

DATA REPORT

Endocrinopathies in a boy with cryptic copy-number variations on 4q, 7q and Xp

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We report a male patient with three copy-number variations (CNVs) and unique phenotype. He carried ~11.2 Mb terminal duplication on 4q, ~13.4 Mb terminal deletion on 7q and ~1.7 Mb interstitial duplication on Xp22.31, which were identified by array-based comparative genomic hybridization. He manifested mental retardation, mild brain anomalies and skeletal deformities ascribable to these CNVs, together with central precocious puberty and mild adrenocorticotrophic hormone overproduction of unknown etiologies.

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Molecular cytogenetic technologies, including array-based comparative genomic hybridization, are useful to identify cryptic copy-number variations (CNVs) in individuals with an apparently normal karyotype.^{1–3} Recent cytogenetic studies have shown that cryptic CNVs account for a certain fraction of the etiologies of congenital disorders.^{1–3} Cryptic CNVs can underlie complex phenotypes that are not explainable by monogenic mutations, although they can also occur as benign polymorphisms.^{2,3}

Here, we report a male patient with unique endocrine abnormalities and additional clinical features, in whom we identified three cryptic CNVs. This study was approved by the Institutional Review Board Committee at the National Center for Child Health and Development and performed after obtaining informed consent. The patient was born to non-consanguineous Japanese parents at 40 weeks of gestation. The patient's parents were clinically normal, whereas two sisters and two of three maternal uncles of the patient had mild mental retardation. Endocrinological assessments were not performed for the family members. At birth, the patient showed mild genital skin pigmentation, mild joint contractures and muscle hypertonia. He had no episodes of hypoglycemia or seizure. His mental development was markedly delayed. He started walking on his own at 6 years of age. At 8 years of age, he was referred to our hospital for the evaluation of early sexual maturation. Physical assessment showed pubic hair of Tanner stage 2, penis of stage 5 and bilateral testes of 8 ml (+9.1 s.d.). He manifested skin pigmentation in the external genitalia, gingiva and fingers. In addition, he showed scoliosis and restricted joint extension of the limbs. He had no facial dysmorphism, except for strabismus, a broad nasal base and platycephaly. He spoke multiple words but no sentences, and was unable to run. His height and weight were 118.2 cm (–1.6 s.d.) and 20.8 kg (–1.1 s.d.), respectively. He had a significantly advanced bone age (14 years and 1 month); his predicted final height was around 130 cm (–7.0 s.d.). Skeletal radiography showed Madelung-like deformity of the forearm

and scoliosis. Brain magnetic resonance imaging detected mild ventricular dilatation and mild atrophy of the anterior lobe. No abnormalities were observed in the hypothalamus or pituitary. Abdominal ultrasonography and computed tomography showed no abnormalities. Endocrine assessment revealed an elevated testosterone level and increased gonadotropin responses to gonadotropin-releasing hormone stimulation, which collectively indicated central precocious puberty (Table 1). He showed a mildly elevated level of insulin-like growth factor 1, which was consistent with the accelerated sexual maturation.⁴ Adrenocorticotrophic hormone (ACTH) levels were elevated before and after corticotropin-releasing factor stimulation, while cortisol levels remained normal (Table 1). These data were consistent with a condition referred to as 'overproduction of ACTH, not associated with Cushing disease' (International Statistical Classification of Diseases and Related Health Problems 10th Revision). Blood levels of glucose and electrolytes were within the normal range. G-banding analysis showed a normal 46,XY karyotype. Array-based comparative genomic hybridization using a human catalog array (4 × 180 k format, Agilent Technologies, Santa Clara, CA, USA) delineated three heterozygous CNVs, i.e., ~11.2 Mb terminal duplication on 4q34.3–35.2, ~13.4 Mb terminal deletion on 7q35–36.3 and ~1.7 Mb interstitial duplication on Xp22.31 (Figure 1; Supplementary Table S1). These CNVs were not found in the database of Genomic Variants (<http://dgv.tcag.ca/dgv/app/home?ref=GRCh37/hg19>) or in the University of California Santa Cruz Genome Browser (<https://genome.ucsc.edu/>). The mother of the boy had the same duplication on Xp22.31, but no other CNV. DNA samples of other family members were not available for genetic testing.

