Paired DNA/RNA genetic testing to uncover a cryptic PTEN pathogenic variant associated with diffuse ganglioneuromatosis

Abstract P-010

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Fox Chase Cancer Center Risk Assessment Program Celebrating 33 Years 1991-2024

BACKGROUND

- Phosphatase and tensin homolog (*PTEN*) hamartoma tumor syndrome (PHTS) is a family of related genetic disorders including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba Syndrome (BRRS), adult Lhermitte-Duclos disease, and autism spectrum disorders
- *PTEN* mutations remain unidentified in up to 40% of individuals with a clinical diagnosis of BRRS, 80% with Proteus syndrome, and 15% with classic CS.
- RNA based genetic testing can clarify clinical actionability of genetic variants indeterminate by DNA. Notably, RNA can aid in the detection of intronic (0-100 bps into the intron) and deep intronic variants (>100 bps into the intron) that may be missed by DNA sequencing of exons
- We review the case of a patient with PHTS associated with a deep intronic *PTEN* variant, and then detail a case series of patients with PHTS associated clinical findings and a PV or suspicious VUS identified by RNA testing.

METHODS

IRB

- The case report and collaborative research (Ambry Genetics) is IRB approved (FCCC IRB #24-9030) Research by Ambry is IRB exempt (issued by WCG).

CASE SERIES

- Patients undergoing RNA testing through Ambry Genetics and found to have an intronic pathogenic variant leading to a splice site variant were identified by laboratory personnel. DNA/RNA testing results were linked to clinical data gathered through a lab test requisition
- Sashimi plots of RNA splice variants were prepared by Ambry Genetics

DNA and RNA-based clinical testing by +RNAinsight

- DNA panel testing was performed on all patients using one of several tests: CustomNext Cancer +RNAinsight, CancerNext +RNAinsight, and CancerNext-Expanded +RNAinsight
- RNA was isolated using standardized methodology and quantified. RNA is converted to complementary DNA (cDNA) by reverse transcriptase polymerase chain reaction (RT-PCR).
- Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed PCR and NGS
- +RNAinsight analyzes transcripts for up to 91 genes. Any transcripts found are compared to a human reference pool.
- The absence or presence of RNA transcripts meeting quality thresholds is incorporated as evidence towards classification of DNA variants.
- Any regions not meeting RNA quality thresholds are excluded from analysis. The results from +RNAinsight are used to provide functional RNA information to further support classification of DNA variants.

INDEX CASE

- 64 yo female referred by community GI due to extensive ganglioneuromatosis of colon and rectum
  - Colonoscopy 4 years prior indicated extensive "hyperplastic/inflammatory" polyposis – likely pathology misread
  - EGD reveals one hyperplastic polyp of duodenum
- Personal history of R breast DCIS at age 42 and L breast ER/PR positive Stage IIA invasive breast cancer at age 53
- Personal history of microinvasive papillary thyroid cancer within multinodular goiter at age 54
- Remote history of condyloma of oral mucosa
- Macrocephalic; OFC = 59.5 cm
- No formal dermatology exam or biopsies:
  - Exam notes tongue papules and possible trichilemmomas of face
  - Extensive acrochordons of axilla/neck
- Germline DNA testing negative (77 gene panel)
- RNA testing reveals LP variant in *PTEN* c.209+2047A>G

FIGURE 1: INDEX PATIENT PEDIGREE

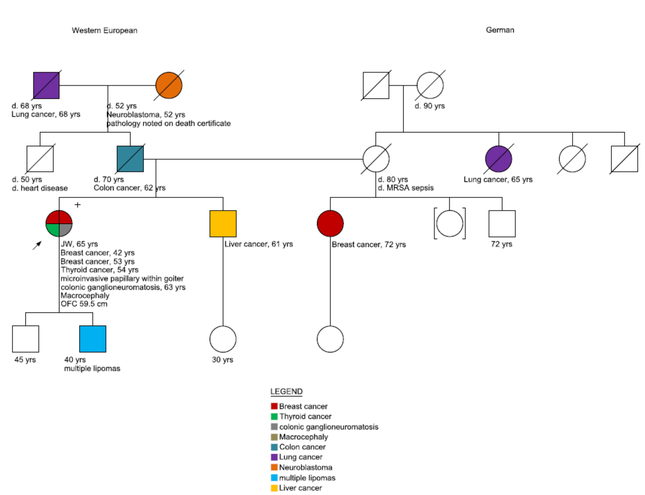
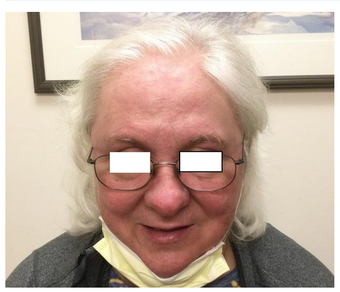
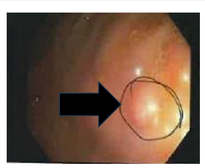
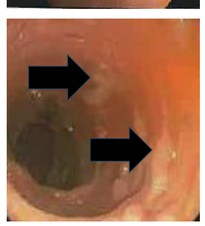


