Reversible hypogonadotropic hypogonadism

Andrew A Dwyer¹, Taneli Raivio²,³ and Nelly Pitteloud¹,⁴

¹Endocrinology, Diabetes and Metabolism Service of the Centre Hospitalier Universitaire Vaudois (CHUV), Rue du Bugnon 46, 1011 Lausanne, Switzerland, ²Faculty of Medicine/Physiology, University of Helsinki, Helsinki, Finland, ³The Children’s Hospital, Helsinki University Central Hospital (HUCH), Helsinki, Finland and ⁴Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Rue du Bugnon 7, 1005 Lausanne, Switzerland

Abstract

Congenital hypogonadotropic hypogonadism (CHH) is characterized by lack of puberty and infertility. Traditionally, it has been considered a life-long condition yet cases of reversibility have been described wherein patients spontaneously recover function of the reproductive axis following treatment. Reversibility occurs in both male and female CHH cases and appears to be more common (~10–15%) than previously thought. These reversal patients span a range of GnRH deficiency from mild to severe and many reversal patients harbor mutations in genes underlying CHH. However, to date there are no clear factors for predicting reversible CHH. Importantly, recovery of reproductive axis function may not be permanent. Thus, CHH is not always life-long and the incidence of reversal warrants periodic treatment withdrawal with close monitoring and follow-up. Reversible CHH highlights the importance of environmental (epigenetic) factors such as sex steroid treatment on the reproductive axis in modifying the phenotype. This review provides an overview and an update on what is known about this phenomenon.

Introduction

In humans, puberty and reproduction are contingent upon the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons. This neuroendocrine activity stimulates the secretion of gonadotropins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) from the gonadotropes that in turn stimulate the gonads to produce sex steroids (testosterone and estradiol) and gametes (sperm and ova). Congenital hypogonadotropic hypogonadism (CHH) is a congenital disorder caused by the deficient secretion or action of GnRH and is clinically characterized by incomplete or absent puberty and infertility (1). Notably, the onset and severity of GnRH deficiency ranges from severe (e.g. complete absence of puberty with cryptorchidism and micropenis) (2) to relatively milder forms as demonstrated by enfeebled GnRH-induced LH secretion and partial pubertal development (3). The latter includes the so-called ‘fertile eunuch’ variant (4) who have sufficient gonadotropin secretion for some testicular growth capable of supporting spermatogenesis yet who

Invited Author’s profile

Professor Nelly Pitteloud is the chief of the Endocrine, Diabetes, and Metabolism service at the University Hospital (CHUV) in Lausanne, Switzerland. Professor Pitteloud is a specialist in reproductive endocrinology, and her translational research focuses on the neuroendocrine control of human reproduction. She is an expert in the genetics of congenital hypogonadotropic hypogonadism and is the chair of the EU-funded COST European Network investigating GNRH deficiency (www.gnrhnetwork.eu).
are hypogonadal and undervirilized. In addition to these reproductive phenotypes, CHH patients present with a range of associated non-reproductive phenotypes including anosmia, cleft lip/palate, hearing loss, and a variety of skeletal anomalies that occur at variable rates (1). Regardless of phenotypic expression or degree of GnRH deficiency, there has been a long-held view that CHH was a permanent and lifelong condition. Over the past 15 years there have been a growing number of reports of reversible CHH challenging this dogma. Importantly, these reversals occur well into adulthood, thus differentiating the phenomenon from constitutional delay of growth and puberty wherein pubertal onset is late but occurs spontaneously (5). This review will summarize the literature on the phenomenon of reversible CHH.

