Search for *ENPP1* Mutation in Infantile Idiopathic Arterial Calcification (IIAC)

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER
at the Permian Basin

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Introduction

A 23-year-old, gravida 2, presented at 9 weeks. An anatomy scan (ultrasound most likely) at 27 weeks, demonstrated abnormal arterial echogenicity and elasticity, suspicious for IIAC. In one study of IIAC patients, 8/11 cases involved a mutation in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) (Rutsch, 2003; Ramjan 2009). IIAC is lethal due to the increased risk of myocardial infarction or congestive heart failure secondary to hypertension (Rutsch, 2008). ENPP1 is involved in the breakdown of adenosine triphosphate, into adenosine monophosphate, and pyrophosphate, specifically outside the cell. More than 40 mutations in ENPP1 gene have been associated with IIAC. These mutations inactivate ENPP1 causing a decrease in the availability of pyrophosphate, a physiologically inhibitor of calcification.

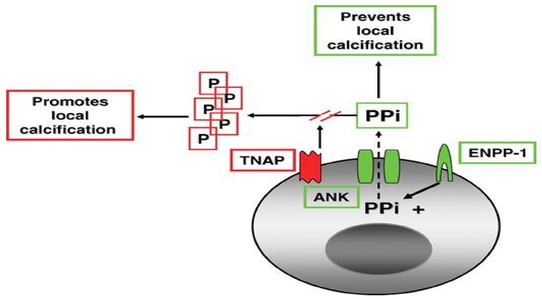


Figure 1: The balance of pyrophosphate (PPi) synthesis and degradation (color 'green' indicates calcification protection; color 'red' indicates calcification induction). Tissue pyrophosphate release is regulated by the following three factors ENPP-1, the transmembrane transporter 'ANK', and the membrane-bound enzyme 'tissue non-specific alkaline phosphatase' (TNAP). **Extracted from: https://doi.org/10.1093/ndt/gfp541**

Objective

To evaluate DNA from amniocytes using primers for ENPP1, to determine the causative mutation.

Materials and Methods

Amniocentesis and capillary sequencing analysis were performed on cultured amniocytes and whole blood from the mother. DNA was isolated using the phenol/chloroform/isoamyl alcohol method. PCR was performed using primer sets that make up the whole ENPP1 gene. The PCR products were sent for cleanup and capillary sequencing (McLabs, San Francisco). The regions of ENPP1 were sequenced: 2669bp-4797bp and 5707bp-6197bp. After delivery, placenta was evaluated by placental histopathology with calcium staining.

Results

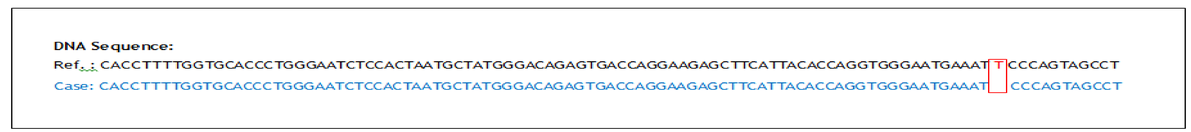
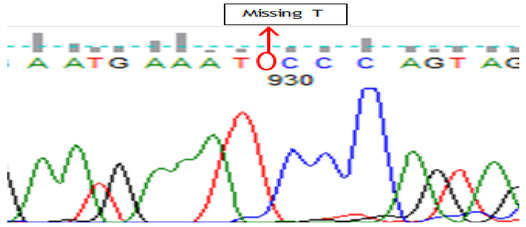


Figure 2: DNA sequence comparison between reference and primer.

Whole Blood From Mother:



Amniotic yte:

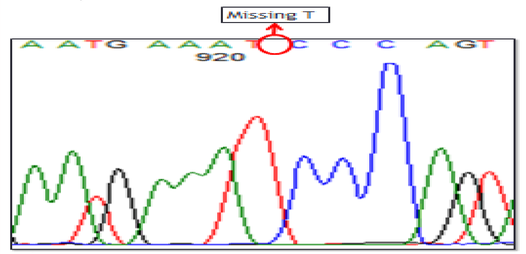


Figure 3: Capillary Sequence from amniocyte and whole blood from mother –derived DNA amplified with primer sets 2669bp-4797bp.

