Severe testotoxicosis phenotype associated with Asp⁵⁷⁸→Tyr mutation of the lutrophin/choriogonadotrophin receptor gene

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Abstract

Testotoxicosis is a form of male precocious puberty caused by heterogeneous activating mutations in the gene for the lutrophin/choriogonadotrophin receptor (LHR). A patient with an unusually early and severe presentation of testotoxicosis, including profound Leydig cell hyperplasia, was found to have a sporadic mutation encoding Asp⁵⁷⁸→Tyr. The severe testotoxicosis phenotype appears to be related to the strongly activating nature of the Tyr substitution.

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Testotoxicosis (MIM176410) is an autosomal dominant, male limited disorder characterised by autonomous Leydig cell function.¹ Signs of precocious puberty usually appear in affected boys at the age of 2 to 3 years. The disorder is caused by at least 10 different missense mutations in the LHR gene that promote agonist independent activation of the receptor, leading to inappropriate intracellular cAMP accumulation.²⁻⁶ The most common mutation encodes substitution of Asp⁵⁷⁸ with Gly (D578G). Interestingly, two unrelated boys with unusually early and severe presentations of testotoxicosis have been shown to have an LHR mutation encoding Asp⁵⁷⁸→Tyr (D578Y).^{4,7,8} We now describe a third sporadic case where the severe testotoxicosis phenotype is associated with the strongly activating D578Y mutation.

The patient presented at the age of 10 months with pubic hair, enlarged phallus, husky cry, and accelerated linear growth. There was no family history of precocious puberty. Serum testosterone was markedly raised at 15 nmol/l (normal adult 11-38 nmol/l), basal and stimulated gonadotrophin levels were prepubertal, and other aetiological factors were excluded. At 16 months of age testosterone was 28 nmol/l, testicular volume was 3 ml, pubic hair was Tanner stage III, height was 4 SD above normal, height velocity was 6.2 SD above normal, and bone age was 5 years. Testicular biopsies performed for diagnostic purposes at 16 months showed bilateral, extensive Leydig cell hyperplasia (fig 1). The cells appeared fully differentiated, with many containing abundant foamy cytoplasm. Within the tubules, spermatogenic activity was evident

and Sertoli cells were well developed. The dense arrangement of Leydig cells filling the interstitial areas is distinctly different from the pattern of patchy Leydig proliferation previously noted in other boys with testotoxicosis.^{9,10}

Several different therapeutic regimens were tried in an effort to control virilisation and growth acceleration. Now, aged 5, the patient is receiving a combination of cyproterone acetate, testolactone, and tamoxifen. Height velocity is 1.5 SD above normal.

Consent was obtained for genetic studies of the LHR. The methods used for mutation analysis have been described previously.² Briefly, genomic DNA was isolated from the patient and his parents and PCR was used to amplify a fragment of exon 11 of the LHR gene encoding amino acid residues 441 to 594. Temperature gradient gel electrophoresis (Qiagen, Chatsworth, CA) showed a heterozygous mutation in the LHR gene of the patient, but not in that of either parent (fig 2). The abnormally migrating PCR product from the patient was used to generate single stranded DNA, and the purified template was then sequenced directly (Sequenase, USB). Sequence analysis showed a heterozygous G to T mutation at nucleotide 1732 of the patient's LHR gene. This mutation encodes substitution of Asp⁵⁷⁸ with Tyr.

Cells expressing the D578Y LHR exhibit an increase in basal cAMP production that is almost twice that produced by the D578G LHR or other known activating LHR mutations.^{4,11} The agonist independent activation produced by D578Y represents 65% of the maximal stimulation produced by the agonist human chorionic gonadotrophin. The D578Y

Figure 1 Extensive Leydig cell hyperplasia in testis biopsy from the 16 month old patient (H&E).

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Figure 2 Temperature gradient gel electrophoresis of PCR products from a normal male (open square), the patient (solid square), and his parents (open symbols). Only DNA from the patient produces an abnormal pattern of bands, indicating the presence of a new heterozygous mutation in this segment of the LHR gene.

mutant is also unique in causing twofold activation of the phosphoinositide hydrolysis pathway.¹¹ Asp⁵⁷⁸ is conserved in all glycoprotein hormone receptors. The fact that substitution with Tyr causes more profound LHR activation than Gly may provide useful insights into normal receptor structure and function. A three dimensional model of the transmembrane receptor bundle places Asp⁵⁷⁸ in the middle of helix 6, engaged in stabilising hydrogen bond interactions with residues in helix 7.¹² We speculate that agonist independent activation is the result of loss of these constraining bonds, and the bulky Tyr side chain may cause additional destabilisation of the inactive receptor conformation by disrupting the packing of adjacent transmembrane helices.^{11 12}

Variation in clinical phenotype resulting from allelic heterogeneity is a well described phenomenon, especially for autosomal recessive diseases involving partial or complete loss of an enzyme or structural protein. Mutations that cause either impairment or inappropriate activation of conformational signalling by heptahelical membrane receptors have only recently been recognised as a cause of human disease, and little is known about the dependence of phenotype on allelic heterogeneity.¹³ In the case of the photoreceptor rhodopsin, patients with an inactivating Pro³⁴⁷→Leu mutation appear to have more severe disease than patients bearing different mutant alleles,^{14 15} and certain mildly activating mutations are the least deleterious.¹⁶ Some naturally occurring mutations of the human thyrotrophin receptor are more strongly activating than others, including some that involve the phosphoinositide hydrolysis pathway.¹⁷ Although no clear association has yet been made between allelic heterogeneity and the severity of thyroid hyperplasia or hyperfunction, somatic thyrotrophin receptor mutations tend to be more activating than those found in cases of hereditary toxic thyroid hyperplasia.^{17 18} In regards to the LHR gene, the recent description of a testotoxicosis kindred in which one 12 year old prepubertal boy was found to have an activating M398T mutation indicates that not all mutant alleles exhibit 100% penetrance.⁵

The age of presentation and degree of Leydig cell hyperplasia and raised serum testosterone in our patient clearly distinguishes him from the majority of familial and sporadic testotoxicosis cases that have been published.^{1 9 10} Furthermore, his mutant LHR exhibits a level of constitutive signalling that is much greater

than that produced by the other mutant receptors associated with testotoxicosis.^{4 11} Our findings confirm and extend those from two other young boys with non-familial testotoxicosis^{4 7 8} and suggest that these unusually early and severe clinical presentations of testotoxicosis are directly related to the strongly activating nature of the de novo D578Y substitution.

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