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ORIGINAL ARTICLE

Lack of association between *DRD2* and *OPRM1* genotypes and adiposity

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BACKGROUND: Dopaminergic and opioid systems are both involved in food intake and appetite control. The dopamine D2 receptor gene (*DRD2*) and the μ-opioid receptor gene (*OPRM1*) therefore represent plausible candidates for association with obesity.
OBJECTIVE: Previous studies of these variants have yielded inconsistent findings, which are likely due to insufficient statistical power. The aim of the current study was to determine whether, in a large population-based sample, there are associations between adiposity and (i) the A1 (T) allele of the Taq1A polymorphism (rs1800497) in *DRD2* and (ii) the G allele of the A118G polymorphism (rs1799971) in *OPRM1*.
STUDY POPULATION: Annual clinic-based measures of body mass index (BMI) and waist circumference were taken from children ($N = 3720$) at 5 measurement time points from ages 7 through to 11 years. BMI was also recorded in their mothers ($N = 2460$) at comparable time points and at pre-pregnancy. All participants were genotyped. Our study was powered (at 80%) to detect per-allele effects on BMI of 0.21 kg m^{-2}.
RESULTS: Our results indicate a lack of association between *DRD2* and *OPRM1* genotypes and adiposity. Combining the data across mothers and children found per-allele effects on BMI of 0.02 kg m^{-2} (95% confidence interval (CI): −0.17, 0.20), $P = 0.9$ for rs1800497 and −0.08 kg m^{-2} (95% CI: −0.29, 0.22), $P = 0.4$ for rs1799971. As a positive control, we also examined the effect of *FTO* genotype over the same time period and confirmed the expected relationship between variability at this locus and higher adiposity.
CONCLUSION: Our findings question existing evidence suggesting associations at *DRD2* and *OPRM1* loci and adiposity. They also highlight the caution required when employing candidate gene approaches to further our understanding of the neurobiology of eating and obesity.

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Keywords: dopamine; opioids; candidate genes; ALSPAC

INTRODUCTION

Before the introduction of genome-wide association studies, the candidate gene approach attempted to identify associations between common genetic variation and a wide range of complex disorders.[1] The identification of candidate genes relies on existing knowledge about the biology of the phenotype under investigation; however, for well-established reasons,[2] this approach has had a high failure rate when judged by the largely accepted notion of effect replication.[3–7] In over 20 years of candidate gene studies, reliable findings have been restricted to type 2 diabetes and only a handful of other phenotypes.[8] Despite this, the value of coupling biological understanding with genetic association analysis should not be underestimated. Indeed, the well-established relationship between variation at the candidate *PPARγ* and type 2 diabetes risk,[8] known to regulate fatty acid storage and glucose metabolism, would have been largely ignored by genome-wide association studies, as its small effect size yielded evidence for association below conventional thresholds for detection in genome-wide analyses.[9] In light of phenomena such as this, there remains a role for the undertaking of well-designed and well-powered examinations of the effects of plausible candidate genes, especially those for which existing evidence is not in agreement.[6]

The increasing worldwide prevalence of obesity and the associated adverse health outcomes highlight the importance of determining its biological, psychological and environmental determinants. Twin studies with adult and child samples indicate a strong genetic contribution to body weight,[10,11] with heritability estimates for body mass index (BMI) as high as 85%.[12] Genome-wide association studies studies have enabled the identification of a large number of genetic loci that are robustly associated with various obesity-related traits.[13–15] Additional genes that are plausible candidates for association with obesity and that are not currently present in the acknowledged lists of genome-wide association signals[13–15] are those that are believed to be important in the neurobiological systems regulating the sensations of reward, and linked to appetite and food intake.[16–18] The dopamine D2 receptor gene, *DRD2*, has been studied in this context, because dopamine is believed to mediate 'wanting' (that is, a non-affective motivational process of appetite).[17,19,20] Previous studies have focused on the single-nucleotide polymorphism (SNP), Taq1A (rs1800497), due to evidence that one or more copies of the minor A1 (T) allele at this

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location is associated with fewer D2 receptors in the striatum.[21,22] However, this has not been consistently found.[23] Furthermore, the Taq1A SNP is now known to be located downstream of *DRD2* in the nearby *ANKK1* gene[24] (we refer to the variant throughout as the *DRD2* Taq1A polymorphism, because this is the nomenclature used in the majority of published studies to date). Nevertheless, a lower density of striatal D2 receptors has been found in the brains of obese people, which suggests that a hypofunctional dopaminergic system may underlie obesity.[25,26] In contrast, the opioid system has an important role in mediating palatability and affective responses to food (that is, 'liking').[19,27] This knowledge has led to an interest in the A118G polymorphism (rs1799971) of the μ-opioid receptor gene, *OPRM1*, and its possible relation with obesity and eating-behavior traits.

