Characterization of *TP53* Sequencing Variants Initially Detected in Peripheral Blood using NGS Analysis

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BACKGROUND

- Individuals with germline pathogenic variants (PVs) in *TP53* have Li-Fraumeni Syndrome (LFS), which is associated with a high cancer risk and early age of diagnosis.
- Previous work has shown that ~40% of *TP53* PVs detected by our laboratory have Next-Generation Sequencing (NGS) allele frequencies between 10–30% and are suspected to be somatic mosaic variants.
- Internal evidence demonstrates that NGS read frequencies for somatic PVs can increase over time to overlap with those observed for true germline heterozygotes (30–70%).
- Given the severe clinical implications of germline PVs in *TP53* and the relatively recent recognition of the prevalence of somatic *TP53* PVs, it has now become critical that apparent germline PVs be confirmed to enable appropriate medical management.
- Here, we present findings from a commercial testing laboratory program that offers confirmatory analysis to individuals with an apparent germline *TP53* PV.

METHODS

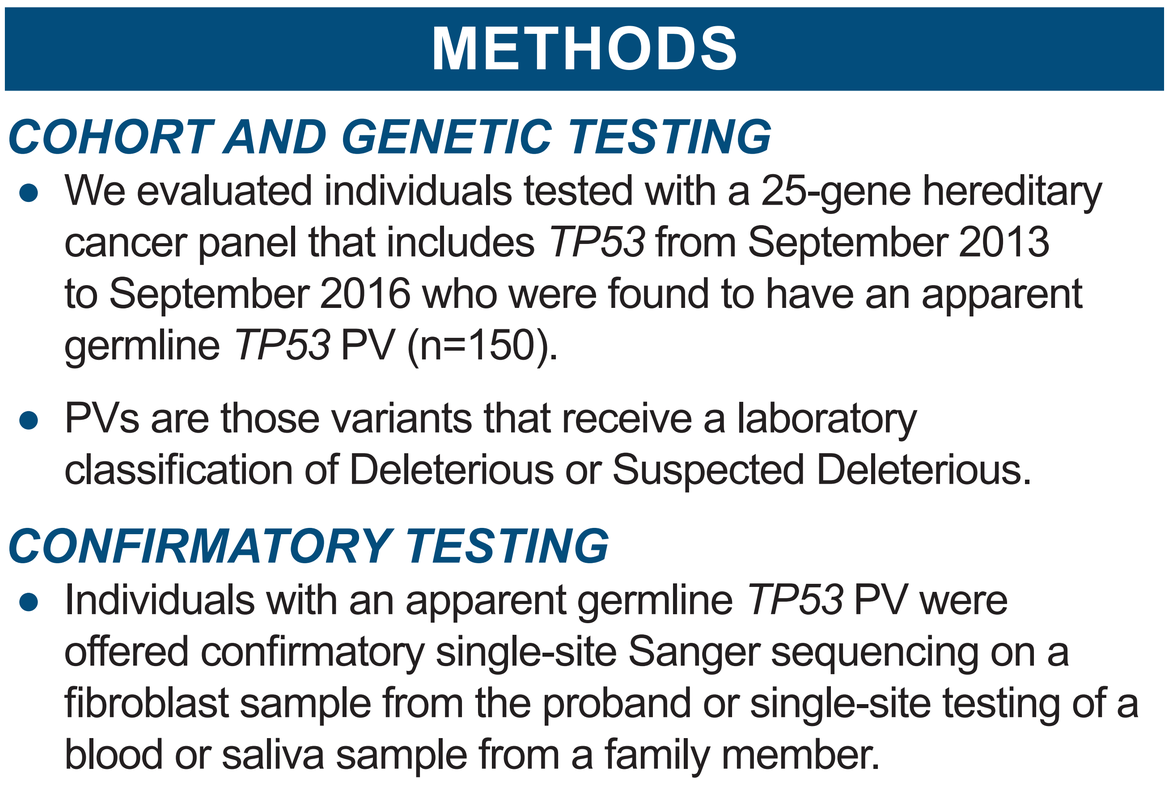
COHORT AND GENETIC TESTING

- We evaluated individuals tested with a 25-gene hereditary cancer panel that includes *TP53* from September 2013 to September 2016 who were found to have an apparent germline *TP53* PV (n=150).
- PVs are those variants that receive a laboratory classification of Deleterious or Suspected Deleterious.

