ANE syndrome caused by mutated RBM28 gene: a novel etiology of combined pituitary hormone deficiency

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Abstract

Objective and design: A homozygous loss-of-function mutation in the gene RBM28 was recently reported to underlie alopecia, neurological defects, and endocrinopathy (ANE) syndrome. The aim of the present study was to characterize the endocrine phenotype of ANE syndrome and to delineate its pathogenesis.

Methods: Detailed neuroendocrine assessment was performed in five affected male siblings harboring the homozygous p.L351P mutation in RBM28.

Results: All five affected patients, aged 20–39 years, displayed absent puberty, hypogonadism, and variable degrees of short stature. Low IGF1 concentration and a lack of GH response to provocative tests in all siblings were consistent with GH deficiency. Low testosterone and gonadotropin levels with absence or low response to GnRH stimulation indicated hypogonadotropic hypogonadism. ACTH deficiency evolved over time, and glucocorticoid replacement therapy was initiated in four patients. Thyroid analysis showed variable abnormal TSH response to TRH stimulation, suggesting hypothalamic compensated hypothyroidism in four subjects and laboratory hypothyroidism (low free thyroxine) in one patient. Low prolactin levels were shown in one case.

Conclusions: The endocrine defects characteristic of ANE syndrome are compatible with variable combined anterior pituitary hormone deficiency (CPHD), which evolves gradually over the years, indicating long-term hormonal monitoring. We propose that defects in the cellular Wnt/β-catenin signaling pathway underlie this endocrinopathy. RBM28 gene defects should be added to the growing list of gene defects associated with syndromic CPHD.

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Introduction

Alopecia, neurological defects, and endocrinopathy (ANE) syndrome is a new autosomal recessive syndrome recently reported by Nousbeck et al. (1). The syndrome is caused by decreased expression of the nucleolar protein RBM28, known to be associated with ribosome biogenesis (2), and is characterized by a distinctive phenotype. Skin involvement includes alopecia of variable severity, resulting from abnormal hair follicle differentiation, multiple facial nevi, and flexural hyperpigmentation (1). Neurological defects comprising moderate to severe mental retardation and progressive motor decline typically begin during the second decade of life, presenting with combined upper and lower motor dysfunction. Additional features of the syndrome include gynecomastia and dental anomalies (1). Endocrine deficiency is a major component of the syndrome. A homozygous p.L351P mutation in the RBM28 gene was identified in five affected siblings with ANE syndrome born to consanguineous parents of Arab–Muslim origin. In a previous study, we showed the absence of mature hair follicles and the presence of dermal cysts in histopathology of a skin biopsy reminiscent of the features seen in mice lacking β-catenin epidermal expression, suggesting a role for β-catenin in skin involvement of patients affected with ANE syndrome (1). In the current study, we examined the biochemical and dynamic hormonal tests in these five affected siblings with variable pituitary hormone deficiencies. We have delineated the pathophysiology of ANE syndrome’s endocrine manifestations and propose a novel etiology for hypopituitarism resulting from interruption of the Wnt/β-catenin pathway.

Patients and methods

Patients

Five affected male siblings of Arab–Muslim descent from a consanguineous family aged 20–39 years and presenting with ANE syndrome were enrolled in the
study. They were previously reported to carry the p.L351P mutation in RBM28. The pedigree and detailed clinical phenotype have been reported previously (1). The present study was approved by the Ha’Emek Medical Center ethics committee.

All patients were clinically examined by the same endocrinologist (Y T-R). Patients’ height, Tanner pubertal stages, penile length, and testicular volume were recorded.

**Hormonal assays**

Basal levels of TSH, free thyroxine (FT$_4$), free triiodothyronine (FT$_3$), insulin-like growth factor 1 (IGF1), GH, ACTH, prolactin (PRL), LH, FSH, and testosterone were measured at fasting at 0800 h. GH excursion was evaluated by clonidine provocative test (samples were taken at intervals of 0, 30, 60, 90, and 120 min). The hypothalamic–pituitary–gonadal axis was studied by GnRH test. LH and FSH levels at basal, 30, 60, 90, and 120 min were measured following i.v. injection of 100 mg GnRH. Hypothalamic–pituitary–adrenal axis was evaluated by ACTH stimulation test (cortisol was measured at 0 and 60 min following i.v. injection of 0.25 mg synacthen). Thyroid function was assessed by sequential sampling of serum TSH at basal, 30, 60, 90, and 120 min following i.v. injection of 100 µg TRH. GH and IGF1, ACTH, and cortisol were measured by Immulite 2000 DPC (Los Angeles, CA, USA). TSH, FT$_4$, FT$_3$, testosterone, PRL, LH, and FSH serum levels were measured by direct automated chemiluminescence assays using an ADVIA Centaur (Bayer Corporation).

