Rare variants in the *MECP2* gene in girls with central precocious puberty: a translational cohort study

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Background

Identification of genetic causes of central precocious puberty have revealed epigenetic mechanisms as regulators of human pubertal timing. MECP2, an X-linked gene, encodes a chromatin-associated protein with a role in gene transcription. MECP2 loss-of-function mutations usually cause Rett syndrome, a severe neurodevelopmental disorder. Early pubertal development has been shown in several patients with Rett syndrome. The aim of this study was to explore whether MECP2 variants are associated with an idiopathic central precocious puberty phenotype.

Methods **In this translational cohort study, participants were recruited from seven tertiary centres from five countries (Brazil, Spain, France, the USA, and the UK). Patients with idiopathic central precocious puberty were investigated for rare potentially damaging variants in the *MECP2* gene, to assess whether MECP2 might contribute to the cause of central precocious puberty. Inclusion criteria were the development of progressive pubertal signs (Tanner stage 2) before the age of 8 years in girls and 9 years in boys and basal or GnRH-stimulated LH pubertal concentrations. Exclusion criteria were the diagnosis of peripheral precocious puberty and the presence of any recognised cause of central precocious puberty (CNS lesions, known monogenic causes, genetic syndromes, or early exposure to sex steroids). All patients included were followed up at the outpatient clinics of participating academic centres. We used high-throughput sequencing in 133 patients and Sanger sequencing of *MECP2* in an additional 271 patients. Hypothalamic expression of Mecp2 and colocalisation with GnRH neurons were determined in mice to show expression of Mecp2 in key nuclei related to pubertal timing regulation.**

Findings **Between Jun 15, 2020, and Jun 15, 2022, 404 patients with idiopathic central precocious puberty (383 [95%] girls and 21 [5%] boys; 261 [65%] sporadic cases and 143 [35%] familial cases from 134 unrelated families) were enrolled and assessed. We identified three rare heterozygous likely damaging coding variants in *MECP2* in five girls: a de novo missense variant (Arg97Cys) in two monozygotic twin sisters with central precocious puberty and microcephaly; a de novo missense variant (Ser176Arg) in one girl with sporadic central precocious puberty, obesity, and autism; and an insertion (Ala6_Ala8dup) in two unrelated girls with sporadic central precocious puberty. Additionally, we identified one rare heterozygous 3′UTR *MECP2* insertion (36_37insT) in two unrelated girls with sporadic central precocious puberty. None of them manifested Rett syndrome. Mecp2 protein colocalised with GnRH expression in hypothalamic nuclei responsible for GnRH regulation in mice.**

Interpretation **We identified rare *MECP2* variants in girls with central precocious puberty, with or without mild neurodevelopmental abnormalities. MECP2 might have a role in the hypothalamic control of human pubertal timing, adding to the evidence of involvement of epigenetic and genetic mechanisms in this crucial biological process.**

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Introduction

Central precocious puberty is defined by the premature development of secondary sexual characteristics due to the early reactivation of pulsatile hypothalamic gonadotropin-releasing hormone (GnRH) secretion. Characteristically, the overall frequency of central precocious puberty is much higher in girls than in boys.[1] The recognition of genetic causes underlying central precocious puberty has increased in the past decade, mainly through high-throughput sequencing studies of familial forms of central precocious puberty.[2,3] To date, loss-of-function mutations in two autosomal imprinted genes *MKRN3* and *DLK1* are the most prevalent known monogenic causes of familial central precocious puberty with the phenotype exclusively associated with paternal transmission.[2–4] Epigenetic regulation of upstream pathways of GnRH neuronal activity is implicated in the pathogenic mechanisms of deficiency of both genes. Identifying the cause of central precocious puberty might allow individuals with precocious puberty to benefit from a precision

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Research in context

Evidence before this study

Precocious puberty is an endocrine disorder that affects children worldwide. In clinical practice, non-treated precocious puberty can lead to adverse outcomes in adulthood including short stature and psychosocial distress. In large population studies, early puberty timing has also been associated with diverse health conditions, such as obesity, type 2 diabetes, hypertension, cardiovascular disease, and cancer risk (breast and endometrial cancers in women and prostate cancer in men).

We searched PubMed from inception to June 15, 2022, without language restrictions, using the search terms "MECP2" AND "precocious puberty" OR "puberty" OR "gonadotropin-releasing hormone (GnRH)" for research articles. We identified three publications that reported early pubertal development (early thelarche and early pubarche) in large cohorts of girls with Rett syndrome due to *MECP2* mutations. In addition, we identified four publications with case reports of patients with Rett syndrome due to *MECP2* mutations that were diagnosed with central precocious puberty. Three studies in animal models discussed a potential influence of Mecp2 in hypothalamic GnRH regulation.

Added value of this study

To our knowledge, this is the first clinical research to investigate whether MECP2 biology is associated with an idiopathic central precocious puberty phenotype, evaluating its potential role in the cause of central precocious puberty. We identified rare heterozygous probably damaging variants in the *MECP2* gene in multiple unrelated girls with sporadic central precocious puberty, with or without mild neurodevelopmental abnormalities, revealing a potential X-linked form of premature pubertal development. *MECP2* emerged as a potential player in the hypothalamic control of human pubertal timing, thus increasing the evidence of epigenetic and genetic mechanisms in this biological process.

