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Pubertal development in ALG6 deficiency (congenital disorder of glycosylation type Ic)

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Abstract

Information on the hypothalamic pituitary ovarian axis in congenital disorders of glycosylation (CDG) females is scarce. Varying hormonal profiles and degrees of virilization in CDG females suggest a spectrum of yet unidentified mechanisms affected by impaired N-glycosylation. We describe an ALG6D woman who completed puberty with normal gonadotropins and testosterone levels, no virilization, and regular menses. Hormonal follow-up of CDG females is necessary to improve our understanding of the role of glycosylation in pubertal development.

Keywords

CDG; Puberty; Glycosylation; Hormone; ALG6; Aromatase

1. Introduction

ALG6 deficiency (ALG6D) (CDG-Ic, OMIM ID:603147) is caused by mutations in the ALG6 gene, which encodes an α -1,3-glucosyltransferase used to add the first glucose to the immature lipid-linked oligosaccharide (LLO) precursor [1–3]. Fewer than 100 cases of ALG6D have been described worldwide, presenting with psychomotor retardation, muscular hypotonia, gross motor delays, seizures, and occasionally protein losing enteropathy [1].

Abnormal protein glycosylation in CDG has been shown to impact the endocrine system as it affects growth, thyroid function, adrenal function, glucose metabolism and pubertal development [4,5]. CDG affected females are usually described with hypergonadotropic hypogonadism and an absence of secondary sexual characteristics [6,7], with only five reported cases of females with CDG (two with Ia, one with Ib, one with Ic, and one with If) to have completed puberty [4,8].

Information on the hormonal profile of the hypothalamic pituitary ovarian (HPO) axis of CDG females is scarce. In two case reports, elevated testosterone and signs of virilization

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were noted in a previously described ALG6D female [8] who developed full sexual characteristics and underwent menarche; and in two adolescent females (CDG 1a) with no pubertal development and hypergonadotropic hypogonadism [9].

We describe an ALG6D (CDG-1c) affected woman who completed puberty with normal gonadotropins and testosterone levels, no signs of virilization, and regular menses. The varying hormonal profiles in these cases suggest that there is a spectrum of mechanisms affected by defects in N-glycosylation.

2. Materials and methods

2.1. Biochemical assays

CLIA-approved analysis of transferrin glycosylation status was performed by Mayo Clinical Laboratories and on a research basis in the author's laboratory.

2.2. Genetic testing and expression studies

Genetic analysis of patient and parents DNA and rescue of ALG6deficient yeast by normal and mutant hALG6 were performed as described in [3].

3. Clinical report

Patient was born term at a normal birth weight. She was hypotonic and had difficulties nursing with an inability to latch. In early infancy, developmental delays were identified and she had ongoing gastrointestinal issues, hyperinsulinemic hypoglycemia, strabismus, cortical blindness, partial agenesis of the corpus callosum and cerebellar dysfunction including dysarthric speech, wide-based gait, past-pointing, and ataxia. Episodes of myoclonus started at age 3.5 years. Puberty was delayed with thelarche at 13 years of age and menarche at 15 years. Siblings had normal timing of menarche.

At 18 years of age, she was referred for evaluation of severe premenstrual symptoms including fatigue, emotional lability, and aggression. Menses were regular, lasting 5 days. She had normal secondary sex characteristics and only a small amount of hair along the linea alba.

4. Results

Laboratory evaluation was normal for the follicular phase of the menstrual cycle (Table 1) with no evidence of elevated androgen levels. A transabdominal pelvic ultrasound showed a normal uterus with a 5 mm endometrial stripe. The ovaries were normal in size with a dominant follicle in the right ovary.

Her constellation of symptoms prompted screening for CDG by analysis of transferrin isoelectric focusing. The isoelectric pattern of glycosylated transferrin appeared normal with mono-oligo/di-oligo ratio 0.016 (reference 0.074) and a-oligo/di-oligo ratio 0.001 (reference 0.022), but this can be intermittently normal in individuals with CDG [10]. Mannose metabolic labeling of her skin fibroblasts showed normal lipid-linked oligosaccharide size. Sequence analysis of the ALG6 gene showed that she was homozygous for p. Tyr131His and that both her parents were heterozygous for the p. Tyr131His mutation. However, complementation analysis of ALG6deficient yeast showed that the p.Tyr131 mutation only partially rescues defective glycosylation and that it is as severe as the most common disease-causing mutation p. Ala333Val, which occurs in over half of all known CDG-1c patients [3].

5. Discussion

Abnormalities in glycosylation of the gonadotropins or their cell-surface receptors causing reduced bioactivity and/or bioavailability appear to be responsible for the well documented delayed or absent sexual maturation in girls with CDG [4]. As the FSH receptor (FSHR) has more glycosylation sites than the unglycosylated LH receptor, the function of FSHR is likely more affected by impaired glycosylation [11].

