Novel compound heterozygous variants in the *NBAS* gene in a child with osteogenesis imperfecta and recurrent acute liver failure

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SUMMARY

Osteogenesis imperfecta (OI) consists of a group of genetically and phenotypically heterogeneous diseases characterised by bone fragility. Recent improvement in gene sequencing methods has helped us identify rare forms of OI that are inherited in an autosomal recessive manner. Paediatric endocrinology was consulted on a newborn girl with multiple fractures and wavy thin ribs noted on X-rays. In addition to the bone phenotype, she also has short stature and recurrent acute liver failure (ALF) episodes triggered by intercurrent illness. Whole exome sequencing revealed two novel compound heterozygous variants in neuroblastoma amplified sequence (*NBAS*) gene. *NBAS* gene codes for a protein that is involved in nonsense-mediated decay pathway and retrograde transport of proteins from Golgi to endoplasmic reticulum. Recognition of pathogenic variants in this gene as a rare cause of autosomal recessive OI and recurrent ALF has important therapeutic implications.

BACKGROUND

Osteogenesis imperfecta (OI) is a group of heterogeneous disease characterised by bone fragility. Studies in the USA suggest a prevalence of 0.3–0.7 per 10 000 births.[1] The incidence of OI is estimated to be around 1:15 000 in the general population. With more novel causative genes being identified, the additive incident may be higher. Various extraskeletal manifestations associated with this condition include dentinogenesis imperfecta, hearing loss, blue sclera and joint hyperflexibility.[2] Pathogenic variants in *COL1A1* and *COL1A2*, which are inherited in an autosomal dominant fashion, account for the majority of causes of OI (types 1–4). The clinical heterogeneity in presentation has been ascribed to the nature (ie, haploid insufficiency or dominant negative) and location of mutation in the collagen gene.

Bardai *et al*[3] report a study of 596 individuals who had been referred to a tertiary care centre for concerns of OI that revealed approximately 80% of the cases were due to defects in *COL1A1* or *COL1A2* genes and only a minority were due to defects in other genes. Almost 3% of patients in the study did not have an identifiable pathogenic variant in the *COL1A1*/*COL1A2* genes. Dysmorphic features or evidence of multisystem disease increase the likelihood of identifying rare genetic defects. Recent improvement in gene sequencing methods has helped us identify rare forms of OI that are inherited in an autosomal recessive manner.

We describe here a neonate who presented with multiple fractures at birth and who developed recurrent acute liver failure (ALF) later on. Chromosomal microarray and initial low bone density slice/panel were negative, but whole exome sequencing revealed a compound heterozygous variants in neuroblastoma amplified sequence (*NBAS*) gene. *NBAS* gene encodes a protein that has been implicated in nonsense-mediated decay (NMD) pathway and retrograde transport of proteins from Golgi apparatus to endoplasmic reticulum (ER).[4] Biallelic inactivating variants in *NBAS* gene have been implicated to cause three syndromes; (1) SOPH (short stature, optic atrophy, Pelger-Huet anomaly) syndrome, (2) the infantile liver failure syndrome type 2 and (3) acrofrontofacionasal dysostosis type 1.[5] Recent reports have implicated pathogenic variants in *NBAS* gene in skeletal fragility.[6] The variants identified in this child have not yet been described in literature.

Interestingly, our case did not have optic nerve hypoplasia or the classical Pelger-Huet anomaly that have been described with this condition. She responded very well to bisphosphonate therapy and has had no further fractures.

CASE PRESENTATION

Paediatric endocrinology was consulted on a newborn with multiple fractures. The female infant was born at 34 weeks of gestation by caesarean section with a birth weight of 1740 g. Her mother had premature rupture of membranes at 21 weeks of gestation, which led to oligohydramnios. Prenatal ultrasound at 28 weeks of gestation revealed short proximal bones of both upper and lower extremities. There was no known teratogen exposure, and family history was non-contributory for any genetic or metabolic bone diseases. Consanguinity was denied. The patient has an older sister who is healthy. The mother had a history of rheumatoid arthritis. After birth, the neonate was admitted to the neonatal intensive care unit for respiratory distress. She was noted to be very fussy on handling and this prompted a skeletal survey. On examination, the neonate was noted to have dysmorphic features including thin and loose skin with visible veins and decreased elasticity. She had frontal bossing, sparse hair and progeroid appearance with large ears, shallow orbits, exophthalmos, flat malar, small mouth, thin upper lip, micrognathia, blue

