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Prenatal testing for variants associated with hereditary cancer risk

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1. Introduction

Prenatal (PN) genetic testing for inherited disorders has been performed for decades. A few of these disorders, such as Fanconi anemia and Bloom syndrome, include increased cancer risk as part of the phenotype, are already recommended for prenatal testing[1], and are associated with increased cancer risk in heterozygous carriers[2,3]. However, the spectrum of genes considered for PN testing is widening due to genetic testing for hereditary cancer risk (HCR) and inclusion of conditions with associated cancer risk in carrier screening panels. Additionally, recent studies implicate heterozygosity for variants in lysosomal storage disease (LSD) genes in HCR etiology[4]. Thus, parents requesting PN testing for familial variants may, either intentionally or inadvertently, obtain cancer-related information for their fetus. Currently, there is no specific professional guidance regarding such testing. In order to determine the prevalence of such testing we reviewed 630 consecutive PN specimens received in our laboratory for familial variant specific testing and identified 150 (24%) with a known or likely HCR component. These specimens were classified into four distinct categories. Our experience assessing PN specimens for variants associated with both a constitutional phenotype and HCR contributes to the points to consider in prenatal testing for hereditary cancer risk.

2. Methods

- Specimens were sent to our laboratory by referring clinicians for genetic testing. Familial variant information and clinical indication for testing was provided on the laboratory test requisition form.
- DNA was isolated from chorionic villus (N=77) and amniotic fluid (N=73) specimens and from blood for positive controls, when available.
- Marker analysis was performed with a multiplex STR system (PowerPlex® 16 System, Promega, Madison, WI) to rule out maternal cell contamination.
- Targeted gene regions were PCR amplified, Sanger sequenced, and assessed using SeqScape® Software v3.0 (Thermo Fisher Scientific, Waltham, MA).
- Positive results were reported using the numbering and nomenclature recommended by the Human Genome Variation Society (HGVS, http://www.hgvs.org/).

3. Results

Table 1 and Figure 1: Thirty-one prenatal specimens were assessed for familial variants in twelve different HCR genes. These include *ATM*, *BLM*, and *FANCC*, which cause autosomal recessive (AR) disorders of DNA repair or instability, *BRAF*, *KRAS*, *MAP2K2*, and *PTPN11*, associated with autosomal dominant (AD) RASopathy, and *APC*, *BTK*, *CREBBP*, *MPL*, and *RET*, each of which cause an AD disorder and are also typically associated with solid tumors or hematopoietic malignancy. The majority of requested variants were classified as pathogenic or likely pathogenic. Three variants of uncertain significance (VUS) had autosomal dominant inheritance and a family history with a previously affected child.

Sequencing results showed that 13 fetuses were positive and heterozygous for a familial variant, and 18 fetuses were negative. No homozygous or compound heterozygous results were obtained, however fetuses heterozygous for AD conditions and male fetuses hemizygous for X-linked conditions are predicted to be affected. Three fetuses had heterozygous positive results for a gene with AR inheritance and for which only one parental variant was requested. Full gene sequencing was performed for two of these cases and no additional variants were identified. The third case had multiple abnormal ultrasound findings and the patient declined additional testing.

Table 2 and Figure 2: There were 119 prenatal specimens assessed for familial variants in 16 different LSD genes. All cases were referred due to risk of being affected with the LSD, based on parental carrier status or other positive family history. The majority of the requested variants were classified as pathogenic or likely pathogenic. In two cases a familial VUS was requested.

Sequencing results showed that 26 fetuses were homo-, hemi-, or compound heterozygous (affected), 54 were heterozygous, and 39 were negative. In both cases where a VUS was requested the fetus was found to be compound heterozygous, one where there was a previously affected child and one with no further information available. Both of these cases were categorized as affected in this study. Of note, while the 54 heterozygous fetuses are not affected with an LSD, they may have an increased cancer risk associated with their carrier status.

4. Conclusions

- 24% of prenatal specimens (150/630) received in our laboratory for variant specific analysis involved genes with a known or possible HCR component.
- Of these 150 specimens, 44% were positive for the familial variant which is or may be associated with increased risk for hereditary cancer.
- These cases fall into four distinct HCR categories:
  1. An autosomal recessive disorder with malignancy as part of the phenotype
  2. An autosomal dominant disorder where the gene is part of an oncogenic pathway
  3. Primarily HCR, but along with an autosomal dominant or X-linked constitutional disorder
  4. An autosomal recessive LSD in which carriers have a possible associated HCR
- Of note, there are additional categories, not present in this data set, which should be considered in the context of prenatal testing for hereditary cancer risk. These may include genes associated with primarily or exclusively hereditary cancer risk, high penetrance, and early infantile to childhood onset, and genes associated with primarily or exclusively hereditary cancer risk with adult onset.
- Our data demonstrate that testing for familial variants associated with cancer risk has entered the prenatal domain and emphasize the need for careful consideration in the interpretation and reporting of variants associated with HCR in the PN setting. These findings may help in developing guidelines for pre- and post-testing genetic counseling, which should include discussion of incomplete penetrance, age of onset, treatment availability, family circumstances, and the benefits of prevention and early detection.