Implications of all the available evidence

Our findings provided further understanding of potential genetic factors involved in the cause of central precocious puberty. Further studies could provide information that might allow individuals with central precocious puberty to benefit from a precision medicine approach, providing them more personalised strategies of follow-up with increased long-term clinical surveillance. In addition, epigenetic and genetic discoveries underlying central precocious puberty might contribute to the development of potential new treatment targets in the future.

medicine approach, providing patients with personalised follow-up strategies with increased long-term clinical surveillance and allowing genetic counselling within families.[4]

X-linked gene defects have long been considered important causes of CNS disorders because these genes are highly expressed in the brain.[5] The potential role of X-linked genes in human pubertal development has been suggested by distinct lines of evidence, including descriptions of early puberty in people with X-chromosome structural variants, enrichment of X-linked differentially methylated regions in methylome profiling of girls at puberty, and genomic association studies in large populations.[6,7] Most X-linked genes are subject to X inactivation in women and girls, a mechanism that ensures dosage compensation between male and female gene products.[5,8]

Methyl-CpG-binding protein 2 (*MECP2*) is an X-linked gene (chromosome Xq28) that encodes a nuclear protein capable of binding to methylated DNA in promoter regions, functioning as a repressor or an activator of gene transcription.[9,10] *MECP2* is widely expressed in human tissues, with the highest expression observed in the brain.[9] Loss-of-function mutations in *MECP2* are usually associated with neurodevelopmental disorders, in particular Rett syndrome.[11] Rett syndrome is a severe neurodevelopmental disorder characterised by the regression of acquired skills between 12 and 30 months old. Its main diagnostic criteria are partial or complete loss of acquired purposeful hand skills and acquired spoken language, absent or abnormal gait, and stereotypic movements principally involving the hands.[12] Additional comorbidities associated with Rett syndrome include epilepsy, scoliosis, and gastrointestinal dysfunction. *MECP2* mutations in patients with Rett syndrome are most commonly due to de novo mutations that arise on the paternal X chromosome.[13] However, broader clinical phenotypes have been described in individuals with *MECP2* mutations, such as mild neurodisabilities, characterised by autism spectrum disorder or intellectual impairment, suggesting that distinct *MECP2* variants might lead to diverse phenotypic consequences.[12,14]

Early pubertal development has been documented in children with Rett syndrome due to *MECP2* mutations.[15,16] An observational American study described early thelarche in 25% and early pubarche in 28% of girls with Rett syndrome; furthermore, menarche occurred earlier in those girls with mutations associated with milder clinical severity.[15] Similarly, mean age of thelarche was 7·1 years (SD 2·5) in a larger cohort of patients with Rett syndrome, which was earlier than the mean age of thelarche in White girls who did not have Rett syndrome (10·0 years [1·8]).[16] Mean age of menarche in girls with Rett syndrome was 12·7 years (2·4), reported as within the normal range (12·9 years [SD 1·2]) in most girls in both studies, leading to a longer time from thelarche to menarche. Nevertheless, central precocious puberty was diagnosed in case reports

of patients with Rett syndrome with *MECP2* mutations.[17] In this study, we aimed to explore whether *MECP2* variants were associated with an idiopathic central precocious puberty phenotype, evaluating the potential role of *MECP2* as a cause of this endocrine disorder.

Methods

Study design and participants

In this translational cohort study, patients with idiopathic central precocious puberty followed up in seven tertiary centres from five countries (Brazil, Spain, France, the USA, and the UK) were investigated for rare potentially damaging variants in the *MECP2* gene. Inclusion criteria were development of progressive pubertal signs, primarily Tanner stage 2 breast development (thelarche) before age 8 years in girls and testicular volume of 4 mL or more before the age of 9 years in boys; precocious menarche in girls aged 9 years of younger; basal or GnRH-stimulated luteinising hormone (LH) concentrations (measured by ultrasensitive assays) within the pubertal range in boys and girls. Additional inclusion criteria were accelerated linear growth, accelerated bone maturation, and absence of pathological lesions of the CNS on MRI. Exclusion criteria were the diagnosis of peripheral precocious puberty and the presence of any recognised cause of central precocious puberty (lesions of the CNS on MRI; known monogenic causes [mutations of *MKRN3*, *DLK1*, *KISS1R*, and *KISS1* genes]; genetic or epigenetic syndromes, and early exposure to sex steroids).[4] The study protocol was approved by the respective local Ethics Committees. Written informed consent was obtained from all patients and their legal guardians (appendix p 2).

Procedures

High throughput sequencing studies were done in patients with central precocious puberty, as part of independent genetic investigations based on multigene sequencing approaches at Sao Paulo University, Sao Paulo, Brazil, and Queen Mary University of London, London, UK. Whole-exome sequencing was mainly done in patients who presented with central precocious puberty and familial form or association with congenital malformations or neurodevelopmental abnormalities (done at Sao Paulo University and Queen Mary University; appendix p 3) and targeted gene sequencing (panel of 746 genes, including *MECP2*) was mainly done in patients who presented with central precocious puberty in association with other clinical features, such as reproductive, metabolic, or growth phenotypes (done at Sao Paulo University; appendix p 3). Additionally, the *MECP2* gene was screened by Sanger sequencing (candidate gene approach) to expand the analysis of *MECP2* to a larger cohort of patients with isolated central precocious puberty (done at Sao Paulo University and Harvard University, Cambridge, MA, USA; appendix pp 3–4). Studies to determine parental origin of variants were done in patients carrying de novo *MECP2* variants, by analysis of linkage between the *MECP2* variants and nearby heterozygous inherited polymorphisms (appendix p 5). X inactivation analyses of blood samples (leukocyte DNA) were done in girls with *MECP2* variants and their mothers (appendix pp 5–6).

All the methods were completed as previously described and were done according to the standard protocol of the manufacturer.[6,18] The genomic positions and the raw data of all experiments were aligned using the Genome Reference Consortium Human Build 38 assembly of the human genome reference. All possible candidate variants were classified according to the American College of Medical Genetics and Genomics (ACMG) standards with five categories of pathogenicity: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. Briefly, the assessment of pathogenicity of the identified variants included mainly population data (minor allele frequency ≤0·01% in public and in-house database), in-silico tools (well established prediction sites, function-structure analysis, and computational tools), and reports of variants in the literature and in well established public archives (appendix p 4). Two public genomic databases were used for population data analyses: the Genome Aggregation Database (gnomAD) and the Online Archive of Brazilian Mutations (ABraOM). To provide additional genetic evidence of the association of MECP2 with central precocious puberty phenotype, an analysis of rare potentially damaging variants across the entire gene was done, comparing allele frequencies between the central precocious puberty cohort (cases) and gnomAD public database (controls). The samples from the central precocious puberty cohort and the gnomAD database were not jointly sequenced or called. Allele frequency differences between groups were analysed by Fisher's exact test. Statistical significance was set at p value less than 0·05 (appendix pp 4–5).

