Development of a Comprehensive, Locus-Specific Patient Database for ENPP1 Deficiency (Generalized Arterial Calcification of Infancy/ Autosomal Recessive Hypophosphatemic Rickets) to Clarify the Clinical Relevance of Individual Variants

Catherine Nester[1], Gus Khursigara[1], Carlos R. Ferreira[2], Lauren M. Chunn[3], Stephanie A. Mercurio[3], Mark J. Kiel[3]

[1] Inozyme Pharma, Boston, MA; [2] National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [3] Genomenon Inc., Ann Arbor, MI

Background

- ENPP1 Deficiency is a rare, genetic, multi-system mineralization disorder caused by loss-of-function variants in the *ENPP1* gene.
- The ENPP1 enzyme is involved in the production of extracellular pyrophosphate (PPi) and adenosine, potent inhibitors of mineralization and neointimal proliferation, respectively.
- Clinical findings in patients with ENPP1 Deficiency include calcification of the soft tissue, pathological skeletal mineralization, and vascular neointimal proliferation and stenosis.
- There is significant heterogeneity in the onset, clinical presentation, and severity of ENPP1 Deficiency. Most patients are diagnosed with generalized arterial calcification of infancy (GACI) and/or autosomal recessive hypophosphatemic rickets type 2 (**Fig 1).**
- The symptoms of ENPP1 Deficiency may be non-specific or resemble other diseases, and genetic testing is a critical diagnostic tool.

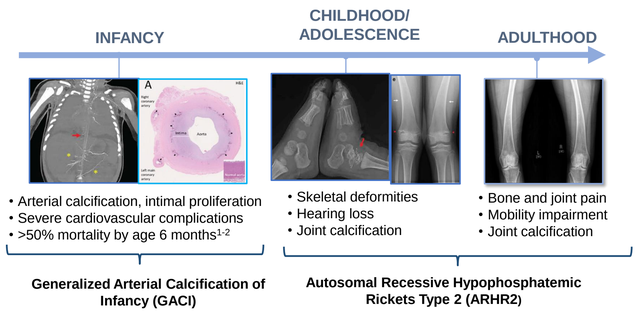

Figure 1. Most common ENPP1 Deficiency phenotypes[1-5]

Objective

To develop a comprehensive database of *ENPP1* variants to better understand this rare disease and increase the diagnostic yield of genetic testing.

Methods

- The patient database was developed by integrating *ENPP1* variants from a comprehensive literature review, and from natural history studies of GACI or ARHR2 patients sponsored by Inozyme Pharma and performed at the National Institutes of Health (NCT03478839) and Münster University Children's Hospital (NCT03758534).
- The comprehensive literature review was performed using Mastermind, a database of variants with evidence cited in the medical literature,[6] and considered all publications indexed from PubMed as of March 25th, 2021.
- Variants were annotated with variant interpretations and detailed clinical and biochemical phenotypes extracted from literature reports and clinical testing submission forms.
- The nomenclature for each entry conforms to the Human Genome Variant Society (HGVS) guidelines.[7]
- Variant interpretations were based on the consensus guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[8]

Results

- The literature revealed 112 GACI and/or ARHR2 patients with at least one *ENPP1* variant allele. An additional 43 unpublished cases, as part of either natural history study or observed separately at the NIH, were included in the analysis, for a total of 155 patients.
- Among these patients, 111 unique *ENPP1* variants and 112 unique genotypes were detected **(Fig 2).**
- The most common disease manifestations in 127 GACI patients and 56 ARHR2 patients (28 of whom also had GACI) are depicted in **Fig 3.**
- The most frequent class of variant found in patients were missense (58/111, 52.3%), followed by numerous truncating variants **(Fig 4a).**
- Of the 111 variants, 70.3% were demonstrably disease-causing based on the aggregated and interpreted evidence (58/111 were pathogenic; 20/111 were likely pathogenic) and 26.1% (29/111) were variants of uncertain significance **(Fig 4c).**
- Of the 49 truncating variants, 43 (87.8%) were categorized as pathogenic. Nearly 60.3% (35/58) of the missense variants were likely pathogenic or pathogenic, and 37.9% were variants of uncertain significance (VUS; 22/58). **(Fig 4d).**
- Notably, the distribution of variant type for all *ENPP1* variants **(Fig 4a)** mirrors the distribution of variant type for pathogenic and likely pathogenic designations **(Fig 4b),** with most variants being missense.
- The distribution of variant types across the protein are depicted in **Figure 5**. Although pathogenic and likely pathogenic variants exist across most regions of the protein, a majority (42/78, 53.8%) are located in the phosphodiesterase domain, followed by those in the nuclease domain (26/78, 33.3%).

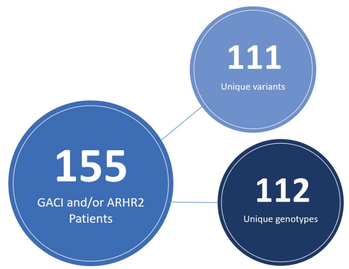

Figure 2. Summary of variant and genotype counts.

