Haploinsufficiency of NKX2-1 in Brain-Lung-Thyroid Syndrome with Additional Multiple Pituitary Dysfunction

Rathi Prasad a Adeline K. Nicholas b Nadia Schoenmakers b John Barton c

a Department of Paediatric Endocrinology, Royal London Hospital, Barts Health NHS Trust, London, UK; b University of Cambridge Metabolic Research Laboratories, Wellcome Trust-Medical Research Council Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK; c Department of Paediatric Endocrinology, Bristol Royal Hospital for Children, University Hospitals Bristol NHS Foundation Trust, Bristol, UK

Established Facts

- Heterozygous point mutations or haploinsufficiency of the transcription factor NKX2-1 is associated with brain-lung-thyroid syndrome.
- Mice homozygous for disruption of Nkx2-1 have absent lung parenchyma, absent thyroid glands, and severe defects of the brain including the ventral forebrain and pituitary.

Novel Insights

- Whilst this case is unique to date, haploinsufficiency of NKX2-1 in humans can be associated with multiple pituitary hormone defects.
- Patients with brain-lung-thyroid syndrome may warrant screening to exclude pituitary pathology at diagnosis or evolving pituitary disease.

Keywords
NKX2-1 · Brain-lung-thyroid syndrome · Hypopituitarism · Congenital hypothyroidism

Abstract
Introduction: Heterozygous mutations or haploinsufficiency of NKX2-1 are associated with the brain-lung-thyroid syndrome incorporating primary hypothyroidism, respiratory distress, and neurological disturbances. Case Presentation: We report a patient presenting in the neonatal period with multiple pituitary hormone deficiency including central hypothyroidism and hypoadrenalism, growth hormone deficiency, undetectable gonadotrophins, and a small anterior pituitary on MRI. CGH microarray revealed haploinsufficiency for NKX2.1 and during subsequent follow-up, she has exhibited the classic triad of brain-lung-thyroid syndrome with undetectable tissue on thyroid ultrasonography. Whilst the role of NKX2-1 is well described in murine pituitary development, this report constitutes the first description of multiple pituitary dysfunction in humans associated with the syndrome and haploinsufficiency NKX2-1. Conclusion: The re-
port highlights a potential need for pituitary screening in patients with established brain-lung-thyroid syndrome and implicates NKX2.1 in human pituitary disease.

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Introduction

Brain-lung-thyroid syndrome describes the triad of primary hypothyroidism, respiratory distress, and neurological impairment associated with heterozygous point mutations or haploinsufficiency of the transcription factor NKX2-1. Penetration of each of the clinical features is variable, and phenotypic heterogeneity of mutations or haploinsufficiency of NKX2-1 is evident (summarised in [1]).

NKX2-1, alternatively known as TITF-1, TTF-1, or T/ebp, encodes a homeodomain containing transcription factor involved in organogenesis and differentiation of the thyroid, lung, and ventral forebrain regions (most particularly the basal ganglia and hypothalamus) [2]. Nkx2-1 expression is noted early in normally developing thyroid gland, lung bronchial epithelium, and specific areas of the forebrain. Mice homozygous for disruption in Nkx2-1 are stillborn with absent lung parenchyma, absent thyroid glands (but normal parathyroids), and severe defects of the brain, particularly the ventral forebrain. NKX2-1 has two transcription activation domains with reported functional redundancy [3].

Conditional knockout of Nkx2-1 in mice leads to impaired thyroid folliculogenesis [4]. Within the thyroid, NKX2-1 is a transcription factor for the genes encoding the TSH receptor, thyroid peroxidase, thyroglobulin, and pendrin essential for thyroid hormone biosynthesis [5–8]. Thus, NKX2-1 plays a role in organogenesis and function of the thyroid gland.

The phenotype of mice with homozygous disruption of Nkx2-1 also implicates the transcription factor in branching of lobar bronchi and thereafter in the regulation of lung-specific genes including surfactant protein genes, and those encoding Clara cell secretory protein and adenosine triphosphate-binding cassette transporter 3 [2, 9].

In all published reports in humans, the hypothyroidism, present in half of patients, is primary in nature ranging between subclinical hypothyroidism, with mild elevation in TSH, to more profound disease associated with atrophy. Respiratory disease, the least prevalent feature, can manifest as neonatal respiratory distress with an increased frequency of pulmonary infections in the first few years of life and carries an associated mortality. The movement disorder predominantly involves choreoathetosis and is found in the majority of patients, with age of onset generally before 5 years of age [10, 11]. This is often preceded by a history of hypotonia and delay in acquisition of motor milestones. Progression of the neurological symptoms in adulthood is rare, with some patients even showing improvement [12]. Speech and intellect is usually reported to be unaffected.