FIGURE 2: COWDEN FEATURES



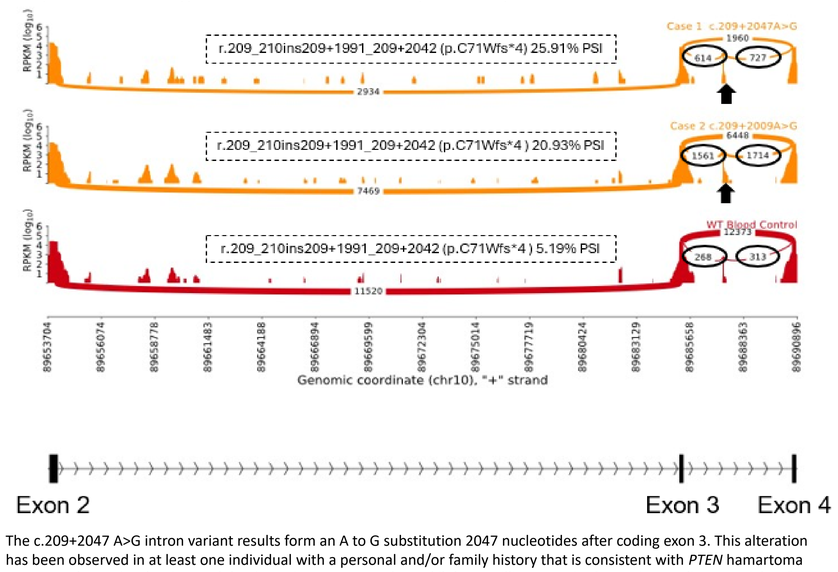




Patient is macrocephalic with OFC of 59.5 cm. Facial trichilemmomas noted on exam (above). Ganglioneuromas in the colon (right)

FIGURE 3: Intronic variant Sashimi plots

Sashimi plots from capture RNA-seq data (+RNAinsight®) represent relevant alignments and exon junction-spanning reads as arcs with read counts as displayed. Variants described using NM_000314.4. Representative wild-type blood control chosen from a pool of samples without an associated *PTEN* alteration.



The c.209+2047 A>G intron variant results form an A to G substitution 2047 nucleotides after coding exon 3. This alteration has been observed in at least one individual with a personal and/or family history that is consistent with *PTEN* hamartoma tumor syndrome (Ambry internal data). Y axis, RPKM = reads per kilobase millions, X axis, Genomic coordinate. Height of nucleotide locations indicates read depth.

