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Full gene sequencing as a follow up to carrier screening: utilization and outcomes

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I. Introduction

Carrier screening facilitates informed reproductive decision making by identifying individuals at risk for having a child affected with a recessive disorder. ACOG Guidelines currently recommend that if a women is found to be a carrier for a specific condition, her reproductive partner should be offered screening[1]. In our laboratory, partners of identified carriers may choose carrier screening via an expanded screening panel or full gene sequencing of just the relevant gene(s). Full gene sequencing can also be used for prenatal testing when the partner of a carrier parent is not available or declines genetic testing.

II. Methods

Retrospective data for 658 adult and 84 prenatal cases received by our laboratory for gene-specific full gene sequencing were reviewed. Next generation sequence analysis was performed using the Agilent® SureSelect® XT enrichment method and the Illumina® next-generation sequencing platform. Regions of interest included all exons and splice junctions for each gene analyzed. Additionally, deep intronic regions were analyzed for known pathogenic mutations when applicable. Positive results were confirmed by Sanger sequencing. Clinical significance of variants was interpreted using internally developed and validated algorithms. Pathogenic, likely pathogenic, and variants of uncertain significance (VUS) were reported.

- To determine the proportion of cases tested as a follow up to carrier screening, clinical indications provided on the test requisition were reviewed (Figure 1).
- Of the 140 genes currently included in our expanded carrier screening, genes requested for gene-specific sequence analysis were summarized (Table 1).
- To assess clinical utility, variants identified by gene-specific full gene sequencing were compared with hypothetical results from expanded carrier screening (Table 2).
- In prenatal samples, variants identified were reviewed (Table 3).
- Follow-up prenatal testing cases sent to our laboratory after (potential) carrier couples were identified by gene-specific full gene sequencing were summarized (Table 4).

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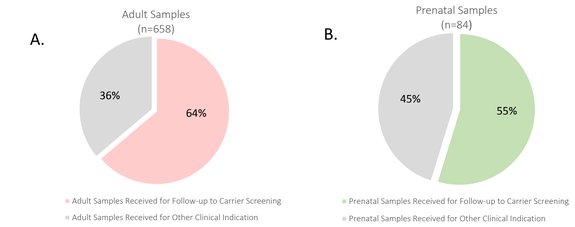
III. Results and Discussion

Figure 1: Proportion of cases tested as a follow-up to carrier screening



Discussion: Of all cases received for gene-specific full gene sequencing: A) 420/658 (64%) of adult cases were partners of individuals who were known or suspected to be carriers based on prior carrier screening; and B) 46/84 (55%) of prenatal cases had one carrier parent or a parent with a VUS based on prior screening.

Table 1: Genes requested for gene-specific sequence analysis as follow-up testing to carrier screening

