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Variants of the dopamine D2 receptor and risperidone-induced hyperprolactinemia in children and adolescents

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Abstract

Objective—To investigate the association between hyperprolactinemia and variants of the dopamine D₂ receptor (*DRD2*) gene in children and adolescents in long-term treatment with risperidone.

Methods—Medically healthy 7–17-year-old patients chronically treated with risperidone but receiving no other antipsychotics were selected for a cross-sectional evaluation. Four *DRD2* variants were genotyped and prolactin concentration was measured. Medication history was obtained from the medical record. The effect of the TaqIA variants of the *DRD2* on the risk of risperidone-induced hyperprolactinemia was the primary outcome measure.

Results—Hyperprolactinemia was present in 50% of 107 patients (87% males) treated with risperidone for an average of 2.9 years. Age, stage of sexual development, and the dose of risperidone independently predicted a higher prolactin concentration, whereas the dose of psychostimulants was negatively correlated with it. However, these four predictors became nonsignificant when risperidone serum concentration was entered into the model. Adverse events potentially related to hyperprolactinemia were more common in participants with elevated prolactin concentration in girls (45%) compared with boys (10%). After controlling for risperidone concentration and the dose of psychostimulants, the TaqIA A1 and the A-241G alleles were associated with higher prolactin concentration, whereas the -141C *Ins/Del* A1 and C957T variants had no significant effect. In addition, adverse events potentially related to hyperprolactinemia were four times more common in TaqIA A1 allele carriers.

Conclusion—Prolactin concentration is closely related to central *DRD2* blockade, as reflected by risperidone serum concentration. Furthermore, the TaqIA and A-241G variants of the *DRD2* gene could be useful in predicting the emergence of hyperprolactinemia and its potential adverse events.

Keywords

adolescents; antipsychotics; children; dopamine receptors; *DRD2*; genetic variants; hyperprolactinemia; risperidone; TaqIA

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Introduction

Antipsychotic medications induce hyperprolactinemia by blocking the dopamine D₂ receptors (*DRD2*) in the anterior pituitary [1,2]. This, in turn, can cause galactorrhea and suppress gonadotropin production, leading to amenorrhea, decreased libido, and infertility [2]. Acutely, these adverse events (AEs) potentially interfere with medication adherence and quality of life. Over more extended treatments, hyperprolactinemia may lead to decreased bone mineral density [1-3].

Risperidone is one of the antipsychotics most likely to cause hyperprolactinemia with up to 71% of the children developing this AE acutely and more than 30% continuing to have it, a year or more later [4,5].

In adults, sex, menopausal status, and antipsychotic type and dose are associated with prolactin concentration [2]. In addition, genetic factors, such as variants of the *DRD2* gene, seem to influence the propensity for antipsychotic-induced hyperprolactinemia. For example, in women with schizophrenia treated with nemonapride or bromperidol, the A1 allele of the TaqIA variant of the *DRD2* gene was associated with a higher prolactin concentration and with a higher ratio of change in prolactin by medication serum concentration [6,7]. Similarly, Young *et al.* [8] described higher prolactin concentration in TaqIA A1 allele carriers receiving antipsychotics.

The interest in the *DRD2* gene stems from the identification of several variants that might alter its function. For example, neuroimaging and postmortem receptor binding studies have revealed that carriers of the TaqIA A1 allele have a lower *DRD2* density in the basal ganglia [9-11]. Furthermore, this allele results in a glycine to lysine substitution in a gene located 9.5kb downstream of the *DRD2* gene and is associated with increased striatal L-amino acid decarboxylase activity [12,13]. In addition, the C957T and -141C *Ins/Del* represent other *DRD2* gene variants linked to decreased messenger RNA stability and *DRD2* synthesis or to reduced gene transcription, respectively [14,15]. Thus, findings across different lines of research converge to suggest a functional role for several *DRD2* variants. This, in turn, might translate into a differential propensity for response to treatment or emergence of AEs.

In this study, we evaluated the association between the TaqIA, C957T, -141C *Ins/Del*, and A-241G variants of the *DRD2* gene and prolactin concentration in youths treated chronically with risperidone. As studies have most consistently linked the TaqIA variants to alteration in *DRD2* density and to antipsychotic-induced hyperprolactinemia in adults with psychosis, our primary hypothesis was that hyperprolactinemia would be more common in the A1 allele carriers. We aimed to replicate and extend findings from studies conducted in adults because of the differential drug metabolism and tolerability of children and adolescents, the active brain development during growth, and the incomplete maturation of the dopaminergic system and its potential susceptibility to long-lasting disruption after a brief postnatal exposure to drugs [16,17]. Finally, the downstream effect of hyperprolactinemia on bone mineralization in children and adolescent might be more dramatic, compared with adults, and possibly irreversible [18,19].