Increased testosterone in CDG females has been attributed to exaggerated LH-driven theca cell function and the lack of FSH-dependent granulosa cell function [9]. If the hypothesis of LH-driven testosterone steroidogenesis is correct, women with an FSHR defect, who have elevated LH and FSH levels and an ovarian phenotype similar to CDG patients, should also have elevated testosterone as did the two CDG-Ia adolescent girls with no pubertal development, ovarian failure and elevated LH and FSH levels [9]. However, females with an FSHR defect have neither increased testosterone levels nor virilization [12]. Further countering LH-driven testosterone steroidogenesis is that the only other CDG-Ic female to have completed puberty [8] had normal LH levels but elevated testosterone along with irregular menses, hirsutism, male pattern baldness and enlarged polycystic ovaries (PCOS). Our ALG6D patient, on the other hand, had regular menses and a normal hormonal profile including testosterone (Table 1). This suggests that some other, not-yet-elucidated, glycosylation-dependent mechanism is involved that can lead to varying degrees of virilization and testosterone levels in CDG females independent of ovarian and adrenal function. One potential explanation could be impaired glycosylation and function of the aromatase enzyme.

Aromatase is a cytochrome P450 enzyme (P450aro) involved in the biosynthesis of estrogens from androgens in many estrogen-responsive tissues (ovaries, adipose tissue, skin, brain, bone, and placenta) with the ovarian follicle being the primary site, driven by FSH stimulation of the FSHR [13]. The aromatase gene contains three putative N-glycosylation sites and unglycosylated forms of the aromatase enzyme have been shown to have reduced catalytic efficiency [14]. Alternative splicing of the aromatase gene (CYP19) at the target tissues and the effect of CDG mutations in glycosylation of aromatase enzyme may lead to variable phenotypes including varying ranges of estrogen, testosterone and gonadotropin levels [15]. Impaired P450aro activity at the ovary and/or adipose tissue may result in elevated testosterone and suboptimal estrogen levels as even genetic variations of the aromatase gene (CYP19) have been shown to be associated with hyperandrogenism and PCOS [16].

We speculate that impaired P450aro activity at the ovary in the previously described CDG-Ic patient with PCOS [8], and impaired activity in adipose tissue in the two CDG-Ia adolescent females with normal-sized ovaries and no signs of follicular activity [9] may explain the virilizing symptoms seen in these cases especially since their elevated testosterone levels were not caused by adrenal hyperfunction as shown by their normal DHEA-S levels.

As the endocrine system depends on glycosylation for stability, binding affinity and ligand specificity of polypeptide hormones, hormone carrier proteins and hormone receptors [2,5], it is important that the ubiquitous role that aromatase and glycosylated hormones and receptors play in the pubertal development of CDG females is evaluated. Systematic evaluation of the HPO axis should be part of the initial and follow-up evaluations including measurement of the gonadotropins, estradiol, testosterone, androstenedione, DHEA-S and inhibins, and pelvic US. Measurement of aromatase enzyme activity in the fat tissue of CDG patients and correlation with the different CDG subtypes and genotypes are also needed.

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Abbreviations

ALG6D	hALG6 deficiency
CDG	congenital disorder of glycosylation
LH	luteinizing hormone
FSH	follicle stimulating hormone
DHEA-S	dehydroepiandrosterone sulfate