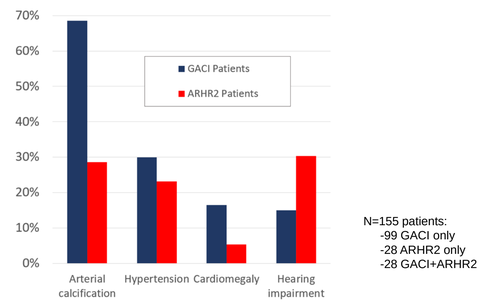

Figure 3. Summary of most frequent disease manifestations

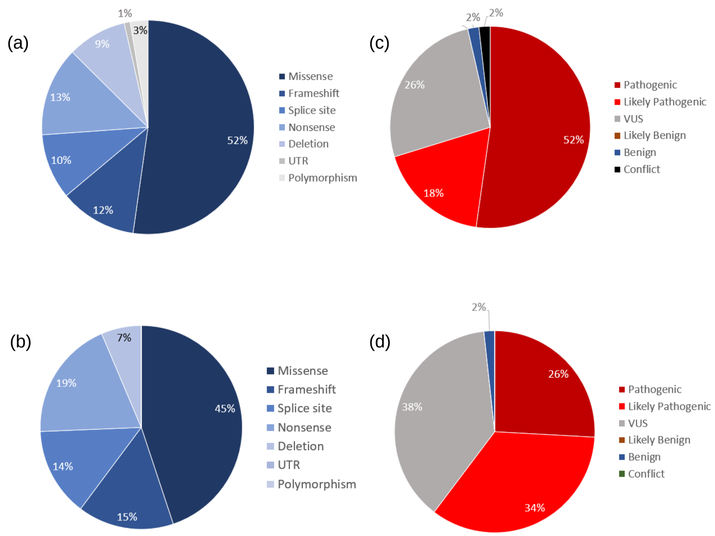

Figure 4. Breakdown of *ENPP1* variants from patient database. (a) All *ENPP1* variants by type. (b) Pathogenic and likely pathogenic variants by type. (c) All variants by pathogenicity call. (d) Missense variants by pathogenicity call.

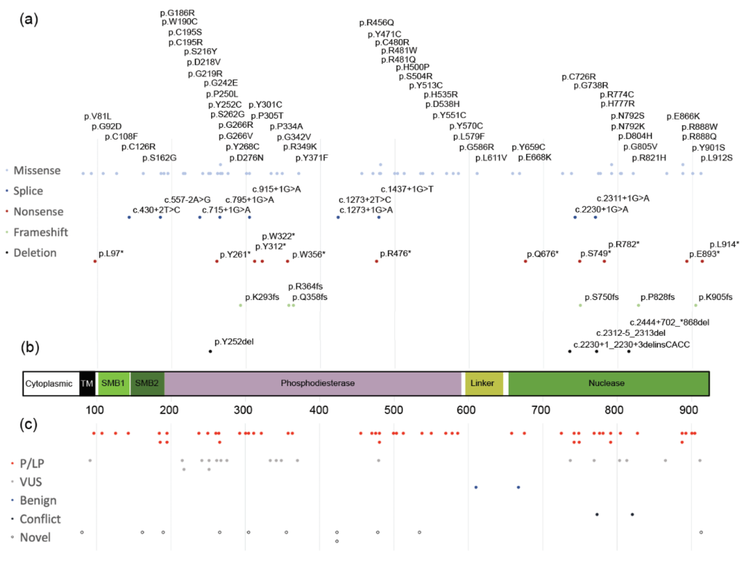

Figure 5. *ENPP1* variant landscape by type and interpretation. (a) Distribution of all variants, color-coded by variant type. (b) Linearized protein structure. (c) Distribution of variants by pathogenicity call.

Conclusions

- This is the largest analysis of *ENPP1* variant data in patients with ENPP1 Deficiency (GACI or ARHR2) and published molecular findings.
- Study identified 78 pathogenic or likely pathogenic variants, representing an 85.7% increase from Clinvar.
- The majority of pathogenic/likely pathogenic variants were located in the phosphodiesterase or nuclease domain of the ENPP1 enzyme, reinforcing that this disease is a consequence of lost enzymatic activity.
- Findings will aid in variant interpretation and facilitate diagnosis of this rare, heterogeneous disorder, while also informing treatment development.

Acknowledgements

The authors would like to thank Dr. Frank Rutsch for his contributions to study design and data acquisition.

References

1. Ferreira CR, Kintzinger K, Hackbarth ME, et al. Ectopic calcification and hypophosphatemic rickets: natural history of ENPP1 and ABCC6 deficiencies. *J Bone Miner Res*. Published online August 5, 2021. doi:10.1002/jbmr.4418
2. Rutsch F, Böyer P, Nitschke Y, et al. Hypophosphatemia, hyperphosphaturia, and bisphosphonate treatment are associated with survival beyond infancy in generalized arterial calcification of infancy. *Circ Cardiovasc Genet.* 2008;1(2):133-140. doi: 10.1161/CIRCGENETICS.108.797704
3. Ferreira CR, Hackbarth ME, Ziegler SG, et al. Prospective phenotyping of long-term survivors of generalized arterial calcification of infancy (GACI). *Genet Med.* 2021;23(2):396-407. doi:10.1038/s41436-020-00983-0
4. Boyce AM, Gafni RI, Ferreira CR. Generalized arterial calcification of infancy: new insights, controversies, and approach to management. *Curr Osteoporos Rep.* 2020;18(3):232-241. doi:10.1007/s11914-020-00577-4
5. Kotwal A, Ferrer A, Kumar R, et al. Clinical and Biochemical Phenotypes in a Family With ENPP1 Mutations. *J Bone Miner Res*. 2020;35(4):662-670. doi:10.1002/jbmr.3938
6. Chunn LM, Nefcy DC, Scouten RW, et al. Mastermind: A Comprehensive Genomic Association Search Engine for Empirical Evidence Curation and Genetic Variant Interpretation. *Front Genet.* 2020;11:577152. Published 2020 Nov 13. doi:10.3389/fgene.2020.577152
7. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Hum Mutat.* 2016;37(6):564-569. doi:10.1002/humu.22981
8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424. doi:10.1038/gim.2015.30