Preliminary experience on testing hereditary gastrointestinal tumor risk using multi-gene panel

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Introduction

The use of next generation sequencing (NGS) in disease risk assessment is now widely employed by clinical laboratories in order to detect genetic risk factors of many conditions, including hereditary cancer. We have developed the Color Test, a 30-gene panel for hereditary cancer risk, including 11 genes associated with increased risk for hereditary gastrointestinal cancer: *APC, BMPR1A, CDH1, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4*, and *STK11*. Of the first 3,311 individuals to receive the Color Test, 125 individuals were found to carry a pathogenic or likely pathogenic variant in one of the gastrointestinal risk related genes. Here, we describe the demographics and results obtained from those 125 individuals.

Methods

The Color Test was designed to assess a panel of 30 genes associated with increased risk for hereditary cancer including breast, ovarian, colorectal, melanoma, pancreatic, prostate, uterine and stomach cancer (*APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A* (*p14ARF* and *p16INK4a*), *CHEK2, EPCAM, GREM1, MITF, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11*, and *TP53*). In *PMS2* exons 12-15 were not analyzed. In several genes, only specific positions known to impact cancer risk were analyzed: *CDK4*: only chr12:g.58145429-58145431 (codon 24), *MITF*: only chr3:g.70014091 (including c.952G>A), *POLD1*: only chr19:g.50909713 (including c.1433G>A), *POLE*: only chr12:g.133250250 (including c.1270C>G), *EPCAM*: only large deletions and duplications including 3' end of the gene, *GREM1*: only duplications in the upstream regulatory region.

The Color Test was performed according to validated laboratory procedures at Color laboratory (Burlingame, CA), under CLIA and CAP regulations. Saliva specimens were collected using the Oragene DX 510 saliva collection device. DNA extraction was performed using standard methods. Library preparation was performed using KapaBiosystems HyperPlus reagents and target enrichment was performed using the Agilent SureSelect XT chemistry. Sequencing was performed on a Illumina NextSeq 500 instrument using the paired-end 150bp, High Output kit.

Sequence reads were aligned against GRCh37.p12 human reference genome with the Burrows-Wheeler Aligner [BWA-MEM][1]. SNVs and indels were called by the HaplotypeCaller module of GATK3[2]. CNVs were detected using dedicated algorithms[3]. The coverage requirements for reporting were ≥20X for each base of the reportable range and ≥50X for 99% of the reportable range. Median coverage typically ranged between 200-300X. Variants were classified according to the guidelines for sequence variant interpretation of the American College of Medical Genetics and Genomics[4]. All variants are evaluated by at least one variant scientist and one ABMGG board certified medical geneticist. Reported variants classified as pathogenic or likely pathogenic were confirmed by a secondary technology (Sanger sequencing, aCGH or MLPA). Ethnicity assignments and personal history of cancer were based on self-reported information.

Results

Table 1. Cohort demographic details

125 individuals were found to have a pathogenic variant in a hereditary gastrointestinal cancer risk associated gene. 11.2% of these individuals had a personal history of colon cancer, and 60.8% had no personal history of cancer. The majority of individuals were women, over the age of 40, and of Caucasian ethnic background.

		Number	Percent
Total		125	100.0%
Gender	Female	96	76.8%
	Male	29	23.2%
Age	18-30	8	6.4%
	31-40	14	11.2%
	41-50	35	28.0%
	51-64	33	26.4%
	≥65	35	28.0%
Ethnicity	Caucasian	63	50.4%
	Ashkenazi Jewish	36	28.8%
	Asian	4	3.2%
	Hispanic	4	3.2%
	African	2	1.6%
	Multiple Ethnicity	6	4.8%
	Unknown	10	8.0%
Personal History of Cancer	Breast Cancer	25	20.0%
	Colon Cancer	14	11.2%
	Melanoma Cancer	5	4.0%
	Ovarian Cancer	4	3.2%
	Pancreatic Cancer	0	0.0%
	Other Cancer	7	5.6%
	No Cancer	76	60.8%

Figure 1. Genes with pathogenic variants associated with hereditary gastrointestinal tumor risk

The number of individuals with a pathogenic/likely pathogenic variant in each gene. 26 individuals had pathogenic variants in Lynch syndrome genes. No pathogenic/likely pathogenic variants were found in the *CDH1, STK11, SMAD4, BMPR1A*, and *PTEN* genes in this cohort.

