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Case Report

Skeletal abnormalities secondary to antenatal etidronate treatment for suspected generalised arterial calcification of infancy

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ABSTRACT

Background: Generalised arterial calcification of infancy (GACI) is a rare disorder characterised by the deposition of hydroxyapatite crystals within the vessel walls. It is associated with a high mortality rate. Bisphosphonates have been used with some success in the treatment of GACI. However, there is a paucity of data on the antenatal use of bisphosphonates for GACI. In this paper, we report development of the skeletal changes suggestive of hypophosphatasia (HPP) in an infant with GACI, whose mother was treated with etidronate during pregnancy.

Case report: A Caucasian infant boy had a suspected antenatal diagnosis of GACI based on the findings suggestive of calcification of the annulus of the tricuspid valve and wall of the right ventricular (RV) outflow tract and main pulmonary artery on foetal echocardiography and the genetic analysis which showed a pathogenic heterozygous mutation in *ABCC6*. Based on these findings, mother was started on etidronate treatment from 26 weeks of gestation. A healthy male baby was delivered at 38 weeks of gestation. Initial postnatal echocardiogram on day 1 of life was normal with good biventricular function; subtle changes suggestive of microcalcifications were detected on the CT angiography. Serum calcium, phosphate, alkaline phosphatase and renal profile were normal. Further, the serum inorganic pyrophosphate (PPi) level was significantly low. Skeletal changes suggestive of HPP were seen on the radiographs. The baby developed cardiac dysfunction on day 4 of life with evidence of ischaemic changes on electrocardiogram (ECG).

Treatment with etidronate was started in view of probable evolving coronary calcifications. Despite treatment with cardiac supportive measures and bisphosphonate, he succumbed to death in the third week of life.

Discussion: We believe, this is the first report of skeletal changes suggestive of HPP, arising secondary to antenatal etidronate (first generation bisphosphonate) used for the treatment of suspected GACI due to a heterozygous *ABCC6* mutation.

1. Introduction

Generalised arterial calcification of infancy (GACI, OMIM # 208000) is a rare autosomal recessive disorder, characterised by the deposition of hydroxyapatite crystals within the vessel wall of the medium and large arteries; leading to fibrous tissue proliferation and blood vessel stenosis (Bolster et al., 2015). It is caused by inactivating mutations in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*; OMIM# *173335) or ATP-binding cassette transmembrane transporter, subfamily C, member 6 (*ABCC6*; OMIM# *603234) genes (Nitschke et al., 2012).

GACI is associated with a high mortality rate, with approximately

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34% of the patients surviving beyond infancy (Rutsch et al., 2008). Those who survive are at risk of developing autosomal recessive hypophosphataemic rickets type 2 (in *ENPP1* mutation), hypertension and cardiovascular sequelae (Levy-Litan et al., 2010; Lorenz-Depiereux et al., 2010; Van der Sluis et al., 2006).

Etidronate, a first-generation bisphosphonate, is considered to be the best choice for treatment of GACI in view of its considerable anti-mineralisation activity (Chong and Hutchins, 2008). Etidronate was in use for the treatment of GACI, even before the genetic basis and pathogenesis of GACI were fully explored (Francis et al., 1969). Infants with GACI have reduced extracellular level of inorganic pyrophosphate (PPi), an inhibitor of hydroxyapatite formation. Etidronate, which is an analogue of PPi, and more recently Pamidronate have been used with some success in the treatment of GACI to reduce the arterial calcifications by blocking the ectopic mineralisation (Ramjan et al., 2009; Edouard et al., 2011; Fleisch et al., 1966).

Etidronate is either excreted unchanged in the urine or is taken up by the bone. Approximately, half the absorbed dose of etidronate gets deposited in the skeleton (Cremers et al., 2005). Once deposited in the bone matrix, it becomes quiescent, until released and ingested by the osteoclasts (Ebetino et al., 2011). A long half-life and an ability to cross the placenta, perhaps, increases the likelihood of the foetus getting exposed to the drug even if it was stopped years before the pregnancy.