Previous studies have examined associations between these variants and adiposity; however, the findings are equivocal. In the *DRD2* literature, there are some reports of a positive association between the A1 allele at rs1800497 and greater adiposity and weight gain.[16,18,28–31] Other studies, however, have failed to replicate the effect and report no association.[32,33] One study found that the A1 allele was more prevalent in obese adults without binge eating disorder than in obese binge eaters.[17] The G allele of the A118G polymorphism in the *OPRM1* gene has been associated with obesity, binge eating, and high-fat and sweet food preferences.[17,34] However, the opposite was reported in a population study of Chinese Uyghurs where the G allele was associated with a 25% reduction in the risk of obesity.[35]

The explanation for the discrepant findings in this area requires scrutiny. Of particular concern is the potential proliferation of 'false-positive' findings, which serve to hinder further understanding of biological and genetic contributions to obesity. Variants that contribute to complex traits have modest effect sizes, and large samples are therefore needed to enable their detection reliably.[36] However, the majority of studies in the obesity literature have employed small sample sizes;[37] the previous work on *DRD2* and *OPRM1*, for example, consists predominantly of small-scale (that is, $Ns<1000$) case–control studies of obesity. This suggests that many studies are underpowered to identify the effect sizes associated with obesity predisposing common variants, and these underpowered studies are prone to finding false-positive associations.[37] There is currently a lack of evidence for effects of *DRD2* and *OPRM* variants on continuous measures of body weight in larger sample sizes. The aim of the current study was thus to determine whether, in a large population-based sample, there are associations between adiposity and (i) the A1 (T) allele of the Taq1A polymorphism (rs1800497) in *DRD2* and (ii) the G allele of the A118G polymorphism (rs1799971) in *OPRM1*. Given that *DRD2* and *OPRM1* variants are hypothesised to influence appetite and food intake, it is plausible that effects on adiposity might appear gradually and, hence, be missed by cross-sectional analyses at single time points. For this reason, our study examined repeated assessments of adiposity over time. As a positive control, we also examined the effect of *FTO* genotype (rs1558902) over the same time periods, because variability at this locus is known to be associated with higher adiposity.[38,39]

SUBJECTS AND METHODS

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a transgenerational prospective observational study investigating influences on health and development across the life course. The children from >13 000 pregnancies and their mothers were recruited between 1990 and 1992 from the Bristol area of United Kingdom. Because of the inclusion of new pregnancies, by the age of 18 years the total sample size was 15 247 pregnancies.[40] The cohort was broadly representative of the general population of United Kingdom at the point of recruitment.[41] A detailed account of the ALSPAC study methodology is provided elsewhere.[41,42] The study website contains details of all data that are available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Parents gave written informed consent for their own and their child's participation. Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Measurements

Our primary outcome measure was child BMI, which was calculated by dividing weight (kg) by height (m) squared. Weight was measured using a Tanita THF 300GS body fat analyser and weighing scales (Tanita UK Ltd, Middlesex, UK). Height was measured to an accuracy of 0.1 cm using a Holtain stadiometer (Holtain Ltd, Pembs, UK). These measurements were taken from the sample of children at five annual clinic visits at ages 7 through 11 years, inclusive. This time period was selected, because preadolescence is recognised to be a critical risk period for the development of obesity.[43] Child waist circumference was also measured at clinic visits at ages 7, 9, 10 and 11 years.

Self-reported height and weight was collected from mothers at pre-pregnancy and at 85, 97, 110 and 145 months (these latter four measurement times correspond to the 7, 8, 9 and 11-year assessment clinics in the children). BMI in the mothers at all time points was computed as described above.

