MC4R rs489693: a clinical risk factor for second generation antipsychotic-related weight gain?

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Abstract

Weight gain is a therapy limiting and very frequent adverse effect of many second-generation antipsychotic (SGA) drugs. The human melanocortin four receptor (MC4R) is a very promising candidate gene possibly influencing SGA-related weight gain. The rs489693 polymorphism near the MC4R gene was associated with SGA-related weight gain in a genome-wide association study. We tried to replicate these results in our independent naturalistic study population. From 341 Caucasian inpatients receiving at least one SGA drug (olanzapine, clozapine, risperidone, paliperidone, quetiapine or amisulpride), carriers homozygous for the rs489693 A-allele (n=35) showed a 2.2 times higher weight increase (+2.2 kg) than carriers of the CC-genotype (+1 kg) after 4 wk of treatment (analysis of covariance, p=0.039). We revealed an even stronger effect in a subpopulation without weight gain inducing co-medication (factor 3.1, +2.8 kg, p=0.044, (n=16 of 169)) and in first episode patients (factor 2.7, +2.7 kg, p=0.017, (n=13 of 86)). Our results confirm the rs489693 A-allele as a possible risk factor for SGA-related weight gain.

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Introduction

Weight gain is an important adverse effect of second-generation antipsychotic (SGA) drug therapy and a leading cause for noncompliance (Weiden et al., 2004; Hugenholtz et al., 2005). Moreover, it is also associated with an increased risk of cardiovascular disease (Kurzthaler and Fleischhacker, 2001): schizophrenic (SCZ) patients have higher incidence of diabetes and hypertension and a 20% shorter lifespan compared with the general population (Newcomer, 2007). Despite these therapy-limiting side effects, SGA drugs are the treatment of choice, because they provide a better safety profile and offer a more effective therapy than first-generation antipsychotic drugs (Kane et al., 2003). Although the field of pharmacogenetics of SGA-related weight gain is rapidly evolving (Lett et al., 2011), no candidate gene has been translated into a clinical benefit for pharmacotherapy. Genome-wide association studies (GWAS) have generated novel candidate genes and identified common variants in the human fat mass and obesity (FTO) gene as first robust associations with body mass index (BMI) and obesity (Frayling et al., 2007; Frayling and Ong, 2011). In 2008, the rs17782313 polymorphism near the melanocortin four receptor (MC4R) gene was identified as a second association signal regarding obesity (Loos et al., 2008). A recently published meta-analysis could confirm the significant association of the rs17782313 polymorphism with the risk of obesity (Xi et al., 2012). The MC4R gene, which is located upstream from the rs17782313 polymorphism, plays a central role in energy homeostasis and is one of the most important causative genes regarding monogenic obesity: over 130 functionally relevant mutations in the human MC4R gene have been identified (Fan and Tao, 2009) and most of them have been shown to lead to either total or partial loss of function (Tao, 2005).
In a recently published report, we showed that the rs17782313 polymorphism is also associated with SGA-related weight gain (Czerwensky et al., 2013). Moreover, Malhotra et al. (2012) identified in a GWAS the rs489693 polymorphism near the MC4R gene in extreme SGA-related weight gain and could confirm this association in three replication cohorts. The inherent biological plausibility of MC4R for weight gain (Malhotra et al., 2012) and the previous findings underline the possible involvement of the region in and around the MC4R gene in SGA-related weight gain. Therefore, we tried to replicate the rs489693 polymorphism as a possible clinical risk factor for SGA-related weight gain in an independent naturalistic study population.

Method

Within the scope of pharmacogenetic studies at the psychiatric department at Klinikum rechts der Isar, 345 inpatients, who received at least one SGA with a medium (risperidone, paliperidone, quetiapine or amisulpride) or high risk (clozapine or olanzapine) of inducing weight gain, were included in our investigation. One subject had to be excluded from BMI analysis because height measurement was missing. We further identified a subpopulation without additional weight gain-inducing co-medication (=adjusted subpopulation). We concentrated on weight gain induced by SGA drugs and tried to avoid bias caused by additional weight gain inducing co-medication, which probably exerts influence on the energy balance system via different pathways. Therefore, patients on co-medication known for its clear influence on weight gain (tricyclic antidepressant, mirtazapine, lithium, valproic acid and paroxetine) were excluded (Drieling et al., 2007). Due to inconsistent data haloperidol, carbamazepine and oxcarbazepine were not considered as weight gain-inducing drugs (Drieling et al., 2007).