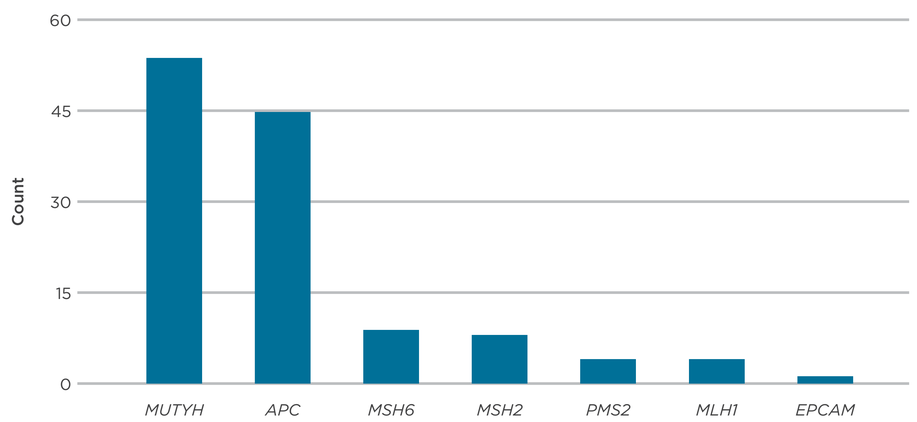

Table 2. Low penetrance alleles

92 alleles known to have lower penetrance, and therefore different screening recommendations than other pathogenic variants, were identified.

Gene	cHGVS	pHGVS	Count
APC	c.3920T>A	p.Ile1307Lys	41
MUTYH	c.1012C>T	p.Gln338*	1
	c.1101delC	p.Arg-368Glyfs*40	1
	c.1186+1G>T		1
	c.1187G>A	p.Gly396Asp	31
	c.1214C>T	p.Pro405Leu	1
	c.536A>G	p.Tyr179Cys	9
	c.734G>A	p.Arg245His	1
	c.933+3A>C		2
	c.934-2A>G		4
Total			**92**

Table 3. Individuals with two concurrent pathogenic/likely pathogenic variants in *MUTYH*

PB-1 is a 54 year old Chinese female with a personal history of ovarian cancer at age 51, 100 polyps found, and a breast biopsy with atypical hyperplasia. She has a family history of melanoma, gastric, stomach, and colorectal cancers.

PB-2 is a 33 year old Hispanic female with a personal history of colon cancer at age 31. She has a family history of breast cancer and melanoma.

PB-3 is a 37 year old Caucasian male with a personal history of colon cancer at age 37, with 20 polyps found.

Proband	Gene	cHGVS	pHGVS
PB-1	*MUTYH*	c.55C>T	p.Arg19*
		c.857G>A	p.Gly286Glu
PB-2	*MUTYH*	c.1187G>A	p.Gly396Asp
		c.721C>T	p.Arg241Trp
PB-3	*MUTYH*	c.1147delC	p.Ala-385Profs*23
		c.536A>G	p.Tyr179Cys

Conclusions

- 125 individuals with a pathogenic variant in a gene associated with hereditary gastrointestinal cancer risk were identified, and only 11.2% of these individuals had a personal history of colon cancer. These positive results could be used to inform preventative actions in the 60.8% of people who do not have a personal history of cancer.
- Pathogenic variants were most commonly identified in *MUTYH* and *APC*, and some of these were low penetrance alleles. The remaining pathogenic variants were identified in Lynch syndrome genes.
- Three individuals carried 2 concurrent pathogenic variants in *MUTYH*. All 3 had a personal history of cancer: 2 individuals had colon cancer and 1 individual had ovarian cancer.
- The majority of individuals in this cohort self-reported as Caucasian and female, indicating a need in the broader community for better outreach and education to minorities and males. Additionally, most of the individuals were over the age of 40, indicating the importance of increasing awareness and uptake of genetic testing within the younger population at an age for which preventative care is more relevant. Taken together, these data reinforce the continued need to increase awareness and broaden access to Hereditary Cancer Risk testing within the general population.

References

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