## Manuscript Information

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Case Report

Haploinsufficiency of NKX2-1 in brain-lung-thyroid syndrome with additional multiple pituitary dysfunction

Rathi Prasad¹, Adeline K Nicholas², Nadia Schoenmakers², John Barton³

1. Department of Paediatric Endocrinology, Royal London Hospital, Barts Health NHS Trust, Whitechapel Road, London, E1 1BB
2. University of Cambridge Metabolic Research Laboratories, Level 4, Wellcome Trust-Medical Research Council Institute of Metabolic Science, Level 4, Box289, Addenbrooke’s Hospital, Cambridge, CB2 0QQ
3. Department of Paediatric Endocrinology, Bristol Royal Hospital for Children, University Hospitals Bristol NHS Foundation Trust, Upper Maudlin St, Bristol BS2 8BJ

Abbreviated title: NKX2-1 haploinsufficiency and pituitary dysfunction

Dr Rathi Prasad

Consultant in Paediatric Endocrinology,

Department of Paediatric Endocrinology, Royal London Hospital, Barts Health NHS Trust, Whitechapel Road, London E1 1BB

Tel: 02035946132

Email: Rathi.Prasad@nhs.net

Key words: NKX2-1, brain-lung-thyroid syndrome, hypopituitarism, congenital hypothyroidism
Established Facts and Novel Insights

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
Abstract

Introduction: Heterozygous mutations or haploinsufficiency of \textit{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 \textit{NKX2.1} and during subsequent follow she has exhibited the classic triad of brain-lung-thyroid syndrome \textbf{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 \textit{NKX2-1}.

Conclusion: The report highlights a potential need for pituitary screening in patients with established brain-lung-thyroid syndrome and implicates \textit{NKX2.1} in human pituitary disease.
**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*. Penetrance 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 athyreosis. 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 D16 of life were undetectable. Her neonatal period was further complicated by episodes of hypoglycaemia with high glucose requirements up to 16.5 mcg/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. Her MRI brain aged 16 weeks
revealed a very small anterior pituitary with a normal stalk and the posterior pituitary was seen in the sella (Figure 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 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[73,893,402-74,297,269]). 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 3 genes, mutations of which are associated with a specific clinical features, included PAX9 (autosomal oligodontia), SEC23A (craniolenticosutural 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.
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 four 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 growth hormone 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 implicating 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
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 NXX2-1 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 NXX2-1 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 NXX2-1, to exclude pathology at diagnosis or even evolving disease during follow up.

Conclusion: We describe, for the first time, haploinsufficiency of NXX2-1 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 NKK2-1 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.

Funding Sources

Wellcome Trust Grant 100585/Z/12/Z (NS) for the study of genetic causes of congenital

hypothyroidism

Author Contributions

RP and JS collated patient information. AN and NS conducted sanger sequencing of NKK2.1. All
authors were involved in preparing the manuscript.
References


Figure Legends

Fig. 1. MRI brain of our patient (T1 weighted images), aged 16 weeks, demonstrates a very small anterior pituitary with a normal stalk and posterior pituitary seen in the sella and an otherwise structurally normal brain.
Figure 1: MRI brain of our patient (T1 weighted images), aged 16 weeks, demonstrates a very small anterior pituitary with a normal stalk and posterior pituitary seen in the sella and an otherwise structurally normal brain.
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<tr>
<th>Patient age</th>
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<th>Radiological results</th>
<th>Clinical findings/ diagnoses</th>
<th>Treatment</th>
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<tr>
<td>D3</td>
<td></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>
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<td>D11</td>
<td>Cortisol &lt; 20 nmol/L</td>
<td></td>
<td>Cortisol deficiency, presenting with hypotension</td>
<td>Hydrocortisone replacement</td>
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<td>D12</td>
<td>TSH 0.49 μu/L (NR 0.27-4.2), FT4 of 0.7 pmol/L (NR 12.0-22.0)</td>
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<td>Central hypothyroidism</td>
<td>Thyroxine replacement</td>
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<tr>
<td>D12</td>
<td>LH&lt;0.5 U/L, FSH &lt; 0.5U/L</td>
<td></td>
<td>Undetectable gonadotrophins</td>
<td>n/a</td>
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<td>D15-D29</td>
<td>Right upper zone lung changes</td>
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<td>Pulmonary infection</td>
<td>Intravenous antibiotics</td>
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<td>Date</td>
<td>Observations</td>
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<td>D18</td>
<td>Insulin 10 mu/L with hypoglycaemia*</td>
<td>Transient Hyperinsulinism</td>
<td>Diazoxide and chlorothiazide treatment started, discontinued by D32</td>
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<td>D18</td>
<td>GH &lt;0.05 mcg/L with hypoglycaemia*</td>
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<td>Growth hormone replacement</td>
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<td>D112</td>
<td>MRI pituitary: small anterior pituitary, with normal stalk and posterior pituitary seen in the sella</td>
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<td>Findings in keeping with anterior pituitary hormone deficiencies seen biochemically</td>
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<td>D115</td>
<td>No thyroid tissue on ultrasonography</td>
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<td>Ongoing thyroxine replacement</td>
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<td>10 months to date (4yr)</td>
<td>Muscle hypotonia and delayed gross motor development</td>
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<td>Input from child development team including regular physiotherapy</td>
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<td>Infancy to date (4 yr)</td>
<td>Recurrent respiratory infections and secretory otitis media</td>
<td>Antibiotics</td>
<td>Bilateral grommets</td>
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Table 1. Chronology of clinical findings/diagnoses and treatment of our patient, together with relevant biochemical and radiological investigations.

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.