These data suggest that the boy carried *de novo* or paternally inherited CNVs on 4q and 7q, and a maternally inherited duplication on Xp (Supplementary Table S1). CNVs on 4q and 7q appear to be pathogenic, because they affected more than 10 Mb regions and have been reported exclusively in individuals

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Table 1. Endocrine findings of the patient at 8 years of age

Hormone	Stimulus (dosage)	Patient		Reference values ^a		Conversion factor to the SI unit
		Baseline	Peak	Baseline	Peak	
Growth hormone (ng/ml)	GRF (20 µg) ^b	1.3	35.1		> 15	1.0 (µg/l)
Luteinizing hormone (mIU/ml)	GnRH (80 µg) ^c	<u>2.8</u>	<u>61.8</u>	0.0–0.4	0.4–6.0	1.0 (IU/l)
Follicle stimulating hormone (mIU/ml)	GnRH (80 µg) ^c	<u>11.1</u>	<u>46.3</u>	0.6–3.0	6.3–15.6	1.0 (IU/l)
Thyroid stimulating hormone (mIU/ml)	TRH (140 µg) ^c	2.3	24.5	0.2–5.4	3.6–26.8	1.0 (mIU/l)
Prolactin (ng/ml)	TRH (140 µg) ^c	7.1	31.5	1.4–11.8		43.48 (pmol/l)
Adrenocorticotropic hormone (pg/ml)	CRF (20 µg) ^b	<u>158</u>	<u>486</u>	10–25	28–131	0.22 (pmol/l)
Cortisol (µg/dl)	CRF (20 µg) ^b	9.1	15.4	6.1–12.3	10.6–26.9	27.59 (nmol/l)
Cortisol (µg/dl)	ACTH (0.2 mg) ^d	7.1	18.5	6.1–12.3	22.7–25.9	27.59 (nmol/l)
17α-Hydroxypregnenolone (ng/ml)	ACTH (0.2 mg) ^d	0.95	2.39	0.26–1.43	1.29–3.92	0.301 (pmol/l)
17-Hydroxyprogesterone (ng/ml)	ACTH (0.2 mg) ^d	0.4	1.8	0.2–0.5	1.1–1.8	0.303 (pmol/l)
Dehydroepiandrosterone sulfate (µg/dl)		12.1		4.41–24.4		0.02714 (µmol/l)
Insulin-like growth factor 1 (ng/ml)		<u>240</u>		114–225		0.131 (nmol/l)
Testosterone (ng/dl)		<u>300</u>		1–13		0.03467 (nmol/l)
Free triiodothyronine (pg/ml)		3.1		3.1–5.1		1.536 (pmol/l)
Free thyroxine (ng/dl)		1.1		1.1–1.6		0.1287 (pmol/l)

Abbreviations: ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; GnRH, gonadotropin-releasing hormone; GRF, growth hormone releasing factor; TRH, thyrotropin-releasing hormone. Hormone values below the reference range are boldfaced and those above the reference range are underlined. Stimulus dosage was administered i.v. ^aReference values in age-matched males. ^bBlood sampling at 0, 15, 30, 60, 90 and 120 min. ^cBlood sampling at 0, 30, 60, 90 and 120 min. ^dBlood sampling at 0, 30 and 60 min.

