This is a repository copy of *A novel homozygous variant in SERPINH1 associated with a severe, lethal presentation of osteogenesis imperfecta with hydranencephaly*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/105483/

Version: Accepted Version

Article:

Marshall, C., Lopez, J., Crookes, L. et al. (2 more authors) (2016) A novel homozygous variant in SERPINH1 associated with a severe, lethal presentation of osteogenesis imperfecta with hydranencephaly. Gene, 595 (1). pp. 49-52. ISSN 0378-1119

https://doi.org/10.1016/j.gene.2016.09.035

Article available under the terms of the CC-BY-NC-ND licence
(https://creativecommons.org/licenses/by-nc-nd/4.0/)

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can’t change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

A novel homozygous variant in SERPINH1 associated with a severe, lethal presentation of Osteogenesis Imperfecta with hydranencephaly

Charlotte Marshall[1], Jaime Lopez[2,3], Laura Crookes[4], Rebecca C Pollitt[4], Meena Balasubramanian[5,6]

[1]Medical School, University of Sheffield

[2]Genetics department, Centenario Hospital Miguel Hidalgo, Aguascalientes, Mexico

[3]Neonatology department, Hospital de la Mujer, Aguascalientes, Mexico

[4]Sheffield Diagnostic Genetics Service, Sheffield Children's NHS Foundation Trust, UK

[5]Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, UK

[6]Highly Specialised Severe, Complex & Atypical OI Service, Sheffield Children's NHS Foundation Trust, UK

Correspondence to:

Dr Meena Balasubramanian

Sheffield Clinical Genetics Service

Sheffield NHS Foundation Trust

Western Bank, Sheffield S10 2TH

Ph- 0114 2717025, Fax- 0114 2737467

E-mail- meena.balasubramanian@nhs.net

Keywords: autosomal recessive, osteogenesis imperfecta, hydranencephaly, lethal presentation

Abstract

Osteogenesis imperfecta (OI) is a genetic disorder characterised by low bone mineral density resulting in fractures. 85-90% of patients with OI carry a variant in the type 1 collagen genes, COL1A1 and COL1A2, which follows an autosomal dominant pattern of inheritance. However, within the last two decades, there have been growing number of variants identified in genes that follow an autosomal recessive pattern of inheritance.

Our proband is a child born in Mexico with multiple fractures of ribs, minimal calvarial mineralisation, platyspondyly, marked compression and deformed long bones. He also presented with significant hydranencephaly, requiring ventilatory support from birth, and died at 8 days of age. A homozygous c.338_357delins22 variant in exon 2 of SERPINH1 was identified. This gene encodes heat shock protein 47, a collagen-specific chaperone which binds to the procollagen triple helix and is responsible for collagen stabilisation in the endoplasmic reticulum.

There is minimal literature on the mechanism of action for variants in SERPINH1 resulting in osteogenesis imperfecta. Here we discuss this rare, previously unreported variant, and expand on the phenotypic presentation of this novel variant resulting in a severe, lethal phenotype of OI in association with hydranencephaly.

Introduction

Osteogenesis imperfecta (OI) is a heritable condition characterised by fragile bones of varying severity (Bishop et al., 2010). Extra-skeletal features are often present, such as blue sclerae, skin hyperextensibility, dentinogenesis imperfecta and hearing loss (Forlino & Marini 2016; Marini et al., 2013).

85- 90% of OI cases are due to an autosomal dominant variant in the genes encoding type I collagen, COL1A1 and COL1A2 (Forlino & Marini 2016). There are several hundred recorded variants in these genes, the most common being a glycine substitution in the collagen triple helix (Lindahl et al., 2015).

More recently, variants in other genes have been identified as causing autosomal recessive forms of OI. Most of these genes are involved in post-translational modification of type I collagen in the endoplasmic reticulum (ER). CRTAP (OMIM *605497), LEPRE1 (OMIM *610339) and PPIB (*OMIM 123841) are genes encoding components of the prolyl-3 hydroxylation complex which undertakes post-translational alterations of the procollagen molecule. The modifications are necessary for folding, secretion and extracellular matrix assembly; pathogenic variants in any of the three genes cause posttranslational over-modification resulting in bone fragility (Barbirato et al., 2015; Duran et al., 2015). Heat shock protein 47 (HSP47) encoded for by SERPINH1 (OMIM *600943), is a collagen-specific chaperone expressed in all collagen-secreting cells. It binds to the procollagen triple helix and is required for the proper assembly of the triple helical procollagen molecules after the modification by the prolyl-3 hydroxylation complex (Ishida et al., 2006; Widmer et al., 2012; Duran et al., 2015). FKBP10 (OMIM 607063) encodes for FKBP65, a type I collagen chaperone present in the ER along with HSP47. Variants in this gene results in two distinct

phenotypes: moderately severe OI and Bruck Syndrome (OI with contractures) (Duran et al., 2015).