The studies on animals have documented detrimental effects of gestational bisphosphonate exposure on foetal survival, birth weight and bone growth (Patlas et al., 1999). However, so far, studies have not reported any serious maternal or neonatal adverse effects in humans following the bisphosphonate use in pregnant females or women of child-bearing age (Rutgers-Verhange et al., 2003).

Hypophosphatasia (HPP) is a rare metabolic bone disease characterised by low levels of tissue non-specific alkaline phosphatase (TNSALP); an enzyme that metabolises PPi. This leads to increased PPi resulting in impaired bone mineralisation (Weiss et al., 1988). Etidronate by being a PPi analogue can lead to mineralisation defects and a clinical and radiological picture similar to hypophosphatasia (Fortuna et al., 1979).

Herein, we report, the occurrence of the skeletal changes suggestive of hypophosphatasia (HPP), secondary to antenatal etidronate (first generation bisphosphonate) used for the treatment of suspected GACI due to an *ABCC6* mutation. We believe, this is the first reported case of antenatal treatment of GACI with bisphosphonate causing such a skeletal change.

2. Case discussion

A Caucasian infant boy, first child of non-consanguineous parents with two healthy half-siblings, had a suspected diagnosis of GACI in the antenatal period.

3. Antenatal course

An antenatal ultrasonography (Voluson (GE) machine) done at 20 weeks of gestation, showed a bright tricuspid valve annulus, suggestive of calcifications. Further foetal cardiology review by a Paediatric cardiologist and antenatal echocardiograms showed persistent echo bright annulus of the tricuspid valve, wall of the right ventricle outflow tract and main pulmonary artery. The tricuspid valve was measured to be slightly smaller than the mitral valve, but was not stenosed. There was also no evidence of any pulmonary stenosis despite the wall of the pulmonary outflow being 'calcified' (Fig. 1a, b). Foetal CT scanning was not done.

3.1. Genetic testing

Amniocentesis was done and genetic testing with Sanger sequencing of *ENPP1* and *ABCC6* showed a heterozygous disease causing variant in *ABCC6* exon 27; C.3775 del. P.(Trp259fs). A second pathogenic variant was not identified within the coding region or conserved splice sites of *ABCC6* but a non-coding variant could not be excluded. There was an extensive multidisciplinary discussion with the parents about the uncertainty of the perinatal outcome given the paucity of literature and extreme variability in the postnatal clinical presentation.