To evaluate population data, we reviewed the *MECP2* associations with human conditions reported in genome-wide association studies at the GWAS catalogue. Additionally, we evaluated the effect of *MECP2* variants in the UK Biobank, a population study with exome sequencing and phenotypic characterisation (appendix p 6).

To show the expression of Mecp2 within GnRH neurons within key hypothalamic nuclei known to be the site of upstream GnRH regulation relevant to pubertal onset in both humans and mice, assays in mice were done. Immunochemistry studies were done from hypothalamic tissue sections of pubertal (postnatal day 38) female mice collected from timed crosses of C57BL/6 mice. Anti-MECP2 (Abcam [253197], Cambridge, UK; rabbit polyclonal; 1:100) and anti-GnRH (Immunostar [20075], Hudson, WI, USA; rabbit polyclonal; 1:1000) primary antibodies were used for tissue expression analysis (appendix p 7)

For the **Genome Aggregation Database** see https://gnomad.broadinstitute.org

For the **Online Archives of Brazilian Mutations** see https://abraom.ib.usp.br

See **Online** for appendix

For the **GWAS catalogue** see https://www.ebi.ac.uk/gwas/

For the **UK Biobank** see https://www.ukbiobank.ac.uk

	Patients with variant in the coding region					Patients with variant in the 3'UTR	
	Patient 1*	Patient 2*	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Genomic position	X:154032331	X:154032331	X:154031336	X:154097642	X:154097642	X:154030331	X:154030331
Variant type	Missense	Missense	Missense	Indel insertion	Indel insertion	3'UTR insertion	3'UTR insertion
cDNA	289C→T	289C→T	528C→A	15_23dup	15_23dup	36_37insT	36_37insT
Protein	p.Arg97Cys	p.Arg97Cys	p.Ser176Arg	p.Ala6_Ala8dup	p.Ala6_Ala8dup	NA	NA
Familial segregation	De novo	De novo	De novo	Inherited from the unaffected mother	Absent in the unaffected mother†	Inherited from the unaffected mother	De novo
Population data (allele frequency)							
gnomAD	0·000005467	0·000005467	Not reported	0·00005002	0·00005002	Not reported	Not reported
ABraOM	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Protein domain	Methyl-CpG-binding domain	Methyl-CpG-binding domain	Intervening domain	N terminal domain	N terminal domain	NA	NA
ACMG classification	Likely pathogenic	Likely pathogenic	Likely pathogenic	VUS	VUS	VUS	VUS
Previous association with Rett syndrome phenotype‡	One case in ClinVar	One case in ClinVar	No	No	No	No	No

All sequence variants are according to the transcript identified as NM_001110792·2 by the Human Genome Variation Society. All genomic positions are according to the GRCh38/hg38 assembly of the human genome reference. ABraOM=Online Archive of Brazilian Mutations. ACMG=American College of Medical Genetics and Genomics standards. gnomAD=Genome Aggregation Database. NA=not applicable. UTR=untranslated region. VUS=variant of uncertain significance. *Patients were monozygotic twin sisters. †DNA and clinical data from the father were not available. ‡Previous association with Rett syndrome phenotype was evaluated by analysis of published literature, ClinVar data, and RettBASE data (appendix p 4).[11-17]

Table 1: **Rare heterozygous variants in *MECP2* identified in seven girls with central precocious puberty**

Outcomes

The main outcomes were the identification of potential factors involved in the control of human pubertal timing and the identification of rare potentially damaging variants in the *MECP2* gene in patients with idiopathic central precocious puberty.

Statistical analysis

No pre-analysis power calculations were done because the number of disease alleles in the collected cohort and of control alleles in the gnomAD database (version 3.1.2) data set (GRCh38) available were fixed (appendix pp 4–5).

The collapsed analysis of variants across the entire *MECP2* gene was done to compare allele frequencies of rare potentially damaging variants in *MECP2* between the central precocious puberty cohort (cases) and the gnomAD public database (controls). We took variants in MECP2 extracted from the gnomAD database, annotated with ANNOVAR, and filtered for rare variants (highest population minor allele frequency ≤0·01%) categorised as pathogenic, likely pathogenic, or uncertain significance. Fisher´s exact test (R studio; version 2022.120+353), was used for analysing allele frequency differences between both groups. Statistical significance was set up at p value less than 0.05.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between June 15, 2020, and June 15, 2022, 404 patients with idiopathic central precocious puberty (383 [95%] girls and 21 [5%] boys; 261 [65%] sporadic cases and 143 [35%] familial cases from 134 unrelated families) were included in the study. Of these patients, 171 (42%) were from Brazil, 105 (26%) from Spain, 72 (18%) from the USA, 34 (8%) from France, and 22 (6%) from the UK. 133 (33%) patients were enrolled for a multigene sequencing approach: 62 patients had whole-exome sequencing and 71 patients had targeted gene sequencing. Additionally, 271 (67%) patients with isolated central precocious puberty were screened for *MECP2* by Sanger sequencing.

We identified four rare heterozygous *MECP2* variants in seven (2%) girls with central precocious puberty (tables 1 and 2; figures 1 and 2). Three of the four *MECP2* variants were identified in five girls (from four unrelated families) by high-throughput sequencing analysis; were located at the coding region; and were predicted to be damaging by several in-silico tools, including a function-structure analysis (appendix p 4). Additionally, a *MECP2* insertion located at the 3□ untranslated region (UTR) was identified in two unrelated girls by Sanger sequencing analysis. *MECP2* variants were classified as likely pathogenic in three (43%) of the seven girls and as VUS in four (57%) girls by ACMG criteria. All variants were mapped to the *MECP2* transcript NM_001110792.2. Detailed genetic findings are described in the appendix (pp 7–13).