We further scrutinized a subsample containing patients with a first episode of antipsychotic treatment with the aim of studying previous treatment as a confounding factor. A detailed study description has been previously published (Czerwensky et al., 2013). As four subjects could not be genotyped the current study population differs marginally from our previously published report: the excluded patients received olanzapine monotherapy in two cases, clozapine and amisulpride therapy in one case and clozapine, amisulpride, ziprasidone and haloperidol treatment in another case. All four subjects were not assigned to the whole and adjusted subpopulation and one subject with olanzapine monotherapy was not assigned to the first episode population. As Malhotra et al. (2012) excluded olanzapine from analyses, we additionally analysed a sub-sample of olanzapine treated patients (n=135) and of all patients treated with other antipsychotics (n=206). No patients admitted to hospital by law or authority direction were included in the study. The study was approved by the local ethics committee and followed the principles of the Helsinki declaration. Patients were informed of the aims of the study and gave written consent, which could be withdrawn at any time.

Preparation of DNA and genotyping was carried out in analogy as described elsewhere (Popp et al., 2003). Real time PCR was performed with the following setup: forward-primer: 5’TITTTCTTCAC- GAATCGCCACAA3’, reverse-primer: 5’TCTGCTGA- ACTGTGCTTGG3’, sensor-probe: 5’LC705-CTGTGTG- CATTAGTCCCCGTGTT-phosphate3’, anchor-probe: 5’fluoresceine- CCAG ATTTGGTCAATACAGG TCATGTCTTAA-phosphate3’, 35× (95 °C-10 s, 58 °C-20 s, 72 °C-30 s), 375 nm forward and 625 nm reverse primers, 45 nm hybridization probes, 100 ng DNA, 2.75 mM MgCl2, 0.5 μl DMSO and 2 μl master hybridization mixture (Roche Diagnostics), total volume 20 μl.

The primers also allowed a restriction fragment length polymorphism (RFLP) analysis with the digestive enzyme BSRI (New England Biolabs, USA) for control and validation of assay performance.

Statistical analyses were performed using SPSS 20.0. Two-tailed p values of 0.05 were considered to be of statistical significance. Owing to the exploratory nature of this study, a correction for multiple testing was not included. Normal distribution was estimated according to the Kolmogorov-Smirnov, Shapiro-Wilk test or Quantile-Quantile-Plot. Weight gain and BMI increase were compared by analysis of variance (ANOVA). To investigate the influence of the rs489693 polymorphism as independent variable on the dependent variable weight gain after 4 wk and to avoid confounding factors, we conducted analysis of covariance (ANCOVA), with the covariates age, gender, SGA drug and baseline weight. The variable SGA drug was coded with 1 for antipsychotics with a high risk of inducing weight (olanzapine and clozapine) and with 2 for antipsychotics with a low risk of inducing weight (all other drugs). We did not differentiate for all antipsychotic drugs as the number of antipsychotics was not robust enough for this approach.
Results

The whole study population (respectively the adjusted and first episode population) consisted of 42% (39/50%) men, of whom 43% (48/42%) were smokers. The mean±S.D. age was 41.3±15.0 (39.7±15.1/37.4±13.6) and the baseline weight (kg) was 75.0±16.6 (74.4±16.4/73.2±15.9). The majority of patients received olanzapine in \( n = 135 \) (\( n = 55/48 \)) of cases.

Of 345 patients, 341 were genotyped successfully and of the 341 patients included in this evaluation, 35 (10.3%) were identified as homozygous for the A-allele, 130 (38.1%) as heterozygous and 176 (51.6%) were homozygous for the C-allele. Both genotyping methods showed 100% concordance in 56 patients tested with both methods.

The distribution of the rs489693 polymorphism was in Hardy–Weinberg equilibrium for both the whole population \( (\chi^2 = 2.2; p=0.14) \), the adjusted subpopulation \( (\chi^2 = 2.2; p=0.14) \) and the first episode population \( (\chi^2 = 0.73; p=0.39) \).

Whole study population

In the whole study population the rs489693 polymorphism had no significant influence on baseline weight. However, AA-genotype carriers displayed a significantly higher baseline BMI (Table 1).

After 4 wk of treatment, carriers of the A-allele gained significantly more percent weight and also showed a higher percent BMI increase than the CC-genotype \( (p=0.046 \text{ and } p=0.034; \text{ Table 1}) \).

First episode population

In the first episode population the rs489693 polymorphism had no significant influence on baseline weight and BMI.

After 4 wk of treatment, AC- or AA-genotype carriers gained significantly more weight and BMI (percent) than the CC-genotype carriers (ANOVA, \( p=0.048 \) for both parameters). Absolute weight gain and BMI increase showed a trend \( (p=0.064 \text{ and } p=0.053) \).

Adjusted subpopulation

In the adjusted subpopulation the rs489693 polymorphism had no significant influence on baseline weight and BMI.