Genotyping

Genotypes at the *DRD2*, *OPRM1* and *FTO* loci were available from genome-wide association studies genotyping data from the ALSPAC collection. A total of 9912 children were genotyped using the Illumina HumanHap550 quad genome-wide SNP genotyping platform by 23andMe, subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK, and the Laboratory Corporation of America, Burlington, NC, USA. Individuals were excluded from further analysis on the basis of having incorrect sex assignments, minimal or excessive heterozygosity (<0.320 and >0.345 for the Sanger data and <0.310 and >0.330 for the LabCorp data), disproportionate levels of individual missingness (>3%); evidence of cryptic relatedness (>10% Identity by descent) and of being non-European ancestry (as detected by a multi-dimensional scaling analysis seeded with HapMap 2 individuals, EIGENSTRAT[44] analysis revealed no additional obvious population stratification and genome-wide analyses with other phenotypes indicate a low lambda). The resulting data set consisted of 8365 individuals. SNPs with a minor allele frequency of <1% and a call rate of <95% were removed. Furthermore, only SNPs that passed an exact test of Hardy–Weinberg equilibrium ($P>5\times10^{-7}$) were considered for analysis.

A total of 9321 ALSPAC mothers were genotyped on the Illumina 660K quad chip at the Centre National de Genotypage, Paris, France. Cleaning in the ALPSAC mothers' sample was similar to that done in the ALSPAC children. Individuals were excluded from further analysis on the basis of having incorrect gender assignments, minimal or excessive heterozygosity, disproportionate levels of individual missingness (>5%), evidence of cryptic relatedness (>10% Identity by descent) and of being non-European ancestry (as detected by a multi-dimensional scaling analysis seeded with HapMap 2 individuals). SNPs with a minor allele frequency of <1% and a call rate of <95% were removed. Furthermore, only SNPs that passed an exact test of Hardy–Weinberg equilibrium ($P>5\times10^{-7}$) were considered for further use.

Genotypes were imputed with Markov Chain Haplotyping software (MaCH 1.0.16)[45] using CEPH (Centre d'Etude du Polymorphisme Humain) individuals from phase 2 of the HapMap project as a reference set (release 22).

Analyses

Analyses were restricted to singleton children. Of the 8365 individuals with genome-wide data, a total of 3720 children (50.9% female) had measurements of BMI at all five time points as per the requirements of the study. Because of fewer (that is, four) measurement time points for waist circumference relative to BMI, the sample size for this outcome measure was slightly larger ($N=4400$, 50.9% female). In addition, a total of 2460 mothers with genome-wide data also had measurements of BMI at all five time points (including pre-pregnancy).

We used the Quanto software (Version 1.2)[46] to calculate that with $N=3720$, we would achieve 80% power at an α-level of 0.05 (two-sided) to

detect a per-allele effect of 0.21 kg m^{-2} on BMI (where mean = 17.6, s.d. = 2.6 kg m^{-2} and minor allele frequency = 0.2 (range 0.2–0.6)).

Dosage data for each SNP of interest were extracted from imputed genotypic data. For subsequent analyses, the data were converted to categorical genotype groups by rounding dosage values, having checked that all imputation quality values (r^2) exceeded 0.99.[45] The relevant genotype groups for each SNP were as follows: A2A2, A2A1 or A1A1 for rs1800497 (in *DRD2*); AA, AG or GG for rs1799971 (in *OPRM1*); TT, AT or AA for rs1558902 (in *FTO*) (Table 1). An additive genetic model was assumed in all cases.

Two types of analysis were conducted on the child data for each SNP. The first analysis examined changes over time. A repeated-measures analysis of variance was conducted where measurement time point was the within-subjects factor and genotype group was the between-subjects factor. Outcome measures were BMI and waist circumference. In this analysis, a main effect of genotype would indicate an overall effect of that particular SNP on the outcome measure. A genotype-by-time interaction would indicate differential changes over time in the outcome measure as a function of genotype group. Where the assumption of sphericity was violated, the Greenhouse–Geisser correction to the degrees of freedom was employed.

The second analysis was a regression analysis in order to predict the per high-risk allele effect on the respective outcome measure. For each SNP, the non-converted dosage data were entered into the model as the predictor variable. The outcome variables were average BMI and average waist circumference; these values were computed by averaging BMI and waist circumference values across all measurement time points.

As a sensitivity analysis, we ran the same analyses in the mothers for each SNP and with BMI as the outcome measure. However, as the mothers are genetically related to the children, these analyses cannot be treated as independent replications. To control for relatedness, we extended this analysis by combining all average BMI data points for mothers and children, and employing an over-conservative mixed model that modelled familial relation as a random effect (STATA command 'xtreg').