Two British monozygotic twin sisters (patients 1 and 2) with central precocious puberty had a heterozygous missense variant in exon 2 (289C→T) of *MECP2,* corresponding to the methyl-CpG-binding domain (MBD) of the protein (Arg97Cys).[19] The Arg97Cys variant was predicted to be damaging in 19 (90%) of 21 in-silico programmes (appendix pp 8–9) and identified at a very low allelic frequency in gnomAD (one [<1%] of

	Patient 1*	Patient 2*	Patient 3†	Patient 4	Patient 5‡	Patient 6	Patient 7
MECP2 variant	Missense substitution Arg97Cys	Missense substitution Arg97Cys	Missense substitution Ser176Arg	Indel insertion Ala6_Ala8dup	Indel insertion Ala6_Ala8dup	3'UTR insertion 36_37insT	3'UTR insertion 36_37insT
Ethnicity	European (UK)	European (UK)	Brazilian	Brazilian	Brazilian	European (Spain)	European (Spain)
Age at pubertal signs, years							
Thelarche	0·7	0·7	..	5·0	5·9	7·6	6·7
Menarche	..	..	8·5	..	..	..	..
Pubarche	..	..	..	7·5	5·9	..	..
At the time of diagnosis							
Chronological age, years	1·2	1·2	..	9·7	5·9	7·9	6·7
Tanner stage	2	2	..	4	2	2	2
Height SDS	–1·7	–1·8	..	–0·1	1·5	2·6	0·8
BMI SDS	–0·1	–0·2	..	1·5	1·3	1·5	–0·7
Bone age, years	..	..	..	12·0	8·5	10·0	9·7
Basal LH, IU/L§	0·7	<0·1	..	<0·6	0·4	0·2	4·1
Peak LH, IU/L§	6·8	4·9	..	13·2	2·4	23·4	18
Basal FSH, IU/L§	2·8	2·2	..	1·7	2·3	1·7	5·5
Peak FSH, IU/L§	86·1	33·1	..	13·5	18·4	17·6	10
Oestradiol, pg/mL§	<11·5	<11·5	..	<13	23·1	5	72
Family history of central precocious puberty	Monozygotic twin sister with CPP	Monozygotic twin sister with CPP	No	No	No	No	No
Neurocognitive phenotypes	Microcephaly¶, speech therapy, and mild difficulties in literacy	Microcephaly¶	Autism and macrocephaly||	No	No	No	No
Other clinical features	Subtle dysmorphisms (short neck, thin upper lip, and up slanting palpebral fissures)	Subtle dysmorphisms (short neck, thin upper lip, and up slanting palpebral fissures)	Hyperphagia with weight gain at age 2 years and obesity at age 10 years	Short stature, irregular cycles, and polycystic ovary (diagnosed by ultrasound) at age 15 years	Irregular cycles, hirsutism, and biochemical hyperandrogenism at age 14 years	No	Small for gestational age ** (birth weight SDS –2·6; birth length SDS –4·2)

FSH=follicle-stimulating hormone. GnRH=gonadotropin-releasing hormone. LH=luteinising hormone. NA=not available. SDS=standard deviation score. *Patients were monozygotic twin sisters. †No time of diagnosis date because the patient was referred to the endocrinology unit at 11·6 years old, reporting precocious menarche at 8·5 years with subsequent regular menstrual cycles, corresponding to a diagnosis of central precocious puberty. ‡Patient presented at 14·0 years old with clinical and biochemical hyperandrogenism; she had undergone endocrine investigation in another institution at 5·9 years old for a presentation of pubarche and thelarche; no management was instituted, and she was lost to follow-up; afterwards, she presented with menarche at 12 years; no hormonal data were available confirming the exact age at central puberty onset. §LH, FSH, and oestradiol concentrations were measured by electrochemiluminescence assay; hormonal criteria for confirming central precocious puberty were pubertal concentrations of basal or GnRH-stimulated LH; cutoff values were 0·3 IU/L or more for basal LH and more than 5·0 IU/L for GnRH-stimulated LH peak; prepubertal ranges were less than 4 UI/L for basal FSH and less than 15 pg/mL for oestradiol. ¶Head circumference in the second percentile (34 cm at 1·2 years old). ||Head circumference on the 97·5th percentile (58 cm at 15 years). **Small for gestational age was defined by birth weight and/or birth length standard deviation score of –2·0 or less for gestational age, according to the Usher & McLean method.

Table 2: **Clinical and hormonal features of seven girls with central precocious puberty and rare heterozygous variants in *MECP2***

182 926 alleles). The position of this variant might be implicated in stabilising the interaction between the MECP2 protein and DNA. Familial segregation analysis revealed that the Arg97Cys variant was a de novo *MECP2* mutation. Biological paternity was confirmed.

One Brazilian girl with sporadic central precocious puberty (patient 3) had a heterozygous missense variant in exon 3 (528C→A) of *MECP2*, corresponding to the intervening domain of the protein (Ser176Arg).[19] The Ser176Arg variant was predicted to be damaging by 15 (75%) of 20 in-silico programmes. Of the 21 in-silico programmes used, 20 had availability for the analysis of the functional domain affected by this variant. The Ser176Arg variant was absent in ABraOM and gnomAD (appendix p 10). The Ser176 was evolutionarily conserved and phosphorylation at this residue highly impaired MECP2 binding to DNA or chromatin in vitro.[20] Familial segregation analysis showed that the Ser176Arg variant was a de novo *MECP2* mutation. Biological paternity was confirmed. Therefore, both missense variants Arg97Cys (patients 1 and 2) and Ser176Arg (patient 3) were predicted to interfere with protein function and were classified as likely pathogenic.