Gene	Disease	Gene	Disease	Gene	Disease
ADA	Adenosine deaminase deficiency	*GCDH*	Glutaric acidemia type 1	*TPP1*	Neuronal ceroid-lipofuscinosis
ASL	Argininosuccinic aciduria	*G6PC*	Glycogen storage disease type Ia	*SMPD1*	Niemann-Pick disease types A and B
ATM	Ataxia-telangiectasia	*SLC37A4*	Glycogen storage disease type Ib	*NPC1*	Niemann-Pick disease type C
SACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay	*AGL*	Glycogen storage disease type III	*PAH*	Phenylalanine hydroxylase deficiency, includes phenylketonuria
BBS1	Bardet-Biedl syndrome, BBS1-related	*GLB1*	GM1 gangliosidosis and mucopolysaccharidosis type IVB	*PHGDH*	Phosphoglycerate dehydrogenase deficiency, PHGDH-related
BBS2	Bardet-Biedl syndrome, BBS2-related	*ALDOB*	Hereditary fructose Intolerance	*PKHD1*	Polycystic kidney disease, autosomal recessive
BBS10	Bardet-Biedl syndrome, BBS10-related	*CBS*	Homocystinuria, CBS-related	*GAA*	Pompe disease
RBB	Beta hemoglobinopathy	*ALPL*	Hypophosphatasia, autosomal recessive	*AGXT*	Primary hyperoxaluria type 1
BLM	Bloom syndrome	*TMEM216*	Joubert syndrome 2	*PCCB*	Propionic acidemia, PCCB-related
ASPA	Canavan disease	*LAMB3*	Junctional epidermolysis bullosa	*DHDDS*	Retinitis pigmentosa 59
CPS1	Carbamoyl phosphate synthetase I deficiency	*GALC*	Krabbe disease	*SLC17A5*	Salla disease
CPT2	Carnitine palmitoyltransferase II deficiency	*HADHA*	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency	*HEXB*	Sandhoff disease
RMRP	Cartilage-hair hypoplasia	*SURF1*	Leigh syndrome, autosomal recessive	*NEU1*	Sialidosis
ASS1	Citrullinemia type I	*BCKDHA*	Maple syrup urine disease type 1A	*ALDH3A2*	Sjogren-Larsson syndrome
MMACHC	Cobalamin C disease	*BCKDHB*	Maple syrup urine disease type 1B	*DHCR7*	Smith-Lemli-Opitz syndrome
MPL	Congenital amegakaryocytic thrombocytopenia	*ACADM*	Medium-chain acyl-CoA dehydrogenase deficiency	*SLC26A2*	Sulfate transporter-related osteochondrodysplasias, includes achondrogenesis type 1B, atelosteogenesis type 2, diastrophic dysp, rec. multiple epiphyseal dysp
PMM2	Congenital disorder of glycosylation type 1a	*ARSA*	Metachromatic leukodystrophy	*SLC22A5*	Systemic primary carnitine deficiency
CFTR	Cystic fibrosis	*MMAB*	Methylmalonic acidemia, MMAB-related	*HEXA*	Tay-Sachs disease
CTNS	Cystinosis	*MUT*	Methylmalonic acidemia, MUT-related	*FAH*	Tyrosinemia type 1
DLD	Dihydrolipoamide dehydrogenase deficiency	*GNPTAB*	Mucolipidosis type II and III, GNPTAB-related	*PCDH15*	Usher syndrome type IF
DPYD	Dihydropyrimidine dehydrogenase deficiency	*MCOLN1*	Mucolipidosis type IV	*CLRN1*	Usher syndrome type IIIA
ADAMTS2	Ehlers-Danlos syndrome type VIIC	*IDUA*	Mucopolysaccharidosis type I	*ACADVL*	Very long-chain acyl-CoA dehydrogenase deficiency
IKBKAP	Familial dysautonomia	*SGSH*	Mucopolysaccharidosis type IIIA	*FKTN*	Walker-Warburg syndrome, FKTN-related
ABCC8	Familial hyperinsulinism, ABCC8-related	*GUSB*	Mucopolysaccharidosis type VII	*ATP7B*	Wilson disease
MEFV	Familial Mediterranean fever	*NEB*	Nemaline myopathy, NEB-related	*PEX1*	Zellweger spectrum disorder, PEX1-related
FANCC	Fanconi anemia group C	*NPHS1*	Nephrotic syndrome, NPHS1-related	*PEX6*	Zellweger spectrum disorder, PEX6-related
GALT	Galactosemia, GALT-related	*NPHS2*	Nephrotic syndrome, NPHS2-related	*PEX12*	Zellweger spectrum disorder, PEX12-related
GBA	Gaucher disease	*PPT1*	Neuronal ceroid-lipofuscinosis	*PEX26*	Zellweger spectrum disorder, PEX26-related

Legend:
Black: Genes sequenced in adult specimens
Green: Genes sequenced in prenatal specimens
Blue: Genes sequenced in both prenatal and adult specimens

Discussion: Of the 140 genes available for individual full gene sequencing, 84 genes were requested as follow-up to carrier screening results.

