least 3 h

of moderate sports per week. After the baseline measurements, individuals had up to 10 sessions with a dietician. The participants were instructed to reduce body weight by $\geq 5\%$ compared to baseline. Nutrition counseling also aimed at reducing intake of calories from fat to $<30\%$ of energy consumed and to increase intake of fibers to at least 15 g per 1,000 kcal. Furthermore, the participants were asked to reduce intake of saturated fat to $\leq 10\%$. This dietary regimen was adapted from the diet intervention used in the Diabetes Prevention Study (16).

Measurement of total adipose tissue and visceral adipose tissue by magnetic resonance (MRI) examinations

The measurement of total adipose tissue and visceral adipose tissue by magnetic resonance examinations in the subjects that underwent lifestyle-intervention was conducted as described earlier (13,17).

MEG measurements and nasal insulin administration

The MEG study with administration of intranasal placebo versus insulin was conducted on 2 days in a single-blind randomized cross-over study. The study started in the morning after an overnight fast. Basal MEG measurements were followed by intranasal insulin or placebo administration. Nasal spray was administered via spray pumps (18) and subjects received either insulin (a total of 160 U) or placebo, as described before (19,20). After 30 min, a second MEG measurement was performed. Blood was taken before and after each MEG measurements to determine plasma glucose, insulin, and C-peptide levels. Magnetoencephalographic signals were recorded with a 275 channel whole-head MEG system (VSM, Medtech, Vancouver, Canada) during a food related visual working memory task (21) (for details on the MEG measurements see (22)). On the basis of our previous study we evaluated induced frequency changes in the theta band (4-8 Hz) (14). From the continuous data single trials with duration of 1,000 ms were extracted starting at the onset of each stimulus. For each trial the power in the theta band was calculated and the average over all trials for each stimulus condition (food vs. non-food) was calculated separately for each measurement and session. Significant differences for the theta band power before and after insulin administration (corrected for placebo effect) in food and non-food condition were determined by using the nonparametric cluster level randomization. For each significant cluster of channels (P value < 0.05 cluster level) an average value over the channels from that cluster was extracted as measure for cerebral insulin resistance, e.g., representing insulin induced change divided by baseline activity. This ensures that only significant cerebral activity changes induced by insulin are further analyzed. All data analysis was performed using the Fieldtrip software package (<http://fieldtrip.fcdonders.nl/>)

Statistical analysis

To analyze the association of five tagging SNPs with anthropometric and metabolic parameters in the cross-sectional cohort, we performed multiple linear regression analysis and adjusted for covariates to identify independent relationships. To analyze gender $\times$ genotype interaction effects on BMI, cross effects were tested by analysis of covariance (ANCOVA) with age as covariate. After Bonferroni correction for multiple comparisons for the five SNPs tested, P values < 0.01 were considered statistically significant.

Changes in body weight and body fat during lifestyle intervention and their association with rs3123554 genotype was analyzed by multiple linear regression analyses with a significance level of $P < 0.05$. The Software package JMP (SAS Institute, Cary, NC) was used. Cerebral insulin sensitivity was quantified by the changes in the theta (4-8 Hz) frequency band after intranasal insulin or placebo spray application in comparison to the basal state. Non-normally distributed variables were logarithmically transformed prior to statistical analysis. In the recessive inheritance model, our overall study cohort was sufficiently powered ($1 - \beta \geq 0.8$) to detect, for the five tagging SNPs, (unadjusted) effect sizes of $\sim 5\%$, the lifestyle subgroup was sufficiently powered to detect effect sizes of $\sim 12\%$, and the MEG subgroup to detect effect sizes of $\sim 35\%$ ($\alpha < 0.05$). Power calculations were performed using the publicly available Quanto software (<http://hydra.usc.edu/gxe>, version 1.2.4, May 2009)

Results

Genotyping and study population

The observed minor allele frequency for SNP rs2501392 was 16%, for rs2229579 11%, for rs3123554 42%, for rs9424398 20%, and for rs4625225 it was 24%. All SNPs were in Hardy-Weinberg equilibrium ($P \geq 0.26$). All cross-sectional data were adjusted for gender and age. Glucose concentrations, insulin secretion, and insulin sensitivity derived from the OGTT were additionally adjusted for BMI. The mean age and BMI of the subjects in the cross sectional cohort was 40 ± 13 years and 30.3 ± 9.7 kg m $^{-2}$, subjects undergoing lifestyle intervention were 46 ± 11 years old and weight 30.1 ± 5.6 kg m $^{-2}$. More detailed subject characteristics of the cross-sectional as well as of subjects taking part in lifestyle intervention and MEG measurements are shown in Supporting Information Table 1.