Here we present a male patient diagnosed with severe bone fragility and hydranencephaly who did not survive past the neonatal period. This was caused by a novel homozygous variant in SERPINH1.

Clinical Summary

The patient was a male, born in Mexico to healthy, non-consanguineous parents. The parents were young at the time of birth: the mother was 15 years of age and the father was 17 years of age. In the second trimester, the pregnancy was complicated by ultrasound findings in the fetus of ascites, pleural effusion, short long bones and cerebral ventriculomegaly, amniocentesis revealed a 46,XY[30] karyotype. The ascites and pleural effusion spontaneously resolved in the third trimester. He was delivered by caesarean section at 36 weeks gestation with a birth weight of 1600 grams (<3 percentile), length of 35 cm (<3 percentile) and head circumference of 32 cm (50 percentile). Apgar scores were 5 at 1 minute and 7 at 5 minutes of age. Continuous assisted ventilation support was required from birth. Physical examination revealed generalised hypotonia, relative macrocephaly, soft skull with enlarged and bulging anterior and posterior fontanelle, short neck, narrow and bell-shaped chest, right undescended testis, flexed and abducted right hip with asymmetrical, short and bowed limbs. Facial dysmorphism included high and prominent forehead, mid-face hypoplasia, deep-set eyes with down-slanting palpebral fissures, blue sclerae, short nose, anteverted nares, retrognathia and bilateral low-set ears. The infant passed away at eight days of age following a prolonged stay in neonatal intensive care with hemodynamic instability and pulmonary haemorrhage.

Radiographs showed narrow chest, multiple fractures of ribs, minimal calvarial mineralisation, platyspondyly, marked compression, osteopenia and deformed long bones, consistent radiologically with type II osteogenesis imperfecta. Cerebral ultrasound reported hydranencephaly, but magnetic resonance study was not undertaken.

Materials and Methods

Genomic DNA was extracted using standard protocols. Clonal sequencing using SureSelect target enrichment (Agilent Technologies) and the Illumina MiSeq platform was performed using a custom designed gene panel: COL1A1; COL1A2; IFITM5; CRTAP; LEPRE1; PPIB; FKBP10; SP7; SERPINF1; SERPINH1; PLOD2; BMP1; TMEM38B; WNT1. Analysis of sequence data using a custom designed bioinformatics pipeline based on the open source workflow by the Broad Institute (http://www.broadinstitute.org/gatk/guide/best-practices) was undertaken using a minimum threshold of 30-fold read depth for exonic sequence and intron/exon boundary. Variants identified were filtered against polymorphism lists and assessed using the Association for Clinical Genetic Science Best Practice Guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics (http://www.acgs.uk.com); no other pathogenic variants were identified in other genes in the panel. Parental testing was performed through targeted analysis by Sanger sequencing.

Results

Genetic analysis was undertaken with informed consent which revealed a homozygous c.338_357delins22 variant in exon 2 of the SERPINH1 (NM_001235.3). This is predicted to result in a frameshift and lead to a premature termination codon 7 amino acids downstream. This change has not been reported previously in SERPINH1. However, similar frameshift variants have been reported in other autosomal recessive genes in OI.

(http://www.le.ac.uk/ge/collagen/). The parents were confirmed to be heterozygous carriers of this variant.

Discussion

This case report adds to the minimal published literature on OI associated with SERPINH1 variants. Here we report a child with OI caused by a homozygous c.338_357delins22 variant in exon 2 in SERPINH1, which is novel in that it results in a premature termination codon. A potential mechanism of action for this variant and how it causes a lethal phenotype is suggested below.

The first identified cases of OI caused by a variant in SERPINH1 were in pedigree Dachshunds (Drogemuller et al., 2009). However, the gene had previously been known in obstetrics, as variants in the promoter region increase the risk of preterm premature rupture of membranes in African-American women (Wang et al., 2006).