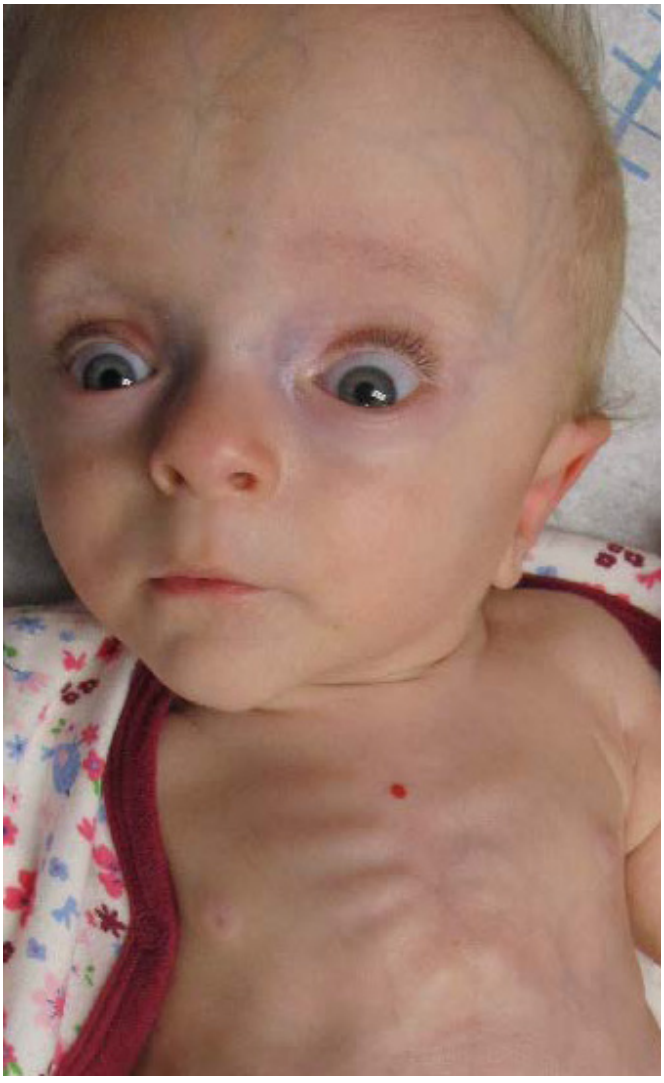

Figure 1 Facial features and pectus excavatum noted at birth.

sclera, narrow chest and pectus excavatum (figure 1). Cardiovascular, pulmonary, abdominal and genitourinary examinations were normal. Extremities examination revealed contracture of thumb bilaterally with normal appearance of palmar creases. The proximal limbs were noted to be short.

INVESTIGATIONS

Preliminary laboratory investigations (table 1) were essentially normal.

Skeletal survey revealed multiple fractures including fractures of mid-right clavicle, distal right tibia metadiaphysis and mid-left fibular diaphysis. Ribs were noted to be wavy and undulating

Table 1 Laboratory data

Laboratory test	Value	Normal range
Cortisol mcg/dL	22.1	4.5–26
IGFBP3 mg/L	2.12	1.39–4.15
PTH pg/mL	23.8	7.0–53.0
Calcium mg/dL	11.1	9.1–11.1
1,25-dihydroxyvitamin D pg/mL	51	15–90
25-hydroxyvitamin D ng/mL	32.7	30–100

PTH, Parathyroid hormone.

