Novel p.Asn628Ser heterozygous mutation in FGFR1 is associated with Hartsfield syndrome and tumoral calcinosis

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Introduction
Hartsfield syndrome (#OMIM 615465) describes the rare co-occurrence of holoprosencephaly (failure of complete separation of the 2 cerebral hemispheres) with ectodactyly, associated with a spectrum of developmental defects including specific pituitary dysfunction.

Novel homozygous and heterozygous mutations in FGFR1 have recently been associated with the condition in six patients (1). Loss-of-function FGFR1 mutations have also been associated with a spectrum of clinical phenotypes including Kalman syndrome, in which gonadotrophin deficiency is also a feature (2). Fibroblast growth factor signalling plays an important role in embryonic development with downstream effects on cell motility, cell survival and cell mitosis. Downstream signalling cascades are FGF-, FGFR- and cell-specific.

Case Report
Holoprosencephaly, Ectodactyly, Pituitary Dysfunction

Our patient, a male infant, born to non-consanguineous Caucasian parents, has several congenital abnormalities including bilateral cleft lip and palate, right sided microtia, bilateral ectodactyly of the hands and feet and semilobar holoprosencephaly (Fig 1). At the age of 5 weeks he was diagnosed with cranial diabetes insipidus and gonadotrophin deficiency.

Tumoral Calcification
Aged 16 months our patient presented with a lesion in the posterior first rib. Histology from the biopsy taken was in keeping with tumoral calcification.

Methods
Direct Sanger sequencing of FGFR1, incisional biopsy (histology consistent with tumoral calcification) and serum for bone biochemistry and C-terminal/ intact FGF23 levels was taken.

Results

c.1883A>G; p.Asn628Ser mutation in FGFR1
Direct Sanger sequencing of FGFR1 in our patient reveals a novel, de novo heterozygous missense mutation, c.1883A>G; p.Asn628Ser (Fig 2A). The p.Asn628Ser mutation affects an amino acid residue located in the ATP binding pocket of the intracellular tyrosine kinase domain, with predicted impairment of FGFR1 kinase activity. Disease-associated mutations in the kinase domain interfere with normal signalling activity in vivo in zebrafish models, with findings supporting a dominant negative mechanism (4).

Biochemistry in keeping with tumoral calcification
Biochemical findings in our patient indicate normo-calcaemia (Corrected calcium 2.44 mmol/L, normal range 2.20-2.70) and hyperphosphataemia (serum phosphate 2.10 mmol/L; normal range 0.9-1.8), with a normal 1, 25 Vitamin D level of 139 pmol/L and normal PTH (2.7 pmol/L), and low fractional excretion of phosphate (0.58%) as is typically seen in familial forms of tumoral calcification.

The circulating factor FGF23 normally promotes excretion of phosphate. C-terminal levels of FGF23 (accounting for both the bioactive intact form of FGF23 and C-terminal fragments present in the circulation) are raised in inherited forms of tumoral calcification, either as a consequence of increased proteolytic processing of FGF23 or end-organ resistance. Raised C-terminal FGF23 levels of 111 relative units/mL (reference range <100) were found in our patient, again supporting a diagnosis of tumoral calcification. Intact FGF23 was 81.7 pg/mL; whilst this is within the normal adult range no paediatric reference range exists. Biochemistry of both parents was within normal limits.

Loss of FGFR1 function potentially contributes to tumoral calcification
For the first time we report tumoral calcification in a patient with Hartsfield Syndrome. Tumoral calcification (#OMIM 211900) can be inherited in an autosomal recessive manner involving loss of function mutations in FGFR2, KLINT1 and GALNT3 (4,5,6). The circulating factor FGF23 promotes phosphate excretion. Klotho directly converts the splice variant FGFR1 (IIIc) into the FGF23-specific receptor (8) (Fig 2B), raising the possibility that an inactivating FGFR1 mutation may contribute to tumoral calcification development. Activating heterozygous missense mutations in FGFR1 are associated with osteophagocytic dysplasia (#OMIM 166250), an autosomal dominant condition where increased serum levels of FGF23 can lead to hypophosphataemia.

Studies have implicated FGFR1 activation in regulating FGF23 gene transcription which may account for the paucity of elevation of FGF23 seen despite increased renal phosphate reabsorption.

Conclusion
This novel mutation in FGFR1 provides further compelling evidence of the association of FGFR1 mutations with Hartsfield syndrome. We also expand the phenotype with the first report of tumoral calcification with mildly raised C-terminal FGF23 levels, which may reflect loss of function of FGFR1.