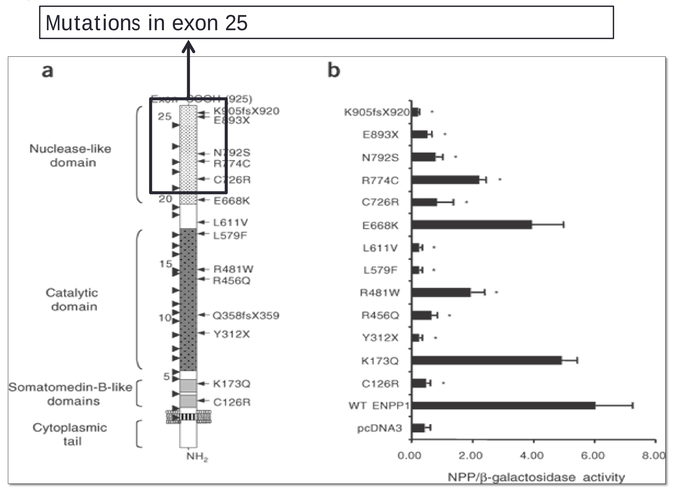


Figure 4: (a) Schematic structure of ENPP1 with positions of the catalytic site Thr256 (boxed) and mutations of ENPP1 identified in individuals with IIAC. Arrowheads on the left side point to boundaries between adjacent exons. (b) NPP activity in SaOS-2 cells transfected with empty vector (pcDNA3), wild-type (WT) ENPP1 and the indicated mutated constructs. Cells were cotransfected with 0.1 mug beta-galactosidase cDNA also in pcDNA3. Values are expressed as ratio of NPP/beta-galactosidase activity and represent the mean plusminus standard error of three independent experiments. *P < 0.0001 versus wild-type ENPP1 (Student's t-test). **Extracted from: doi:10.1038/ng1221**

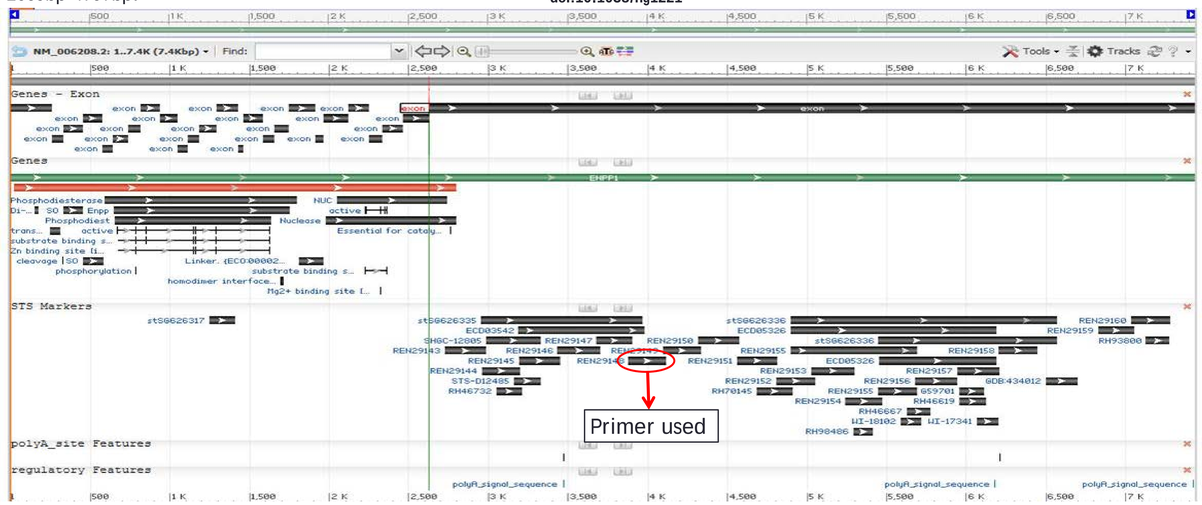


Figure 5: Overview of genomic structure of gene encoding ENPP1, open reading frame, and functional activity.
Source: https://www.ncbi.nlm.nih.gov/

Discussion

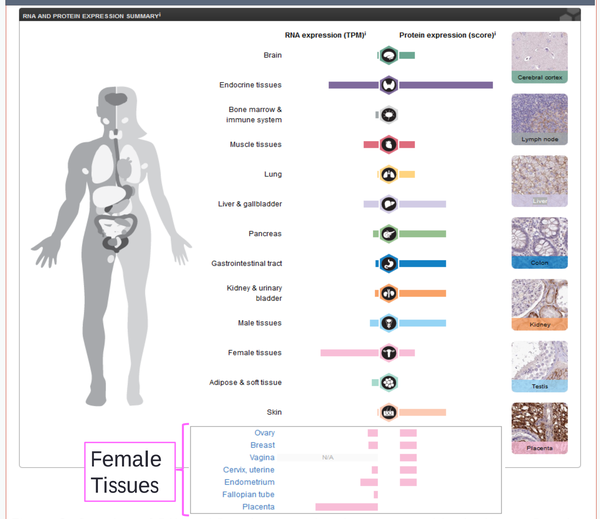


Figure 6: Overview of RAN and Protein expression data generated in the Human Protein Atlas project. Analyzed tissues are divided into color-coded groups according to which functional features they have in common. RNA-seq results generated in HPA are reported as number of transcripts per million (TPM). Protein expression scores are based on a best estimate of the "true" protein expression from a knowledge-based annotation,

Pathology related to *ENPP1* mutation: Ossification of the posterior longitudinal ligament of the spine, arterial calcification of infancy, Diabetes mellitus, non-insulin-dependent, hypophosphatemic rickets, Cole disease, and obesity.

In our study, placental pathology demonstrated decidual vasculopathy with medial hypertrophy of the spiral arterioles, multiple infarcts with villous ischemia, dystrophic calcifications and microgranular calcifications around villous capillaries.

Conclusion

We were able to demonstrate, using SNP Microarray, multiple contiguous regions of allele homozygosity across many chromosomes which confirmed our suspicions of a rare case of IIAC. In the regions of *enpp1 which* we have sequenced: 2669bp-4797bp and 5707bp-6197bp. The sequence shows a deletion of a T at 4849bp in Exon 25.

References

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