Two unrelated Brazilian girls with sporadic central precocious puberty (patients 4 and 5) carried a heterozygous insertion of nine base pairs in exon 1 (23_24insCGCCGCCGC) of *MECP2*, corresponding to the amino (N) terminal domain of the protein (Ala6_Ala8dup).[19] The insertion was within an amino acid sequence specific to the NM_001110792.2 transcript.[21] It was located at

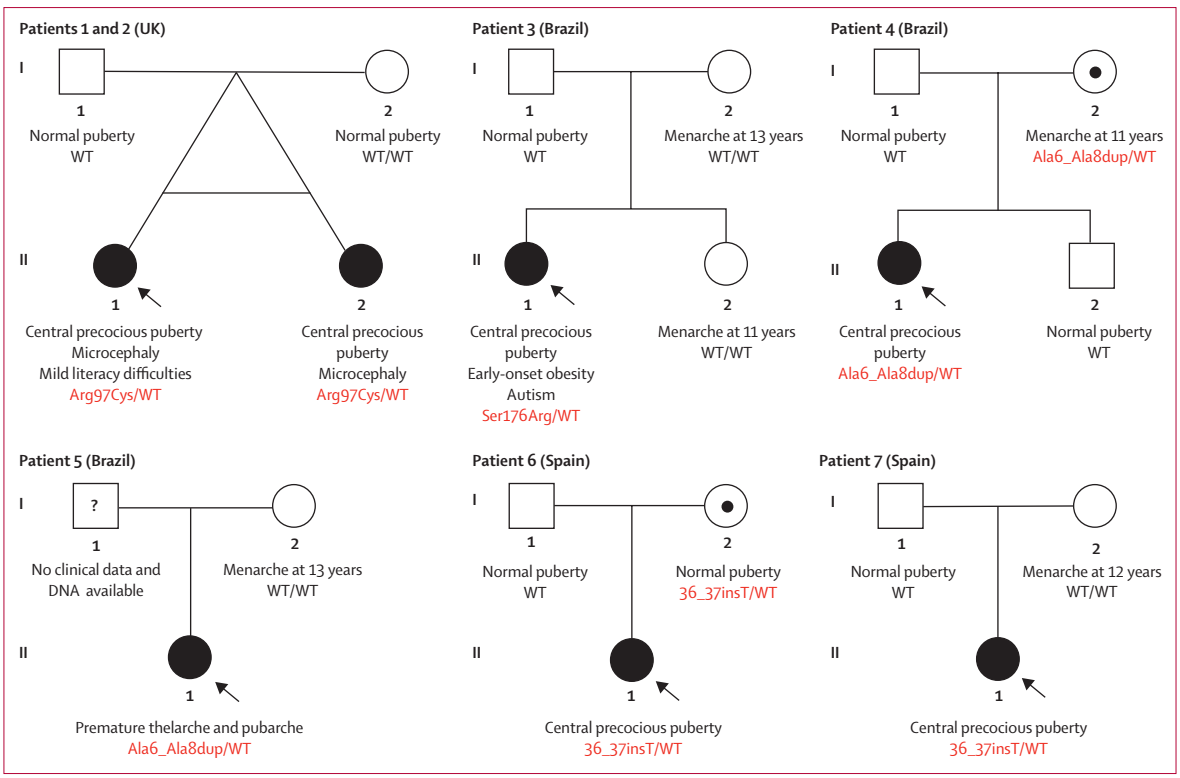

Figure 1: **Pedigrees of patients with central precocious puberty associated with rare heterozygous *MECP2* variants**
Squares are male family members, circles are female family members, black symbols are clinically affected family members, symbols with black internal circles are asymptomatic carriers, and symbols with a question mark are family member whose phenotype is unknown and arrows the proband in each family. Pubertal characteristics are shown for each individual; neurodevelopmental disorders are shown, when identified. The *MECP2* genotype is shown for individuals whose DNA was available for genetic studies. WT=wildtype.

a highly conserved region, consisting of a polyalanine tract. Previous studies suggested that expansion of alanine repeats could alter protein subcellular localisation, which could cause retention of MECP2 within the cytoplasm.[22] This insertion was absent in ABraOM and identified at a very low allelic frequency in gnomAD (three [<1%] of 59 976 alleles). Familial segregation analysis showed that patient 4 inherited the Ala6_Ala8dup variant from her unaffected mother, whereas it was absent in the mother of patient 5; clinical data and DNA from patient 5's father were not available. Therefore, the Ala6_Ala8dup variant was classified as of uncertain significance with major pathogenic evidence.

Two unrelated Spanish girls with sporadic central precocious puberty (patients 6 and 7) had a heterozygous insertion (36_37insT) in the 3□UTR of *MECP2*. This insertion was absent in ABraOM and gnomAD, and it was in a region that is highly conserved across mammals. Previous studies showed that the *MECP2* 3□UTR has multiple polyadenylation sites and microRNA binding sites.[23] Familial segregation analysis showed that patient 6 inherited the insertion from her unaffected mother and patient 7 had a de novo insertion with biological paternity confirmed. The 36_37insT variant was classified as of uncertain significance.

We studied the parental origin of the de novo *MECP2* variants (patients 1, 2, 3, and 7). Based on the presence of an informative heterozygous polymorphism (rs3027928), we could determine that the Ser176Arg variant arose on the paternal X chromosome in patient 3. The remaining patients had no informative polymorphisms near the de novo *MECP2* variants to specifically determine the parental origin.

According to available databases, these four rare *MECP2* variants identified in girls with central precocious puberty have not been associated with Rett syndrome phenotype, except for one patient with the Arg97Cys variant.[24]

The allele frequency of rare potentially damaging *MECP2* variants identified in girls with central precocious puberty (0·91%) was significantly higher than that identified in women from the gnomAD database (0·09%; unadjusted p=0·00002; table 3).

In the analyses of the UK Biobank, in 222 283 women and girls with both exome sequencing data and information on recalled age at menarche, we found 18 women with one of the three *MECP2* variants identified in our cohort (Arg97Cys variant [n=one]; Ala6_Ala8dup variant [n=12]; 36_37insT variant [n=five]). The mean age at menarche of these 18 women was 13·0 years (SD 1·6), not statistically different from the

Biobank average (12·9 years [1·6] years; p=0·92). However, one woman with the Ala6_Ala8dup variant had menarche when she was 10 years old.