The effect of the lifestyle intervention on glucose metabolism, body composition, and weight loss was reported previously (23). As expected, lifestyle intervention resulted in a significant reduction of body weight, body fat and improvement of glucose metabolism.

Association of genetic variation in the CNR2 locus with body weight

We investigated five tagging SNPs of the *CNR2* locus for association with anthropometric parameters (Table 1). All SNPs were tested in the additive inheritance models. After correction for multiple comparisons, SNP rs3123554 showed a significant association with weight ($P_{\text{add}} = 0.0062$, Table 1). Because the minor allele of SNP rs3123554 showed a recessive effect (the power of the recessive model was 0.82 versus 0.69 in the additive model; based on the default effect size = square root of the sum of squares for the hypothesis divided by N) we also analyzed the recessive model and found a significant association of the minor allele with lower BMI, body weight and bioimpedance-derived total body fat (all $P_{\text{rec}} \leq 0.0078$, Table 1). In addition, SNP rs4625225 showed a nominal association with weight, total body fat, and waist-to-hip ratio (WHR) (all $P_{\text{add}} \leq 0.0448$, Table 1), but did not pass correction for multiple comparisons. Moreover, we analyzed associations of the five SNPs with parameters of glucose metabolism. Some associations with insulin secretion parameters were detected, however, none of them passed correction for multiple comparisons (Supporting Information Tables 2-4).

TABLE 1 Associations of *cnr2* SNPs with anthropometric parameters

SNP	rs3123554			P_{add}	P_{rec}
Genotype	GG	AG	AA		
<i>N</i>	677	939	361		
BMI (kg m ⁻²)	30.5 ± 0.4	30.7 ± 0.4	29.0 ± 0.5	0.0142	0.0041
Weight	88.9 ± 1.0	88.7 ± 0.9	84.9 ± 1.5	0.0062	0.0021
Total body fat (%)	33.1 ± 0.5	33.2 ± 0.4	31.2 ± 0.6	0.0586	0.0078
waist-to-hip ratio (WHR)	0.87 ± 0.004	0.86 ± 0.003	0.86 ± 0.005	0.6	0.2

SNP	rs9424398			P_{add}
Genotype	GG	GT	TT	
<i>N</i>	1295	621	77	
BMI (kg m ⁻²)	30.08 ± 0.26	30.57 ± 0.38	30.13 ± 1.0	0.3
Weight	87.66 ± 0.77	88.55 ± 1.10	89.26 ± 3.14	0.2
Total body fat (%)	32.32 ± 0.35	33.71 ± 0.49	32.18 ± 10.97	0.0934
waist-to-hip ratio (WHR)	0.87 ± 0.008	0.87 ± 0.004	0.88 ± 0.01	0.0661

SNP	rs2501392			P_{add}
Genotype	CC	CG	GG	
<i>N</i>	1426	516	50	
BMI (kg m ⁻²)	30.1 ± 0.2	30.7 ± 0.4	29.7 ± 8.2	0.4
Weight	87.7 ± 0.7	89.0 ± 1.3	86.1 ± 3.5	0.4
Total body fat (%)	32.5 ± 0.33	33.8 ± 0.5	31.5 ± 1.5	0.1
waist-to-hip ratio (WHR)	0.87 ± 0.007	0.87 ± 0.004	0.87 ± 0.01	0.1

SNP	rs2229579			P_{add}
Genotype	CC	CT	TT	
<i>N</i>	1599	368	25	
BMI (kg m ⁻²)	30.20 ± 0.23	30.08 ± 0.49	29.04 ± 1.61	0.4
Weight	87.96 ± 0.69	88.32 ± 28.9	86.2 ± 5.08	0.4
Total body fat (%)	32.82 ± 0.32	32.54 ± 0.60	31.78 ± 2.62	0.1
waist-to-hip ratio (WHR)	0.87 ± 0.007	0.88 ± 0.005	0.86 ± 0.02	0.1

SNP	rs4625225			P_{add}
Genotype	TT	CT	CC	
<i>N</i>	1144	731	115	
BMI (kg m ⁻²)	29.91 ± 0.27	30.66 ± 0.35	30.78 ± 0.89	0.0689
Weight	86.96 ± 0.80	89.36 ± 28.35	89.64 ± 2.66	0.0448
Total body fat (%)	32.21 ± 0.37	33.67 ± 12.15	32.32 ± 1.03	0.0235
waist-to-hip ratio (WHR)	0.87 ± 0.01	0.87 ± 0.003	0.88 ± 0.01	0.0326

Data represent means ± SEM. For statistical analysis, data were ln-transformed. BMI was adjusted for gender and age. P_{add} — P values for the additive inheritance model; P_{rec} — P -values for the recessive inheritance model. BMI—body mass index; SNP—single nucleotide polymorphism.