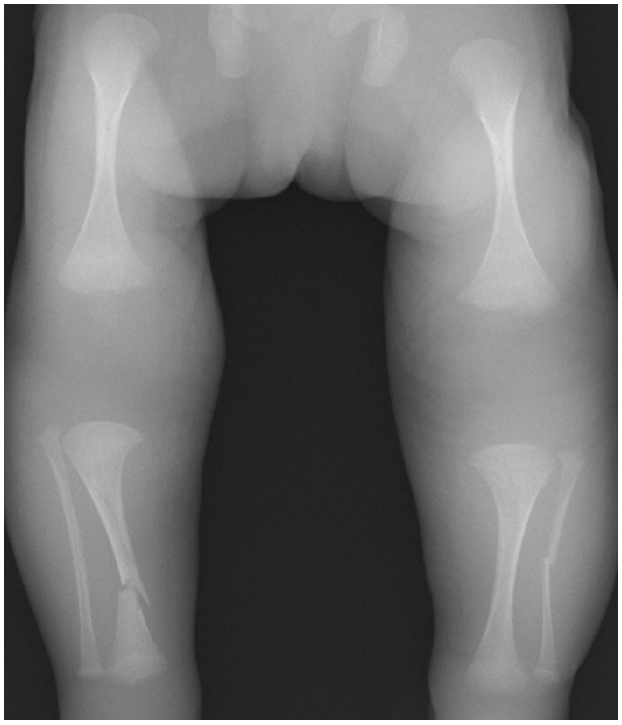

Figure 2 Skeletal survey at birth.

(figures 2 and 3). No Wormian bones were noted. CT and MRI of the brain were normal and revealed normal myelination. Audiology work up was within normal limits, and an echocardiogram did not reveal any structural heart defect. Dual energy x-ray absorptiometry (DXA) scan at 3 months of age revealed a total body less head bone mineral density of 0.246 g/cm^2 and AP spine (antero-posterior lumbar spine) bone mineral density at 26 months of age of 0.289 g/cm^2.

DIFFERENTIAL DIAGNOSIS

Given the dysmorphic features and increased skin elasticity, the differential diagnosis included Cole-Carpenter syndrome, cutis laxa, rare types of Ehlers-Danlos syndrome or geroderma osteodysplasticum. An exome slice was done to evaluate the genes *ALPL, B3ALTG, BMP1, COL1A1, AOL1A2, CREB3L1, CRTAP,*

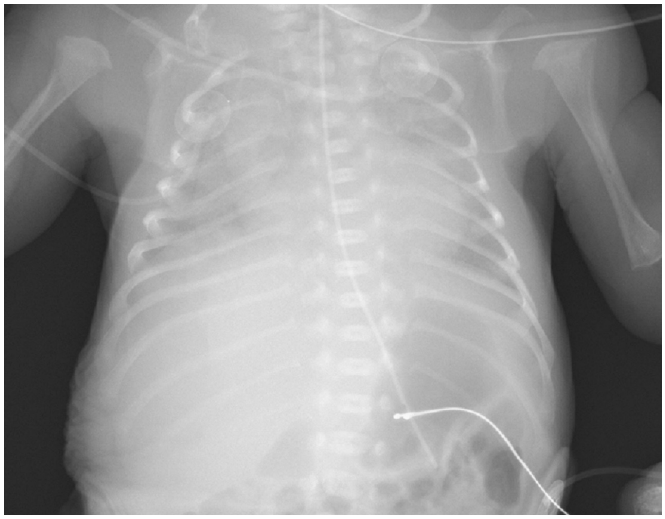

Figure 3 Chest X-ray at birth.

Table 2 Liver function tests in our patient

Age (months)	Peak SGOT U/L (17–64)	Peak SGPT U/L (12–48)	Ammonia µg/dL (34–76)	Bilirubin total mg/Dl (0.3–1.2)	GGT U/L (7–76)	INR (0.9–1.2)
5	240	275		0.2	17	1.4
17	>10 000	10 010	96	0.9	201	2.5
32	7242	9526			159	2.8

GGT, Gamma-glutamyl transferase; INR, International normalized ratio; SGOT, serumglutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

EFEMP2, FKB10, GORAB, IFITM5, P3H1, LEPRE1, P4HB, PLOD2, PP1B, PYCR1, SEC24D, SERPINF1, SERPINH1, SP7, SPARC, TMEM38B, WNT1 (GeneDx). This was negative. Whole exome sequencing revealed a compound heterozygous variant in the *NBAS* gene; (p.R517C, c.1549 C>T) inherited from father, which is considered a likely pathogenic variant and (p.E151K, c451 G>A) inherited from mother, which is reported as a variant of uncertain significance by the laboratory mainly because this is a novel finding. However, the R517C variant is a non-conservative amino acid substitution, which is likely to impact secondary protein structure and in silico analysis predicts this to be damaging to protein structure and function. The E151K variant is a non-conservative amino acid substitution, which is likely to impact protein structure and function. In silico analysis was inconsistent in its predictions. Neither of these variants have been reported previously as a pathogenic or a benign variants. She did not have skin biosy, bone biopsy or liver biopsy done for collagen analysis.