Of the men and boys from the UK Biobank, five had three *MECP2* variants (Arg97Cys variant [n=one]; Ala6_Ala8dup variant [n=one]; and 36_37insT variant [n=three]. In the UK Biobank, voice breaking was recalled in three categories (appendix p 6) and these five individuals were in the group with an average age of voice breaking. The Ser176Arg variant was not identified in any individual, confirming its very rare frequency. In the publicly available UK Biobank data, high confidence protein truncating variants in *MECP2* were identified in seven females and no males; these variants had no significant associations with age at menarche (p=0·12), BMI (p=0·89), height (p=0·97), or testosterone concentrations (p=0·078). Data on signs of pubertal onset (thelarche, testicular enlargement, and pubarche) and polycystic ovary syndrome were not available in the Biobank.

Additionally, we evaluated *MECP2* for associations with human conditions in genome-wide association studies. To date, no locus near *MECP2* has been associated with measured pubertal traits.[25]

Studies of X inactivation pattern in blood samples of ten women and girls (six girls [patients] and four women [mothers]) identified an extreme skewing in patient 1 (appendix pp 9, 14). No other extreme skewed X inactivation was identified in blood samples of the remaining women and girls evaluated.

Precocious thelarche was identified by physical exam as the first sign of premature pubertal development in six [86%] of the seven girls with *MECP2* variants (table 2). The median age of onset of breast development was 5·4 years (IQR 6·2). The remaining girl (patient 3) came for the first endocrine visit with a report of precocious menarche at age 8·5 years. Bone age, when available, showed a typical advancement in relation to chronological age (≥2 years). None had family history of premature sexual development.

Patients 1, 2, and 3, who had missense likely pathogenic *MECP2* variants (Arg97Cys [patients 1 and 2] and Ser176Arg [patient 3]), had central precocious puberty and neurobehavioral phenotypes. These three girls were comprehensively assessed with clinical geneticists and neuropaediatricians, but criteria for the diagnosis of a recognised syndrome, including Rett syndrome, were not identified. Patient 1 had microcephaly and mild difficulties in literacy, while patient 2 had microcephaly. Patient 3 had autism spectrum disorder (confirmed by standardised neuropsychological evaluation), macrocephaly, and hyperphagia followed by early-onset obesity. By contrast, the unrelated girls with Ala6_Ala8dup (patients 4 and 5) and the 36_37insT (patients 6 and 7) had no evident neurodevelopmental abnormalities. Both girls with Ala6_Ala8dup developed irregular menstrual cycles and other findings resembling polycystic ovary syndrome as young adults. All clinical findings are detailed in the appendix (pp 7–13).

Immunohistochemistry and immunofluorescence showed abundant staining for Mecp2 in multiple relevant hypothalamic nuclei, including arcuate, suprachiasmatic, and paraventricular nuclei, and in the median eminence, in pubertal female mice (figure 3). Double labelling experiments showed colocalisation of Mecp2 and Gnrh in more than 70% of GnRH neuronal cells visualised.

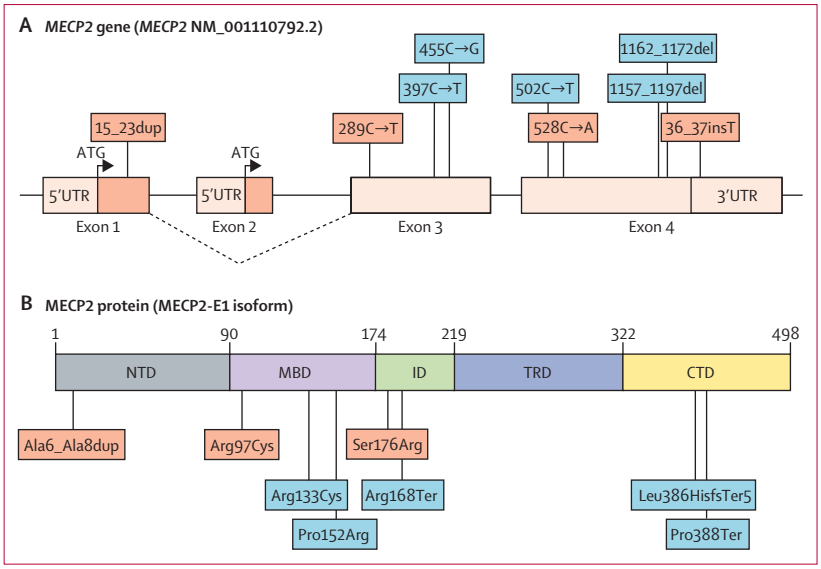

Figure 2: **Schematic representation of the *MECP2* gene structure (four exons) and the MECP2 protein (five protein domains)**

The *MECP2* gene is comprised of four exons; the MECP2 protein contains five protein domains. The MECP2-E1 isoform is derived from alternate splicing of exon 2 (shown in dashed lines), and it corresponds to the NM_001110792.2 transcript. In our study, four heterozygous *MECP2* variants (shown in light red) were identified in seven girls with central precocious puberty, of whom three girls had missense mutations (two in the MBD and one in the ID), and four girls had *MECP2* insertions. In previous studies, five heterozygous *MECP2* variants (shown in blue) were identified in six girls with Rett syndrome diagnosed with central precocious puberty.[17] Of whom, two girls with Rett syndrome with central precocious puberty had missense mutations in the methyl-binding domain and four girls had truncating mutations (one in the intervening domain and three in the CTD). CTD=carboxy-terminal domain. ID=intervening domain. MBD=methyl-binding domain. NTD=amino-terminal domain. TRD=transcriptional repression domain.

	Number of individuals	Number of potentially damaging alleles†	Total number of alleles evaluated	Allele frequency (%)	Unadjusted p value‡
Girls with central precocious puberty	383	7	766	0·9	0·00002
Women and girls from the gnomAD	35750	69	71500	0·1	1 (ref)

*Data from the Genome Aggregation Database were extracted from the version 3.1.2 dataset (GRCh38), containing data from genomes of diverse ancestries. The comparison between cases and controls included only women, because men are hemizygous for the *MECP2* gene and there was no *MECP2* variant identified in boys with central precocious puberty from the current study. †Potentially damaging *MECP2* variants were defined as rare variants (Minimal allele frequency ≤0·01%) that were categorised as pathogenic, likely pathogenic, or variant of uncertain significance by the American College of Medical Genetics and Genomics criteria. ‡Statistical significance was set at p value less than 0·05.

