Stephany Tandy[1], Kelly Gonzalez[1], Layla Shahmirzadi[1], Wendy K Chung[2], Rebecca Sisson[2], Jessica Mester[3], Charis Eng[3], Amie Blanco[4], Jonathan Terdiman[4], Tina Pesaran[1], Frances Oh[1], Timothy Vo[1], Sha Tang[1], Chia-Ling Gau[1]
1) Ambry Genetics Corporation, Aliso Viejo, CA
2) Columbia University, New York, NY
3) The Cleveland Clinic , Cleveland, OH
4) UCSF, San Francisco , CA

The Ever Expanding Clinical Phenotype Associated With *PTEN* Gene Mutations: Two Remarkably Different Cases Involving the *PTEN* Mutation p.D24G Detected by Sanger Sequencing and Exome Sequencing Analyses.

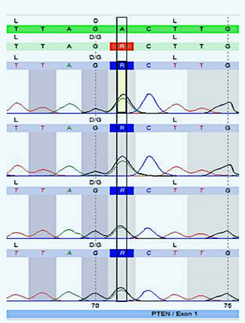
BACKGROUND

- Heterozygous mutations in the *PTEN* gene are associated with *PTEN* hamartoma tumor syndrome (PHTS), which includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS) and autism spectrum disorder.
- PHTS presents with variable expression and is characterized by a wide spectrum of clinical features.
- Numerous *PTEN* gene mutations have been reported in the literature, however there is currently minimal information about known alterations occurring at codon 24 (p.D24G, p.D24H, and p.D24Y).
- Here we present two unrelated individuals with strikingly different clinical phenotypes that both carry the *PTEN* pathogenic mutation p.D24G

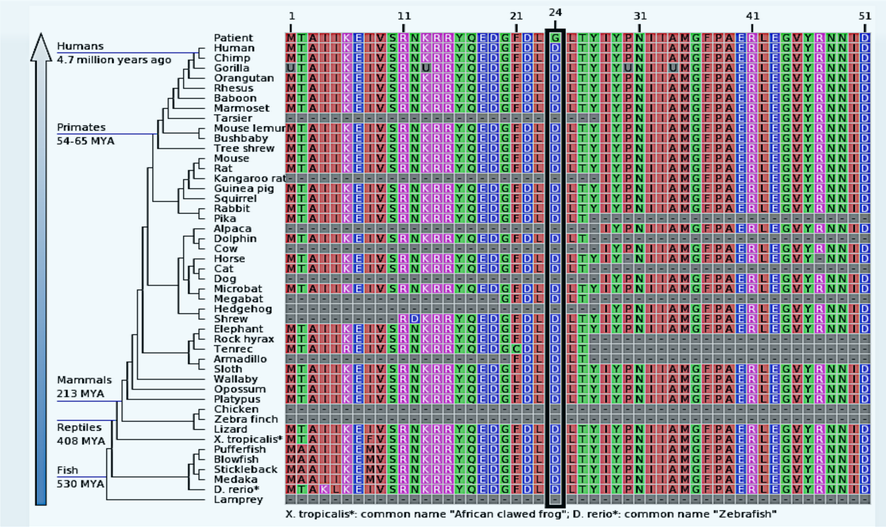
Family #1

- Individual diagnosed with multiple primary cancers
  - Leukemia at age 46
  - Breast cancer at age 49
  - Endometrial cancer at age 56
- Endometrial tumor analysis showed microsatellite stability and normal IHC
- Family history included an individual with bladder cancer in 60s and uterine cancer in 70s. Another relative was diagnosed with stomach cancer at 40
- *PTEN* mutation detected by Sanger sequencing
- Single site analysis clinical confirmation for the Cleveland Clinic

Proband #1: p.D24G



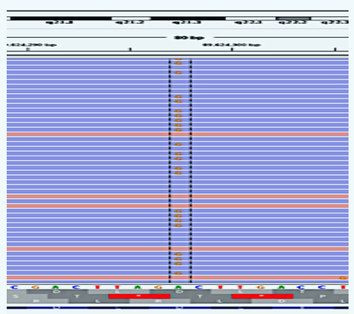
Amino Acid D24 Is Highly Conserved Across Evolution



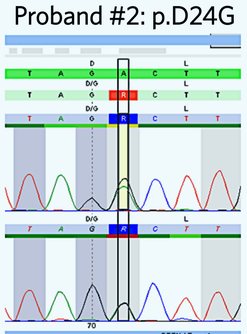
Family #2

- 2 year old Ashkenazi Jewish male whose clinical phenotype was highly atypical for PHTS
  - macrocephaly with frontal bossing
  - short stature
  - developmental delays
  - failure to thrive
- The family history was negative for similar phenotypes
- de novo *PTEN* mutation detected by exome sequencing and confirmed by Sanger sequencing
- No other mutations were identified

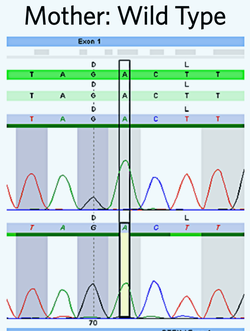
Proband #2: p.D24G



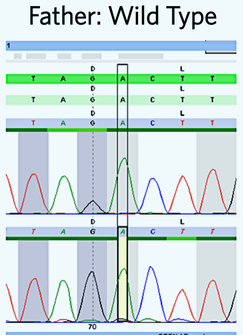
Proband #2: p.D24G



Mother: Wild Type



Father: Wild Type



METHODS

➢*PTEN* Single Site Analysis

Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized kit and quantified by agarose gel electrophoresis. Regions of gDNA corresponding to the *PTEN* gene are selectively amplified by polymerase chain reaction (PCR) and then sequenced in both sense and anti-sense directions.

➢Whole exome sequencing:

Trio samples from the proband and parents of family #2 or were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).

Bioinformatics annotation, filtering of variants, and Family history Inheritance-based Detection (FIND): HGMD, OMIM, the Single Nucleotide Polymorphism database (dbSNP) (Sherry, 2001), 1000 genomes, HapMap data (International HapMap, 2003) and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering included the removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection).

RESULTS

- Pathogenic mutation p.D24G (c.71A>G) in coding exon 1 of the *PTEN* gene

Take-Home Points

- The variability in phenotypic presentation observed in these two cases not only adds to the wide phenotypic spectrum of PHTS, but also provides more information about the p.D24G mutation.
- These cases also demonstrate the power and validity of exome sequence analysis and its ability to end the diagnostic odyssey.

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