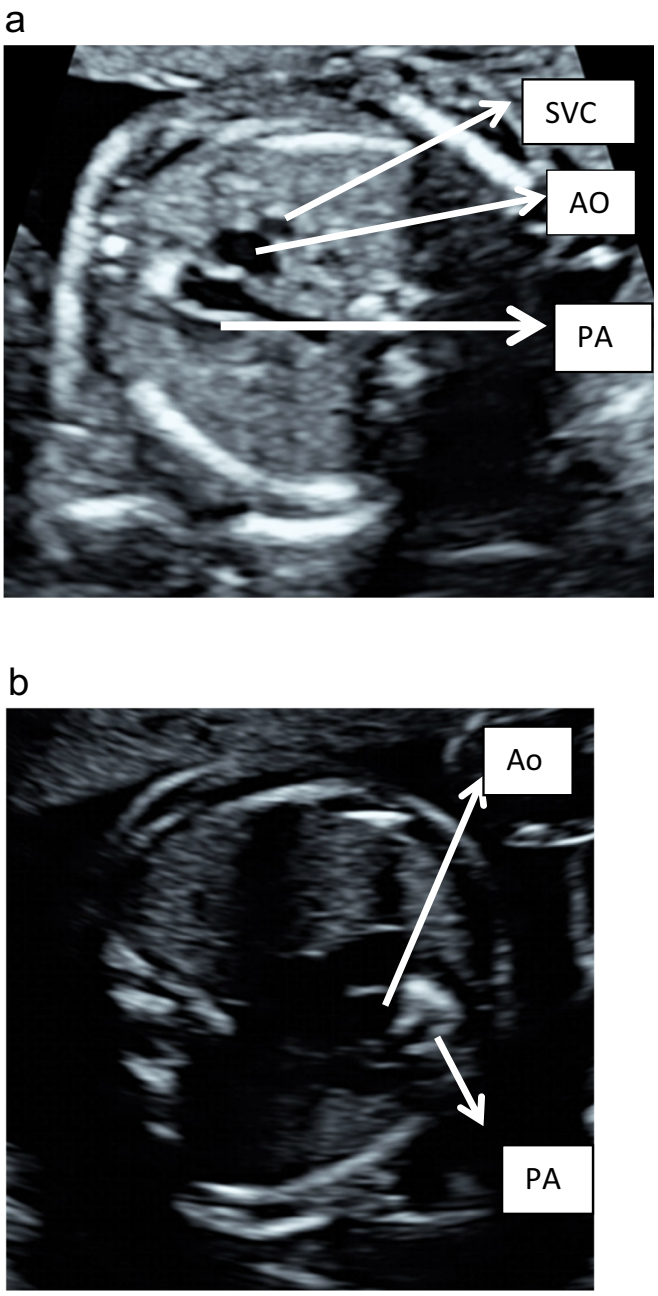

Fig. 1. a: Fetal Echocardiogram in the 3vessel outlet view (3VV) demonstrating the superior vena cava (SVC), Aortic arch (Ao) and Pulmonary trunk (PA). Note that the wall of the pulmonary trunk is bright (evidence of possible calcification) as compared to the aortic arch and SVC.
b: Fetal echocardiogram with significantly reduced 2D gain settings demonstrating the pulmonary outflow in the modified oblique sagittal view. Note the bright wall of the pulmonary trunk (PA) indicating evidence of possible tissue calcification. The wall of aortic root (Ao) in centre is dark indicating no wall calcification seen.

3.2. Antenatal treatment

Based on the genetic and scan findings, mother was started on etidronate (EHDP; ethane-1-hydroxy-1, 1-diphosphonic acid, also known as 1-hydroxyethylidine-bisphosphonate) treatment at a dose of 20 mg/kg/day in two divided doses (1200 mg/day) from 26 weeks of gestation. Mother was monitored with serum vitamin D, bone, liver and renal profile, PTH and urine calcium and creatinine while on etidronate treatment. All these were normal. She tolerated the medication well and reported no side-effects. She was also advised not to take any vitamin D during the pregnancy but have normal calcium intake. Regular foetal scans showed persisting calcification, marginally smaller size of the tricuspid valve and size of the right ventricle, but no new areas of brightness were identified.

4. Postnatal course

The mother was induced at 38 weeks and achieved uneventful vaginal birth without any intrapartum or postpartum complications. Baby (male; birth weight of 3 kg) was admitted to the special care baby unit, reviewed by cardiology team and then transferred to local children's hospital for further imaging and endocrinology input. Initial echocardiogram (GE Vivid E9, E11 and Philips) on day 1 of life and day 3 of life showed a structurally and functionally normal heart. There was no evidence of echo bright walls of the pulmonary outflow, nor of the annulus of the tricuspid valve. The initial electrocardiogram (ECG) was also normal.

Apart from the subtle changes indicative of micro-calcifications in the wall of inferior abdominal aorta, proximal common iliac arteries, iliac veins and lower inferior vena cava (IVC) on CT angiography, no evidence of aortic, pulmonary or coronary calcification was seen. Incidentally, markedly abnormal bony changes were detected on CT angiography, with cupping and splaying of the anterior rib ends and metaphyses of the long bones. Subsequently, a limited skeletal survey was undertaken.