Table 3: **Comparison of allele frequencies of rare potentially damaging *MECP2* variants between girls with central precocious puberty and women from the gnomAD public database***

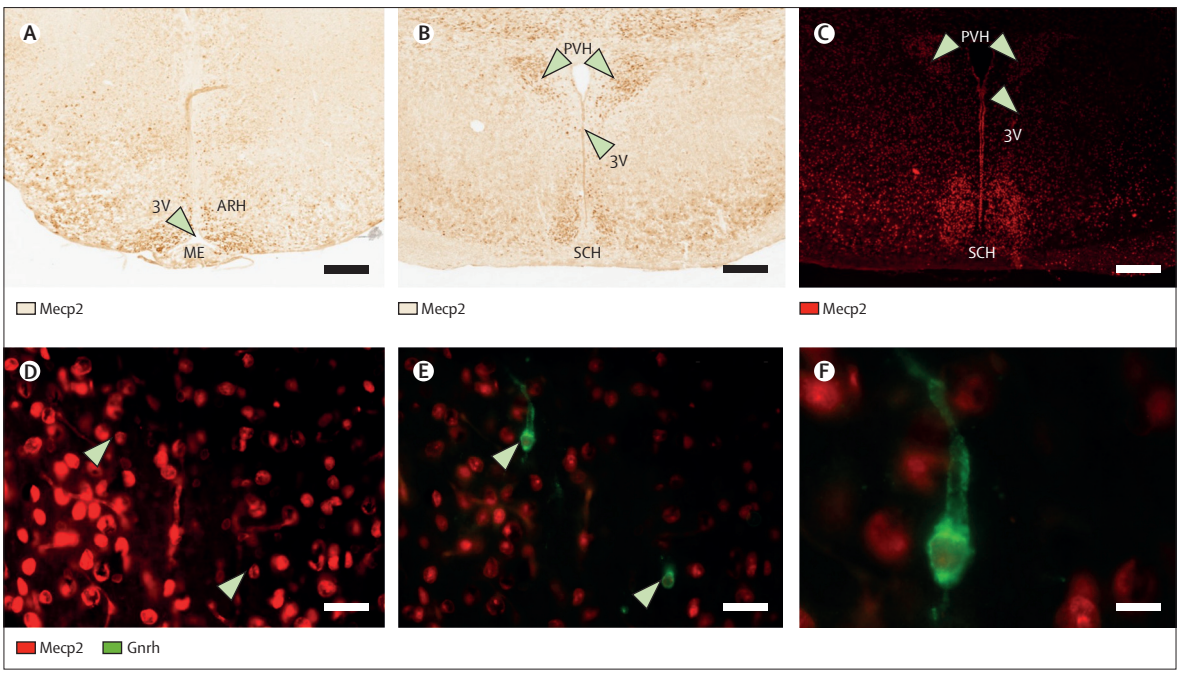

Figure 3: **Tissue expression of Mecp2 and Gnrh in postnatal (day 38) female mouse hypothalamus.**
Mecp2 is expressed in key regions for GnRH neuronal function. Immunohistochemistry analysis revealing Mecp2 localisation in the arcuate nucleus and median eminence of the hypothalamus (A) and the paraventricular hypothalamic nucleus, parvicellular division, and suprachiasmatic nucleus (B) at postnatal day 38 in female mice (coronal section). Immunofluorescent staining for Mecp2 confirms localisation to the same regions of the hypothalamus (C, D). Gnrh and Mecp2 might be colocalised within the paraventricular nucleus (E, F). Scale bars in A–C: 200 µm; D, E: 50 µm; F: 10 µm. Images are representative of experiments done at least three independent times. Arrowheads point to Gnrh neurons (D, E). 3V=third ventricle. ARH=arcuate nucleus. ME=median eminence. PVH=paraventricular hypothalamic nucleus. SCH=suprachiasmatic nucleus.

Discussion

Loss-of-function mutations in two maternal imprinted genes (*MKRN3* and *DLK1*) have been identified as the most frequent causes of familial central precocious puberty, indicating an essential role for DNA methylation in the mechanisms underlying pubertal timing.[2,3] Both genes are located at crucial regions of imprinting disorders that might be associated with central precocious puberty (*MKRN3* at chromosome 15q11–13 of Prader-Willi syndrome and *DLK1* at chromosome 14q32.2 of Temple syndrome).[6] Our results suggest a potential X-linked form of central precocious puberty associated with rare variants in *MECP2*, a key component of the human DNA methylation machinery, the inactivation of which has previously been associated with Rett syndrome.[26] We identified seven girls (from six unrelated families) with central precocious puberty carrying four rare heterozygous *MECP2* variants (Arg97Cys, Ser176Arg, Ala6_Ala8dup, and 36_37insT). None of these girls with central precocious puberty and *MECP2* variants developed Rett syndrome.

The MECP2 protein belongs to the methyl-binding domain family, which in mammals are mediators of DNA methylation, a major epigenetic mechanism that occurs predominantly in the context of CpG dinucleotides.[26] The known functions of MECP2 involve repression or activation of gene transcription and regulation of chromatin structure,[9,10,26] playing a role in the epigenetic regulation of target gene expression.[10] Growing evidence has shown the participation of epigenetic mechanisms in the hypothalamic control of puberty development. Animal studies proposed that MBD3, another protein of the methyl-CpG-binding domain family, is a substrate for the ubiquitin activity of MKRN3. The MKRN3–MBD3 axis regulates the methylation status of *GNRH1* promoter, directly regulating pubertal timing.[27]