**Pathophysiology and genetics of CHH**

CHH is typically diagnosed during the minipuberty of infancy or in adolescence and early adulthood when patients fail to undergo spontaneous pubertal development (1). It is typically classified as normosmic CHH (when the sense of smell is intact) or Kallmann syndrome (KS) when associated with anosmia. To date, more than 2 dozen genetic loci have been identified to underlie (KS) when associated with anosmia. To date, more than 20 genes have been implicated in CHH that can impair the action (or secretion) of GnRH, resulting in normosmic CHH. In contrast, mutations in genes impacting the migration of GnRH neurons from the olfactory placode during development (ANOS1, SEMA3A, IL17RD, SOX10 and FEZF1) (15, 16, 17, 18, 19, 20, 21) result in KS. KAL1, recently renamed ANOS1, was the first X-linked gene implicated in CHH (15, 16, 22, 23). Although mutations in X-linked genes may help account for the fact that there are 3–5 times more males diagnosed with CHH than females (24), mutations in ANOS1 only account for ~5% of cases (25). The striking sex discordance may simply represent a bias of ascertainment as females may be under-diagnosed and treated empirically with oral contraceptives. Regardless, as our understanding of the molecular basis of CHH has accelerated the genetics of CHH has become increasingly complex. For instance, incomplete penetrance and variable expressivity can be observed in CHH pedigrees wherein family members harboring the identical mutation exhibit different phenotypes (26, 27). Part of this phenomenon can be explained by the interaction of mutations in two or more genes/proteins (oligogenicity) as has been reported in several studies (19, 25, 28). Further, some patients with reversible CHH harbor mutations in genes implicated in CHH, (29) including cases of oligogenicity (30). Such reversal cases highlight the importance of environmental (i.e. epigenetic) modifiers on the reproductive axis.

**CHH reversibility**

CHH has been thought to be a congenital and a lifelong disorder. Once diagnosed, CHH treatment is typically initiated in the form of low-dose sex steroid treatment to develop the secondary sexual characteristics (31) yet pubertal induction can also be effectively achieved using either pulsatile GnRH therapy (32, 33) or exogenous gonadotropin therapy (34). All of these treatment options are similar in that they are effective in normalizing serum sex steroid levels.

The notion that CHH patients could spontaneously recover function of the HPG axis was first raised in a 1975 case report abstract by Rezvani and *et al.* (35). This was followed by a report of reversal and proven paternity in a fertile eunuch male (36). In the subsequent two decades, additional case reports emerged documenting reversals (37) including fertility/conception (38, 39, 40) and two series were published demonstrating pulsatile LH secretion in a subset of CHH men following cessation of treatment (41, 42). The first estimate of prevalence of reversal came from Quinton and *et al.* (43) who depicted five cases among a retrospective cohort of 76 CHH patients revealing a prevalence of 5%. Authors went on to recommend that patients with a testicular volume of 6 ml or greater (i.e. partial pubertal development) undergo biochemical reassessment, particularly if TV increases to 8 ml or greater without gonadotropin therapy (34). All of these treatment options are similar in that they are effective in normalizing serum sex steroid levels.

There is some debate regarding the clinical distinction between constitutional delay of puberty (CDP) and CHH reversal. Delayed puberty is statistically defined (i.e. 2–2.5 S.D. beyond the mean and thus ~2% of the population) (5). Both CHH and CDP are characterized by delayed pubertal onset. In CDP, puberty eventually starts spontaneously, while CHH patients typically will not initiate spontaneous pubertal development or have a stalled puberty. For example, when an adolescent male has failed to undergo puberty by age 17 (+4 S.D. for age) (45) the clinical probability of congenital GnRH deficiency (CHH) is very...
high. Notably, as 10–15% of CHH cases will reverse later in life there appears to be a clinical overlap between CDP and CHH with these entities existing on a spectrum of GnRH deficiency. Despite the clinical overlap, relatively little shared genetic basis has been identified between CHH and delayed puberty (46, 47).