The radiograph of the chest showed diffusely sclerotic bones with cupping and expansion of the anterior rib ends bilaterally (Fig. 2a). Radiographs of the right upper and lower limbs were suggestive of diffuse sclerosis, and fraying and irregularity of the metaphyses; with sparing of the elbow and proximal tibia metaphyses (Fig. 2b, c). The radiographs also revealed the typical radiolucent protrusions (“tongues”) extending from the metaphyses towards the bone shafts. These changes are similar to those seen in perinatal and infantile onset hypophosphatasia (HPP).

Subsequently, cardiac dysfunction developed on day 4 of life with initial symptoms of tachycardia and impaired cardiac function on echocardiogram with ischaemic changes on ECG.

Serum calcium, phosphate, alkaline phosphatase and renal profile were normal. FGF23 was not measured. Serum lactate steadily increased and troponin T level was significantly elevated (> 10,000 ng/l; normal 0-14 ng/l). A serum inorganic pyrophosphate (PPi) analysis showed a level of 0.438microM, which was estimated to be 33% of healthy adult control levels. Because of the limited availability of PPi assays and normative data for paediatric population, the results were compared with the adult reference values.

The baby was treated with IV Sodium Thiosulphate infusions (based on advice from the Children's Hospital of Philadelphia team) starting from day 6 of life.

In view of the deteriorating cardiac function, a possibility of evolving coronary calcifications was kept; and a dose of oral etidronate was given in the second week of life. Etidronate was later changed to pamidronate, since the baby was put nil by mouth in view of deteriorating clinical condition.

Despite treatment with the cardiac supportive measures, sodium thiosulphate and bisphosphonate, he succumbed to death on day 19th of life due to worsening myocardial ischaemia secondary to poor