Table 2. Distribution of results among 420 adult partners of known carriers

Result in adult partners	Full gene sequencing result	Hypothetical expanded carrier screening result
Pathogenic or likely pathogenic variant	12	12
VUS	19	0
Negative (no variants, or benign or likely benign variants)	389	408
Total	420	420

Discussion:

- All pathogenic or likely pathogenic variants identified by full gene sequencing would have been identified by the expanded carrier screening performed in our laboratory.
- All 19 VUS identified by full gene sequencing would not have been reported and/or identified by the expanded carrier screening performed in our laboratory.
- Based on variant categories of pathogenic, likely pathogenic, and VUS, 31 couples were identified as being potentially at risk for having an affected child.

Table 3: Distribution of results among 46 prenatal diagnostic testing cases

Prenatal result	Full gene sequencing result
One pathogenic or likely pathogenic variant	25
One pathogenic and one VUS	1
One VUS	1
Negative (no variants, or benign or likely benign variants)	19
Total	46

Discussion:

- The two VUS identified by full gene sequencing would not have been reported and/or identified by the expanded carrier screening performed in our laboratory
- 25 fetuses inherited only the known parental variant and were predicted to be at least a carrier of the relevant disorder.
- One fetus inherited both a parental mutation and a VUS from the other parent and was predicted to be at least a carrier.

Table 4: Summary of follow up prenatal diagnosis performed at our laboratory for (potential) carrier couples subsequent to partner testing by gene-specific full gene sequencing

Couple Number	Gene(s)	Parent 1 Carrier Screening Result(s)	Partner (Parent 2) Full Gene Sequencing Result(s)	Follow-up Prenatal Testing Result(s)
1	*PMM2*	c.422G>A (p.R141H)	c.422G>A (p.R141H)	Positive for one copy of c.422G>A (p.R141H)
2	*HEXA*	c. 1274_1277dupTATC (p.Y427fs)	c.1073+1G>A	Positive for one copy each of c.1073G>A and c.1274_1277dupTATC
3	*CFTR*	c.3454G>C (p.D1152H)	c.1521_1523delCTT(p.F508del)	Pregnancy 1-positive for one copy of c.1521_1523delCTT (p.F508del) Pregnancy 2-positive for one copy of c.1521_1523delCTT (p.F508del)
	MMACHC	c.271dupA (p.R91fs)	c.641G>A (p.214H)	Pregnancy 2-negative
4	*MMACHC*	c.331C>T (p.R111*)	c.844C>A (p.P282T)	Positive for one copy each of c.331C>T (p.R111*) and c.844 C>A (p.P282T)
5	*HADHA*	c.1528G>C (p.E510Q)	c.-17G>A	Positive for one copy each of c.1528G>C (p.E510Q) and c.-17G>A

Legend: Variant of uncertain significance

Discussion:

- This table summarizes diagnostic results for the six prenatal cases tested at our laboratory as a follow up for five couples in which the partner was identified as a (potential) carrier by gene-specific full gene sequencing.
- Three fetuses were identified as carriers of one parental mutation and one fetus inherited both parental mutations.
- Two fetuses inherited both parental variants, including the parental VUS identified by gene-specific full gene sequencing.
- No follow up information was available for the remaining 26 (potential) carrier couples identified by gene-specific full gene sequencing.

IV. Conclusions

- Gene-specific full gene sequencing is requested by healthcare professionals as a follow-up to carrier screening.
  - In our laboratory, 64% of all adult cases received for gene-specific full gene sequencing were for partner carrier testing.
  - In our laboratory, 55% of all prenatal cases received for gene-specific full gene sequencing were for diagnostic testing as follow up to carrier screening for one parent.
- Full gene sequencing that assesses the presence of rare pathogenic variants and VUS, in contrast to targeted genotyping, provides a better negative predictive value for couples.
- We observe that prenatal testing was requested by healthcare professionals for both pathogenic variants and variants of uncertain significance.

V. Reference

1. Carrier screening in the age of genomic medicine. Committee Opinion No. 690. American College of Obstetricians and Gynecologists. Obstet Gynecol 2017; 129:e35-40