**Results**

Clinical features and hormonal studies are summarized in Table 1. All patients were short compared to target height (mean $-2.2\pm1.5$, range $-0.86$ to $-4.0$ s.d., target height 179.5 cm (0.72 s.d)). Bone age assessments confirmed that they completed their growth. They had very low serum IGF1 levels (mean $4.5\pm1.6$, range $3.3–7.2$ nmol/l) and lacked a GH response to provocative test, consistent with the diagnosis of GH deficiency (GHD; Table 1). All five male patients displayed absent or markedly delayed pubertal development. All patients except patient 5 had variable degrees of bilateral gynecomastia. Hormonal studies revealed very low basal levels of serum testosterone and gonadotropins combined with either blunted or no increase in LH and FSH response to GnRH stimulation, consistent with hypogonadotropic hypogonadism.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Mean ± s.d. (range)</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>5M</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39</td>
<td>36</td>
<td>30</td>
<td>27</td>
<td>20</td>
<td>30.4 ± 7.5 (20–39)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>150</td>
<td>166</td>
<td>167</td>
<td>169</td>
<td>148</td>
<td>160 ± 10 (148–169)</td>
<td>Target height, 179.5</td>
</tr>
<tr>
<td>Height (s.d.)</td>
<td>–3.7</td>
<td>–1.3</td>
<td>–1.16</td>
<td>–0.86</td>
<td>–4.0</td>
<td>–2.2 ± 1.5 (–4.0 to –0.86)</td>
<td>0.72</td>
</tr>
<tr>
<td>Tanner stage (T,P,Pu)</td>
<td>1.1,1</td>
<td>1.3,3</td>
<td>1.1,1</td>
<td>1.3,2</td>
<td>1.1,1</td>
<td>13.8 ± 3.6 (10.7–19.3)</td>
<td>Normal puberty</td>
</tr>
<tr>
<td>Penile length (cm)</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>9.5</td>
<td>4</td>
<td>15–20</td>
<td></td>
</tr>
<tr>
<td>Testicular volume (ml)</td>
<td>2.5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>&gt;26</td>
<td></td>
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<tr>
<td>Prolactin (mU/l)</td>
<td>48.0</td>
<td>380.9</td>
<td>194.6</td>
<td>107.6</td>
<td>108.4</td>
<td>168 ± 130 (48–380)</td>
<td>58–489</td>
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<tr>
<td>Testosterone (nmol/l)</td>
<td>0.7</td>
<td>4.2</td>
<td>0.8</td>
<td>4.2</td>
<td>0.7</td>
<td>21.9 ± 1.9 (0.7–4.2)</td>
<td>8.3–28.6</td>
</tr>
<tr>
<td>IGF1 (nmol/l)</td>
<td>4.89</td>
<td>3.67</td>
<td>3.25</td>
<td>3.63</td>
<td>7.19</td>
<td>4.5 ± 1.6 (3.3–7.2)</td>
<td>15.5–62</td>
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<tr>
<td>FT$_4$ (pmol/l)</td>
<td>16.7</td>
<td>13.1</td>
<td>8.5</td>
<td>14.8</td>
<td>15.4</td>
<td>13.7 ± 3.2 (8.5–16.7)</td>
<td>9.9–22.8</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>13.3</td>
<td>10.8</td>
<td>10.7</td>
<td>15.1</td>
<td>19.3</td>
<td>13.8 ± 3.6 (10.7–19.3)</td>
<td>0–46</td>
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<td>Clonidine test</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal GH (mIU/l)</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>0–13</td>
<td></td>
</tr>
<tr>
<td>Peak GH (mIU/l)</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.56</td>
<td>&gt;26</td>
<td></td>
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<tr>
<td>ACTH test</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal cortisol (mmol/l)</td>
<td>249</td>
<td>420</td>
<td>39</td>
<td>54</td>
<td>97</td>
<td>172 ± 162 (39–420)</td>
<td>&gt;276</td>
</tr>
<tr>
<td>Peak cortisol (mmol/l)</td>
<td>497</td>
<td>662</td>
<td>186</td>
<td>262</td>
<td>409</td>
<td>303 ± 189 (186–662)</td>
<td>&gt;552</td>
</tr>
<tr>
<td>TRH test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Basal TSH (mIU/ml)</td>
<td>4.0</td>
<td>3.1</td>
<td>9.5</td>
<td>7.5</td>
<td>5.0</td>
<td>5.8 ± 2.6 (3.1–9.5)</td>
<td>0.35–5.4</td>
</tr>
<tr>
<td>Peak TSH (mIU/ml)</td>
<td>11.0</td>
<td>12.8</td>
<td>28.3</td>
<td>28.8</td>
<td>16.0</td>
<td>19.4 ± 8.6 (11–28.8)</td>
<td>5–20</td>
</tr>
<tr>
<td>GnRH test</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Basal LH (mIU/ml)</td>
<td>0.5</td>
<td>1.7</td>
<td>1.8</td>
<td>1.8</td>
<td>0.5</td>
<td>1.3 ± 0.7 (0.5–1.8)</td>
<td>1.1–7.4$^a$</td>
</tr>
<tr>
<td>Peak LH (mIU/ml)</td>
<td>1.2</td>
<td>3.8</td>
<td>3.1</td>
<td>5.8</td>
<td>0.7</td>
<td>1.9 ± 2.0 (0.3–5.8)</td>
<td>10.4–34.4$^a$</td>
</tr>
<tr>
<td>Basal FSH (mIU/ml)</td>
<td>0.81</td>
<td>5.0</td>
<td>2.24</td>
<td>1.17</td>
<td>0.30</td>
<td>1.9 ± 1.9 (0.3–5.0)</td>
<td>0.3–4.8$^a$</td>
</tr>
<tr>
<td>Peak FSH (mIU/ml)</td>
<td>1.3</td>
<td>5.8</td>
<td>3.0</td>
<td>2.73</td>
<td>0.3</td>
<td>2.6 ± 2.1 (0.3–5.8)</td>
<td>12.2–19.9$^a$</td>
</tr>
</tbody>
</table>