References

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Tables + Figures

Table 1. Categorized list of the twelve hereditary cancer risk genes in which variants were assessed, the genetic disorder caused by the variant, the predominantly associated cancer risk for each, and the number and status of prenatal cases.

Gene	Primary Genetic Disorder	Inheritance	Common Associated Cancer Risks	# Affected	# Carriers	# Negative	Total # Cases
ATM	Ataxia-telawngiectasia	AR	Leukemia, lymphoma, breast cancer	0	1	1	2
BLM	Bloom syndrome	AR	Leukemia, osteosarcoma, multiple other cancers	0	1	1	2
FANCC	Fanconi anemia type C	AR	Acute myeloid leukemia, multiple other cancers	0	4	1	5
BRAF	Noonan, LEOPARD, cardiofaciocutaneous syndromes	AD	Melanoma, lung cancer, colorectal cancer, others	1	NA	0	1
KRAS	Noonan syndrome	AD	Childhood leukemias, multiple solid tumors	1	NA	1	2
MAP2K2	Noonan syndrome	AD	Childhood leukemias, multiple solid tumors	0	NA	1	1
PTPN11	Noonan syndrome	AD	Childhood leukemias, multiple solid tumors	3	NA	8	11
APC	Familial adenomatous polyposis	AD	Colorectal cancer, small bowel cancer	1	NA	1	2
BTK	Bruton agammagloninemia	XL	Leukemia, lymphoma, multiple solid tumors	1	0	0	1
CREBBP	Rubenstein-Taybi syndrome	AD	Leukemia, lymphoma	0	NA	2	2
MPL	Essential thrombocytopenia	AD	Myeloproliferative disorders, acute myeloid leukemia	0	NA	1	1
RET	Hirschsprung disease	AD	Multiple endocrine neoplasia type 2	0	NA	1	1
			Totals:	**7**	**6**	**18**	**31**

AR = autosomal recessive, AD = autosomal dominant, XL = X-linked, NA = not applicable

Figure 1. Number of prenatal cases positive (affected or carriers) or negative for familial variants in hereditary cancer risk genes for the first three categories.

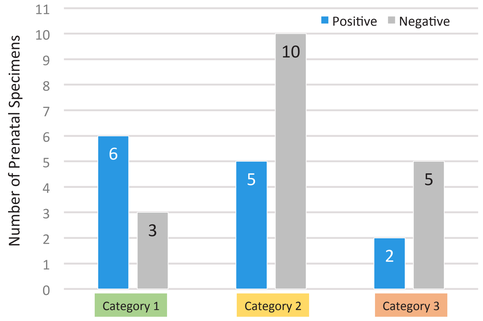
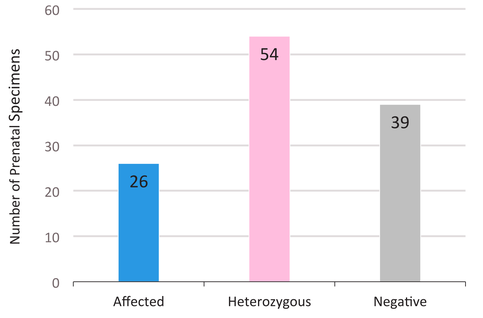


Table 2. List of the 16 lysosomal storage disease genes in which variants were assessed, the genetic disorder caused by the variant, and the number and status of prenatal cases.

Gene	Lysosomal Storage Disease	# Affected	# Carriers	# Negative	Total # Cases
ARSA	Metachromatic leukodystrophy	2	5	1	8
GAA	Glygogen storage disease type II (Pompe disease)	5	9	4	18
GALC	Globoid cell leukodystrophy (Krabbe disease)	2	7	6	15
GALNS	Muchopolysaccaridosis IVA (Morquio A syndrome)	0	2	0	2
GBA	Gaucher disease	4	14	3	21
GLB1	Muchopolysaccaridosis IVB	2	1	2	5
HEXA	Tay-Sachs disease	4	9	4	17
HEXB	Sandhoff disease	0	0	2	2
IDS	Muchopolysaccaridosis II (Hunter syndrome)	3*	0	0	3
IDUA	Muchopolysaccaridosis I (Hurler, Scheie and Hurler-Scheie syndromes)	1	4	8	13
MAN2B1	Alpha-mannosidosis	0	0	2	2
MCOLN1	Mucolipidosis IV	0	1	0	1
NPC1	Neimann-Pick type C disease	0	0	1	1
SGSH	Muchopolysaccaridosis IIIA (Sanfilippo A syndrome)	2	1	1	4
SMPD1	Niemann-Pick disease type A and B	1	1	4	6
SUMF1	Multiple sulfatase deficiency	0	0	1	1
	Totals:	**26**	**54**	**39**	**119**

* Hemizygous for X-linked disease

Figure 2. Number of prenatal cases affected (homo-, hemi-, or compound heterozygous), heterozygous carriers, or negative for familial variants in category 4, lysosomal storage disease genes.



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