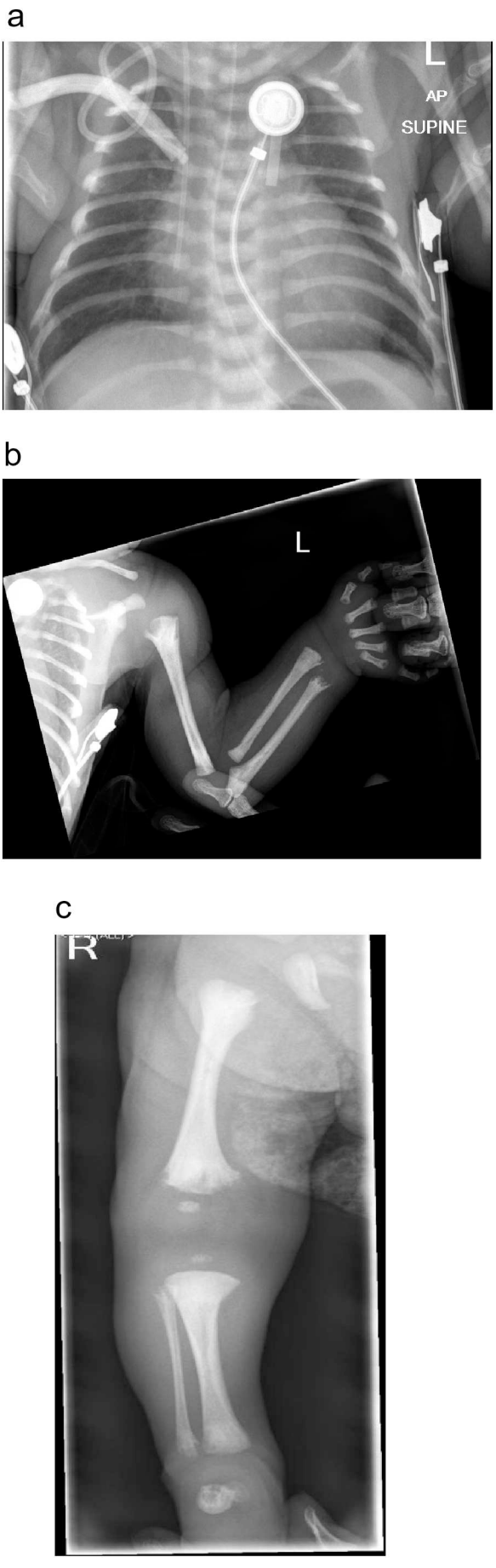

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Fig. 2. a: X-ray Chest showing diffusely sclerotic bones with cupping and expansion of the anterior rib ends bilaterally.
b: X-ray left upper limb showing diffuse sclerosis, fraying and irregularity of the metaphysis; with sparing of the elbow metaphysis.
c: X-ray right lower limb showing diffuse sclerosis, fraying and irregularity of the metaphysis; with sparing of the proximal tibia metaphysis.

coronary perfusion. Parents did not consent for the post-mortem study. Post natal Trio exome sequencing in the UK and also from Ghent genetics laboratory showed the same finding as before in the baby's and one of the parent's sample. Whole genome sequence was not done. Since, the parents did not wish to know who was carrying the gene, no further investigations were performed on the parents. Also, both the parents were fit and well from self-reported medical history.

5. Discussion

We believe this to be the first report of skeletal changes suggestive of hypophosphatasia (HPP), arising secondary to antenatal etidronate used for the treatment of suspected GACI due to a heterozygous *ABCC6* mutation.

Majority of GACI (autosomal recessive disorder) cases, are caused by bi-allelic deactivating mutations in *ENPP1* (GACI 1) (Rutsch et al., 2003), which encodes for ectonucleotide pyrophosphatase/phosphodiesterase 1, an enzyme responsible for the generation of PPi; a potent inhibitor of hydroxyapatite crystal formation in the vessel walls (Goding et al., 2003). A similar phenotype (GACI 2) is reported due to the loss-of-function mutation of *ABCC6*; an ATP-binding cassette transmembrane encoding gene (Le Boulanger et al., 2010). But, the precise role of *ABCC6* in the pathogenesis of GACI is unclear (Nitschke et al., 2012). Serum and urine levels of inorganic pyrophosphate are reported to be lower in these patients as compared to healthy controls (Rutsch et al., 2001).

Although, our patient had a monoallelic mutation, the findings on imaging and the clinical course along with the low PPi level increases the likelihood of GACI. Further, monoallelic mutations in *ABCC6* have been shown to manifest with a similar clinical picture as GACI patients with biallelic mutations (Nitschke et al., 2012; Votava-Smith et al., 2017). However, it is still possible that the other mutation was in the intron or the regulatory region which was not a part of gene sequencing. Copy number variation was not found in our analysis using MLPA. Nitschke et al. have identified 14 patients with GACI with pathogenic mutations in *ABCC6* (biallelic mutations in 8 and monoallelic mutations in 6) (Nitschke et al., 2012). Severity of the condition does not correlate with the genotype (Ruf et al., 2005). A broad phenotypic variation ranging from a spontaneous resolution in childhood to an early death despite adequate treatment with the bisphosphonates has been reported in the siblings carrying identical mutations (Le Boulanger et al., 2010).

Etidronate, a first generation bisphosphonate, is a synthetic PPi analogue and leads to the cessation of the hydroxyapatite crystal formation and elimination of radiographically evident arterial calcifications in GACI (Meradji et al., 1978). It typically takes from two weeks to two years for radiological resolution of arterial calcifications (Meradji et al., 1978). Recurrence of arterial calcifications has not been reported (Marrott et al., 1984; Sholler et al., 1984). In-vitro studies have shown that bisphosphonates accumulate within the vessel walls, suggesting their direct effect on the calcifications (Lomashvili et al., 2009). They might also influence differentiation of arterial smooth muscle (Rutsch et al., 2008; Li et al., 2016). This may explain the presence of only micro-calcifications on postnatal angiogram in our patient who received antenatal etidronate treatment. It is difficult to comment if the calcifications could have disappeared independent of EHDP treatment given to the mother. However, the clinical deterioration was due to coronary insufficiency raising the possibility of persisting coronary calcification/narrowing.

