# Detection of Somatic Variants in Peripheral Blood Lymphocytes Using a 25-Gene Hereditary Cancer Panel

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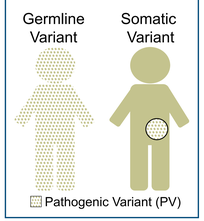
## BACKGROUND

- Next Generation Sequencing (NGS) can determine the proportion of variant DNA in a sample by quantifying the read frequency for each variant.
- The vast majority of variants identified by NGS have read frequencies of ~50%, consistent with these variants being present in the germline.
- NGS can also detect significant deviations from this 50% read frequency that may indicate a variant is not present in the germline, but may be somatic (Figure 1).
- With the increased use of NGS in hereditary cancer testing, somatic variants will be more frequently detected and may complicate the interpretation of test results.
- Here we report on the detection of potentially somatic variants.

***Figure 1. Somatic vs Germline Variants***




## METHODS

### *COHORT*

- 222,098 consecutive individuals who underwent clinical genetic testing with a 25-gene hereditary cancer panel were assessed.
- Individuals were ascertained based on clinical suspicion of hereditary cancer risk. Clinical information was obtained from the provider completed test request form.

### *GENETIC TESTING*

- The gene panel included *APC*, *ATM*, *BARD1*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *SMAD4*, *STK11*, and *TP53*.
- PVs are those variants that receive a laboratory classification of Deleterious or Suspected Deleterious.
- Variants were evaluated based on the NGS read frequency (Table 1). Additional information was considered, if available.

***Table 1. NGS Interpretation***

| Read Frequency | Typical Interpretation (in the absense of additional information/considerations) |
|---|---|
| 30% – 70% | Germline |
| 10% – 30% | Likely Somatic |
| <10% | Not evaluated |

### *STATISTICAL ANALYSIS*

- The clinical characteristics of individuals found to carry a likely somatic PV were compared to those with a germline PV and those with no PV.
- Fisher's Exact tests were performed to determine the differences between the prevalence of likely somatic PVs by age division.
- False Discovery Rate controlling adjustments were used for multiple comparisons. Adjusted p values < 0.05 were considered statistically significant.

## RESULTS

- 137 (0.06%) individuals were identified as carrying a likely somatic variant (Table 1).
- The majority of likely somatic PVs were in *TP53* (n=73, 52.5%) (Table 1).
  - Likely somatic PVs accounted for 38.8% of all PVs identified in *TP53* (Figure 2).
- Likely somatic PVs were relatively common in *ATM* (n=20, 14.4%) and *CHEK2* (n=27, 19.4%).
  - This accounted for only 1.36% and 1.47% of all PVs in *ATM* and *CHEK2*, respectively (Figure 2).
- 21/137 (15.3%) individuals with a likely somatic PV had a second, germline PV.
  - 3 individuals had likely somatic and germline PVs in the same gene (*TP53* – 1, *ATM* – 2).
- The mean age at testing for individuals found to carry a likely somatic PV was 59.3 years.
  - This is older than individuals with germline PVs (49.1 years) or no PVs (48.3 years).

***Table 1. Distribution of Likely Somatic PVs****

| Gene | Likely Somatic PVs (N) | % of PVs in Gene |
|---|---|---|
| *TP53* | 73 | 38.83% |
| *CHEK2* | 27 | 1.47% |
| *ATM* | 20 | 1.36% |
| *BRCA2* | 6 | 0.18% |
| *BRCA1* | 3 | 0.10% |
| *NBN* | 3 | 0.71% |
| *APC* | 2 | 0.88% |
| *CDH1* | 1 | 1.15% |
| *MSH6* | 1 | 0.17% |
| *PALB2* | 1 | 0.09% |
| *PMS2* | 1 | 0.16% |
| *STK11* | 1 | 6.25% |
| **Total** | **139** | **0.71%** |

*Included individuals with >1 likely somatic PV
NOTE: Likely somatic PVs were not identified in *BARD1, BMPR1A, BRIP1, CDK4, CDKN2A, EPCAM, MLH1, MSH2, MUTYH* (biallelic), *PTEN, RAD51C, RAD51D, SMAD4*.