Patients and methods

Participants

Participants, 7–17 years old, treated with risperidone for 6 months or more, irrespective of diagnosis or indication, were selected from psychiatric outpatient clinics. Concomitant treatment with psychotropic medications, such as psychostimulants and antidepressants, was allowed except for antipsychotics other than risperidone. Medical conditions that could

interfere with normal pituitary function led to exclusion as did metabolic, autoimmune, and hormonal diseases, except for minor thyroid stimulating hormone elevation [20]. Pregnant females and those receiving hormonal contraception were not eligible.

Procedures

This study was approved by the local institutional review board. Assent was obtained from children and consent from adolescents and all parents or legal guardians.

Race and ethnicity were based on self-report and the psychiatric diagnoses on chart review. Treatment information was extracted from the medical and psychiatric records. All dosages of psychostimulants were expressed in methylphenidate equivalents for amphetamines (x2) [21]. To evaluate the validity of the psychiatric diagnoses made by the participants' mental healthcare providers, a best-estimate diagnosis was generated in 49 participants based on a combination of a review of the psychiatric record and a standardized interview of the parent and the youth (if 11 years old or older) using the National Institute of Mental Health Diagnostic Interview Schedule for Children [22]. Agreement between the clinical diagnosis and the research-generated best-estimate diagnosis was compared, using κ statistic [23], for five diagnostic categories: attention deficit hyperactivity disorder, disruptive behavior disorder, pervasive developmental disorder, tic disorder, and mood disorder. The κ values were 0.63, 0.66, 0.73, 0.61, and 0.51, respectively, reflecting moderate to substantial agreement. The lower agreement for mood disorders likely reflects the transient nature of this disorder, in contrast to the others, making it less likely for the symptoms to remain present upon selection.

Pubertal stage was evaluated by a physical exam and self-assessment [24]. During this exam, before any laboratory results became available, the participants and their parent(s) were queried about the presence of AEs potentially related to hyperprolactinemia (Table 1).

In 83% of the participants, a morning fasting blood sample was obtained to measure thyroid stimulating hormone, prolactin, and trough risperidone and 9-OH-risperidone serum concentrations (referred to as risperidone concentration, henceforth). In the remaining participants, a nonfasting blood sample was collected. Prolactin was measured by electrochemiluminescence immunoassay. On the basis of the upper range of normal of this assay, hyperprolactinemia was defined as a prolactin level of greater than 18.4 ng/ml in males and greater than 24.1 ng/ml in females. In our work, reported elsewhere [3], we have found prolactin concentrations above these cutoffs to negatively affect bone mineral density in boys in this sample.

Genotyping for the TaqIA, C957T, -141C *Ins/Del*, and A-241G variants of the *DRD2* gene was done using polymerase chain reaction (PCR) and sequencing primers that were designed with Pyrosequencing SNP Primer Design Version 1.01 software (<http://www.pyrosequencing.com>). PCR reactions were done in a 30 μ l volume with 1.5 mmol/l Mg^{2+} , 10 μ mol of each forward and reverse primer, according to the following specifications: initial denaturation time of 5 min at 95°C followed by 45 cycles of 95°C for 30 s, primer specific annealing temperatures for 30 s, 72°C for 30 s, and a final extension of 10 min at 72°C. Genotyping was done with Pyrosequencing Technology [25]. PCR products were visualized by electrophoresis on 1.8% agarose gels stained with ethidium bromide before pyrosequencing.

Statistical analysis

Prolactin values were log transformed to normalize the data residuals. Initially AQ2, multiple linear regression was used to investigate, in the total sample ($n=107$), whereas age, stage of sexual development, sex, treatment with selective serotonin reuptake inhibitors (SSRIs), dose

of psychostimulants and risperidone per kilogram of body weight, and duration of risperidone treatment independently predicted prolactin concentration. Then, to investigate the role of the different *DRD2* variants in risperidone-induced hyperprolactinemia, we entered each genetic group, individually, in the model while controlling for the significant covariates identified in the initial step. Finally, we used logistic regression to test for the association of hyperprolactinemia, defined categorically as described earlier, with the TaqIA A1 carrier state, controlling for the same significant independent covariates [26]. The adjusted odds ratios (OR) for hyperprolactinemia along with 95% confidence intervals (CI) were then computed.

Owing to racial/ethnic differences in the distribution of the *DRD2* variants [15], we restricted the genetic analysis to nonHispanic Caucasians ($n=90$). The homozygous wild genotypes for the TaqIA, -141 *Ins/Del*, and A-241G variants were uncommon ($\leq 3\%$, see Results). Consequently, a recessive mode of inheritance was used in the analyses. In contrast, both a recessive and a codominant mode were tested for the C957T variant.