In a retrospective study, Rutsch et al., have described survival in 11 of 17 patients with GACI treated with bisphosphonate and 8 of 26 untreated patients (Rutsch et al., 2008). Similar to previous studies on etidronate treatment in children with GACI, a dose of 20 mg/kg/day was used antenatally and in our patient (Rutsch et al., 2008; Van der Sluis et al., 2006; Li et al., 2016; Cheng et al., 2005; Tran and Boechat, 2006).

GACI is not reported to be associated with generalised skeletal changes. However, features of hypophosphatemic rickets have been observed at varying ages, in the surviving patients with GACI 1, with or without the treatment with bisphosphonates (Levy-Litan et al., 2010; Lorenz-Depiereux et al., 2010). Otero et al. have reported skeletal changes suggestive of HPP in a child with GACI 1, following prolonged postnatal etidronate treatment (Otero et al., 2013). In our patient, skeletal survey showed radiological features consistent with those seen in the infants with perinatal and infantile onset HPP. His clinical features, a normal serum alkaline phosphatase level and a low serum level of PPi make this diagnosis very unlikely. Also the ALPL gene was included in the exome sequencing but no likely causative variant was identified.

None of the animal studies with etidronate or limited literature on gestational bisphosphonates use in humans report any skeletal abnormalities in foetus or newborns (Rutgers-Verhange et al., 2003). Similarly, no skeletal change was reported in a 3-year-old girl who was started on etidronate therapy from day 8 of life for GACI 2 due to a heterozygous *ABCC6* mutation (Ali et al., 2018).

Irreversible inhibition of alkaline phosphatase (ALP) by etidronate is known to occur (Fortuna et al., 1979). Although, there is a paucity of literature on antenatal use of etidronate for GACI, there are case reports of HPP like changes on prolonged or high dose use of etidronate in postnatal cases, hence our impression that the HPP like changes are secondary to antenatal etidronate use in our patient.

Limitations of our study include the lack of further clinical and genetic details of the parents and half-siblings, and lack of post-mortem study of the baby.

In conclusion, we report radiological changes like those seen in perinatal and infantile onset HPP, resulting from antenatal treatment of a GACI patient with etidronate. While we do not believe that HPP like skeletal changes contributed to demise of our case, we recommend radiological screening for HPP like skeletal changes in infants who have been treated with bisphosphonates in utero.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Author contribution

All the authors except NA were involved in patient management. NA did literature review and drafted the initial manuscript. All authors contributed and critically reviewed the manuscript. RR would act as guarantor of the paper.

Declaration of competing interest

None.

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References

Ali, S.A., Ng, C., Votava-Smith, J.K., Randolph, L.M., Pitukcheewanont, P., 2018. Bisphosphonate therapy in an infant with generalized arterial calcification with an *ABCC6* mutation. Osteoporos. Int. 29 (11), 2575–2579.

Bolster, F., Ali, Z., Southall, P., Fowler, D., 2015. Generalized arterial calcification of infancy—findings at post-mortem computed tomography and autopsy. Forensic Sci. Int. 254, e7–e12.

Cheng, K.S., Chen, M.R., Ruf, N., Lin, S.P., Rutsch, F., 2005. Generalized arterial calcification of infancy: different clinical courses in two affected siblings. Am. J. Med. Genet. A 136 (2), 210–213.

Chong, C.R., Hutchins, G.M., 2008. Idiopathic infantile arterial calcification: the spectrum of clinical presentations. Pediatr. Dev. Pathol. 11, 405–415.

Cremers, S.C., Pillai, G., Papapoulos, S.E., 2005. Pharmacokinetics/pharmacodynamics of bisphosphonates: use for optimisation of intermittent therapy for osteoporosis. Clin. Pharmacokinet. 44, 551–570.

