Associations between the *LEP* -2548G/A promoter and baseline weight and between *LEPR* Gln223Arg and Lys656Asn variants and change in BMI z in Arab children and adolescents treated with risperidone

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Abstract

Data on baseline (antipsychotic-naïve) age, weight, and height and change in these over three subsequent follow up time points up to 313.6 days (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 subsamples, results are reported from the white Arabs (N=144). Age and gender-normed BMI-standardised 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 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, compared to G/G). rs1137101 (G/G, P=0.018, OR=4.13 compared to 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 on risperidone, whilst being the first to report such associations in those of Arab ethnicity.

Key words (MeSH terms): Antipsychotic Agents; Weight Gain; Child; Adolescent; Serotonin; 5-HT2C

Abbreviations:
Attention deficit hyperactivity disorder (ADHD)
body mass index (BMI)
leptin gene (*LEP*)
leptin receptor gene (*LEPR*)
analysis of variance (ANOVA)
odds ratio (OR)
polymerase chain reaction (PCR)
random effects ordered logistic regression (REOLR)
serotonin5-HT2C receptor gene (*HTR2C*)
single nucleotide polymorphism (SNP)

Introduction

Antipsychotics are now relatively commonly used in children and adolescents for a variety of indications, including mood disorders, disruptive behavior disorders, developmental disorders, and psychosis [1-4]. While weight gain on antipsychotics has received greater attention in adults, it is also a relatively common adverse effect in children and adolescents [1]. The distribution of such weight gain tends to be central (abdominal), which is associated with dysregulation of adipokines, including leptin, ghrelin, and adiponectin. Such dysregulation may be associated with cognitive impairment, as well as with long-term adverse health outcomes (diabetes, cardiovascular disease, and some forms of cancer) [5-7]. Risperidone is the most frequently prescribed atypical antipsychotic in children and adolescents [1-4].

There are clear inter-individual differences in the magnitude of weight gain in patients treated with antipsychotics. Underlying such inter-individual differences are both genetic and environmental factors such as diet and sedentary lifestyle, and the interaction between these [8,9]. The leptin (*LEP*), leptin receptor (*LEPR*), and serotonin 5-HT_{2C} receptor (*HTR2C*) genes are among those with the strongest evidence for association with antipsychotic-induced weight gain [10-16], including specifically in risperidone-treated patients [17-20].

Markers rs7799039, rs1137101, and rs1805094 are single nucleotide polymorphisms (SNPs) in the *LEP* promoter and *LEPR* with previously identified relevant associations [21,22], although results have been variable [23-26]. SNP rs7799309 (-2548G/A) is located in the *LEP* promoter. In a study of the *LEP* -2548G/A variant in risperidone-treated children and adolescents, A allele carriers showed a steeper rate of increase in weight gain and the G/G genotype carriers were 2 times less likely to be overweight [17]. This marker has also been associated with a measure of metabolic dysfunction, cholesterol/high density cholesterol ratio, in adult male patients using atypical antipsychotics who were at a relatively early phase (less than a year) in treatment [24]. Other studies in adults did not find any association between rs7799309 and obesity after three months of antipsychotic use [27] or weight gain after antipsychotic use [28]. To our knowledge, there is only one study that examined rs7799309 and antipsychotic related weight gain in children and adolescents, which reported that A allele carriers had steeper weight gain [17]. This study was done in a white Arab sample with attention deficit hyperactivity disorder (ADHD) as the most frequent diagnosis. The *LEPR* rs1137101 and rs1805094 (into which rs8179183 has been merged) are functional variants encoding amino acid changes: the former (c.668A>G) encoding a glutamine to arginine substitution at amino acid 223 (Q223R) and the latter (c.1968G>C) a lysine to asparagine substitution at amino acid 656 (K656N). In a study of the *LEPR* Q223R

in 200 adult patients treated for a psychotic disorder with antipsychotics, in females, the average body weight was 13.6 kg more (95% CI, 1.11-26.1) in the *LEPR* 223QQ group compared with the *LEPR* 223RR group [27]. In a previous study of 13 SNPs and weight profile in olanzapine and risperidone-treated patients [26], the strongest genetic association for the risperidone-treated group was found with rs8179183 (the minor allele C being protective against weight with a frequency of 20% in those with a weight of 40–60 kg, and approaching 0% for those with weight above 100 kg). We herein report genetic association analysis of above SNPs in the *LEP* and *LEPR* as well as of the *HTR2C* rs1414334 in white Arab children and adolescents treated with risperidone, with a variety of diagnoses including ADHD.