M, male; T, testes; P, penis; Pu, pubic hair.

$^a$Adult male.

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ACTH stimulation tests yielded variable responses ranging from a normal increase in serum cortisol (case 2), a partial response (cases 1 and 5), and a blunted response (cases 3 and 4). Basal ACTH levels were not elevated, indicating central adrenal insufficiency. Repeated ACTH stimulating tests over 1 year in patients 3 and 4 revealed decreased peak cortisol from 453 to 185 and from 323 to 235 mmol/l respectively, thus confirming a progressive course of adrenal impairment. Patient 3 was admitted due to clinical signs of adrenal insufficiency. Replacement therapy with oral hydrocortisone was initiated in patients 1, 3, 4, and 5 with clinical improvement.

Basal TSH levels were moderately elevated in patients 3 and 4. Normal FT4 serum levels were shown in all subjects apart from patient 3, who had low FT4 concentrations. TRH stimulation study yielded an inappropriate TSH response, with delayed peak response (case 4) and TSH levels remaining abnormally elevated at 90 and 120 min. Mean PRL serum concentrations were low, but only below the normal male range in case 1. Brain magnetic resonance imaging of patient 5 demonstrated a severely hypoplastic thin anterior pituitary gland at the bottom of the sella turcica and an ectopic posterior pituitary located at the proximal level of the pituitary stalk. This finding was previously reported (1).

**Discussion**

We report on five male siblings with ANE syndrome caused by a p.L351P mutation in the RBM28 gene who displayed endocrine features consistent with combined anterior pituitary hormone deficiency (CPHD). Thus, ANE syndrome should be considered in the differential diagnosis of CPHD.