As our primary hypothesis was concerned with the TaqIA variants of the *DRD2* gene, we divided the sample based on A1 allele carrier state. Though not statistically significant, fewer TaqIA A1 allele carriers received psychostimulants (Table 2), which can suppress prolactin release because of their dopamine-agonist activity [27]. To test whether this potential confound affected our results, we matched the two TaqIA genetic groups on the number of patients treated with psychostimulants and, in those treated, on the daily dose of psychostimulants per kilogram of body weight.

Differences across the two TaqIA genetic groups were compared using Student's *t*-test for continuous variables and Fisher's Exact test for categorical ones. The Wilcoxon's rank sum test was used whenever the *t*-test assumption of normality was violated. The McNemar's test was used to compare the rate of hyperprolactinemia and the Wilcoxon's signed rank test to compare prolactin concentration across the two matched TaqIA genetic groups. All tests were two tailed with the statistical significance set at $\alpha=0.05$. All analyses were conducted using SAS version 9.1.3 (SAS Institute Inc, Cary North Carolina, USA).

Results

A total of 109 participants were enrolled. Two boys, one from each TaqIA genetic group, had a risperidone concentration less than 0.5ng/ml and were excluded from further analyses because of suspected medication nonadherence. In the remaining 107 participants (87% males), although externalizing disorders were the most prevalent psychiatric conditions, comorbidity was common (Table 2). Risperidone was administered to target irritability and aggression in 76% ($n=81$) of the sample, tic disorder in 10% ($n=11$), severe impulsivity in 8% ($n=8$), sleep problems in 3% ($n=3$), obsessive compulsive disorder in 2% ($n=2$), and mood symptoms in one child. The dose prescribed is consistent with published trials [28]. In addition to risperidone, participants were most often taking psychostimulants (65%), $\alpha 2$ -agonists (52%), and SSRIs (32%), concurrently.

The median prolactin level was 18.7ng/ml (interquartile range: $13.4\text{--}28.5$) with 50% of the participants exhibiting hyperprolactinemia as defined earlier. With a cutoff of 25.7ng/ml , regardless of sex, age, or pubertal status [29], 34% ($n=36$) of the sample had hyperprolactinemia.

Using multiple linear regression, we tested whether stage of sexual development, sex, SSRI treatment, dose of psychostimulants and risperidone per kilogram of body weight, or duration of risperidone treatment were associated with prolactin concentration in the entire sample ($n=107$). We found that the dose of risperidone (partial $R^2=0.1$, $P=0.0009$) and Tanner stage (partial $R^2=0.07$, $P<0.003$) positively predicted prolactin concentration, whereas the dose of

psychostimulants (partial $R^2=0.04$, $P<0.03$) was negatively associated with it. None of the other covariates were significant. When age was substituted for Tanner stage, comparable results were found with age accounting for 3.5% of the variance in prolactin concentration (partial $R^2=0.035$, $P<0.04$). When risperidone concentration was substituted for the dose, it accounted for 39% ($P<0.0001$) of the variance in prolactin concentration with Tanner stage (or age) and the dose of psychostimulants becoming nonsignificant. Similar results were found when these analyses were restricted to males (females were a small proportion of the total sample).

As illustrated in Table 1, of 99 participants queried, 14% reported AEs potentially related to hyperprolactinemia. Of the 11 queried girls, five (45%) reported at least one AE compared with 10% ($n=9$) of boys (Fisher's Exact $P=0.008$). Although most participants did not report any AE potentially related to hyperprolactinemia, 23% ($n=12$) of those with hyperprolactinemia endorsed at least one AE compared with 4% ($n=2$) of those without (Fisher's Exact $P=0.01$). In fact, all the males and the three girls who reported at least one AE had hyperprolactinemia (Table 1).

Owing to racial/ethnic differences in the distribution of the different *DRD2* variants [15], we restricted the genetic analyses to nonHispanic Caucasians who formed 84% ($n=90$) of the sample. The genotype frequencies were as follows: TaqIA A2A2 63% ($n=57$), A1A2 33% ($n=30$), and A1A1 3% ($n=3$); C957T CC 24% ($n=22$), CT 44% ($n=40$), and TT 31% ($n=28$); -141 *Ins/Del* Ins/Ins 77% ($n=69$), Ins/Del 22% ($n=20$), and Del/Del 1% ($n=1$); and A-241G AA 87% ($n=78$), AG 13% ($n=12$), and GG 0%. All were in Hardy—Weinberg equilibrium. Using the Haploview program [30] (Fig. 1), TaqIA and -141C *Ins/Del* were found to be incomplete, but AQ3nonsignificant ($D'1$, $R^2=0.04$, LOD AQ4score 0.79), linkage disequilibrium (LD). Significant LD was detected between C957T and TaqIA ($D'0.76$, $R^2=0.17$, LOD score 3.39) and C957T and -141C *Ins/Del* ($D'0.86$, $R^2=0.12$, LOD score 2.69). A-241G was not in LD with any of the three other variants.