Case Report

Our patient, a female infant, the first baby born to non-consanguineous, White/Black Caribbean parents was identified to have profound hypopituitarism in the early neonatal period in addition to undetectable tissue on thyroid ultrasonography (see Table 1 for clinical chronology). She was born in poor condition with significant respiratory distress requiring resuscitation and ventilation. She had pulmonary hypertension requiring nitrous oxide and ionotropic support, including hydrocortisone in the first week of life. She was also diagnosed with a patent ductus arteriosus, which was conservatively managed. On initial weaning off hydrocortisone, she was hypotensive and her serum cortisol was found to be undetectable (<20 nmol/L). She additionally had profound central hypothyroidism with serum TSH of 0.49 mU/L (NR 0.27–4.2), FT4 of 0.7 pmol/L (NR 12.0–22.0), requiring thyroxine replacement. Her gonadotrophins at day 16 of life were undetectable. Her neonatal period was further complicated by episodes of hypoglycaemia with high glucose requirements up to 16.5 mg/kg/min, this despite being on stress doses of hydrocortisone. She had transient hyperinsulinism (requiring a brief period of treatment with diazoxide and chlorothiazide). She was found to be growth hormone (GH) deficient, GH of <0.05 mcg/L during a hypoglycaemia screen (corresponding blood glucose 2.3 mmol/L). Hypoglycaemia resolved on initiation of GH treatment and on continuing hydrocortisone replacement. Pituitary dysfunction to date appears to be isolated to the anterior pituitary with no evidence of diabetes insipidus. MRI of the brain at the age of 16 weeks revealed a very small anterior pituitary with a normal stalk and the posterior pituitary was seen in the sella (Fig. 1). Her ophthalmology review in infancy was normal with no clinical evidence of optic nerve hypoplasia.

During follow-up, she has demonstrated significant muscular hypotonia associated with delayed gross motor development (walking independently just prior to her second birthday) and easy fatigability. Her gait remains unsteady with frequent falls particularly during intercurrent infections. Her language and fine motor development has been age-appropriate. She has not yet developed any involuntary movements. She has suffered recurrent upper respiratory infections with a persistent mucopurulent nasal discharge and chronic secretory otitis media for which she required bilateral grommet insertion (see Table 1 for clinical chronology).

Genetic analysis included a CGH microarray which revealed 2 de novo deletions, a 4.9-Mb deletion from the long arm of chromosome 14 (q13.2-q21.1 [35, 975, 495–40, 890, 854]) and a 404-kb deletion from the short arm of chromosome 3 (p12.3-p13 [73, 975, 40, 890, 854]).

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The former deletion contains 21 HGNC (HUGO Gene Nomenclature Committee) curated genes, 4 of which have OMIM Morbid entries. These include NKX2-1 associated with choreoathetosis, primary hypothyroidism, and neonatal respiratory distress. In view of this finding, our patient went on to have thyroid ultrasonography which failed to detect any tissue. The remaining three genes, mutations of which are associated with specific clinical features, included PAX9 (autosomal oligodontia), SEC23A (cranio lenticosutural dysplasia), and MIPOL (mirror image polydactyly); none of which are in keeping with our patient’s phenotype. The latter deletion on the short arm of chromosome 3 is of unknown significance. Direct Sanger sequencing of NKX2-1 reveals no coding region mutations on the other allele.

### Table 1. Chronology of clinical findings/diagnoses and treatment of our patient, together with relevant biochemical and radiological investigations

<table>
<thead>
<tr>
<th>Patient age</th>
<th>Biochemical results</th>
<th>Radiological results</th>
<th>Clinical findings/diagnoses</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>Cortisol &lt;20 nmol/L</td>
<td>Diffuse granular shadowing in both lungs consistent with respiratory distress syndrome</td>
<td>Neonatal respiratory distress Persistent pulmonary hypertension of newborn</td>
<td>Ventilatory support: IPPV until D7 initially with inhaled nitric oxide and inotropic support, CPAP until D15, high flow oxygen until D25</td>
</tr>
<tr>
<td>D11</td>
<td>TSH 0.49 mU/L (NR 0.27–4.2), FT4 of 0.7 pmol/L (NR 12.0–22.0)</td>
<td>Cortisol deficiency, presenting with hypotension</td>
<td>Hydrocortisone replacement</td>
<td></td>
</tr>
<tr>
<td>D12</td>
<td>LH &lt;0.5 U/L, FSH &lt;0.5 U/L</td>
<td>Central hypothyroidism</td>
<td>Thyroxine replacement</td>
<td></td>
</tr>
<tr>
<td>D15–D29</td>
<td>Right upper zone lung changes</td>
<td>Undetectable gonadotrophins</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>D12</td>
<td>Insulin 10 mU/L with hypoglycaemia*</td>
<td>Transient hyperinsulinism</td>
<td>Diazoxide and chlorothiazide treatment started, discontinued by D32</td>
<td></td>
</tr>
<tr>
<td>D12</td>
<td>GH &lt;0.05 mcg/L with hypoglycaemia*</td>
<td>Growth hormone deficiency</td>
<td>Growth hormone replacement</td>
<td></td>
</tr>
<tr>
<td>D115</td>
<td>MRI pituitary: small anterior pituitary, with normal stalk and posterior pituitary seen in the sella</td>
<td>Findings in keeping with anterior pituitary hormone deficiencies seen biochemically</td>
<td>Ongoing thyroxine replacement</td>
<td></td>
</tr>
</tbody>
</table>

Clinical features in italics are those that correspond to the classically described features of brain-lung-thyroid syndrome. IPPV, intermittent positive pressure ventilation; CPAP, continuous positive airway pressure; TSH, thyroid-stimulating hormone; FT4, thyroxine; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone. * Hypoglycaemia with blood glucose of 2.3 mmol/L.