Materials and methods

Sample

The inclusion criteria were children and adolescents aged 18 years or under who were taking risperidone. The following patients were excluded: those with anorexia or bulimia nervosa, those taking more than one antipsychotic drug, those taking other medications that could affect weight gain (e.g., corticosteroids, valproic acid, or methylphenidate), and those with concurrent medical conditions that could affect weight gain (e.g., diabetes, Cushing's syndrome, or renal disease).

Ethical approval was obtained from the Department of Neurology, King Fahd Hospital of the University of Damman in Saudi Arabia. All procedures were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study information sheets and consent forms were provided in multiple versions: for parents or guardians, and in age-appropriate versions so that participants could also understand (children under 8 years, 8-11 years, and 12-15 years; and adolescents aged 16-18 years; with assent forms additionally for those over 8 years of age). Eligible participants were identified from the pediatric neurology clinic of the Department of Neurology, King Fahd Hospital of the University of Damman. All were seen at the hospital by the researcher (NBA) with the responsible physician. NBA or the physician provided verbal translations for the study information sheets and consent forms as required. Informed consent was obtained from the parents or guardians for all participants included and, in children with capacity, assent was additionally obtained. Data on the following were extracted from patient clinical records: age, gender, date of birth, ethnicity, diagnosis, risperidone dose, dates of patient visits from baseline until the last visit recorded, weight and height. Clinic visits were recorded from baseline (first date prescribed risperidone, all patients being antipsychotic naïve at baseline, visit 0) to the third visit (visit 3); the time between visits

was 3 to 6 months depending on the patient's condition.

Age and gender-normed BMI-standardised z scores (BMI z) were generated using the lmsGrowth program, designed for use with growth references based on the LMS method [29]. In brief, the LMS method summarises BMI data in terms of three smooth age specific curves called L (lambda), M (mu), and S (sigma). The M and S curves correspond to the median and coefficient of variation of body mass index at each age whereas the L curve allows for the substantial age dependent skewness in the distribution of body mass index. The values for L, M, and S can be tabulated for a series of ages. Patient ID, date of birth, age, sex, height and weight were inputted into the program and BMI Standard Deviation Score (SDS-BMI, or BMI z) was calculated [29].

Genetic analysis

DNA was extracted from buccal swabs at the Institute of Psychiatry, Psychology and Neuroscience, London, UK as previously described [30]. Genotyping for five single nucleotide polymorphisms (SNPs), the -2548G/A promoter SNP (rs7799039) for *LEP*, and SNPs in *LEPR*, K109R (rs1137100), Q223R (rs1137101), K656N (rs1805094), and the *HTR2C* rs1414334 C/G intronic polymorphism, was performed using TaqMan SNP Genotyping Assays on a ViiA™ 7 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems/Life Technologies/Thermofisher, Canada) at the University of Alberta, Canada. Although the D' between rs1137101 and 1137100 was high at 0.84 and that between rs1137010 and rs1805094 also high at 0.86, the r^2 between these pairs of markers in our data was <0.5 (0.15 and 0.08 respectively), and therefore all markers were taken forward for analysis. While K109R (rs1137100) was originally selected for genotyping, owing to the relatively high linkage disequilibrium between this marker and rs1137101 (D'>0.0.84), data from this marker were not included in this analysis.

Statistical analysis

STATA 15.1 was used to conduct the analyses. One-way analysis of variance (ANOVA) was initially used to examine the effects of potential covariates on baseline weight (in kilograms). Linear regression was performed for baseline outcome variables – weight (kg) and BMI z, to explore which one of the two should be used for the repeated measures analysis. The linear regression model was as follows: baseline weight as the dependent variable and the following as independent variables: age in years, gender, diagnosis (psychosis versus the rest), height and genotype. Separate regressions were run for each of the four markers analyzed.

For the longitudinal analysis, preliminary linear mixed model analyses of change in BMI z revealed a poor distribution of the residuals. BMI z was therefore converted into an ordinal variable for ordered logistic regression as

follows: 0, 1, 2 and 3 for BMI z scores <-2, -2 to 1.99, 2 to 2.99, and >/= 3, respectively, based on previous literature [31]. Analysis of change in BMI z thus categorized over visits was conducted using random effects ordered logistic regression. Random effects ordered logistic regression in STATA is an efficient method to test longitudinal trends in an ordered variable [32]. The predictors in all such analyses were: genotype, baseline BMI z and diagnosis. To assess the effect of genotype over visits on BMI z, an interaction term between genotype as a factorial predictor and visit as a continuous predictor was employed, adjusting for baseline BMI z, with each genotypic analysis being run separately. In the random effects ordered logistic regression STATA analysis settings, subjects were used for the 'Panel ID variable' and visit was the 'time variable'. Results are reported using the odds ratio (OR) and *p* value. All results were not adjusted for multiple testing and are therefore reported as nominally significant.