As shown in Table 2, there were no demographic or clinical differences between the two TaqIA genetic groups except for patients with the A2A2 genotype being more likely to have attention deficit hyperactivity disorder ($P<0.006$).

Prolactin concentration was higher in the TaqIA A1 allele carriers ($P=0.03$). Furthermore, whether defined based on the laboratory upper normal limit for males and females or using a single cutoff of prolactin concentration greater than 25.7 ng/ml [29], the rate of hyperprolactinemia was 1.5–2 times higher in the A1 allele carriers compared with participants with the A2A2 genotype ($P\leq0.04$). We found similar results when we matched the two genetic groups on the daily dose of psychostimulant per kilogram of body weight. In fact, the median prolactin concentration was 24.0 ng/ml in the A1 allele carriers and 16.2 in the A2A2 group (Wilcoxon's signed rank $S=114$, $P=0.04$) and the rate of hyperprolactinemia was 65% ($n=22$) in the A1 allele carriers versus 34% ($n=11$) in the A2A2 group (McNemar's $S=7.1$, d.f.=1, $P=0.01$).

We then used multiple linear regression to predict prolactin concentration adjusting for the TaqIA genetic group as well as the significant covariates identified earlier (i.e. age or Tanner stage, the dose of risperidone or its serum concentration, and the dose of psychostimulants). As expected, the daily dose of risperidone per kilogram of body weight increased and that of psychostimulants decreased prolactin concentration. We also found a trend for prolactin to increase with age ($F(1,85)=2.7$, $P=0.1$) and with the TaqIA A1 allele carrier state [adjusted least squares means of log prolactin 3.2 ng/ml in A1 allele carriers versus 2.9 in noncarriers, $\beta=0.24$, $F(1,85)=3.2$, $P<0.08$]. This genetic effect accounted for 2.8% of the variance in log prolactin concentration ($\Delta R^2=0.028$). Similar results were obtained when the stage of sexual

development, instead of age, was included in the model. When the risperidone concentration was substituted for the oral dose, the TaqIA effect became significant ($\beta=0.24$, $F(1,78)=4.3$, $P=0.04$, $\Delta R^2=0.030$) while the opposite was true for the dose of psychostimulants ($F(1,78)=2.5$, $P=0.1$). However, neither age nor Tanner stage significantly contributed to the model ($P>0.4$). Finally, we used logistic regression to compare the rate of hyperprolactinemia across the two TaqIA genetic groups while controlling for the concentration of risperidone and the dose of psychostimulants per kilogram of body weight. We found that the adjusted odds of developing hyperprolactinemia for A1 allele carriers were 3.1 times (OR: 95% CI: 1.1–9.7, Wald $\chi^2=4.0$, $P<0.05$) greater than for participants with the A2A2 genotype. The risperidone concentration also increased the probability of hyperprolactinemia (Wald $\chi^2=14.8$, $P<0.0001$), whereas the dose of psychostimulants had no effect ($P=0.3$). When the analyses were restricted to boys, the pattern of the results remained unchanged.

Next, we explored the effects of the -141C *Ins/Del*, C957T, or A-241G variants on prolactin concentration using multiple linear regression and controlling for the serum concentration of risperidone and the dose of psychostimulants per kilogram of body weight. Although the former continued to have a strong positive effect on prolactin, neither psychostimulants nor the -141C *Ins/Del* variant had a significant effect ($P>0.1$ and $P=0.6$, respectively). When C957T was substituted for -141C *Ins/Del*, we again found a strong effect of risperidone concentration ($P<0.001$) but no effect of psychostimulants ($P>0.2$). In addition, there was a trend for the three genotypes to predict prolactin, with the CT genotype having the highest concentration ($F(2,77)=2.6$, $P=0.08$, $\Delta R^2=0.037$). With a recessive mode of transmission, C957T had no significant effect ($P=0.4$). In contrast, after controlling for the serum concentration of risperidone ($F(1,78)=52.2$, $P<0.0001$) and the dose of psychostimulants ($F(1,78)=5.6$, $P=0.02$), having the AA genotype of the A-241G variant was associated with a strong protective effect ($\beta=-0.53$, $F(1,78)=10.0$, $P=0.002$). This genetic effect accounted for 6.6% of the variance in prolactin concentration ($\Delta R^2=0.066$). When both TaqIA and A-241G genetic groups were introduced into the regression model predicting prolactin, both independently contributed to the model [$\beta=0.23$, $F(1,77)=4.1$, $P<0.05$, $\Delta R^2=0.026$ and $\beta=-0.51$, $F(1,77)=9.7$, $P<0.003$, $\Delta R^2=0.062$, respectively] in addition to risperidone serum concentration and the dose of psychostimulants. Finally, after controlling for the concentration of risperidone and dose of psychostimulants, we found a synergistic effect of the TaqIA and A-241G variants on prolactin concentration ($F(3,76)=5.1$, $P=0.003$) (Fig. 2). In fact, compared with those participants with the TaqIA A2A2/-241AA joint genotype, participants with the A1 allele/AA genotype experienced a mean increase in prolactin (log transformed) of 7% ($n=28$, $t=1.5$, $P=0.1$) whereas those with the A2A2/-241G allele genotype experienced a 14% mean increase in prolactin ($n=7$, $t=1.8$, $P=0.07$). In participants with the A1/-241G alleles joint genotype, the mean prolactin concentration was 31% higher ($n=5$, $t=3.6$, $P=0.0006$).