Interaction effects of gender with genetic variation in the CNR2 locus on body weight

To assess gender specific effects of *CNR2* tagging SNPs we pre-formed ANCOVA. In additive model, we found nominal gender

interaction effects of rs3123554 and rs2229579 on BMI ($P = 0.0215$ and $P = 0.0169$, respectively). SNP rs3123554 showed significant gender interaction on BMI in the recessive model ($P = 0.0076$).

TABLE 2 Associations of *cnr2* SNPs rs3123554 with anthropometrics, metabolic parameters, stratified by gender

SNP rs3123554 Genotype	Female			<i>P</i> _{add}	<i>P</i> _{rec}	Male			<i>P</i> _{add}	<i>P</i> _{rec}
	GG	AG	AA			GG	AG	AA		
<i>N</i>	454 (0)	622 (1)	235 (2)			229	320	126		
BMI (kg m ⁻²)	30.8 ± 0.4	31.1 ± 0.4	28.5 ± 0.5	0.0008	0.0002	29.8 ± 0.6	29.5 ± 0.5	30.2 ± 0.9	0.6	0.6
WEI (kg)	84.9 ± 1.2	85.8 ± 1.1	78.4 ± 1.5	0.0004	<0.0001	96.8 ± 1.9	93.9 ± 1.6	97.1 ± 3.0	0.4	0.7
Body fat (%)	37.9 ± 0.5	37.9 ± 0.5	35.2 ± 0.7	0.0043	0.001	23.6 ± 0.6	24.0 ± 0.5	23.5 ± 0.69	0.8	0.9
WHR	0.83 ± 0.004	0.84 ± 0.002	0.83 ± 0.004	0.4	0.8	0.9 ± 0.006	0.9 ± 0.005	0.93 ± 0.01	0.2	0.1

Data represent means ± SEM. For statistical analysis, data were ln-transformed. Data was adjusted for gender and age. *P*_{add}—*P* values for the additive inheritance model; *P*_{rec}—*P* values for the recessive inheritance model; BMI—body mass index; SNP—single nucleotide polymorphism.

As shown in Table 2, SNP rs3123554 was significantly associated with BMI, weight and percentage of body fat in women only. rs12744386, rs6424115, rs2179395, rs2256179, rs4237. None of these SNPs revealed significant association with BMI (all *P* ≥ 0.7).

Replication of rs3123554’s effect on BMI

For replication of SNP rs3123554’s effect on BMI, we interrogated the publicly available GIANT database. SNP rs3123554 was not covered by the GWAS arrays used by the GIANT consortium. Therefore, we searched for SNPs in high linkage disequilibrium (*r*² > 0.8) using SNAP freeware. We identified seven proxy SNPs that were covered by the arrays: rs10917431, rs4649119,

The minor allele of SNP rs3123554 is associated with decreased weight loss during live style intervention and lower cerebral insulin sensitivity

The association of rs3123554 with body weight prompted us to study this SNP in further detail. For further investigation, the additive and recessive models were applied. During lifestyle intervention, the carriers of the minor allele displayed lower reduction in BMI (*P*_{add} = 0.03, *P*_{rec} = 0.008, Figure 1A). Homozygous carriers of the minor allele lost 1.4 (±3.8 SD) kg, whereas carriers of the major allele lost 2.7 (±4.0 SD) kg after 9 months of lifestyle intervention. The MRI data showed that all subjects were able to reduce their total adipose tissue (from 28.6 ±0.8 l before to 25.89 ± 0.7 l after intervention). Visceral fat was reduced from 3.3% ± 0.1% to 2.9% ± 0.1% body weight. There was no significant difference in reduction of total adipose tissue between genotype groups, however we found a trend toward a less pronounced decrease in the visceral fat depot in carriers of the minor allele (*P*_{rec} = 0.06).