The seven girls identified in our study with rare heterozygous *MECP2* variants had a sporadic form of central precocious puberty. This clinical form is in line with the identification of de novo variants in four of these girls, a pattern considered as strong evidence for pathogenicity. In patient 3, the de novo Ser176Arg variant was shown to arise from the paternal X-chromosome, a mechanism that might partly explain the female predominance of *MECP2* mutations.[13] In two other girls, the *MECP2* variants were inherited from their unaffected mothers, a pattern of clinical variability that has been shown in women and girls with defects in genes subject to X inactivation.[8,12] Mild neurobehavioral abnormalities were identified in girls with de novo missense *MECP2* variants (patients 1, 2, and 3). Such cases might be considered syndromic forms of central precocious puberty and thus represent part of the spectrum of neurodevelopmental disorders related to *MECP2*.[14] The

girls with central precocious puberty and *MECP2* insertion mutations (patients 4, 5, 6, and 7) did not manifest clear neurobehavioural symptoms, suggesting milder clinical pictures. This clinical heterogeneity could be related to the severity of *MECP2* variants, where the more damaging the variant, the more complex the phenotype. The phenotypic spectrum of patients with *MECP2* mutations is highly variable, ranging from Rett syndrome to other *MECP2*-related disorders, or to unaffected carrier mothers in rare familial cases.[26] This variable expressivity could be partly attributed to the tissue-specific patterns of X chromosome inactivation that could result in distinct MECP2 expression profiles in the brain, leading to heterogeneous phenotypes observed in individuals carrying the same mutations.[28] Our findings of rare heterozygous *MECP2* variants in girls with central precocious puberty, including two unaffected carrier mothers, might be secondary to this phenomenon.

An extreme skewing of X inactivation was identified in one girl carrying the Arg97Cys variant (patient 1). This finding implied that one of the X chromosomes was preferentially inactivated in this patient, at least in the blood. Skewed X chromosomes inactivation can be observed in a small proportion of healthy women due to stochastic processes, but skewed X inactivation also can be associated with clinical conditions caused by the presence of a pathogenic sequence variant in the X chromosome, such as Rett syndrome due to *MECP2* mutations.[29]

Our association testing of rare variants showed a significantly higher frequency of rare potentially damaging *MECP2* variants in girls with central precocious puberty than in controls, indicating an enrichment of *MECP2* variants in the central precocious puberty cohort and strengthening a putative association of *MECP2* with the central precocious puberty phenotype. Although UK Biobank data did not identify an association between precocious menarche and rare protein truncating variants in *MECP2*, data on age at thelarche and pubarche were not available from the Biobank study. This result might suggest a stronger effect of MECP2 deficiency on pubertal onset than completion (menarche), as is observed in cohorts of women and girls with Rett syndrome due to *MECP2* mutations.[15,16] In addition, the identification of women and girls with normal mean age at menarche carrying three distinct *MECP2* variants in the Biobank also suggested a possible reduced penetrance or variable expressivity, which could be modulated by distinct patters of X inactivation or by compensatory mechanisms. In genome-wide association studies no locus near *MECP2* was associated with measured pubertal traits to date, nor with neurological conditions often associated with *MECP2* mutations.

We showed that Mecp2 is highly expressed in key areas of the hypothalamus responsible for GnRH function in female mice and colocalised with Gnrh in most Gnrh neurons in these regions. A similar result was reported in a study showing coexpression of MECP2 with GnRH and kisspeptin in the hypothalamus of ewes.[30] The exact mechanism by which MECP2 might influence hypothalamic GnRH secretion is not yet known. A potential intermediary factor is *FXYD1*, expression of which was increased in the brain of a mouse *Mecp2* knockout model.[31] Rodent studies suggested that *Fxyd1* promoted Gnrh neuronal excitability, facilitating the advent of puberty.[16] These results suggested that loss of *MECP2* might lead to precocious pubertal timing potentially due to increased *FXYD1* activity. Alternatively, *MECP2* might influence hypothalamic GnRH secretion via d-aminobutyric acid (GABA), a major inhibitory factor of GnRH secretion.[1] Animal studies have shown that MECP2 is crucial for normal function of GABA-releasing neurons.[32] Mecp2 deficient mice have disruption of the central balance between excitatory and inhibitory inputs, marked by a GABAergic pathway deficit. In this model, disruption of *MECP2* regulation might lead to precocious pubertal onset due to decreased GABAergic inhibition of GnRH secretion. Despite the novelty of our findings, functional studies of the identified VUS and the action of *MECP2* in the reproductive axis were not provided to address this question, which is a limitation of our study.

Investigation of genetic causes has been applied in children with central precocious puberty presenting with the familial form or in association with multiple phenotypes, especially neurodevelopmental abnormalities.[4] Because genomic investigation is extended to larger cohorts of patients with central precocious puberty, the recognition of key factors involved in the neuroendocrine control of pubertal timing is likely to increase. Although Rett syndrome has been associated with abnormal pubertal development, MECP2 function has not previously been linked to the timing of puberty in patients with previous diagnosis of idiopathic central precocious puberty. Our findings identifying rare heterozygous *MECP2* variants in multiple unrelated girls with central precocious puberty, with or without mild neurodevelopmental abnormalities, suggested a potential X-linked form of premature pubertal development.

Contributors

APMC, SRH, and ACL conceptualised and designed the study. APMC, FR, DS, MEdM, LGG, MPP, RB, RE-A, AE-M, APA, JA, and VNB collected the clinical data. APMC, FRT, LG, LRM, AP, JER, CES, SSC, AD, LRK, and JRBP did the technical or computational experiments. APMC, LRM, LLG, MK, ACK, AALJ, AD, KKO, JRBP, APA, UBK, SRH, and ACL analysed and interpreted the genetic data. APMC, LRM, SRH, and ACL verified the data. All authors had access to raw data. APMC wrote the original draft. LLG, MK, ACK, AALJ, APA, UBK, JA, BBM, VNB, SRH, and ACL critically reviewed the manuscript. APMC, SRH, and ACL edited the final version. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Anonymised data will be made available upon reasonable request for academic use and within the limitations of the informed consent.

Proposals should be made to the corresponding author. Data will be shared according to the data protection regulations of each hospital and country participating in the study.

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