Ebetino, F.H., Hogan, A.M., Sun, S., Tsoumpra, M.K., Duan, X., Triffitt, J.T., Kwaasi, A.A., Dunford, J.E., Barnett, B.L., Oppermann, U., Lundy, M.W., Boyde, A., Kashemirov, B.A., McKenna, C.E., Russell, R.G., 2011. The relationship between the chemistry and biological activity of the bisphosphonates. Bone 49, 20–33.

Edouard, T., Chabot, G., Miro, J., Buhas, D.C., Nitschke, Y., Lapierre, C., Rutsch, F., Alos, N., 2011. Efficacy and safety of 2-year etidronate treatment in a child with generalized arterial calcification of infancy. Eur. J. Pediatr. 170, 1585–1590.

Fleisch, H., Russell, R.G., Straumann, F., 1966. Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature 212 (5065), 901–903.

Fortuna, R., Anderson, H.C., Carty, R., Sajdera, S.W., 1979. Enzymatic characterization of the chondrocytic alkaline phosphatase isolated from bovine foetal epiphyseal cartilage. Biochim. Biophys. Acta 570 (2), 291–302.

Francis, M.D., Russell, R.G., Fleisch, H., 1969. Diphosphonates inhibit formation of calcium phosphate crystals in vitro and pathological calcification in vivo. Science 165 (899), 1264–1266.

Goding, J.W., Grobben, B., Slegers, H., 2003. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. Biochim. Biophys. Acta 1638, 1–19.

Le Boulanger, G., Labreze, C., Croue, A., Schurgers, L.J., Chassaing, N., Wittkampf, T., et al., 2010. An unusual severe vascular case of pseudoxanthoma elasticum presenting as generalized arterial calcification of infancy. Am. J. Med. Genet. A 152A, 118–123.

Levy-Litan, V., Hershkovitz, E., Avizov, L., Leventhal, N., Bercovich, D., Chalifa-Caspi, V., Manor, E., Buriakovsky, S., Hadad, Y., Goding, J., Parvari, R., 2010. Autosomal-recessive hypophosphatemic rickets is associated with an inactivation mutation in the *ENPP1* gene. Am. J. Hum. Genet. 86 (2), 273–278.

Li, Q., Kingman, J., Sundberg, J.P., Levine, M.A., Uitto, J., 2016. Dual effects of bisphosphonates on ectopic skin and vascular soft tissue mineralization versus bone microarchitecture in a mouse model of generalized arterial calcification of infancy. J. Invest. Dermatol. 136 (1), 275–283.

Lomashvili, K.A., Monier-Faugere, M.C., Wang, X., Malluche, H.H., O'Neill, W.C., 2009. Effect of bisphosphonates on vascular calcification and bone metabolism in experimental renal failure. Kidney Int. 75, 617–625.

Lorenz-Depiereux, B., Schnabel, D., Tiosano, D., Häusler, G., Strom, T.M., 2010. Loss-of-function *ENPP1* mutations cause both generalized arterial calcification of infancy and autosomal-recessive hypophosphatemic rickets. Am. J. Hum. Genet. 86 (2), 267–272.

Marrott, P.K., Newcombe, K.D., Becroft, D.M., Friedlander, D.H., 1984. Idiopathic infantile arterial calcification with survival to adult life. Pediatr. Cardiol. 5 (2), 119–122.

Meradji, M., de Villeneuve, V.H., Huber, J., de Bruijn, W.C., Pearse, R.G., 1978. Idiopathic infantile arterial calcification in siblings: radiologic diagnosis and successful treatment. J. Pediatr. 92 (3), 401–405.

Nitschke, Y., Baujat, G., Botschen, U., Wittkampf, T., du Moulin, M., Stella, J., et al., 2012. Generalized arterial calcification of infancy and pseudoxanthoma elasticum can be caused by mutations in either *ENPP1* or *ABCC6*. Am. J. Hum. Genet. 90, 25–39.