***Figure 2. Proportion of Likely Somatic PVs by Gene***

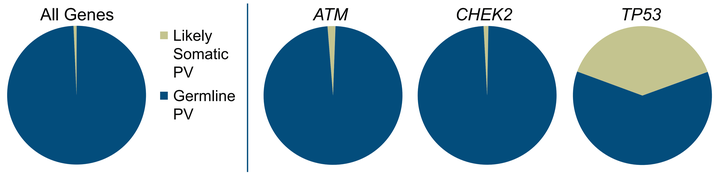
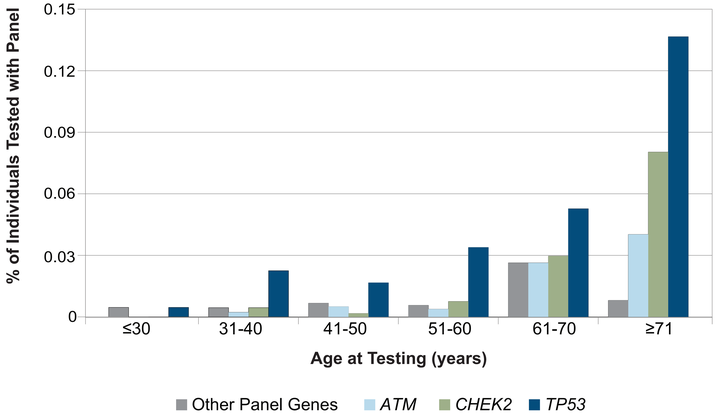



***Figure 3. Likely Somatic PVs Identified by NGS Stratified by Age***




- The frequency of likely somatic variants identified by NGS increases with age, especially for *TP53*, *ATM*, and *CHEK2* (Figure 3).
- Individuals who underwent panel testing over the age of 60 had a statistically higher probability of carrying a likely somatic PV than those tested before age 60 (p<0.001).
  - This remained significant even when *TP53*, *ATM*, and *CHEK2* were excluded (p=0.014).
- 87.9% of individuals with likely somatic PVs had a personal incidence of cancer (Table 2).
  - This is higher than individuals with germline PVs (62.9%) and those with no PVs (44.0%).

***Table 2. Personal Cancer History***

| | No Cancer | Affected |
|---|---|---|
| **Total – All Genes** | | |
| Likely Somatic | 14 (12.1%) | 102 (87.9%) |
| Germline | 5,577 (37.1%) | 9,440 (62.9%) |
| ***TP53*** | | |
| Likely Somatic | 7 (10.6%) | 59 (89.4%) |
| Germline | 13 (12.3%) | 93 (87.7%) |
| ***ATM*** | | |
| Likely Somatic | 4 (33.3%) | 8 (66.7%) |
| Germline | 576 (41.8%) | 802 (58.2%) |
| ***CHEK2*** | | |
| Likely Somatic | 1 (4.3%) | 22 (95.7%) |
| Germline | 738 (42.8%) | 985 (57.2%) |

NOTE: 21 individuals with a likely somatic PV were excluded due to a second, germline PV (*TP53* – 7, *ATM* – 7, *CHEK2* – 3); 147 individuals with a germline PV were excluded due to a second, germline PV (*TP53* – 1, *ATM* – 59, *CHEK2* – 87)

## CONCLUSIONS

- The detection of likely somatic PVs in this cohort was rare (0.06%), with the majority (86.3%) occurring in *TP53*, *ATM*, and *CHEK2*.
- The identification of likely somatic PVs in *ATM*, *CHEK2* and *TP53*, which are known to be disrupted early in tumorigenesis, support previous studies showing that somatic variants in peripheral blood are associated a 12.9-fold increased risk or hematologic cancer.[1]
- Relative to the overall testing cohort, individuals with likely somatic PVs were older and had a higher incidence of cancer.
- Together with previous studies showing accumulation of somatic variants with age[1,2] and chemotherapy-enhanced clonal expansion of blood cells carrying somatic *TP53* PVs,[2] this suggests that somatic variants identified with NGS may be due to amplification of a randomly acquired variant following chemotherapy for an existing cancer diagnosis.

## REFERENCES

1. Genovese G, et al. *N Engl J Med*. 2014;371:2477-2487.
2. Wong TN, et al. *Nature*. 2015;518:552-555.

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