**Etiology of CHH reversal**

The etiology of reversal is still unclear. In males, reversal was indicated by testicular growth while on testosterone replacement therapy while in females, fertility (i.e. spontaneous pregnancy) has been a key indicator of axis recovery (30, 48, 49). Interestingly, the common basis among all the cases of reversal was a normal sex steroid milieu for months following sex steroid replacement, exogenous gonadotropin therapy or pulsatile GnRH therapy. As such, some reports have proposed that the reversal phenomenon could be related to the androgen-driven upregulation of genes involved in the regulation of GnRH secretion (50). Interestingly, reversal has been noted in patients with severe GnRH deficiency, i.e. in those with a history of cryptorchidism and/or micropenis at birth (29, 49, 51). Moreover, some reversal patients lack olfactory structures (44, 52) pointing to the possibility that recovery of GnRH neuronal function may not always be dependent on intact olfactory structures (53). Interestingly, hypothalamic progenitor cells in rat can give rise to GnRH neurons, suggesting that postnatal genesis of GnRH neurons can occur in certain circumstances (54). Importantly, mouse studies demonstrate that fetal tissue from the preoptic area of wild-type mice injected into the 3rd ventricle of hpg mice can develop into functional GnRH neurons (55). This is relevant for when considering reversible CHH as relatively few GnRH neurons are necessary for inducing pulsatile LH secretion (56). Further, murine studies suggest that the GnRH system is highly plastic as environmental stimuli such as sexual interactions can rescue GnRH function in some instances (57). Further, epigenetic influences seem also to play a critical role in the onset of puberty (58), and thus potentially in the reversal process.

**Genetic basis of CHH reversibility?**

Unexpectedly, patients with CHH who undergo reversal can harbor CHH mutations – indicating that the effects of a genetic defect can be overcome. The first genetic report of a CHH patient with reversal harboring a homozygous mutation in GNRHR was in 2001 (59). Subsequently, several patients with CHH reversal were identified with mutations in other genes reported in CHH including ANOS1 (60), CHD7 (44), FGFR1 (29, 30, 44, 49, 61), HS6ST1 (62), NSMF (49), PROKR2 (30, 52, 63) and TAC3/TACR3 (51) (Table 1). A recent report of reversal cases identified CHH mutations in (45%) of probands (17/38). Mutations were identified in FGFR1 (5/38, 13%), GNRHR (3/38, 8%), TACR3 (3/38, 8%), PROKR2 (2/38, 5%) as well as TAC3 and HS6ST1 (one each, 3% respectively) (49). In considering all reports in the literature, mutations in GNRHR appear to be the leading genetic causes among cases of reversal occurring in both homozygous (48, 59, 64, 65, 66) and compound heterozygous (44, 66) forms. Additionally, a digenic CHH case has been documented wherein a proband exhibiting reversal carries heterozygous mutations in both FGFR1 and PROKR2 (30). Interestingly, although mutations in ANOS1 (KAL1) and CHD7 occur in 5–15% of CHH cases, only two reversals have been found to harbor a mutation in these two penetrant genes (Table 1). Importantly, ascertaining the genetic basis for reversible CHH is challenging as not all published reports have systematically examined all CHH genes. Therefore, comprehensive and systematic studies on larger cohorts of reversible CHH are needed to identify genetic signatures predicting reversal.

**CHH reversible among other ethnic groups**

In addition to the previous reports of reversible CHH in Caucasian probands, reversal cases have now been reported in other ethnic groups. In 2013, a group of Indian investigators reported case descriptions of nine CHH men who underwent reversal and the recent retrospective study identifying 18 reversals among a large Chinese cohort (n=354, 5%). The observed frequency among Chinese is lower than other reports yet this may simply reflect different definitions of reversal. In both reports, all cases were males and had received either exogenous testosterone replacement therapy or human chorionic gonadotropin injections (67, 68). Further, like our prospective study (29), the common denominator among the reversal cases was a normalized sex steroid milieu.

**Fragility of the reversal state**

An important recent development is the growing appreciation that reversal may not be lasting (44, 49, 50, 65, 68). Indeed, some patients who recover HPG axis function slip back into a hypogonadal state. While the mechanism(s)
Table 1  Mutations in CHH genes identified in cases of reversal.