TABLE 1: Intronic and deep intronic variant cases

FAM #	PRO AGE	PRO SEX	PRO TUMOR	*PTEN* PV	CLASS	CC/PEDS	OTHER PV/VUS	TESTED FAMILY	OTHER FEATURES
1	30	F	Pap thyroid (25), salivary (31), breast (38)	c.493-20_497del25	P	Pro 8	None	Moth (-), Sis (-)	None
2	60	F	Breast (42, 53)	c.209+2047A>G	VLP	Pro 22	None		Proband: Macro-c, GI ganglio-n, Goiter, thyroid nodule
3	40	F	Breast/DCIS (41)	c.209+2047A>G	VLP	Pro 20			Proband: Polyps(20+), goiter, macro-c
4	50	F		c.209+2047A>G	VLP	Pro 57	VUS *NF1* c.4760A>C; VUS *GALNT12* c.704A>G	Daugh (+)	Proband: Adenomas (multi), neurofibromas, CAL spots, tinnitus, goiter Relative: Ganglio-n, macro-c, penile freckling, lipoma
5	10	M		de novo VUS c.210-12C>G; IVS3-12	VLP	Ped 5			Proband: Macro-c, cerebellar tonsillar ectopia, FTT
6	30	F	Ovarian (28)	c.1027-1852A>G	VLP	Pro 0		Mother (-)	Relative: Lipoma
7	40	F	Uterine (44)	c.1027-1852A>G	VLP	Pro 6	VUS *SDHC* c.14T>C		
8	60	F	Breast (63)	c.1027-1852A>G	VLP	Pro 2			Skin (61)
9	40	M		c.1027-1852A>G	VLP	Pro 22		Daugh (+), Son (+)	Proband: Macro-c, colon polyps (sessile, hyperplastic), thyroid adenoma, penis freckling, Ruvalcaba-Myhre-Smith
10	70	F		c.1027-1852A>G	VLP	Pro 0	VUS *NF1* c.4396C>T	Son (-)	
11	60	M	Prostate, Gleason 9 (66)	c.1027-1852A>G	VLP	Pro 0, Sister 7, Daugh 6	VUS *NF1* c.3625G>T	Sis (+), Daugh (+), Niece (-), Sis (-)	Proband: Polyps (multiple), macro-c (daughter), skin tag (sister)
12	30	F	Ovary Sertoli-Leydig (28)	c.1027-1852A>G	VLP	Pro 0		Sis (-)	
13	40	F	Uterine (46)	c.1027-1852A>G	VLP	Pro 6		Daugh (-)	
14	60	M	CRC (56)	c.493-9349A>G	VLP	Pro 6			Proband: Adenomas (10+), Sessile serrated polyps (5+)
15	40	M		c.493-9349A>G	VLP	Pro 17 (Peds 5)	*BRCA2* PV c.2330dup; VUS *GALNT12* c.702 C>G		Proband: CAL spots, nevus, penile freckles, thyroid nodules, macro-c, learning disability, BRRS
16	40	F		c.209-2009A>G	VUS	Pro 1			Proband: Fibroid uterus, thyroiditis
17	30	M	Bladder (29)	c.210-791T>G	VUS	Pro 0			
18	40	F	Ovary (32)	c.210-791T>G	VUS	Pro 0			

RESULTS

- Including the index case, 18 total patients /families with *PTEN* intronic splice site variants were identified (see **Table 1**)
- 12 probands had cancer diagnoses (4 breast, 2 uterine, 1 papillary thyroid, 2 ovarian, 1 ovary-Sertoli-Leydig, 1 salivary, 1 bladder, 1 CRC, 1 prostate adeno)*1 proband had 3 cancers
- Also reported in families: macrocephaly (n=7), ganglioneuromas (N=2), penile freckling (n=2) and café-au-lait spots (N=2), developmental delay/learning disability (N=2), goiter (n=3) and thyroid nodules (N=3), neurofibromas (N=1)
- 1 family had a clinical diagnosis of FAP, 1 FAP/BRRS, and 1 BRRS in infancy
- 3 families had concurrent *NF1* variants/VUS (c.4760A>C, c.4396C>T, c.3625G>T) and one family had a concurrent *BRCA2* pathogenic variant (c.2330dup)

LIMITATIONS

- Personal and family history data collected on the clinical laboratory test requisition form is limited, unverified and sometimes not provided

CONCLUSIONS

- Significant opportunity exists to improve the yield of germline genetic testing in PTHS with RNA
- Identification of cryptic PTEN variants not detected by DNA with limited intronic probe coverage illustrates the added value of RNA
- More expansive testing of clinical CS and other clinical PHTS families can help better refine the prevalence of intronic/splice variants and the clinical phenotype associated with these variants

COLLABORATIVE RESEARCH

- We are developing a collaborative research study with Ambry Genetics to testing clinical CS/clinical PHTS with RNA testing.
- Participation will involve a genetics provider completing an online clinical criteria checklist, and if eligibility is met, electronic informed consent from the patient, a brief clinical survey for the provider, and a blood specimen sent to Ambry.
- Results will be clinical grade and released directly to the provider upon completion

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