Otero, J.E., Gottesman, G.S., McAlister, W.H., Mumm, S., Madson, K.L., Kiffer-Moreira, T., Sheen, C., Millán, J.L., Ericson, K.L., Whyte, M.P., 2013. Severe skeletal toxicity from protracted etidronate therapy for generalized arterial calcification of infancy. J. Bone Miner. Res. 28 (2), 419–430.

Patlas, N., Colomb, G., Yaffe, P., et al., 1999. Transplacental effects of bisphosphonates on foetal skeletal ossification and mineralization in rats. Teratology 60, 68–73.

Ramjan, K.A., Roscioli, T., Rutsch, F., Sillence, D., Munns, C.F., 2009. Generalized arterial calcification of infancy: treatment with bisphosphonates. Nat. Clin. Pract. Endocrinol. Metab. 5, 167–172.

Ruf, N., Uhlenberg, B., Terkeltaub, R., Nurnberg, P., Rutsch, F., 2005. The mutational spectrum of *ENPP1* as arising after the analysis of 23 unrelated patients with generalized arterial calcification of infancy (GACI). Hum. Mutat. 25 (1), 98.

Rutgers-Verhange, A., deVries, T., Torringa, M., 2003. No effects of bisphosphonates on the human fetus. Birth Defects Res. A Clin. Mol. Teratol. 67, 203–204.

Rutsch, F., Vaingankar, S., Johnson, K., Goldfine, I., Maddux, B., Schauerte, P., Kalhoff, H., Sano, K., Boisvert, W.A., Superti-Furga, A., Terkeltaub, R., 2001. PC-1 nucleoside triphosphate pyrophosphohydrolase deficiency in idiopathic infantile arterial calcification. Am. J. Pathol. 158 (2), 543–554.

Rutsch, F., Ruf, N., Vaingankar, S., Toliat, M.R., Suk, A., Höhne, W., et al., 2003. Mutations in *ENPP1* are associated with 'idiopathic' infantile arterial calcification. Nat. Genet. 34, 379–381.

Rutsch, F., Böyer, P., Nitschke, Y., Ruf, N., Lorenz- Depierieux, B., Wittkampf, T., et al., 2008. GACI Study Group: Hypophosphatemia, hyperphosphaturia, and bisphosphonate treatment are associated with survival beyond infancy in generalized arterial calcification of infancy. Circ. Cardiovasc. Genet. 1, 133–140.

Sholler, G.F., Yu, J.S., Bale, P.M., Hawker, R.E., Celermajer, J.M., Kozlowski, K., 1984. Generalized arterial calcification of infancy: three case reports, including spontaneous regression with long-term survival. J. Pediatr. 105 (2), 257–260.

Tran, K.H., Boechat, M.I., 2006. Idiopathic infantile arterial calcification: imaging evaluation and the usefulness of MR angiography. Pediatr. Radiol. 36 (3), 247–253.

Van der Sluis, I.M., Boot, A.M., Vernooij, M., Meradji, M., Kroon, A.A., 2006. Idiopathic infantile arterial calcification: clinical presentation, therapy and long-term follow-up. Eur. J. Pediatr. 165 (9), 590–593.

Votava-Smith, J.K., Pitukcheewanont, P., Randolph, L.M., Chmait, R.H., 2017. Generalized arterial calcification in a recipient twin: discordant foetal hemodynamics result in differing phenotypes in monozygotic twins with an *ABCC6* mutation. Foetal Diagn. Ther. 41 (3), 234–236.

Weiss, M.J., Cole, D.E., Ray, K., Whyte, M.P., Lafferty, M.A., Mulivor, R.A., Harris, H., 1988. A missense mutation in the human liver/bone/kidney alkaline phosphatase gene causing a lethal form of hypophosphatasia. Proc. Natl. Acad. Sci. U. S. A. 85 (20), 7666–7669.