It is known that mutant mice deficient in SERPINH1 suffer from defective collagen synthesis and die at around Day-11 of development with abnormal epithelial tissues and ruptured blood vessels (Nagai et al., 2000; Drogemuller et al., 2009). In humans, fibroblasts without HSP47 produce collagen fibrils that are abnormally long and thin (Ishida et al., 2006). The collagen chaperone appears to have several roles in the precise manufacture of the collagen molecule.

HSP47 is a collagen-specific chaperone found in ER and encoded by SERPINH1. The main function of HSP47 is to stabilise folded collagen and Serpinh1 KO mice are embryonic lethal. HSP47 binds directly onto the triple helix region of procollagen molecules after they have undergone the first modification in the ER by the prolyl-3 hydroxylation complex. There it functions as a stabilisation mechanism for the molecule and marks the folded collagen for transportation to the Golgi (Forlino and Marini, 2015). HSP47 also aids quality control, as

imperfect collagen molecules are not marked for movement out of the ER (Nagai et al., 2000). It then dissociates from the triple helix either in the cis-Golgi or the ER-Golgi intermediate compartment, perhaps mediated by the Golgi's lower pH. HSP47 is transported back to the ER to repeat the process (Widmer et al., 2012).

At body temperature, mature collagen is thermally unstable: the procollagen triple helix spontaneously forms its native conformation at temperatures several degrees below body temperature, but not at 37°c. To do this, it requires the attachment of HSP47 to stabilise the molecule (Makareeva and Leikin, 2007). If insufficient HSP47 is present within the cell, the secreted type I collagen denatures quickly at body temperature when exposed to proteases (Christiansen et al., 2010; Widmer et al., 2012). It is thought that the binding of HSP47 prevents local unfolding of the triple helical regions at body temperature: this does not occur in OI, as there are reduced levels of the chaperone (Widmer et al., 2012).

In cells without HSP47, the secretion of type I collagen to the Golgi is delayed, resulting in an accumulation of insoluble procollagen aggregates within the endoplasmic reticulum. This delayed secretion may be due to the abnormal structure of the collagen produced (Ishida et al., 2006). Alternatively, it may be caused by a lack of HSP47 on the binding sites of the triple helix: procollagen has a tendency to form lateral aggregates and the chaperones may inhibit this (Widmer et al., 2012).

When there is deficient or no HSP47, the collagen produced by the fibroblasts is inadequate (Ishida et al., 2006) and causes bone to be inefficiently mineralised. Hence, a bone fragility phenotype arises and bone fragility results. Given the truncating variant in our patient reported here, it is likely that there is a quantitative defect in Heat shock protein 47 (HSP47) encoded for by SERPINH1 resulting in defective collagen contributing to severe bone fragility and perinatal lethality.

Other cases where variants in SERPINH1 have resulted in an OI phenotype include three patients with missense pathogenic variants in the gene: 2 siblings were diagnosed with moderately severe OI (c.710T>C) after infancy (Duran et al., 2015) and one other patient suffered from severe OI before passing away from respiratory distress (c.233T>C) (Christiansen et al., 2010). Both variants resulted in an amino acid substitution within the gene, which impaired its function. Additionally of note, consanguinity was present in both the families.

However, our proband had a truncating variant in the SERPINH1 gene resulting in a lethal OI phenotype. It seems likely that because the outcome of the variant is a premature termination codon, there would be resulting nonsense mediated decay of the abnormal mRNA. This would suggest that there may be less HSP47 within the ER with resultant loss of function causing a more severe phenotype: as with less HSP47, the collagen molecules would be more irregularly structured and unstable.

Conclusions and Summary

The multiple roles of HSP47 within the ER, and the phenotype that is a consequence of its deficiency, show that the chaperone is vital for the proper production of collagen. This report adds to the minimal published literature on the severe, lethal OI phenotype associated with SERPINH1 variants. This is the first report of a truncating variant in SERPINH1 and adds to the growing number of OI presentations caused by recessive gene variants.