Statistical analyses were conducted using SPSS version 18 and STATA version 12.

RESULTS

Child sample

Body mass index. For rs1800497 (in *DRD2*), there was no association between the genotype group and BMI ($F(2, 3717) = 0.53$, $P = 0.59$) and no genotype-by-time interaction ($F(3.9, 7340) = 0.53$, $P = 0.72$) (Figure 1a). Regression analysis found no difference in BMI per copy of the high-risk A1 allele; B = −0.03 kg m^{-2} (95% confidence interval (CI): −0.17, 0.12).

For rs1799971 (in *OPRM1*), there was no association between genotype group and BMI ($F(2, 3717) = 2.3$, $P = 0.1$) and no genotype-by-time interaction ($F(4.0, 7341) = 0.44$, $P = 0.78$) (Figure 1b). Regression analysis found no difference in BMI per copy of the high-risk G allele; B = −0.11 kg m^{-2} (95% CI: −0.29, 0.06).

Table 1. Genotype distribution groups in the child and mother samples

SNP	*Genotype group*	*Child sample (%)*	*Mother sample (%)*
rs1800497 (in *DRD2*)	A2A2	64.4	65.8
	A2A1	31.6	30.4
	A1A1	4.0	3.7
rs1799971 (in *OPRM1*)	AA	75.2	74.1
	AG	22.8	24.1
	GG	1.9	1.8
rs1558902 (in *FTO*)	TT	35.6	36.7
	AT	48.1	47.9
	AA	16.3	15.4

Abbreviations: SNP, single-nucleotide polymorphism; *DRD2*, dopamine D2 receptor gene; *OPRM1*, μ-opioid receptor gene.

In contrast, for rs1558902 (in *FTO*) there was an association between genotype group and BMI ($F(2, 3717) = 12.3$, $P = 4.64 \times 10^{-6}$) and a genotype-by-time interaction ($F(4.0, 7371) = 11.9$, $P = 1.55 \times 10^{-9}$) (Figure 1c). Regression analysis confirmed the association between each additional A allele at rs1558902 and BMI; B = 0.31 kg m^{-2} (95% CI: 0.18, 0.43).

Waist circumference. For rs1800497 (in *DRD2*), there was no association between genotype group and waist circumference ($F(2, 4397) = 0.37$, $P = 0.69$) and no genotype-by-time interaction ($F(4.5, 9884) = 0.68$, $P = 0.63$) (Figure 2a). Regression analysis found no difference in waist circumference per copy of the high-risk A1 allele; B = −0.05 cm (95% CI: −0.42, 0.33).