Finally, after controlling for hyperprolactinemia status (Wald $\chi^2=4.1$, $P=0.04$), the odds of developing at least one AE potentially related to hyperprolactinemia was 4.4 times greater in TaqIA A1 allele carriers compared with A2A2 participants (OR: 95% CI: 1.1–76.0, Wald $\chi^2=4.0$, $P<0.05$). However, although hyperprolactinemia status continued to increase the risk for AE (Wald $\chi^2=5.0$, $P<0.03$), the A-241G variants did not affect this risk, likely because of the small number of A-241G allele carriers ($n=11$). Similar analyses were not conducted with the other variants, owing to their lack of effect on prolactin concentration, or with the joint genotypes because of the small size of the different genetic subgroups.

Discussion

To our knowledge, this is the largest study to investigate the role of several variants of the *DRD2* gene in chronic antipsychotic-induced hyperprolactinemia in children and adolescents. Prolonged hyperprolactinemia may interfere with bone mineralization [3], which, in turn, could

increase lifetime morbidity and mortality, particularly when it develops during childhood and adolescence, a period of accelerated bone mass accrual [18,19]. After an average of 3 years of risperidone treatment, 34–50% of the sample exhibited hyperprolactinemia. Moreover, the TaqIA A1 and A-241G alleles were associated with higher prolactin concentrations, with the A1/-241G alleles joint genotype resulting in a 31% higher concentration compared with the genotype without either alleles. Furthermore, the TaqIA A1 carrier state also increased the risk for hyperprolactinemia-related AEs.

Before discussing the results, we review the study limitations. First, the sample is heterogeneous, reflecting the variety of psychiatric conditions where risperidone is prescribed. In addition, the diagnoses were based on chart review and prolactin measurements were conducted cross-sectionally, in a naturalistically treated sample. When we compared the chart-based diagnoses to best-estimate research diagnoses made in nearly half the sample, there was moderate-to-substantial agreement. This is likely because of the fact that more than 90% of our participants were selected from a teaching hospital where AQ5DSM-based diagnoses are generated after an extensive evaluation and are reviewed systematically on a regular basis. As our overall aim was to investigate the safety of long-term treatment with risperidone in children and adolescents, we designed this study to reduce the risk of patients discontinuing the medication because of acute AEs or lack of efficacy. High attrition is a characteristic of most long-term psychiatric studies[4,31,32]. The possible impact of attrition on the role of the *DRD2* variants in hyperprolactinemia is unknown but should not detract from the significance of our findings. In fact, our data implicate the TaqIA and the A-241G variants in risperidone-induced hyperprolactinemia specifically during long-term treatment. Furthermore, attrition did not seem to selectively affect a particular genotype as the allele frequencies remained in Hardy—Weinberg equilibrium, at rates comparable with those observed in the other short-term pediatric placebo-controlled study [4]. Another shortcoming of this study is the unequal, though not statistically significant, proportion of participants receiving psychostimulants across the two TaqIA genetic groups. Owing to their dopamine-agonist activity, this difference could explain the lower rate of hyperprolactinemia in the A2A2 participants [27]. In contrast, in those treated with psychostimulants, the A1 allele carriers tended to receive a higher dose, potentially attenuating the effect of this allele on prolactin (Table 2). We addressed this confound by matching the two genetic groups on the number of patients treated with psychostimulants and, in those treated, on the daily dose of psychostimulants. We also controlled for the daily dose of psychostimulants in the other analyses. In fact, although psychostimulants did predict prolactin concentration in the model that included the dose of risperidone, this effect disappeared when the serum concentration of risperidone was substituted for the oral dose. In contrast, the genetic effect persisted. Although we did not correct for multiple comparisons because our primary analysis involving the TaqIA variant was hypothesis-driven, the highly significant finding of the A-241G variants withstands a Bonferroni correction ($\alpha=0.05/4=0.013$). Another challenge intrinsic to longitudinal studies relates to medication nonadherence, a common problem in chronic diseases. We tried to minimize this effect by excluding patients with undetectable risperidone concentration. Furthermore, we confirmed that our findings applied to boys but could not do the same with girls or with ethnic/racial minority groups because of small sample sizes. In addition, because of the low frequency of certain genotypes, we could not test a codominant mode of inheritance for three of the four genotypes. Therefore, our findings require replication in a larger and more racially/ethnically diverse sample. This would also allow testing the role other genes might have in antipsychotic-induced hyperprolactinemia, such as genes transcribing alternative dopaminergic receptors or membrane drug transporters. As the contribution of additional genetic variants is clarified, a more comprehensive model for antipsychotic-induced hyperprolactinemia will be developed.