Results

Of the 181 Arab patients approached, all provided consent, and usable data were available for 162 (144 white Arabs, 18 black Arabs). As the minor allele frequencies were significantly different in the black Arabs than in the white Arabs (data not shown), and, given the relatively small number of black Arabs, these were excluded from further analyses. The diagnostic distribution in the 144 white Arabs (98 boys, 46 girls) was as follows: 23 (15.97%) had autism, 65 (45.14%) had ADHD, 50 (34.72%) had a psychotic disorder (schizophrenia/schizoaffective disorder/bipolar disorder/psychosis not otherwise specified), and the number of subjects with a diagnosis of disruptive behavioral disorders or aggression, and of developmental disorders were 3 (2.08%) and 3 (2.08%), respectively. The mean age was 12.58 years (SD 4.99), and there was a significant difference in age between the different diagnostic groups ($P<0.001$). The mean ages of those with autism, ADHD, psychotic disorder, disruptive behavior disorders, and developmental disorders were 12.01, 10.26, 15.92, 16.43 and 7.69 years, respectively. The mean number of days between baseline, and first, second and third follow-up visits was 106.7 (95% CI 102.1-111.4), 209.7 (CI 202.9-216.5), and 313.6 (CI 303.5-323.7), respectively.

The genotyping call rate was 100%. All SNPs were in Hardy-Weinberg equilibrium ($P=0.58$, 0.82, 0.21, 0.18, and 0.42 for rs7799039, rs1137101, rs1805094, rs1137100, and rs1414334 respectively (with the Hardy-Weinberg *P* value for the latter being calculated from the subgroup of girls). As rs1414334 is an X- linked SNP, genotypes for boys were coded as 0 and 1 and for girls as 0, 1, or 2.

On examination by ANOVA of potential covariates (height, age, gender, and diagnosis) to include in the linear regression model of *baseline* weight, there was a significant effect of height ($F= 598.04$, $P<0.0001$) and of diagnosis

(F=9.53, *P*<0.0001), age (F=357.8, *P*<0.0001) and gender (F=4.27, *P*=0.04) on baseline weight. Risperidone dose was not included in this baseline analysis as all patients were *drug-naïve* at baseline. Owing to the significant difference in age between the different diagnostic groups, graphical visual inspection of weight by the diagnostic group was performed, which showed that those with a psychotic disorder had a higher baseline weight. The mean weight of those with a psychotic disorder was 68.20 kg (95% CI 65.89 to 70.50), while that of the rest was 48.26 kg (95% CI 46.04 to 50.48).

Linear regression analyses of baseline weight by genotype showed a nominally significant effect of rs7799039 A/G genotype (*P*=0.035, β=-3.62) with a trend for A/A genotype (*P*=0.097, β=-4.34), both being associated with lower baseline weight compared to G/G (Table 1). The proportion of the variance in weight (adjusted R^2) accounted for by the model was high at 81%, and the residual distribution good (Figure 1). There was no effect of the other genotypes: for rs1414334, no difference between C/G (*P*=0.666, β=1.25) and C/C genotypes (*P*=0.216, β=2.55) versus the G/G genotype; for rs1137101, no difference in baseline weight for A/G (*P*=0.625, β=-0.848) and G/G (*P*=0.614, β=-1.41) genotypes compared to A/A; for rs1137100, no difference in baseline weight for A/G (*P*=0.528, β=-1.36) compared to A/A; and for rs1805094, no difference in baseline weight for C/G (*P*=0.898, β=-0.228) and C/C (*P*=0.918, β=0.417) genotypes compared to G/G. By contrast, although linear regression of baseline BMI z (without gender, age and height, since BMI z takes these variables into account) gave a significant *P* value for the A/G genotype for rs7799039 (*P*=0.049, β=-0.604), the model adjusted R^2 was low at 3.5%, and the residual distribution poor (data not shown).