<table>
<thead>
<tr>
<th>Gene</th>
<th>HUGO ID</th>
<th>Reference sequence</th>
<th>Nucleotide change*</th>
<th>Amino acid change</th>
<th>Mutation type</th>
<th>ExAC</th>
<th>Poly-Phen2</th>
<th>SIFT</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOS1</td>
<td>6211</td>
<td>NM_000216</td>
<td>c.223_224insC</td>
<td>p.Val75Alafs*11</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(45)</td>
</tr>
<tr>
<td>CHD7</td>
<td>20626</td>
<td>NM_017780.2</td>
<td>c.151C&gt;T</td>
<td>p.Gln51*</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(43)</td>
</tr>
<tr>
<td>FGFR1</td>
<td>3688</td>
<td>NM_023110</td>
<td>c.91 +2T &gt; A</td>
<td>n/a</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.296A&gt;G</td>
<td>p.Tyr99Cys</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.716T&gt;C</td>
<td>p.Ile239Thr</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.1279G&gt;T</td>
<td>p.Val427Leu</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.1809C&gt;A</td>
<td>p.Cys603*</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.1864C&gt;T</td>
<td>p.Arg622*</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.317A&gt;G</td>
<td>p.Gln106Arg</td>
<td>Homozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(44, 52, 55)</td>
</tr>
<tr>
<td>GNRHR</td>
<td>4421</td>
<td>NM_000406.2</td>
<td>c.317A&gt;G</td>
<td>p.Gln106Arg</td>
<td>Homozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(44, 52, 55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.416G&gt;A</td>
<td>p.Arg139His</td>
<td>Heterozygous 0.02%</td>
<td>Deleterious</td>
<td>Deleterious</td>
<td>(43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.1279G&gt;A</td>
<td>p.Arg262Gln</td>
<td>Heterozygous 0.20%</td>
<td>Deleterious</td>
<td>Deleterious</td>
<td>(43)</td>
<td></td>
</tr>
<tr>
<td>H56ST1</td>
<td>5201</td>
<td>NM_004807</td>
<td>c.1210A&gt;G</td>
<td>p.Asp262Gln</td>
<td>Homozygous 0.02%</td>
<td>Deleterious</td>
<td>Deleterious</td>
<td>(53, 54)</td>
<td></td>
</tr>
<tr>
<td>NSMF</td>
<td>29843</td>
<td>NM_001130969</td>
<td>c.587G&gt;A</td>
<td>p.Asp196His</td>
<td>Heterozygous 0.00%</td>
<td>Tolerated</td>
<td>Tolerated</td>
<td>(48)</td>
<td></td>
</tr>
<tr>
<td>PROKR2</td>
<td>15836</td>
<td>NM_144772</td>
<td>c.604A&gt;G</td>
<td>p.Asp202Gly</td>
<td>Heterozygous 0.02%</td>
<td>Tolerated</td>
<td>Tolerated</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.743G&gt;A</td>
<td>p.Arg248Gln</td>
<td>Heterozygous 0.00%</td>
<td>Tolerated</td>
<td>Tolerated</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.820T&gt;A</td>
<td>p.Val274Asp</td>
<td>Homozygous 0.00%</td>
<td>Deleterious</td>
<td>Deleterious</td>
<td>(49)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.991G&gt;A</td>
<td>p.Ile331Met</td>
<td>Heterozygous 0.60%</td>
<td>Tolerated</td>
<td>Tolerated</td>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>TAC3</td>
<td>11521</td>
<td>NM_013251</td>
<td>c.60delG</td>
<td>p.Gly20fs*39</td>
<td>Homozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(51)</td>
</tr>
<tr>
<td>TACR3</td>
<td>11528</td>
<td>NM_001059</td>
<td>c.824G&gt;A</td>
<td>p.Trp275*</td>
<td>Heterozygous 0.02%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.766C&gt;T</td>
<td>p.Tyr256His</td>
<td>Homozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.294G&gt;C</td>
<td>p.Trp98Val</td>
<td>Heterozygous</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(51)</td>
</tr>
</tbody>
</table>

HUGO, Human Genome Organization. * cDNA numbering begins with the start ATG (Met); n/a, not applicable (the heterozygous mutation in intron 2 of FGFR1 destroys the conserved donor splice site); ExAC, Exome Aggregation Consortium (frequency of mutation in database of > 60 000 unrelated individuals) http://exac.broadinstitute.org/; Poly-Phen2, polymorphism phenotyping v2 (in silico prediction program) http://genetics.bwh.harvard.edu/pph2/; SIFT, sorting intolerant from tolerant (in silico prediction program) http://sift.jcvi.org/.