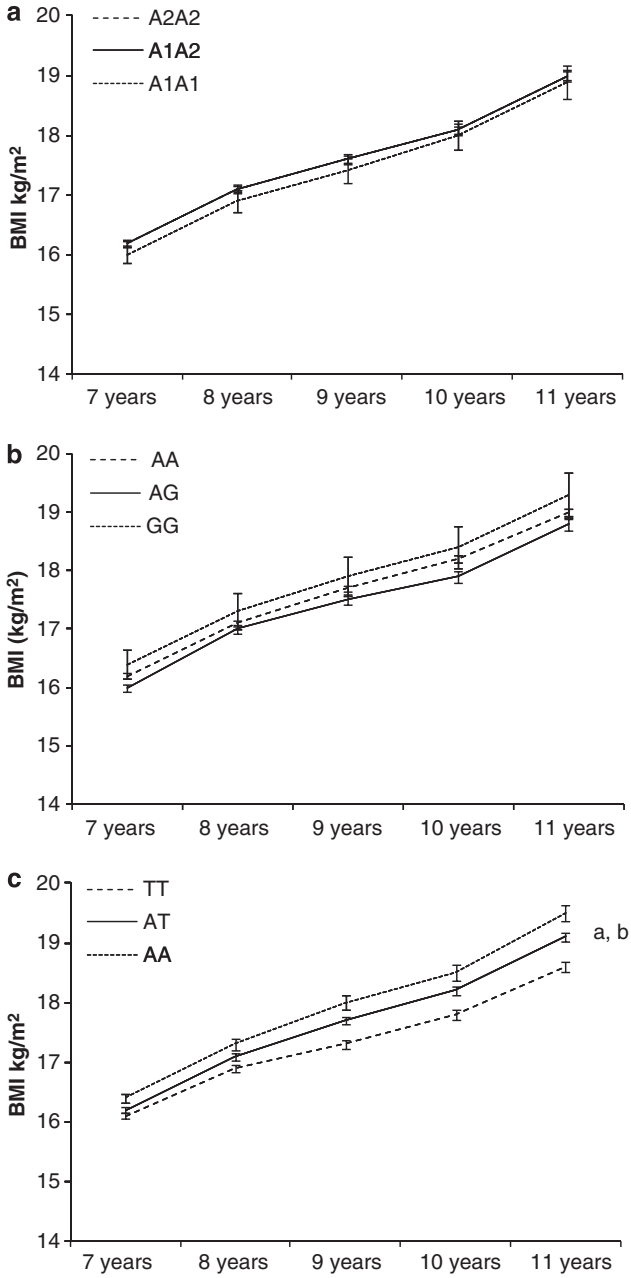

Figure 1. Mean BMI in children at each assessment age by genotype group for rs1800497 (in *DRD2*) (**a**), rs1799971 (in *OPRM1*) (**b**) and rs1558902 (in *FTO*) (**c**). Bars represent ± 1 s.e. of the mean. [a]Main effect of genotype group, $P = 4.64 \times 10^{-6}$. [b]Genotype-by-time interaction, $P = 1.55 \times 10^{-9}$.