[Figure 1 near here]

In the random effects ordered logistic regression analysis of change in BMI z category over time, we observed the following: a significant effect of rs1137101 G/G (*P*=0.018, OR=4.13) genotype compared to A/A but not of A/G (*P*=0.826, OR=1.06) compared to A/A. A significant effect of rs1805094 C/G (*P*=0.042, OR=0.547) and a trend level effect of C/C (*P*=0.083, OR=0.27) genotype compared to G/G was also seen. Given the previous literature [26] and this pattern in our data, we grouped C/G and C/C genotypes together, and re-ran the ordered logistic regression, which resulted in a *P* value of 0.019 and an OR of 0.55. In the rs7799039 analysis, a trend level effect for individuals of A/G genotype (*P*=0.068, OR=1.72) compared to G/G was seen, with no such trend being seen for the A/A genotypic group (*P*=0.678, OR=0.84). For rs1414334, there was no significant effect of C/G (*P*=0.431, OR=0.72) or C/C (*P*=0.431, OR=0.79) compared to G/G genotype. Similarly, for rs1137100, there was no significant

effect for A/G (*P*=0.128, OR=1.85) compared to A/A All results are for genotype by visit interaction for change in BMI z category over time.

[Insert Tables 2 and 3 about here]

Discussion

We observed a nominally significant association between *LEP* rs7799039 and baseline weight; with the A/G genotype being nominally associated (*P*=0.035) with lower baseline weight and the A/A genotype having a trend level association (*P*=0.097). On sensitivity analysis entering all diagnoses into the model, the *P* values were similar (0.037 and 0.12 for A/G and A/A genotypes respectively). In the present study, the rs7799039 A/G genotype also had a trend level association (*P*=0.068) with increase in BMI z category over the three follow-up time points (which also remained similar at *P*=0.074 when entering all diagnoses into the model). In a previous study of risperidone in this age group by Calarge and colleagues [17], rs7799039 genotypes containing the A allele were associated with more weight gain. Although Calarge et al. did not find an association with baseline weight, of note, the ethnicity of their sample was different to ours (74 patients, 84% non-Hispanic Caucasian, 12% African American, 3% Hispanic, 1% Other).

In the random effects ordered logistic regression, we observed nominally significant effects for the *LEPR* Gln223Arg and Lys656Asn variants. The rs1137101 G/G (*P*=0.018, OR=4.13) genotype encoding 223QQ increased odds of BMI z increase compared to A/A; on sensitivity analysis entering all diagnoses rather than the dichotomized (psychosis versus the rest) variable, this result remained similar (*P*=0.021, OR=3.91). Moreover, this finding is consistent with previous literature. Meta-analytic evidence suggests that rs1137101 [G/G] increases the odds of type 2 diabetes mellitus, and other studies suggest a role for the same in obesity [33,34]. For rs1805094 (previously rs8179183), C-allele carriers (*P*=0.019, OR=0.51) had reduced odds of BMI z increase compared to G/G; on sensitivity analysis entering all diagnoses, the results were the same (*P*=0.019, OR=0.51). Interestingly, this is consistent with the results of Ruano et al. (2007) in which the strongest association with weight profile (out of 29 SNPs tested in 13 candidate genes) in the risperidone-treated group was with rs8179183 C-allele carriers (encoding 656N), who, as in our sample, were relatively protected against weight gain compared to rs8179183 [G/G] [26].

We included children treated with only one antipsychotic medication (risperidone), all of which were antipsychotic naïve at baseline, unlike other studies where adults treated with a variety of antipsychotics were pooled

into a single analysis [26,27,35,36]. This is first report of associations of genetic markers with weight gain in Arab children and adolescents treated with risperidone. Of note, there is a relative paucity of publicly available data on DNA sequence variation in Arabs and therefore any contribution to genetic associations in this population is valuable; the fact that our findings are largely consistent with those of previous investigators despite differing ethnicities is noteworthy.

The limitations of this study include sample size, gender distribution (low proportion of girls), lack of a measure of compliance, and lack of availability of a measure of activity level. Limited sample sizes increase the risk not only of finding a spurious positive association (a type I error) but also of missing genetic associations with small effect sizes (a type II error). However, a power analysis using Quanto 1.2.4 [37] revealed that for a sample size of 130, with a SNP minor allele frequency of 0.28-0.29 (frequency range for the rs1137101), there was 80% power using an odds ratio of 1.60 (in fact substantially less than our odds ratio for this marker) for the risk genotype in an additive model with alpha set at 0.05 (without adjustment for multiple testing); our sample was therefore sufficiently powered to generate a result of nominal significance. We have not adjusted for multiple testing and hence report our findings as of nominal significance. An adjustment for multiple testing such as Bonferroni would be too conservative, as a Bonferroni correction assumes independence of tests conducted. In fact, the variants are both statistically (in terms of linkage disequilibrium) and functionally significantly related (including the *HTR2C* polymorphism being associated with circulating levels of leptin [38]). Another potential criticism could be the focus on baseline weight rather than baseline BMI z score for the baseline analysis. We have justified this by presentation of the adjusted R^2 for both. Of relevance to BMI z calculations, the Centers for Disease Control and Prevention 2000 growth charts do not include Arab ethnicity, which may explain why the use of the weight variable, adjusting for age, gender, height and diagnosis was appropriate for the baseline analysis in our sample.