Several studies have suggested that the rate of risperidone-induced hyperprolactinemia decreases over time but remains significant [4,5,33-35]. Consistent with these reports, a

sizeable proportion of our sample continued to have hyperprolactinemia. Owing to the wide range of duration of exposure to risperidone (0.5–8.3 years), it is possible that this AE will resolve in some of our participants. We, however, found no correlation between the total duration of risperidone treatment and prolactin (Spearman's $r=0.07$, $n=107$), suggesting that after an initial period, the concentration stabilizes regardless of the total duration of maintenance treatment.

Fourteen percent of the sample endorsed at least one AE potentially related to hyperprolactinemia. This rate is higher than those reported in two large pediatric longitudinal studies [4,5], possibly because of the older age of our participants and their longer treatment with risperidone. In addition, we explicitly asked about these AEs, which might have prompted patients to endorse symptoms they may not have reported spontaneously. Moreover, participants with hyperprolactinemia were six times more likely to endorse an AE.

Owing to the potential clinical significance of our findings, however, some clarifications are necessary. The reliable and valid assessment of these symptoms in children is challenging [4, 5]. For example, in those patients who complained of gynecomastia and were examined by a physician, no increase in breast tissue was noted. Two factors could lead to the endorsement of AEs that do not necessarily result from hyperprolactinemia. First, some of these symptoms occur during normal development [5]. In fact, more than 40% of males could develop pubertal gynecomastia [36] and a sizeable number of females exhibit menstrual irregularities during the first 2 years after menarche [37]. As such developmental changes usually normalize within 2 years of the onset of puberty, longitudinal follow-up is paramount. This is being pursued by our group in a follow-up study currently underway. Second, excessive weight, often associated with risperidone [24] makes the accurate diagnosis of gynecomastia more challenging because of the increase in the adipose tissue component of the breast [36]. Interestingly, however, BMI z -score did not vary across the two TaqIA genetic groups ($t=0.2$, d.f.=88, $P=0.8$) even though only A1 allele carriers reported gynecomastia. We also found no correlation between BMI z -score and prolactin (Spearman's $r=0.04$). Finally, querying about libido is also of unclear reliability in this age group. In total, these challenges, which do not necessarily apply to adults, add to the complexity of the decision-making process in children when evaluating the clinical implications of hyperprolactinemia.

As reported here and elsewhere, several clinical factors correlate with prolactin concentration during antipsychotic treatment [2]. For example, we found that prolactin rises with age or sexual development, likely signaling the maturation of the central dopaminergic system and the hypothalamic-pituitary-gonadal axis [38–40]. In addition, psychostimulants exerted a suppressing effect on prolactin but only before the risperidone concentration was taken into account. Once the latter was entered into the regression model, it alone explained 39% of the variance in the prolactin concentration with the other potential covariates becoming nonsignificant. This probably reflects the more proximal nature of the serum concentration as a marker of *DRD2* blockade compared with the oral dose.

Though sexual development (or age), dose of psychostimulants, and dose of risperidone (or serum concentration) are all closely and independently correlated with prolactin, more than 60% of the variance in prolactin concentration remains unaccounted for. This suggests that genetic factors may contribute to one's susceptibility to antipsychotic-induced hyperprolactinemia. In fact, as predicted, compared with the A2A2 genetic group, the TaqIA A1 allele carriers were two to three times more likely to develop hyperprolactinemia, after controlling for the dose of risperidone (or its serum concentration) and of psychostimulants. In contrast, the AA genotype of the A-241G variants exerted a protective effect. As a result, prolactin concentration gradually escalated with the different possible genotype combinations.