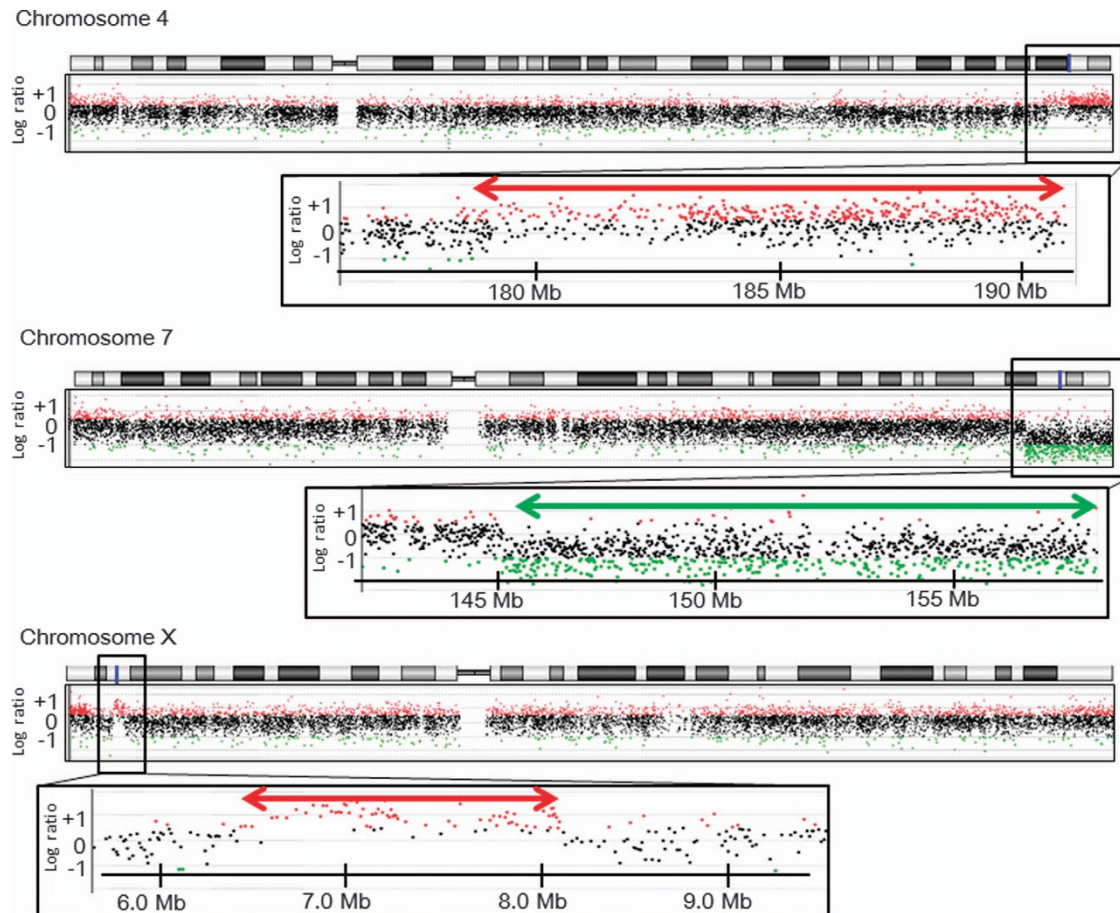


Figure 1. Cryptic copy-number variations (CNVs) in the patient. The black, red and green dots denote signals indicative of the normal, the increased ($\log \text{ratio} \geq +0.5$) and the decreased ($\log \text{ratio} \leq -1.0$) copy-numbers, respectively. Genomic positions correspond to the human genome reference assembly (UCSC Genome Browser, hg19, build 37). The red and green arrows indicate the duplicated and deleted regions, respectively. The names of the genes affected by the CNVs are shown in Supplementary Table S1.

with developmental defects.^{5–14} Indeed, previous studies have shown that terminal duplications of 4q result in various clinical abnormalities, including mental retardation, short stature, microcephaly, facial dysmorphism and finger anomalies.^{5,6} Of these, mental retardation is likely to be associated with copy-number gain of *GLRA3* or *GPM6A*,^{7,8} whereas craniofacial features and minor limb abnormalities can be ascribed to *HAND2* duplication.⁹ Terminal deletions of 7q are known to cause haploinsufficiency of *SHH* that leads to holoprosencephaly, mental retardation, single incisor and limb anomalies.¹⁰ This 7q35–36 region also harbors several genes such as *CNTNAP2*, *EZH2*, *KCNH2* and *PRKAG2*, whose haploinsufficiency has been associated with congenital malformations and/or neurological abnormalities.^{11–14} In contrast, the pathogenicity of the relatively small duplication on Xp22.3 remains unclear; Li *et al.*¹⁵ reported that CNVs at Xp22.3 are shared by 0.37% of individuals with neuropsychological disabilities and/or congenital anomalies and by 0.15% of healthy controls. Taken together, the mental retardation and mild brain anomalies, and probably skeletal deformities as well, of our patient are ascribable to one or more of these three CNVs. As molecular analysis was not performed for patient's relatives except for the mother, it remains unknown whether these CNVs underlie mental retardation in his sisters and uncles. Furthermore, endocrinological evaluation was not performed for the patient's mother with the same Xp22.3 duplication, and therefore the association between the CNV and hormonal abnormalities remained unknown. Although the boy lacked most of the characteristic features of 4q terminal duplications and 7q terminal deletions, this can be explained by a relatively incomplete penetrance or variable expressivity of these features.^{7,10} In particular, the lack of holoprosencephaly in this patient is consistent with the broad phenotypic spectrum of *SHH* haploinsufficiency.¹⁶ Alternatively, this patient may have somatic mosaicism.

The patient manifested additional clinical features that have not been reported in patients with 4q duplications, 7q deletions or Xp22.3 duplications. The most remarkable findings were central precocious puberty and mild ACTH overproduction. The origin of elevated ACTH levels in this case has yet to be studied. The hormone data are indicative of ACTH resistance; however, the patient showed no signs of glucocorticoid deficiency characteristic of ACTH resistance.¹⁷ Furthermore, it remains unclear whether precocious puberty and ACTH overproduction are independent events. Precocious puberty has not been described in patients with ACTH overproduction due to *MC2R* or *MRAP* mutations,¹⁷ although ACTH-dependent precocious pseudopuberty was observed in an infant with adrenal hypoplasia due to *DAX1* mutations,¹⁸ and ACTH is known to stimulate testosterone production in the neonatal mouse testis.¹⁹ The CNVs in our patient included no known genes involved in the regulation of pituitary or adrenal hormones. However, since central precocious puberty and ACTH overproduction are genetically heterogeneous conditions,^{17,20} hitherto unidentified causative genes may reside within these CNVs. Further studies are necessary to clarify the etiology of the unique phenotype of the patient.

In summary, we identified a boy with three cryptic CNVs. The boy manifested mental retardation, mild brain anomalies and skeletal deformities that are ascribable to the CNVs on 4q, 7q and/or Xp, together with endocrine abnormalities of unknown genetic origin.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.592>, <http://dx.doi.org/10.6084/m9.figshare.hgv.594>, <http://dx.doi.org/10.6084/m9.figshare.hgv.596>.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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