### Discussion

Our patient’s phenotype is in keeping with brain-lung-thyroid syndrome associated either with haploinsufficiency of NKX2-1, as in our patient’s case, or as heterozygous point mutations in NKX2-1, first described in 5 individuals with the clinical triad in 2002 [13].

Currently aged 4 years, our patient has or has had evidence of the triad associated with this syndrome with the additional finding of pituitary dysfunction. Hypopituitarism is not a recognized feature of the condition, and multiple pituitary hormone deficits have not been described in the context of NKX2-1 deficiency. However, a father and daughter were recently reported who harboured a
heterozygous *NKX2-1* nonsense mutation in association with empty sella and either hypogonadotrophic hypogonadism or GH deficiency [14]. MRI findings of cystic pituitary masses or appearances of empty sella turcica are described in 6 previously published cases associated variably with either point mutations or chromosomal deletions incorporating *NKX2-1* [13, 15–17]. In these cases, however, pituitary function is reportedly normal. A more recent case series of 25 patients with NKX2-1 deficiency reported the additional presence of hypothalamic symptoms in some of their patients including temperature dysregulation and dysrhythmic sleep [1]. The authors also noted that the affected patients had a tendency to lower height SDS and propose potential disruption of the hypothalamic-pituitary-growth axis, though this was not formally evaluated.

There are no other known genes within our patient’s deleted chromosomal regions that are associated with a pituitary phenotype. The pituitary gland is derived from an evagination of the ventral diencephalon, forming the infundibulum and posterior lobe of the pituitary, with an invagination of the oral ectoderm which forms Rathke’s pouch, later the anterior pituitary. In rodent embryos, *Nkx2-1* is expressed in the developing ventral diencephalon, but it is not present in Rathke’s pouch, the precursor to the anterior pituitary [18]. However, in the absence of *Nkx2-1*, both structures are absent indicating the transcription factor in the development of the entire pituitary [2]. Further study highlights the particular importance of activation domain 1 of NKX2-1 for complete pituitary development [19]. Point mutations in NKX2-1 are not routinely sought in patients with hypopituitarism.

Patterning of the ventral forebrain impacts generation of the organising centre of the pituitary which is characterised by expression of bone morphogenetic protein 4 (*Bmp4*), fibroblast growth factor 8 (*Fgf8*), and *Fgf10* [20]. This “pituitary organiser” is in turn necessary for generation of Rathke’s pouch. Hetero/homozygous mutations or chromosomal deletions of *FGF8* are described variably with hypogonadotrophic hypogonadism, holoprosencephaly, septo-optic dysplasia, and Moebius syndrome [21, 22]. Interestingly, in the diencephalon of *Nkx2-1* null mouse mutant embryos, *Bmp4* expression is maintained however *Fgf8* expression is not detectable, suggesting that the loss of Rathke’s pouch through apoptosis in the mutants is a consequence of reduced *Fgf8* expression in the pituitary organiser [23].

Considering the hypothalamo-pituitary findings in the mouse models, it is therefore possible that haploinsufficiency of *NKX2-1* contributes to our patient’s additional neuroendocrine dysfunction by similar mechanisms. This phenotype of multiple pituitary deficit is unique to our patient in the literature and certainly suggests that further genetic mutations or gene haploinsufficiency are involved. Direct Sanger sequencing of *NKX2-1* reveals no coding region mutations on the other allele. This is not entirely surprising as one would expect this to be lethal in view of the early lethality seen in mouse models homozygous for disrupted *Nkx2-1*. Findings in our patient do however raise the question as to whether pituitary function should be routinely screened in patients with heterozygous point mutations or haploinsufficiency of *NKX2-1*, to exclude pathology at diagnosis or even evolving disease during follow-up.

**Conclusion**

We describe, for the first time, haploinsufficiency of *NKX2-1* associated with brain-lung-thyroid syndrome in a patient with additional, multiple pituitary hormone deficits. This further expands the phenotypic heterogeneity of the syndrome and, in view of the important role of the transcription factor in murine hypothalamo-pituitary development, implicates NKX2-1 in the aetiology of pituitary disease in humans.
Statement of Ethics

Genetic studies were undertaken with consent of the patient’s parents and the genetic study protocol was approved by Cambridge South REC (MREC 98/5/24). The patient’s parents have given their written informed consent to publish their case (including publication of images).

Disclosure Statement

The authors have no conflicts of interest to declare.

References


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Author Contributions

R.P. and J.S. collated patient information. A.K.N. and N.S. conducted sanger sequencing of NKX2.1. All authors were involved in preparing the manuscript.