Although, to our knowledge, this is the first study to evaluate the role of the A-241G variants in antipsychotic-related hyperprolactinemia, three studies have identified a link between the TaqIA A1 allele and increase in prolactin in adults with schizophrenia treated with antipsychotics [6-8]. Furthermore, in healthy Chinese-Canadian men, Aklilu *et al.* [41] found carriers of this variant to predict a faster increase in prolactin after a single dose of perphenazine. In contrast, no association was found between TaqIA variants and risperidone-induced hyperprolactinemia in institutionalized Japanese adults with schizophrenia or in children with autism [4,42]. Nonetheless, our findings partially agree with data from this latter study where C957T and -141C *Ins/Del* were also found not to affect prolactin concentration. Possible factors that could explain the partial divergence in the results include our enrolment of a more heterogenous clinical group of older age, receiving other psychotropics concomitantly with risperidone. For example, 32% of our participants were prepubertal compared with 81% in the study by Anderson *et al.* [4]. In addition, we focus on long-term prolactin concentration, whereas they restricted their genetic analysis to the change in prolactin at week 8 after the onset of risperidone treatment [4]. As the vast majority of children develops risperidone-induced hyperprolactinemia acutely [5], it might be argued that genetic factors are more critically implicated in the long-term risk for this AE to emerge where more interindividual variability is observed. Furthermore, in most of our participants, prolactin was measured in the morning, whereas Anderson *et al.* [4] collected a nonfasting sample between 9λ:λ00 and 15λ:λ00λh. Finally, we control for dose of risperidone and its serum concentration in the analysis. This is most important as these two variables account for a large part of the variance in prolactin concentration and can mask the smaller genetic effect.

The TaqIA locus has been proposed as a potential marker for antipsychotic-induced hyperprolactinemia because of the reduced *DRD2* density in A1 allele carriers [10,43,44]. As a result, when treated with a *DRD2* antagonist, these individuals may have a relatively higher receptor occupancy at a given drug serum concentration, thereby increasing their propensity for hyperprolactinemia [10,43,44]. Although most studies suggest that the A1 allele carrier state is associated with lower *DRD2* density, less research has involved the other variants. The *Ins/Ins* genotype of the -141C *Ins/Del* variant has been associated with higher *DRD2* promoter activity; however, one imaging study reported a paradoxically lower *DRD2* density in participants with this genotype and another one was negative [9,14,45]. In contrast, the 957T allele has been linked to reduced mRNA stability, translating into lower *DRD2* receptor availability [15,46]. However, neither the C957T nor -141C *Ins/Del* variants were found to influence the *DRD2* gene expression in a recent study [47]. Finally, the A-241G had the largest effect on prolactin concentration in this study. This could not be explained by an association with the TaqIA variant as they are not in LD. Both variants independently predicted prolactin concentration when simultaneously introduced into the regression model, and they had a synergistic effect in the joint genotypes analysis. Although Arinami *et al.* [14] failed to show an effect of the A-241G variants on *DRD2* gene promoter activity, the A-241G allele has been associated with a faster response to antipsychotic treatment in adults with schizophrenia [48]. This finding could be seen as consistent with our results as a lower *DRD2* density would be associated with a relatively larger receptor occupancy, resulting in a better response to treatment and higher prolactin concentration [6,48]. We have started administering rating scales to measure clinical response and intend to investigate whether hyperprolactinemia and response to treatment are associated with the *DRD2* genotypes. Nonetheless, our finding should be considered preliminary because of the relatively small number of G allele carriers and the exploratory nature of the investigation.

Equally significant, after controlling for hyperprolactinemia, the TaqIA A1 allele carrier state was also associated with a higher rate of AEs potentially related to the hormonal abnormality. If our findings are replicated and as further work is conducted to identify genetic predictors of

AEs, it might eventually become possible to more specifically tailor treatments to match each patient's strengths and vulnerabilities.

Conclusion

The last decade has seen a dramatic increase in the use of antipsychotic medications in children [49]. Although these medications have received FDA's approval for pediatric use in acute psychosis, mania, and irritability and aggression in autistic disorder, they are frequently prescribed off-label to target symptoms such as irritability and insomnia [50,51]. Owing to their potential to cause weight gain, metabolic, and hormonal abnormalities, clinicians must prescribe them judiciously, especially in children and adolescents, and only after nonpharmacological interventions as well as more benign psychotropics have been tried [52-54]. When unavoidable, close monitoring of patients who receive antipsychotics is critical [24,29].

Our findings add to the increasing body of evidence confirming the persistence of hyperprolactinemia in a sizeable number of children and adolescents in extended therapy with risperidone. They also suggest that variants of the *DRD2* gene may contribute to this risk and to the emergence of AEs potentially related to hyperprolactinemia. Current guidelines do not recommend universal monitoring of prolactin concentration during antipsychotic treatment in children and adolescents [1]. However, measuring prolactin is indicated when galactorrhea or amenorrhea develop, especially when these AEs do not resolve with dose reduction or medication discontinuation [1]. In such cases, appropriate referrals are indicated.

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Fig. 1.

Pairwise linkage disequilibrium (LD) analysis across the four *DRD2* variants. Light grey: LD (moderate-to-high D') between C957T and TaqIA and between C957T and -141C Ins/Del. White: almost no significant LD between the variants. Empty white: complete, but nonsignificant, LD between TaqIA and -141C Ins/Del.