TREATMENT

She had persistent respiratory distress and poor feeding after birth. Due to history of multiple fractures, she was started on pamidronate infusion. She received her first dose as an inpatient. Laboratory work up prior to the start of infusion revealed elevated liver enzymes and mild coagulopathy (table 2), which prompted gastroenterology consult.

Her coagulopathy improved with one dose of vitamin K and after that she received her first pamidronate infusion, which she tolerated well. Pamidronate infusion dose was 4.5 mg/kg per year (Omaha protocol, written communication). Subsequent doses were given in the outpatient clinic. Marked improvement in her respiratory distress and oral intake was noted after her first pamidronate dose. Repeat skeletal survey at 10 months of age revealed healing old fractures with no new fractures. At 17 months of age, she was admitted to the intensive care with fever, respiratory distress and ALF from respiratory syncytial virus. Her liver dysfunction improved with supportive management with vitamin K. Work up for causes of ALF in an infant-like infection, vascular, drug-induced, metabolic, alpha 1 antitrypsin deficiency, autoimmune and immunological causes was essentially normal. Again at 32 months of age, she was readmitted with second episode of ALF (laboratory tests as per table 1) following 3 days of fever. She recovered completely following conservative management.

OUTCOME AND FOLLOW-UP

She is currently 4 years old and continues to be on pamidronate infusions per our protocol. No new fractures have occurred since the ones described in her newborn period. She does not have cognitive delay and at 27 months of age could speak in complete sentences.

DISCUSSION

The improved molecular technology has been identifying more and more biallelic pathogenic variants, that is, autosomal recessive forms that are associated with bone fragility. Pathogenic variants in *NBAS* gene were initially described as a cause of short stature with facial dysmorphism and Pelger-Huet anomaly (SOPH) in the isolated Yakut population in Asia.[7] Pelger-Huet anomaly refers to an abnormal nuclear shape of neutrophil granulocytes. Other features that were described in this condition include decreased skin elasticity, a progeroid face and optic atrophy. While bone fragility was not reported in the initial description of this condition, Balasubramanian *et al*[6] described two cases with compound heterozygous variants in *NBAS* gene with bone fragility. Slender bones with osteopenia were described in both those patients. Similarly, Capo-Chichi *et al*[8] described pathogenic variants in

Table 3 The varied clinical manifestations described with pathogenic variants in *NBAS* gene and the features seen in our patient

Abnormality	Case reports	Our patient
Osteoporosis	Balasubramanian *et al*[6] and Segarra *et al*[9]	Multiple fractures noted at newborn period Started on pamidronate infusions in infancy and tolerated it well with no subsequent fractures noted
Other bone anomalies like acrofrontofacionasal dysostosis type 1, skeletal dysplasia	Palagano *et al*[4] and Staufner *et al*[18]	Slender wavy ribs
Liver dysfunction	Regateiro *et al*[19] and Li *et al*[20]	Acute liver failure during illness
Immune dysfunction	Segarra *et al*[9]	None
Cardiac dysfunction	Haack *et al*[14]	None
Short stature	Maksimova *et al*[7]	At 27 months of age her weight Z-score is at −7 and height Z-score is at −3.9
Pelger-Huet anomaly	Maksimova *et al*[7]	Not seen
Metabolic abnormalities including ketotic hypoglycaemic	Balasubramanian *et al*[6] and Segarra *et al*[9]	Not seen
Eye manifestations: optic atrophy	Maksimova *et al*[7] and Park and Lee[21]	Not seen
Neurological manifestations: developmental delay, thin corpus callosum, axial hypotonia	Severe developmental delay, truncal hypotonia, distal hypertonia, microcephaly[4]	None seen

Table 4 Comparison of other reports of pathogenic variants in *NBAS* gene associated with skeletal fragility with our case