After 4 wk of treatment, carriers of the AA-genotype showed higher weight gain and BMI increase; however, only BMI gain (absolute and percent) reached significance (Table 1). In contrast to the whole and first

Table 1. ANOVA for the rs489693 polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Whole study population</th>
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<tbody>
<tr>
<td></td>
<td>AA ( n=35 )</td>
<td>AC ( n=130 )</td>
<td>CC ( n=176 )</td>
</tr>
<tr>
<td>Baseline weight (kg)</td>
<td>79.5(73.1–85.9)</td>
<td>73.0(70.2–75.8)</td>
<td>75.6(73.2–78.0)</td>
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<tr>
<td>Weight after 4 wk (kg)</td>
<td>81.4(75.1–87.7)</td>
<td>74.9(72.1–77.6)</td>
<td>76.6(74.3–78.9)</td>
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<tr>
<td>Absolute weight gain (4 wk-baseline in kg)</td>
<td>1.8(0.7–3.0)</td>
<td>1.9(1.3–2.5)</td>
<td>1.0(0.6–1.5)</td>
</tr>
<tr>
<td>Relative weight gain after 4 wk (% of baseline)</td>
<td>2.7(1.1–4.4)</td>
<td>2.9(2.1–3.8)</td>
<td>1.6(1.0–2.3)</td>
</tr>
<tr>
<td>Baseline BMI (kg/m²)</td>
<td>27.1(24.7–29.3)</td>
<td>24.7(24.0–25.5)</td>
<td>25.8(25.0–26.6)</td>
</tr>
<tr>
<td>BMI after 4 wk (kg/m²)</td>
<td>27.6(25.4–29.8)</td>
<td>25.4(24.6–26.1)</td>
<td>26.1(25.4–26.9)</td>
</tr>
<tr>
<td>Absolute BMI gain (4 wk-baseline in kg/m²)</td>
<td>0.6(0.2–1.0)</td>
<td>0.7(0.5–0.8)</td>
<td>0.3(0.2–0.5)</td>
</tr>
<tr>
<td>Relative BMI gain after 4 wk (% of baseline)</td>
<td>2.7(1.1–4.4)</td>
<td>2.9(2.1–3.8)</td>
<td>1.6(0.9–2.7)</td>
</tr>
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</table>

Values in parentheses are 95% confidence interval.

Adjusted subpopulation

|                     | AA \( n=16 \)          | AC \( n=59 \)       | CC \( n=94 \)       |
| Baseline weight (kg)| 80.3(69.7–91.0)        | 71.3(67.6–75.0)      | 75.3(71.8–78.7)      |
| Weight after 4 wk (kg)| 83.1(72.9–93.3)        | 73.1(69.5–76.7)      | 76.2(72.8–79.6)      |
| Absolute weight gain (4 wk-baseline in kg)| 2.7(1.2–4.2)       | 1.8(0.8–2.8)        | 0.9(0.3–1.5)        |
| Relative weight gain after 4 wk (% of baseline)| 4.0(1.6–6.4)       | 2.9(1.4–4.4)        | 1.4(0.6–2.3)        |
| Baseline BMI (kg/m²)     | 26.7(22.9–30.5)        | 24.2(23.1–25.4)      | 25.8(24.7–27.0)      |
| BMI after 4 wk (kg/m²)   | 27.6(23.9–31.2)        | 24.8(23.7–26.0)      | 26.1(25.0–27.2)      |
| Absolute BMI gain (4 wk-baseline in kg/m²)| 0.9(0.4–1.4)       | 0.6(0.3–0.9)        | 0.3(0.1–0.5)        |
| Relative BMI gain after 4 wk (% of baseline)| 4.0(1.6–6.4)       | 2.9(1.4–4.4)        | 1.3(0.5–2.1)        |

Values in parentheses are 95% confidence interval.
episode population the adjusted population showed the expected continuous increase of weight and BMI from CC via AC to AA.

To illustrate the weight increase for all three study populations and to avoid bias effects caused by covariates, we calculated the estimated marginal means (adjusted means) for weight gain after 4 wk from our ANCOVA model with the rs489693 genotype as independent variable. This model resulted in significant p-values for the rs489693 genotype in all three study populations: Carriers of the homozygous A-allele had, dependent on study population, a 2.2–3.1 times higher increase in weight gain than the CC-genotype (Fig. 1). Moreover, we compared these results with Malhotra’s replication cohort 1 (only clozapine treated patients) and our results of the rs17782313 polymorphism, another very promising point mutation near the MC4R gene.

In contrast to our primary publication (ANOVA) and for better comparability Fig. 1 shows the results of an ANCOVA. We decided to compare with Malhotra’s cohort 1 since the majority of our study population received olanzapine, an SGA with a similar risk to clozapine for inducing weight gain (Allison et al., 1999). The ANCOVA of the subsamples of olanzapine and not olanzapine treated patients revealed a significant influence of the rs489693 polymorphism on weight gain after 4 wk for the non-olanzapine group (p=0.041, estimated marginal means: AA=1.78 kg, AC=1.54 kg, CC=0.56 kg). The olanzapine group also displayed higher weight gain for A-allele carriers (estimated marginal means: AA=2.91 kg, AC=2.09 kg, CC=1.80 kg), but this analysis did not reach significance. Comparing the two polymorphisms near the MC4R gene, both point mutations showed a comparable increase of weight and are in strong linkage disequilibrium (LD) (D’ = 0.293, r² = 0.632).