Regarding measurements of cerebral insulin sensitivity, subjects showed a statistically significant increase in theta band power after intranasal administration during the working memory task for food condition. In homozygous carriers of the minor allele compared to carriers of the major allele (*P*_{rec} = 0.0402, adjusted for BMI), we observed a decreased effect of insulin on theta activity (Figure 1B). When applying the same approach to the placebo measurements, there was no change in theta activity, regardless of the *CNR2* genotype.

Genotype × gender interactions were not tested in the lifestyle intervention and MEG study due to the limited sample sizes.

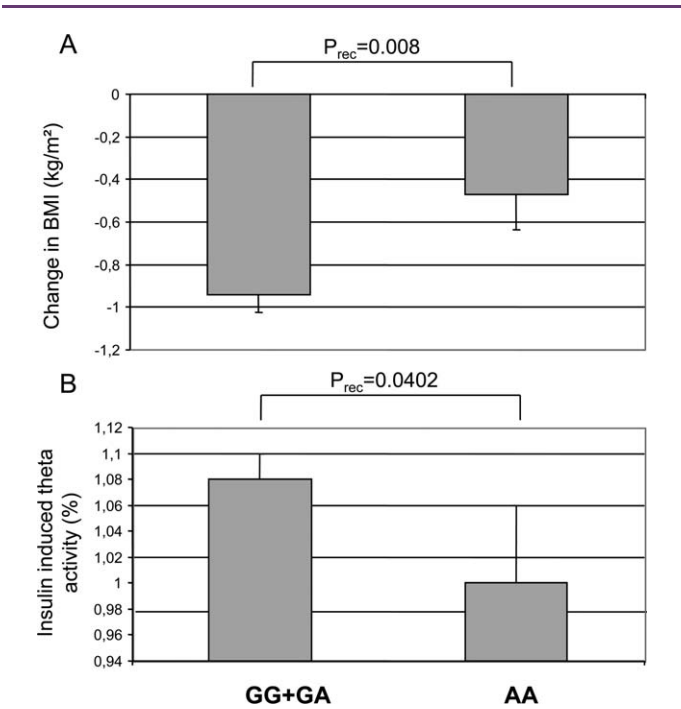


FIGURE 1 A. Changes in BMI during the lifestyle intervention. Homozygous carriers of the minor allele of rs3123554 (AA) lost significantly less weight than carriers of the major allele (GG+GA) (*P* = 0.008). Data are presented as mean ± SEM. B. Insulin effect on theta activity in the genotype groups: In homozygous carriers of the minor allele of rs3123554 (AA), the effect of insulin on theta activity was significantly reduced as compared to carriers of the major allele (GG+GA) (*P* = 0.0403, adjusted for BMI). As measures for cerebral insulin sensitivity, we divided theta activity after insulin spray application by theta activity before insulin spray. The power differences are shown. Data are presented as mean ± SEM.

Conclusions

In this study, we were able to show an association of the minor allele of SNP rs3123554 within the *CNR2* gene with lower BMI and body fat in a cross-sectional cohort.

To strengthen this finding, we interrogated the publicly available genome-wide analyses from the GIANT consortium database for associations of our SNP with BMI. Because the same SNP could not

be found in the database, we searched SNPs that are in complete linkage with rs3123554. Unfortunately, this interrogation failed to reveal a significant association of the SNPs with BMI.

Yet, that our finding could not be replicated in GIANT, prompted us to further investigate our data by stratifying in different phenotypes. Interestingly, we found the significant association of the SNP with BMI to be limited to females in the cross sectional cohort (Table 2). This points to the well known interaction between the cannabinoid system and sex hormones. Unfortunately, data with stratification by gender is not publicly available anywhere—gender differences in our cohort might be a reason why we were not able to replicate our finding in the data provided. Furthermore, the GIANT data lack adjustment for age which could introduce a bias.

Because obesity is a major risk factor, one main target of lifestyle interventions for the prevention of T2DM is loss of body weight. Aiming to perform personalized medicine, gene-environment interactions have become of more and more interest, and in this context, SNPs associated with diabetes and/or obesity risk have been identified to influence the weight loss during intervention programs (24-26). This prompted us to further investigate the effects of SNP rs3123554 within *CNR2* on weight loss during lifestyle intervention. Because of the relatively low number of participants we refrained from stratifying the group by gender in the further analysis. Surprisingly, we found that the minor allele of rs3123554—that was associated with lower body weight in the cross sectional cohort—led to a significantly reduced loss of body weight during lifestyle intervention, and this effect was independent of the body weight at baseline.