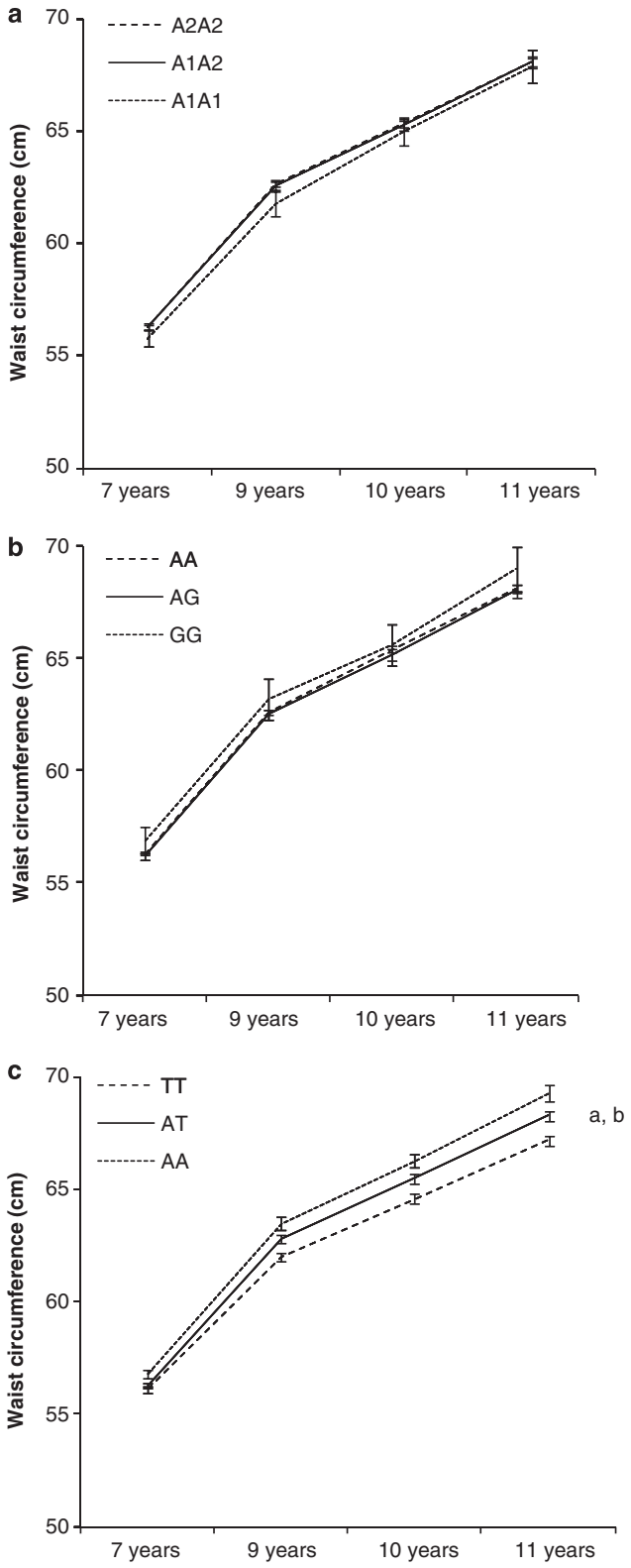

Figure 2. Mean waist circumference in children at each assessment age by genotype group for rs1800497 (in *DRD2*) (**a**), rs1799971 (in *OPRM1*) (**b**) and rs1558902 (in *FTO*) (**c**). Bars represent ± 1 s.e. of the mean. [a]Main effect of genotype group, $P = 8.0 \times 10^{-6}$. [b]Genotype-by-time interaction, $P = 3.7 \times 10^{-8}$.

For rs1799971 (in *OPRM1*), there was no association between genotype group and waist circumference ($F(2, 4397) = 0.45$, $P = 0.64$) and no genotype-by-time interaction ($F(4.5, 9884) = 0.26$, $P = 0.92$) (Figure 2b). Regression analysis found no difference in waist circumference per copy of the high-risk G allele; B = −0.08 cm (95% CI: −0.52, 0.36).

In contrast, for rs1558902 (in *FTO*), there was an association between genotype group and waist circumference ($F(2, 4397) = 11.8$, $P = 8.0 \times 10^{-6}$) and a genotype-by-time interaction ($F(4.5, 9914) = 9.3$, $P = 3.7 \times 10^{-8}$) (Figure 2c). Regression analysis confirmed the association between each additional A allele at rs1558902 and waist circumference; B = 0.79 cm (95% CI: −0.48, 1.11).

Mothers sample

Body mass index. For rs1800497 (in *DRD2*), there was no association between genotype group and BMI ($F(2, 2457) = 1.8$, $P = 0.16$) and no genotype-by-time interaction ($F(6.1, 7445) = 1.0$, $P = 0.44$) (Figure 3a). Regression analysis found there to be no difference in BMI per copy of the high-risk A1 allele; B = 0.24 kg m^{-2} (95% CI: −0.04, 0.51).

For rs1799971 (in *OPRM1*), there was no association between genotype group and BMI ($F(2, 2457) = 0.6$, $P = 0.54$) and no genotype-by-time interaction ($F(6.1, 7445) = 1.2$, $P = 0.29$) (Figure 3b). Regression analysis found no a difference in BMI per copy of the high-risk G allele; B = −0.18 kg m^{-2} (95% CI: −0.49, 0.14).

In contrast, for rs1558902 (in *FTO*), there was an association between genotype group and BMI ($F(2, 2457) = 5.71$, $P = 0.003$). There was no genotype-by-time interaction ($F(6.1, 7446) = 0.64$, $P = 0.7$) (Figure 3c). Regression analysis confirmed the association between each additional A allele at rs1558902 and BMI; B = 0.38 kg m^{-2} (95% CI: 0.16, 0.60).

Combined sample

Combining all average BMI data across mothers and children into a joint data set yielded a working panel of 6233 participants. In a mixed model, including familial relation as a random effect, there was no evidence of association between variation at either rs1800497 (in *DRD2*) or rs1799971 (in *OPRM1*) and average BMI. The change in average BMI per high-risk A1 allele at rs1800497 was 0.02 kg m^{-2} (95% CI: −0.17, 0.20), $P = 0.9$, and was −0.08 kg m^{-2} (95% CI: −0.29, 0.22), $P = 0.4$, per high-risk G allele at rs1799971. In contrast (and consistent with previous results), there was evidence for association between variation at rs1558902 (in *FTO*) and average BMI, where each copy of the high-risk A allele was associated with an increase in BMI of 0.28 kg m^{-2} (95% CI: 0.13, 0.42), $P < 0.0001$.