Conclusion

Our investigation of baseline weight and change in BMI z and relevant genetic variants in Arab children and adolescents treated with risperidone revealed associations with functional variants in the leptin pathway. Specifically, baseline weight was nominally associated with SNP rs7799039 encoding *LEP* -2548G/A, while change in BMI z category was nominally associated with *LEPR* 223QQ (rs1137101) and 656N (rs1805094/rs8179183) variants, the latter replicating an earlier report of this variant being protective against risperidone-associated weight gain. Further replication and extension to more diverse demographic groups is desirable. Specifically, we recommend further

studies of this and other variants in the *LEP* promoter and *LEPR* versus baseline weight and BMI z category on treatment with risperidone and other antipsychotics in individuals of Arab and other ethnicities in this age group and in adults.

Given the association between markers in the leptin pathway and relevant Mendelian genetic disorders, it would also be interesting to see if markers in the leptin pathway were associated with persistent weight gain on psychotropics despite interventions aimed at reducing weight gain, and, in a larger sample, whether such markers are associated with weight gain to an unhealthy extent (to an at least obese level). More thorough analysis of this gene including sequencing and haplotype analysis might result in the identification of other functionally relevant variants in this ethnic group. Although often difficult in practice, replication studies should ideally use protocols with antipsychotic monotherapy, especially for children and adolescents in their first psychotic episode, and, like this one, ideally commence the study when patients are antipsychotic naïve. Additionally, extending the analysis to other antipsychotics and to more genes, as well as conducting more complex analyses including consideration of gene–environment interactions including epigenetics and gene–gene interactions models could shed further light on relevant biological mechanisms [39]. As patients and their caregivers are certainly interested in preventing and ameliorating antipsychotic-associated weight gain and predicting who will respond well to interventions for this and who will not, further collaborative research efforts in this area are indicated.

Conflict of Interest

KJA reports consultancy services for Otsuka Canada Pharmaceutical Inc., and Lundbeck Canada and a research fellowship grant to a trainee from Jannsen Inc., Canada.

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Table 1 Linear regression showing the nominally significant association between baseline weight and rs7799039 genotype (with G/G as the reference genotype)

	β	t	*P*	95% CI	
rs7799039					
A/G	-3.62	-2.13	**0.035**	-6.97	-0.266
A/A	-4.34	-1.67	0.097	-9.48	0.801
Age (years)	0.001	0.9	0.372	-0.001	0.004
Gender	0.383	0.21	0.833	-3.19	3.96
Diagnosis	-2.50	-1.32	0.188	-6.24	1.24
Baseline height	0.71	7.90	**<0.001**	0.533	0.889

Table 2 Ordered logistic regression analysis of BMI z category, showing a nominally significant rs1137101 by time interaction

	Odds Ratio	*z*	*P*	95% CI	
rs1137101					
A/G	0.857	-0.19	0.852	0.169	4.34
G/G	0.163	-1.16	0.245	0.008	3.48
Time	2.00	3.61	<0.001	1.37	2.92
rs1137101*Time					
A/G	1.06	0.22	0.826	0.612	1.85
G/G	4.13	2.37	**0.018**	1.28	13.38
Diagnosis	2.95	1.53	0.127	0.734	11.82
Baseline BMI z	21.15	7.54	<0.001	9.57	46.75

Table 3 Ordered logistic regression analysis of BMI z category, showing a nominally significant rs1805094 (previously rs8179183) by time interaction for the C/G genotype and a nominal trend for the C/C genotype

	Odds Ratio	*z*	*P*	95% CI	
rs1805094					
C/G	1.63	0.57	0.568	0.304	8.71
C/C	1.58	0.22	0.824	0.028	90.43
Time	2.86	5.47	<0.001	1.96	4.17
rs1805094*Time					
C/G	0.547	-2.04	**0.042**	0.307	0.978
C/C	0.269	-1.74	**0.083**	0.061	1.19
Diagnosis	2.80	1.46	0.145	0.70	11.21
Baseline BMI z	19.75	7.63	<0.001	9.17	42.51

Fig. 1 Standardized normal probability plot of post regression residuals for baseline weight with age in years, gender, diagnosis (psychosis versus the rest), height and genotype (rs7799039) as predictors