We further analyzed the effect of SNP rs3123554 on cerebral insulin action using MEG and intranasal insulin application. Central insulin action is an important regulator of energy homeostasis and body weight (11,27). In contrast to the insulin effects in peripheral tissues, high cerebral insulin sensitivity rather facilitates body weight loss during life style interventions (13). We found that carriers of the minor allele showed lower theta activity after nasal insulin application, representing decreased cerebral insulin sensitivity (12). Because this brain insulin sensitivity determines effectiveness of lifestyle intervention in terms of weight loss (13), one could speculate that the altered cerebral insulin sensitivity in carriers of the minor allele of rs3123554 may at least be partially responsible for their inability to loose as much weight as major allele carriers.

The endocannabinoid system is known to influence several aspects of energy balance including eating behavior (28). Because of its expression in the brain, CB1 was thought to be mainly responsible for these effects. However, after referring to the CB2 as peripheral receptor for a long time, functional CB2 receptors have been identified in different cells of the brain including the hippocampus (7-10), but the physiological role still remains largely unclear. Most recently, a putative role of cerebral CB2 receptors in the modulation of body weight was found in the animal model—central CB2 overexpression leads to a lean phenotype in mice (29). Moreover, CB2 activation in humans also seems to influence eating behavior (30), maybe due to its central effects. Our finding of an association of a SNP within the *CNR2* gene and altered central insulin action gives further evidence of a cerebral role of the CB2 receptor in humans.

Cerebral theta activity is mainly generated in the hippocampus (31); the hippocampus has been shown to contribute to food-related

reward in obese women (32) and theta activity has also been attributed to voluntary movements (33). Interestingly, theta rhythm in the hippocampus has been linked to human memory and self-directed learning (34) which might be of special importance during life style interventions. Interestingly, we previously observed that genetic variation in some other genes associated with obesity and/or weight loss (MCR4, FTO, IRS1) show alterations in theta or beta activity of the brain in response to insulin (35,36). Of interest, we now observed a reduced insulin effect related to *CNR2* SNP rs3123554 in the theta band. It has to be stressed that this effect was not determined in spontaneous activity as in our former study, but during a memory task. This adds further evidence that theta activity is especially affected by insulin.

The result of lower body weight in carriers of the minor allele of SNP rs3123554 being accompanied by decreased body weight loss during lifestyle intervention may be surprising. As mentioned, CB2 is expressed in cells of the immune system, but also present in organs important for the control of metabolism like liver, adipose tissue and skeletal muscle (37-39). Although speculative, there is the possibility that the body weight effect in the cross-sectional cohort is due to peripheral metabolic/homeostatic mechanisms, whereas during lifestyle intervention, altered cerebral activity involved in self-directed learning and activation of the reward system might come to the fore.

SNP rs3123554 is located 4 kb downstream of the *CNR2* gene and 1.5 kb upstream of the *FUCA1* gene. Mutations in *FUCA1* are associated with a rare autosomal recessive lysosomal storage disease called fucosidosis, which is characterized by progressive psychomotor deterioration, angiokeratoma and growth retardation (40). Other than that, hardly anything is known about the function of *FUCA1*. Although we cannot entirely rule out that the effects observed in our study might be caused by alterations in this genes' product, so far, there is no indication of *FUCA1* being involved in any aspects of bodyweight regulation or metabolism.

The main limitation of this study is the low number of subjects undergoing measurement of cerebral insulin sensitivity, especially in the context of genetic analysis. This low number, especially of homozygous carriers in the recessive model, might increase the risk of reporting false positive results. However, we are not aware of any larger available study with measurement of brain insulin action that could be interrogated for replication of our findings. Moreover, the detailed mechanisms behind the weight effect in the cross-sectional cohort and during lifestyle intervention is still unclear. To verify our results and to more intensively study the interaction between the endocannabinoid system and insulin action in the brain, further studies in bigger cohorts or/and in animal models are needed.

In conclusion, our data suggest that carriers of the minor allele of SNP rs3123554 near the *CNR2* gene loose less body weight during lifestyle intervention; the lower cerebral insulin sensitivity in these persons might contribute to this negative outcome, supporting our previous findings. In contrast to the central effect, the lower BMI found in the cross sectional study population in female carriers of the same allele may be due to peripheral metabolic mechanisms. In the context of individualized medicine, identifying SNPs that are associated with obesity but at the same time may even lead to a better outcome regarding weight loss during intervention could be of

special interest for prevention programs since carriers of such polymorphisms would benefit most from such efforts. [O](#)

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