* oligogenic reversal case of a proband harboring mutations in both FGFR1 and PROKR2. Mutations are reported using HGVS nomenclature http://www.hgvs.org/mutnomen/.
for relapse remain unclear, emotional, metabolic or psychiatric stressors have been implicated in some cases of relapse into hypogonadotropic hypogonadism (49, 50). It is tempting to postulate that although these patients were able to sustain normal HPG function for a period of time, the recovered system may be enfeebled and vulnerable to stressors. From this perspective, these CHH cases share some similarities with hypothalamic amenorrhea (69). To conclude, reversal and relapse underscore an important clinical caveat as there is need for ongoing monitoring of these cases of reversal. While definitive guidelines are lacking for monitoring reversal cases, ongoing surveillance is advisable and annual follow-up seems a prudent recommendation (Box 1).

Box 1 Key points
1. CHH is clinically heterogeneous representing a spectrum for GnRH deficiency
2. Recovery of HPG axis function occurs in 10–15% of the cases following normalizing the sex steroid milieu
3. Increase in testicular size on testosterone is the most common sign of reversal in males
4. Patients should undergo supervised treatment washouts to assess for recovery (every 2 years)
5. Individuals experiencing reversal should be advised on sperm banking
6. Reversal patients should have ongoing monitoring to assess for relapse
7. More studies are needed to clarify reversals in female CHH patients

Conclusion
The traditional dogma that CHH is necessarily a lifelong condition has given way to the notion that 10–15% of patients will undergo a reversal of their condition. It is notable that those patients who recover function of their reproductive axis include cases of severe GnRH deficiency and those who harbor mutations in known CHH loci. The common denominator among the reported reversals has been a normalized sex steroid milieu following treatment. There are several key clinical points including the importance of periodic supervised treatment washouts to assess for CHH reversibility (every 1–2 years). Specifically, any indication of increased testicular volume in males on testosterone treatment or spontaneous menses in females off treatment should be considered as a likely indicator of reversal. Secondly, patients should be counseled on appropriate use of birth control to avoid the possibility of unwanted pregnancy in the event of reversal. Lastly, it appears that some patients undergoing reversal may be prone to waxing and waning of their reproductive function secondary to stressors. Thus, reversal patients warrant ongoing monitoring and it seems prudent to counsel male reversal patients on the possibility of sperm banking for fertility preservation in the event that they slip back into a hypogonadal state and their spermatogenesis is interrupted.

To conclude, reversal in CHH is a true phenomenon that has important clinical implications. To date, several questions remain regarding the etiology as well as clinical and genetic predictors of reversal CHH. It is tempting to hypothesize that this reversibility could occur in other developmental disorders.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This work was supported by the Swiss National Science Foundation and by COST Action BM1105.

References
2. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT & Crowley WF Jr. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. Journal of Clinical Endocrinology and Metabolism 2002 87 152–160. (doi:10.1210/jcem.87.1.8131)
28 Rezvani I, DiGeorge AM, Rutano J & Snyder PJ. Delayed puberty and anosmia: Coincidence or Kallmann variant? [abstract] *Pediatric Research* 1975 **9** 244.


60 Beneduzi D, Trabrab E, Min L, Jorge AA, Garmes HM, Renk AC, Fichna M, Fichna P, Arantes KA, Costa EM et al. Role of...


Received 20 October 2015
Revised version received 14 January 2016
Accepted 20 January 2016