DISCUSSION

The aim of this study was to determine whether associations exist between *DRD2* and *OPRM1* genotypes and adiposity in a large population-based sample (ALSPAC). Notably, our study included clinic-based assessments of BMI and waist circumference in the child sample, which is of particular importance given the inaccuracies and response biases that can be associated with self-reported measurements.[37]

Despite the availability of a relatively large collection of well-phenotyped samples, we failed to provide reasonable evidence that variation at *DRD2* and *OPRM1* is associated with BMI and waist circumference in children. We similarly found a lack of association over a wider age range when including the mothers of ALSPAC participants. Combining the data across mothers and children indicated per-allele effects on BMI of 0.02 kg m^{-2} (95% CI: −0.17, 0.20) for rs1800497 (in *DRD2*) and −0.08 kg m^{-2} (95% CI: −0.29, 0.22) for rs1799971 (in *OPRM1*). As a positive control, our analyses also examined the effect of *FTO* genotype over the same time period in children and mothers. Here we found that each copy of the high-risk A allele at rs1558902 was associated with an increase in BMI of 0.28 kg m^{-2} (95% CI: 0.13, 0.42) in the combined sample.

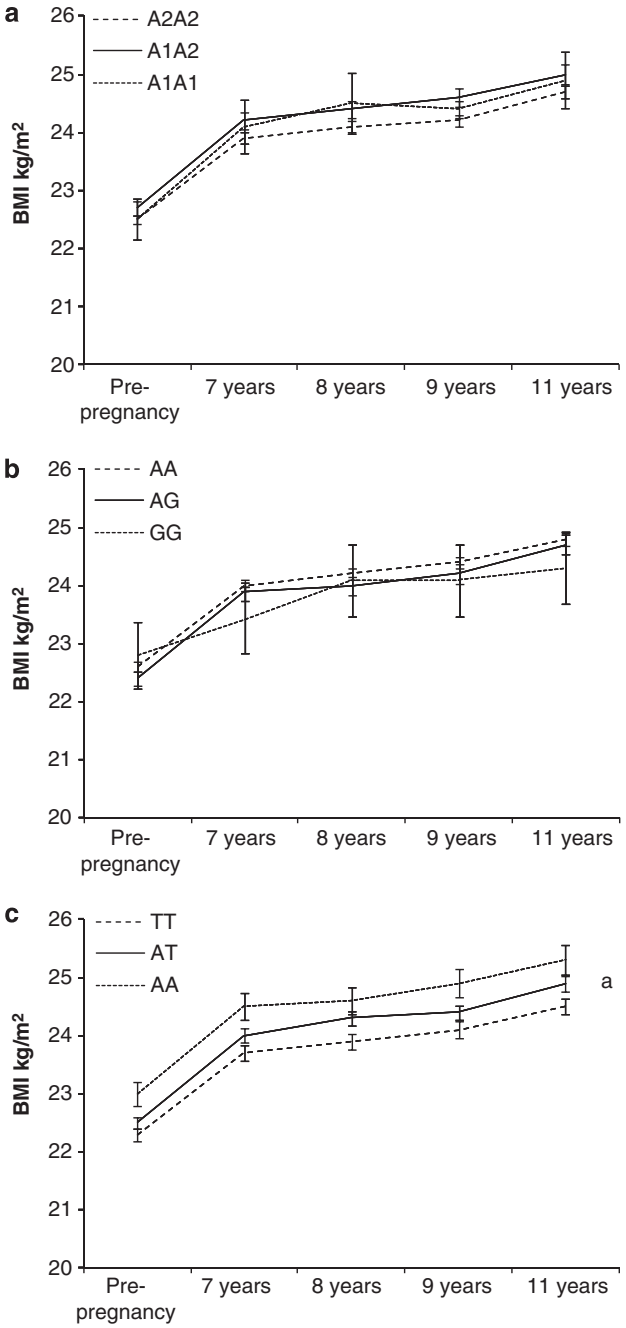

Figure 3. Mean BMI in mothers at each assessment age by genotype group for rs1800497 (in *DRD2*) (**a**), rs1799971 (in *OPRM1*) (**b**) and rs1558902 (in *FTO*) (**c**). Bars represent ± 1 s.e. of the mean. [a]Main effect of genotype group, $P = 0.003$.

We therefore corroborated the well-established finding that variability at the *FTO* locus is associated with higher adiposity.[38,39] This is important, because it confirms that the null results with respect to *DRD2* and *OPRM1* genotypes are unlikely to reflect design flaws or anomalies in our sample.

In accordance with our previous work,[39] we show in our child sample that the adiposity-enhancing effect of *FTO* genotype became stronger with age. Although BMI was highest in the AA group, followed by the AT group and then the TT group, these group differences became more pronounced over time (as evidenced by the genotype-by-time interaction, Figure 1c). The interaction between genotype and time was similarly found in the waist circumference data in the child sample (Figure 2c), but was not seen in the mothers (Figure 3c). These findings provide important insight into the developmental trajectory of genetic associations during the high-risk pre-adolescent period.

