Incidental germline findings from tumor molecular profiling for precision oncology: is it common and how to manage?

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BACKGROUND

- Complex molecular profiling via NGS is gaining recognition as an in vitro companion diagnostic aid in clinical decision-making
- High information yield provided by NGS frequently results in "incidental", or secondary findings outside primary DNA analysis indication
- Germline variants associated with cancer hereditary syndromes are major type of secondary findings during companion diagnostics via NGS. Such variants are frequently missed in complex molecular profiling reports
- There are no guidelines covering questions of management, reporting and clinical application of incidental germline findings
- Here, we report our first-hand experience with NGS analysis of a large population of cancer patients. We present the statistics on identified genetic alterations and their interpretations, along with a detailed dissection of methodological obstacles faced in course of the identification of such incidental findings

METHODS

- 183 unselected adult patients were referred for comprehensive molecular profiling at the discretion of their oncologists
- Tumor-only (FFPE) sequencing was performed. All patients were profiled on the Comprehensive Cancer Panel (Ion Torrent). For 132 patients, additional sequencing of BRCA1/2, ATM, and CHEK2 genes was performed. Analysis was focused only on variants in genes potentially associated with the development of hereditary cancer syndromes (37 genes)
- The discrimination between somatic and likely-germline missense mutations was performed employing ISOWN (Kalatskaya et al. 2017) with further manual curation and manual tools
- Following ACMG guidelines, clinical interpretation of germline variants or variants of uncertain origin was performed to classify them into pathogenic (PV), likely pathogenic (LPV), benign (BV), likely benign (LBV) variants, or variants of uncertain significance (VUS)

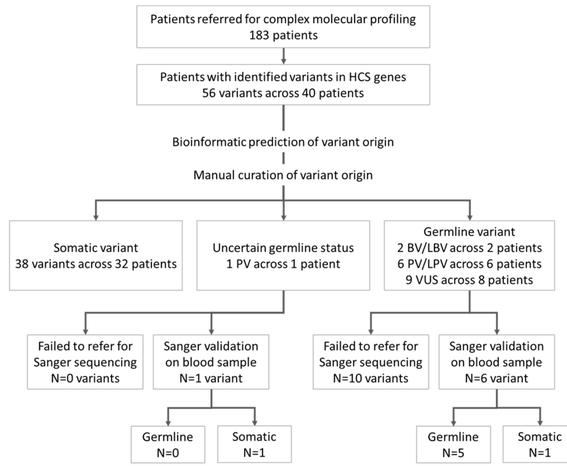
RESULTS

Table 1. Patient characteristics

Total patients	183
Age (years) at disease manifestation	
<40	14 (7.7%)
40-49	19 (10.4%)
50-59	21 (11.5%)
60-69	23 (12.6%)
70-79	11 (6.0%)
≥ 80	2 (1%)
Unknown	93 (50.8%)
Sex, Female, n (%)	108 (59%)
Disease stage, n (%)	
I	7 (3.8%)
II	15 (8.2%)
III	12 (6.6%)
IV	26 (14.2%)
Not allowed to collect / not reported/ unknown	123 (67.2%)
Primary tumor site, n (%)	
Colon and rectum	34 (18.6%)
Pancreatic	24 (13.1%)
Lung	18 (9.8%)
Ovary / Fallopian tube	16 (8.7%)
Breast	15 (8.2%)
Stomach	11 (6.0%)
Cervix	8 (4.4%)
Skin / melanoma	8 (4.4%)
Soft tissue	7 (3.8%)
Other, including unknown primary	42 (22.9%)

- In total, from a sample of 183 patients, we detected 56 unique variants
- Mutations found were classified as somatic, germline homozygous, germline heterozygous, or variants of uncertain origin based on machine learning algorithms (ISOWN) followed by manual validation or, for indel variants, based on manual validation only
- ISOWN predictions were concordant with the results of manual validation for the 41 (97%) missense variants, including 10 germline and 31 somatic variants.
- The most commonly mutated gene was TP53, which accounted for 48.2% of all the detected variants. All of the variants in TP53 were somatic, based on the results of both ISOWN and manual validation.

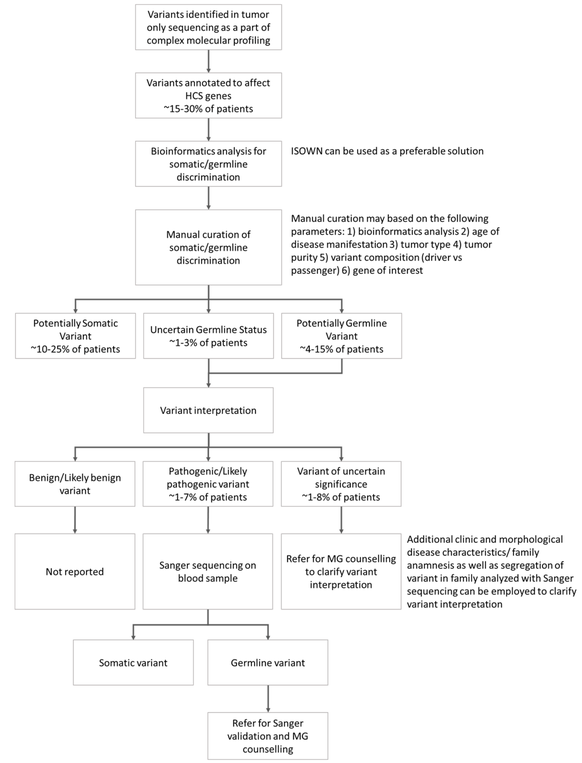
Figure 1. Study design and major results of variant detection and validation



*PV - pathogenic variant, LPV - likely pathogenic, BV - benign, LBV - likely benign, VUS - variant of uncertain origin.

- Mutations in DDR genes (ATM, BLM, BRCA1, BRCA2, MLH1, MSH6, NBN, PMS2) accounted for up to 40% of the variants. The majority of observed variants were detected in patients with colorectal (35.7% of all variants), gynecological (21.2%), and pancreatic (12.5%) cancers.
- A total of 38 variants across 32 patients were classified as somatic
- A total of 17 potentially-germline variants were detected in 14 (8%) patients

Figure 2. Proposed framework for managing patients with detected variants in Hereditary Cancer Syndrome (HCS) associated genes. MG - medical genetics



Conclusion

- Routine tumor molecular profiling revealed potentially-germline variants in 14 (8%) patients with various tumor types referred for tumor molecular profiling
- While the prediction of the variant origins may be done by computational tools, manual curation of the tumor-only sequencing results is paramount
- We suggest adding an additional category of "variants of uncertain origin", which is of use when determining the origin of the sequencing variants
- We highlight the importance of Sanger sequencing in patients' normal tissue for validation of the origin of PV/LPV/VUS variants that are either potentially germline, or of uncertain origin