References

1. Grunewald S, Imbach T, Huijben K, Rubio-Gozalbo ME, Verrips A, de Klerk JB, Stroink H, de Rijk-van Andel JF, Van Hove JL, Wendel U, Matthijs G, Hennet T, Jaeken J, Wevers RA. Clinical and biochemical characteristics of congenital disorder of glycosylation type Ic, the first recognized endoplasmic reticulum defect in N-glycan synthesis. *Ann Neurol*. 2000; 47:776–781. [PubMed: 10852543]
2. Korner, C.; Thiel, C.; Hoffmann, G. Congenital disorders of glycosylation. In: Sarafoglou, K.; Hoffmann, G.; Roth, K., editors. *Pediatric Endocrinology and Inborn Errors of Metabolism*. McGraw Hill; New York: 2009. p. 339-352.
3. Westphal V, Xiao M, Kwok PY, Freeze HH, Westphal V, Xiao M, Kwok PY, Freeze HH. Identification of a frequent variant in ALG6, the cause of congenital disorder of glycosylation-Ic. *Hum Mutat*. 2003; 22:420–421. [PubMed: 14517965]
4. Miller BS, Freeze HH. New disorders in carbohydrate metabolism: congenital disorders of glycosylation and their impact on the endocrine system. *Rev Endocr Metab Disord*. 2003; 4:103–113. [PubMed: 12618564]
5. Miller BS, Khosravi MJ, Patterson MC, Conover CA, Miller BS, Khosravi MJ, Patterson MC, Conover CA. IGF system in children with congenital disorders of glycosylation. *Clin Endocrinol (Oxf)*. 2009; 70:892–897. [PubMed: 19207313]
6. Jaeken J, Vanderschueren-Lodeweyckx M, Casaer P, Snoeck L, Corbeel L, Eggermont E, Eeckels R. Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG-deficiency, increased serum arylsulphatase A and increased CSF protein: a new syndrome? *Pediatr Res*. 1980; 14:179.
7. Stibler H, Blennow G, Kristiansson B, Lindehammer H, Hagberg B. Carbohydrate-deficient glycoprotein syndrome: clinical expression in adults with a new metabolic disease. *J Neurol Neurosurg Psychiatry*. 1994; 57:552–556. [PubMed: 8201322]
8. Sun L, Eklund EA, Van Hove JL, Freeze HH, Thomas JA, Sun L, Eklund EA, Van Hove JLK, Freeze HH, Thomas JA. Clinical and molecular characterization of the first adult congenital disorder of glycosylation (CDG) type Ic patient. *Am J Med Genet A*. 2005; 137:22–26. [PubMed: 16007612]
9. de Zegher F, Jaeken J. Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. *Pediatr Res*. 1995; 37:395–401. [PubMed: 7596677]
10. Dupre T, Cuer M, Barrot S, Barnier A, Cormier-Daire V, Munnich A, Durand G, Seta N. Congenital disorder of glycosylation Ia with deficient phosphomannomutase activity but normal plasma glycoprotein pattern. *Clin Chem*. 2001; 47:132–134. [PubMed: 11148191]
11. Davis DP, Rozell TG, Liu X, Segaloff DL. The six N-linked carbohydrates of the lutropin/choriogonadotropin receptor are not absolutely required for correct folding, cell surface expression, hormone binding, or signal transduction. *Mol Endocrinol*. 1997; 11:550–562. [PubMed: 9139799]

12. Aittomaki K, Herva R, Stenman UH, Juntunen K, Ylostalo P, Hovatta O, de la Chapelle A. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J Clin Endocrinol Metab.* 1996; 81:3722–3726. [PubMed: 8855829]
13. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev.* 1994; 15:342–355. [PubMed: 8076586]
14. Jo Corbin C, Mapes SM, Lee YM, Conley AJ. Structural and functional differences among purified recombinant mammalian aromatases: glycosylation. N-terminal sequence and kinetic analysis of human, bovine and the porcine placental and gonadal isozymes. *Mol Cell Endocrinol.* 2003; 206:147–157. [PubMed: 12943997]
15. Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, Dattani MT, Achermann JC, Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, Dattani MT, Achermann JC. Variable phenotypes associated with aromatase (CYP19) insufficiency in humans. *J Clin Endocrinol Metab.* 2007; 92:982–990. [PubMed: 17164303]
16. Petry CJ, Ong KK, Michelson KF, Artigas S, Wingate DL, Balen AH, de Zegher F, Ibanez L, Dunger DB. Association of aromatase (CYP 19) gene variation with features of hyperandrogenism in two populations of young women. *Hum Reprod.* 2005; 20:1837–1843. [PubMed: 15802318]

Table 1

Hormonal evaluation of CDG females from previous studies and our patient*.

	Ia [9]	Ia [9]	Ic [8]	Ic*
D4A	77 ng/dL (72–143)	89 ng/dL (72–143)	201, 234 ng/dL (60–245)	101, 180, 200 ng/dL (50–430)
DHEA-S	169 mcg/dL (48–239)	206 mcg/dL (48–239)	38 mcg/dL (60–255)	81, 168, 216 mcg/dL (35–430)
17-OHP			42 ng/dL (15–70)	39 ng/dL (20–170)
T	74.5 ng/dL (b74.5)	109.5 ng/dL (b74.5)	76, 101 ng/dL (10–55)	36, 46, 47 ng/dL (14–75)
Free T			14, 16 pg/mL (1.1–6.3)	11 pg/mL (1–15)
SHBG			0.7, 1.0, 1.1 mcg/dL (1.0–3.0)	0.9 mcg/dL (0.9–3.9)
LH	↑	↑	2.4, 1.1 IU/L (2–15 F, 0.6–19 L)	2.5, 3.4, 3.4 IU/L (1.9–12.5 F)
FSH	↑	↑	1.5, 0.8 IU/L (3–20 F, 1–12 L)	4.1, 4.3, 4.9 IU/L (2.5–10.2 F)
E2	b13.6 pg/mL	b13.6 pg/mL		b10, 26, 42 pg/mL (11–165 F)
P				0.8 ng/dL (0.15–1.4)
Menarche	N/A	N/A	15 years	15 years
Menses	N/A	N/A	Scanty	Regular

Abbreviations: D4A—androstenedione, DHEA-S—Dehydroepiandrosterone-Sulfate, 17-OHP—17-hydroxyprogesterone, T—Testosterone, SHBG—sex hormone binding globulin, LH—luteinizing hormone, FSH—follicle stimulating hormone, E2—estradiol, P—progesterone.