The findings from previous studies on the associations between *DRD2* and *OPRM1* genotypes and adiposity are equivocal.[16–18,28–35] Sample size is the likely source of these discrepancies, with many of the aforementioned studies comparing small groups of obese subjects with population controls. The study by Spitz *et al.*,[29] for example, reported that 58% of obese subjects possessed the A1 allele compared with 35% of non-obese subjects; however, sample sizes were just 37 and 139 for the two groups, respectively. Blum *et al.*[16] conducted many of their analyses on an even smaller number of participants ($N = 11$ in the 'obese with severe comorbid substance disorder' group). Interestingly, the study with the largest sample size ($N = 1187$) found no association between *DRD2* genotypes and BMI.[32] Less research has considered associations between *OPRM1*, adiposity and eating behaviours, but studies to date have similarly employed modest sample sizes ($Ns < 1000$).[17,34,35] In contrast, our study was powered to detect per-allele effects down to 0.21 kg m^{-2}, which is comparable with other SNPS that are associated with BMI,[13] while being considerably smaller than that for *FTO*. Our study thus helps to resolve ambiguous findings in this literature by indicating that in a large and well-powered sample, there is a lack of association between *DRD2* and *OPRM1* genotypes and adiposity.

Low statistical power is a pervasive problem in genetic and neuroscientific studies. A recent examination of neuroscience meta-analyses indicated a median statistical power of just 21%.[47] As power (that is, sample size) decreases, the proportion of 'significant' results that are false positives is greatly increased.[48] Furthermore, it is commonly believed that an effect detectable with a smaller sample size will be automatically found with a larger one; however, this assumption is erroneous[49] and it has been shown that a possible false-positive rate of almost 97% can appear within candidate gene studies at the $P \leq 0.05$ level.[5] The candidate gene approach is further compounded by publication bias, which has been shown to exist in the literature on the *DRD2* Taq1A polymorphism and alcoholism.[50] A recent review by Li and Meyre[37] highlighted the problems associated with insufficient statistical power in the obesity literature, which suggests that many of the findings reported to date are false positives. Taken together, it is clear that precise replications of genetic associations are needed[5] in order to prevent against the propagation of a biased body of evidence in the public domain.

A further factor likely to be responsible for the overinterpretation of results surrounding *DRD2* and *OPRM1* is assumed functional effects of genetic variation at these loci. However, it is now known that the Taq1A SNP does not fall within the *DRD2* coding region, but is located downstream in the nearby *ANKK1* gene and is of unknown impact.[24] Similarly, there is disagreement in the literature regarding the physiological effects of the A118G polymorphism of *OPRM1* and whether it is associated with a gain in the μ-opioid receptor function,[51] or, alternatively, a loss of function.[52] In this context, it is not clear how the continued study of these variants will advance our knowledge of the neurobiology of appetite control and obesity.

Despite this, there remains an important role for the undertaking of well-designed and sufficiently powered genetic association studies.[2] Their findings advance our understanding of the genomic architecture of complex traits and provide novel insight into the biological mechanisms that underpin diseases. This advances knowledge of the pathophysiology of disorder and indeed the natural aetiology of complex traits, and, theoretically, may yield information important for intervention. In some cases, analyses of these loci with the addition of environmental information has also had the capacity to comment on the

presence of likely gene × environment interaction and the notion that despite relatively high heritability, complex trait gene effects can indeed be altered by exogenous factors.[53–54] In addition, the identification of genetic variants associated with risk factors of interest (for example, tobacco and alcohol consumption, and obesity) can enable their use as instrumental variables in Mendelian randomisation analyses in order to determine the causal pathways involved in complex diseases.[55] This approach has been used in our previous work to elucidate causal associations between smoking and BMI,[56] and between BMI and ischaemic heart disease, C-reactive protein levels and blood pressure.[57–59]

In conclusion, this study found a lack of association between *DRD2* and *OPRM1* genotypes and adiposity. The findings highlight the caution required when employing candidate gene approaches and emphasise the need for appropriately powered and well-replicated studies in order to further our understanding of biological and genetic contributions to obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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