Fig. 2. Adjusted least squares mean of prolactin concentration (log transformed) as a function of the TaqIA and A-241G joint genotypes of the dopamine D₂ receptor gene in children and adolescents in long-term treatment with risperidone.

Table 1
Adverse events potentially related to hyperprolactinemia induced by risperidone

	Boys (n=88)	Girls (n=11)	Tanner stages n at I/II/III/IV/V	A1 carrier state ^a (%)	Hyperprolactinemia ^b (%)
λGynecomastia, n (%)	7 (8)	—	0/2/1/2/2	100	100
Breast tenderness	3 (3)	2 (22)	2/1/1/1/0	80	100 boys, 0 girls
Galactorrhea	0	1 (11)	0/0/0/0/1	0	100
Menarche	—	4 (44)	0/0/1/2/1	25	75
Irregular cycles	—	3 (33)	0/0/1/1/1	33	67
Amenorrhea	—	0	0/0/0/0/0	0	0
Menorrhagia	—	0	0/0/0/0/0	0	0
Oligomenorrhea	—	2 (22)	0/0/0/1/1	0	100
≥1 Adverse events	9 (10)	5 (56)	2/2/2/4/4	71	100 boys, 60 girls

^a A1 carrier state reflects the percentage of patients with each of the adverse events who carried at least one A1 allele of the TaqIA variants.

^b Hyperprolactinemia reflects the percentage of patients with each of the adverse events who also had hyperprolactinemia, defined as a prolactin level of greater than 18.4ng/ml in males and greater than 24.1ng/ml in females.

Table 2

Demographic, clinical, and laboratory characteristics of nonHispanic Caucasians in the two TaqIA genetic groups

Characteristics	A1A1/A1A2 genotypes (N=33)	A2A2 genotype (N=57)	Statistical analysis	P value
λAge, mean±SD, years	12.5±2.5	11.8±3.0	<i>t</i> =0.6, d.f.=88	<i>P</i> =0.5
Male, <i>n</i> (%)	30 (91)	50 (88)	Fisher's Exact	<i>P</i> =0.7
Pubertal status, % at Tanner stage I, II, III, IV, V	27/21/12/27/12	35/19/9/26/11	Wilcoxon <i>S</i> =1571	<i>P</i> =0.5
Psychiatric diagnoses				
Attention deficit hyperactivity disorder, <i>n</i> (%)	23 (70)	53 (93)	Fisher's Exact	^b <i>P</i> <0.006
Disruptive behavior disorder, <i>n</i> (%)	21 (64)	37 (65)	Fisher's Exact	<i>P</i> =1.0
Mood disorder, <i>n</i> (%)	9 (27)	13 (23)	Fisher's Exact	<i>P</i> =0.6
Anxiety disorder, <i>n</i> (%)	11 (33)	22 (39)	Fisher's Exact	<i>P</i> =0.7
Tic disorder, <i>n</i> (%)	8 (24)	10 (18)	Fisher's Exact	<i>P</i> =0.6
Pervasive developmental disorder, <i>n</i> (%)	8 (24)	11 (19)	Fisher's Exact	<i>P</i> =0.6
Psychotic disorder, <i>n</i> (%)	1 (3)	1 (2)	Fisher's Exact	<i>P</i> =1.0
Pharmacotherapy				
Risperidone dose, median (quartiles), (mg/kg/d)	0.03 (0.02–0.04)	0.02 (0.01–0.04)	Wilcoxon <i>S</i> =1658	<i>P</i> =0.2
Risperidone treatment duration, median (quartiles), years	3.1 (1.5–5.0)	2.9 (1.2–3.9)	Wilcoxon <i>S</i> =1575	<i>P</i> =0.5
Risperidone level, median (quartiles), ng/ml	8.3 (6.2–16.8)	7.3 (4.3–14.1)	Wilcoxon <i>S</i> =1281	<i>P</i> =0.2
Psychostimulants, <i>n</i> (%)	17 (52)	40 (70)	Fisher's Exact	^c <i>P</i> =0.1
Psychostimulants dose, mean±SD, (mg/kg/d)	1.4±0.6	1.2±0.4	<i>t</i> =1.8, d.f.=55	^c <i>P</i> =0.08
Prolactin concentration				
Prolactin, median (quartiles), ng/ml	24.0 (17.8–37.8)	17.0 (12.8–26.1)	Wilcoxon <i>S</i> =1769	^b <i>P</i> =0.03
Elevated prolactin, <i>n</i> (%) ^a	22 (67)	24 (42)	Fisher's Exact	^b <i>P</i> =0.03
Prolactin >25.7 λng/ml, <i>n</i> (%)	16 (49)	15 (26)	Fisher's Exact	^b <i>P</i> =0.04

^aDefined as a prolactin level of greater than 18.4λng/ml in males and greater than 24.1λng/ml in females.

^b Statistically significant findings.

^c Statistically significant events.