Bone phenotype	Other features	References
Neonatal fractures	Acute liver failure, dysmorphic facial features	Our case
Neonatal fractures	Developmental delay	Capo-Chichi *et al*[8] Mégarbané *et al*[22]
Neonatal fractures	Severe developmental delay, hypogammaglobulinaemia	Sunwoo *et al*[23]
Fracture in early childhood, skeletal dysplasia	Reduced natural killer cells	Staufner *et al*[18] and Segarra *et al*[9]
Fractures early childhood, low bone density (patient 1)	Hypogammaglobulinaemia, ketotic hypoglycaemic	Balasubramanian *et al*[6]
Fractures early childhood (patient 2)	Intellectual disability, optic atrophy	Balasubramanian *et al*[6]

NBAS gene in three siblings who presented with recurrent ALF and early-onset osteoporosis. Two of these siblings had neonatal fractures, while the third had severe osteoporosis with a Z-score below 7 SDS.[8] Other features reported in this condition include recurrent liver failure, immune dysfunction and optic atrophy.[9] A recent report by Carli *et al*[5] provided an excellent review of genotype and phenotype correlations in patients with *NBAS* pathogenic variants.

We have summarised the clinical features seen in our patient compared with other reports with *NBAS* pathogenic variants (table 3). Our patient was noted to have liver dysfunction during periods of viral illness but has not suffered from either frequent infections suggestive of immune dysfunction or optic atrophy so far.

The NBAS protein is implicated in the NMD pathway. This is a highly conserved pathway that leads to degradation of mRNA that harbours premature termination codons.[10] It is also involved in the Golgi to ER retrograde transport as protein encoded by this gene forms a subunit of the syntaxin 18 complex, which is involved in the Golgi–ER transport.[11] Recent reports suggest that it is involved in skeletal morphogenesis by : (1) Golgi–ER retrograde transport and thus its loss is associated with defects in protein glycosylation, in addition to (2) accumulation of truncated proteins that may interfere with cellular function.[4] Li *et al* and Staufner *et al*[12 13] recently published genotype–phenotype correlations on a large number of patients depending on which part of the NBAS protein structure was affected (β-propeller, sec39, C-terminal or the grey zone). The variant (p.R517C, c1549 C>T) inherited from father which is considered a likely pathogenic variant is in the grey area between β-propeller and sec 39, far away from the C-terminal. Since it is in the grey area, the laboratory called it non-conserved. The variant (p.E151K, c451 G>A) inherited from mother is in the β-propeller region of the gene. Based on the clinical presentation of our patient and review of literature, pathogenic variants in *NBAS* gene can present as either OI type 1 or 1V from a severity standpoints. A summary of other reports of pathogenic variants in *NBAS* gene with skeletal fragility is summarised in table 4.

Case reports from Germany included individuals with biallelic variants in NBAS leading to isolated recurrent ALF with no other extra hepatic manifestations. This pattern of ALF, onset in infancy and precipitated by febrile illness, is now termed infantile liver failure syndrome type 2.[14] Liver involvement in this condition most commonly presents as recurrent ALF triggered by febrile illnesses,[8 15 16] but there are case reports demonstrating mildly persistent liver dysfunction in the form of elevated transaminases. Similar to our case report, Cousin *et al*[17] described case series of biallelic RINT1 alterations causing multisystem disorder including recurrent ALF and skeletal abnormalities. In all published case series with pathogenic variants in infantile liver failure syndrome type 2 from *NBAS 2*, majority of them had a complete recovery of their episodic liver dysfunction with conservative management.

Learning points

We report this unique case of severe bone fragility and recurrent acute liver failure (ALF) in a child with variants in both alleles of the *NBAS* gene. This case adds to the expanding evidence of available literature regarding the role of NBAS in skeletal fragility and liver dysfunction and expands our knowledge on the pathogenic genetic changes associated with dysfunction in this protein.

- Pathogenic variants in *NBAS* gene should be considered in the aetiology for a child who presents with bone fragility and recurrent ALF.
- Patients with pathogenic variants in *NBAS* gene and bone fragility seem to respond well to antiresorptive therapy.
- ALF seen in this condition responds well to conservative therapy in the majority of cases.
- The phenotypic spectrum described with pathogenic variants in this gene is varied.

Contributors SK was involved in the initial work up, treatment and follow up of the child. She initiated the draft of the manuscript and data analysis. AR was involved in writing the manuscript, revision and editing manuscript. SP was the liver specialist taking care of this child and provided input on interpretation of data and treatment. AT is a geneticist and helped us in the interpretation of genetic test results and contributed to write the genetic aspect of the disease.

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