Discussion

We report a significant impact of the rs489693 polymorphism on SGA-related weight gain in all three study populations. According to our ANCOVA model, patients homozygous for the A-allele had a significantly higher increase in weight parameters than carriers of the CC-genotype. Moreover, AA-genotype carriers displayed higher baseline weight and BMI than AC- or CC-genotype carriers. This effect, however, only reached significance for baseline BMI in the whole study population.

Our observation regarding weight increase confirms a recently published GWAS of SGA-related weight gain (Malhotra et al., 2012). Although Malhotra et al. (2012) revealed higher weight gain for each genotype, the proportion between the AA- and the CC-genotype in weight gain (factor 2.9) is comparable to our analysis. Longer study period (6 wk vs. 4 wk) with clozapine as the only SGA, which has the highest potential of inducing weight gain (Allison et al., 1999) or unknown confounding factors may explain the stronger weight increase in the Malhotra et al. (2012) population. Moreover, subjects in Malhotra’s clozapine cohort were undergoing their first exposure to SGAs, which could have led to a stronger increase of weight than in pretreated patients, who already may have reached their plateau of weight increase. We, however, did not observe a more pronounced weight increase in our first episode population.

The observation regarding baseline BMI is in contrast to Malhotra et al., who could not detect a significant association between the rs489693 polymorphism and baseline BMI. The latter is surprising, because the rs489693 polymorphism is in strong LD with the rs17782313 polymorphism, which has been robustly associated with baseline weight and BMI. Therefore, an influence of the rs489693 polymorphism on baseline weight and BMI is not unlikely.

The exclusion of additional weight gain-inducing co-medication resulted in higher estimated marginal means for weight gain, pointing to a bias effect caused by unbalanced distributed co-medication (Fig. 1). The strong LD between the rs17782313 and the rs489693 polymorphism and the comparable effects in the Malhotra cohort underline the involvement of these point mutations in SGA-related weight gain. At present it is not clear which polymorphism is a better predictor of SGA-related weight gain, as the physiological mechanisms of these SNPs are unclear and linkage disequilibrium with other mutations cannot be excluded.

Although not all parameters reached significance in ANOVA, a nearly significant trend was observed for all weight parameters. Moreover, ANCOVA of weight gain revealed significant results in all three study populations, indicating that confounding factors like baseline weight or gender could have biased our results. This fact could explain why heterozygote patients of the whole study population showed the highest BMI increase and weight gain in ANOVA, whereas carriers of the AA-genotype revealed the highest weight modifications in the adjusted ANCOVA model. The subsample of only olanzapine-treated patients showed comparable results to the other investigated populations in ANCOVA, but did not reach significance, possibly due to small sample size or unknown confounding factors.
A limitation of our study is that it consists of nearly 75% patients, which had been exposed to SGA medication before admission to hospital, both in the whole study and adjusted subpopulation. Further analyses of first episode patients revealed comparable effects for the AA-genotype. We, however, observed an unexpected high weight increase for the heterozygous AC-genotype comparable to the AA-genotype.
This probably spurious effect was not observed in the ANCOVA of the other investigated study populations.

Another limitation is the naturalistic and retrospective study design with a heterogeneous SGA drug therapy. On the other hand, the naturalistic study design better reflects clinical practice and thus helps to confirm the clinical relevance of the studied polymorphisms.

To summarize, we could replicate the rs489693 A-allele as a possible risk factor and the region around the MC4R gene as a very promising area for SGA-related weight gain. As early weight gain during olanzapine treatment appears to be a good predictor of substantial long-term weight gain (Kinon et al., 2005; Lipkovich et al., 2008), identifying high risk patients before pharmacotherapy could be a next step into individualized antipsychotic therapy. Further studies are needed to elucidate the clear role and mechanism of this and other polymorphisms near and in the MC4R gene.

Acknowledgments

None.

Statement of Interest

F. Czerwensky declares no conflict of interest. W Steimer has received speaker fees from Roche Diagnostics and DELAB and consultancy honoraria from Abbott. S. Leucht has received honoraria for consulting/advisory boards from Alkermes, Bristol-Myers-Squibb, EliLilly, Janssen, Johnson & Johnson, Medavante, Roche; lecture honoraria from AstraZeneca, Bristol-Myers-Squibb, EliLilly, Essex-Pharma, Janssen, Johnson & Johnson, Lundbeck Institute, Pfizer and SanofiAventis, and EliLilly has provided medication for a trial with SL as the primary investigator.

References