CPHD refers to a rare heterogeneous group of conditions, in which there is a true deficiency of at least two anterior pituitary hormone axes. It may have a purely pituitary phenotype or may be associated with extra pituitary features (syndromic CPHD) (3). Mutations in at least ten genes encoding both signaling molecules and transcription factors have been implicated in various forms of inherited hypopituitarism. These genes are HESX1, PROP1, POU1F1, LHX3, LHX4, TBX19, SOX2, SOX3, TBCE, and OTX2 (4–6). Non-syndromic CPHD has been found to be caused by mutations in PROP1 and POU1F1 (7–9), while mutations in the other genes typically cause a syndromic subtype of CPHD, featuring septo-optic dysplasia (HESX1) (10), short stiff neck (LHX3) (11), cerebellar anomalies (LHX4) (12, 13), mental retardation (SOX3) (14), anophthalmia, esophageal atresia, and genital anomalies (SOX2) (15, 16), hypoparathyroidism-retardation and dysmorphism (TBCE) (5), and microphthalmia and anophthalmia (OTX2) (6). Associated neuroradiological findings, including anterior pituitary hypoplasia, absent infundibulum, and an ectopic posterior pituitary, may present in 50% of patients with CPHD (4).

The spectrum of pituitary hormone deficiencies may vary in ANE syndrome. It encompasses complete GH and gonadotropin deficiency, variable degrees of ACTH deficiency, and mild TSH and PRL abnormalities. Unfortunately, we were unable to trace any previous records documenting anterior pituitary function before the age of 20 years in any of our patients. Nevertheless, we can assume that GHD had a juvenile onset with a progressive course, allowing three of our five patients to achieve a final height within the low range of the normal spectrum, albeit significantly below their target height. Testosterone deficiency as seen in our patients may result in low GH peak following provocative tests. Yet, the complete deficiency of GH is in favor of primary pituitary cause, rather than a secondary effect of testosterone shortage.

Hyponadotropic hypogonadism in ANE syndrome appears to present before or in the second decade, resulting in absent puberty. ACTH insufficiency is assumed to evolve during the second or even third decade of life, resulting in slowly progressive, mildly symptomatic adrenal insufficiency. The development of adrenal insufficiency of central origin, clearly seen in repeated ACTH stimulation tests in the same subject over time, supports the need for long-term monitoring for evolving pituitary hormone deficiencies to prevent clinical adrenal insufficiency.

TSH dysfunction is reflected by mildly elevated basal TSH and sustained TSH response to TRH stimulation, indicating hypothalamic hypothyroidism. Replacement therapy with l-T4 was indicated in only one patient. The RBM28 protein has recently been shown to be a component of the mammalian spliceosomal small nuclear ribonucleoproteins (2), and to play a major role in ribosome assembly (1). It suggests that deficiency of this protein may have pleiotropic effects, as seen in ANE syndrome. Moreover, abnormal ribosome assembly and/or defective global splicing alterations may result in variable expression of the disease phenotypes, as exemplified by the cutaneous (1) and endocrine features observed in the kindred reported in this study.

Meticulous examination of skin biopsies from our patients revealed the absence of mature follicles and the presence of dermal cysts (1) reminiscent of the features seen in humans and mice affected by a large spectrum of genetic defects, all known or suspected to result in defective stabilization of epidermal β-catenin expression (17, 18). Accordingly, β-catenin expression is defective in the skin of ANE syndrome patients (1). β-catenin is a key protein of the Wnt signaling pathway which plays a pivotal role in gene expression, cell adhesion, and tissue development (19). Activation of the Wnt pathway results in cytosolic β-catenin accumulation and its translocation into the nucleus, where it engages in gene activation and the cell cycle (20). Interestingly, recent data have established a major role for the Wnt/β-catenin signaling pathway in regulating pituitary development and growth (21). These studies
demonstrated that β-catenin plays an essential role in transducing the GnRH signal by interacting with multiple DNA-binding proteins in gonadotropes (22–24). In addition, in a mouse model, β-catenin acts as a cofactor for a number of pituitary transcription factors that control pituitary cell specification and exert both stimulatory (prop1) in regulating expression of pit-1 and inhibitory (Hexs1) effects (21, 25, 26).

In summary, we show that the endocrinopathy in ANE syndrome is characterized by variable anterior pituitary hormone deficiencies, which evolve over the years resulting in syndromic CPHD. We propose that dysfunction of the Wnt/β-catenin signaling pathway may underlie the pituitary hormone deficiencies reported in the present study in ANE syndrome.

**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.

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**References**