Figure Legends:

Figure 1a demonstrating relative macrocephaly, high and prominent forehead, mid-face hypoplasia, deep-set eyes with down-slanting palpebral fissures, short nose, anteverted nares, retrognathia and bilateral low-set ears. There was also evidence of short neck, narrow and

bell-shaped chest, flexed and abducted right hip with asymmetrical, short and bowed limbs and need for ventilatory support soon after birth

Figure 1b radiograph demonstrating multiple fractures of ribs, minimal calvarial mineralisation, platyspondyly, marked compression, osteopenia and deformed long bones, consistent radiologically with type II Osteogenesis Imperfecta. Figure 1c Cerebral ultrasound demonstrating severe ventriculomegaly and remnants of brain tissue.

Figure 2a-b radiograph demonstrating bowed limbs, multiple fractures and severe osteopenia

Acknowledgements:

We thank the family for their consent to publish this report.

References:

Barbirato, C., M. Trancozo, M. G. Almeida, L. S. Almeida, T. O. Santos, J. C. Duarte, M. R. Reboucas, V. Sipolatti, V. R. Nunes, and F. Paula, 2015, Mutational characterization of the P3H1/CRTAP/CypB complex in recessive osteogenesis imperfecta: Genet Mol Res, v. 14, p. 15848-58.

Bishop N, 2010, Characterising and treating osteogenesis imperfecta. Early Hum Dev, 86(11):743-6.

Christiansen, H. E., U. Schwarze, S. M. Pyott, A. AlSwaid, M. Al Balwi, S. Alrasheed, M. G. Pepin, M. A. Weis, D. R. Eyre, and P. H. Byers, 2010, Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta: Am J Hum Genet, v. 86, p. 389-98.

Drogemuller, C., D. Becker, A. Brunner, B. Haase, P. Kircher, F. Seeliger, M. Fehr, U. Baumann, K. Lindblad-Toh, and T. Leeb, 2009, A missense mutation in the SERPINH1 gene in Dachshunds with osteogenesis imperfecta: PLoS Genet, v. 5, p. e1000579.

Duran, I., L. Nevarez, A. Sarukhanov, S. Wu, K. Lee, P. Krejci, M. Weis, D. Eyre, D. Krakow, and D. H. Cohn, 2015, HSP47 and FKBP65 cooperate in the synthesis of type I procollagen: Hum Mol Genet, v. 24, p. 1918-28.

Forlino A, Marini JC, 2016, Osteogenesis imperfecta. Lancet, 387(10028):1657-71.

Marini JC, Blissett AR, 2013, New genes in bone development: what's new in osteogenesis imperfecta. J Clin Endocrinol Metab, 98(8):3095-103.

Ishida, Y., H. Kubota, A. Yamamoto, A. Kitamura, H. P. Bächinger, and K. Nagata, 2006, Type I collagen in Hsp47-null cells is aggregated in endoplasmic reticulum and deficient in N-propeptide processing and fibrillogenesis: Molecular Biology of the cell, v. 17, p. 2346-2355.

Lindahl, K., E. Åström, C.-J. Rubin, G. Grigelioniene, B. Malmgren, Ö. Ljunggren, and A. Kindmark, 2015, Genetic epidemiology, prevalence, and genotype–phenotype correlations in the Swedish population with osteogenesis imperfecta: European Journal of Human Genetics.

Makareeva, E., and S. Leikin, 2007, Procollagen triple helix assembly: an unconventional chaperone-assisted folding paradigm: PLoS One, v. 2, p. e1029.

Nagai, N., M. Hosokawa, S. Itohara, E. Adachi, T. Matsushita, N. Hosokawa, and K. Nagata, 2000, Embryonic Lethality of Molecular Chaperone Hsp47 Knockout Mice Is Associated with Defects in Collagen Biosynthesis: The Journal of Cell Biology, v. 150(6), p. 1499-1506.

Wang, H., S. Parry, G. Macones, M. D. Sammel, H. Kuivaniemi, G. Tromp, G. Argyropoulos, I. Halder, M. D. Shriver, R. Romero, and J. F. Strauss, 3rd, 2006, A functional SNP in the promoter of the SERPINH1 gene increases risk of preterm premature rupture of membranes in African Americans: Proc Natl Acad Sci U S A, v. 103, p. 13463-7.

Widmer, C., J. M. Gebauer, E. Brunstein, S. Rosenbaum, F. Zaucke, C. Drogemuller, T. Leeb, and U. Baumann, 2012, Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition: Proc Natl Acad Sci U S A, v. 109, p. 13243-7.