CONFIRMATORY TESTING

- Individuals with an apparent germline *TP53* PV were offered confirmatory single-site Sanger sequencing on a fibroblast sample from the proband or single-site testing of a blood or saliva sample from a family member.

Figure 1. Possible Outcomes of Confirmatory Testing

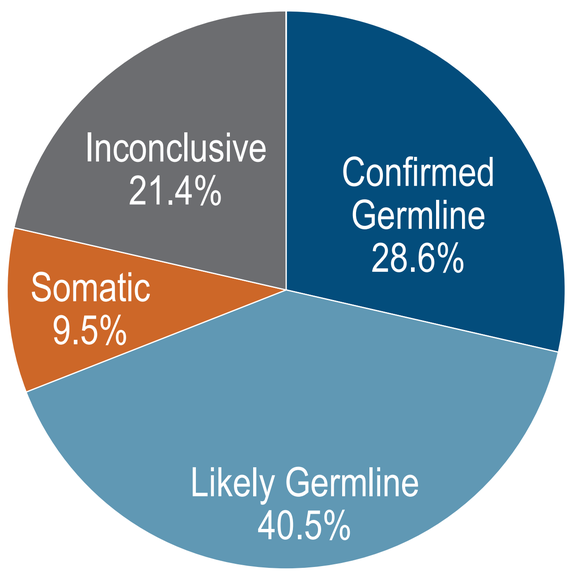


*Mutation may be post-zygotic mosaic in proband

RESULTS

- 42 individuals with *TP53* PVs have participated in additional testing.
  - 21 (50.0%) family testing
  - 19 (45.2%) fibroblast testing
  - 2 (4.8%) family testing and fibroblast testing
- This testing has provided additional evidence regarding the PV origin (germline or somatic) for 33 (78.6%) individuals thus far (Figure 2).
- The clinical presentation of the 4 patients with confirmed somatic PVs is shown in Table 1.
- As part of this testing program, 23 family members were found to have a germline or likely germline PV in *TP53*.

Figure 2. Results of Confirmatory Testing



Test Result	Family Testing	Fibroblast Testing
Germline	12	1
Likely Germline	4	13
Somatic	0	4
Inconclusive	7	3
Total*	23	21

*2 patients underwent family and fibroblast testing (1 with a germline result; 1 with an inconclusive result)

Table 1. Clinical Presentation of Patients with Somatic PVs

Allele Frequency	Age at Testing	PHx	FHx
39.9%	49	None	Maternal Aunt - EC, 30s
34.5%	67	EC, 55	Maternal Relatives - BC, OC, PC; 50s & 60s Maternal Uncle - CRC, 48
29.3%	47	BC, 43	Not Specified
56.5%	49	None	Maternal Aunt - OC, 35; Maternal Aunt - BC, 37

BC, Breast Cancer; CRC, Colorectal Cancer; EC, Endometrial Cancer; PC, Prostate Cancer; OC, Ovarian Cancer

CONCLUSIONS

- In this ongoing program, we have demonstrated that *TP53* PVs detected with NGS read frequencies consistent with an inherited PV can be either germline or somatic in origin.
- The confirmation of germline *TP53* PVs in patients with a clinical presentation inconsistent with LFS may have a significant impact on medical management decisions, while confirmation of a somatic PV may prevent inappropriate patient care.
- Overall, this demonstrates the value of confirmatory testing in individuals with apparent germline *TP53* PVs.

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