Ambry Genetics™

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Diagnostic Exome Sequencing Suggests Digenic Inheritance in a Cobalamin Metabolism Disorder

BACKGROUND

- Diagnostic exome sequencing is being applied clinically resulting in elevated diagnostic yield from genetic testing (~33-50%).
- Exome testing is useful in generating hypotheses for novel disease-associated genes or patterns of inheritance.
- One challenge in DNA-based diagnosis is the availability of an appropriate sample. Genetic disease leading to spontaneous abortion, fetal demise, or early neonatal or childhood death often results in a missed opportunity to collect and store appropriate material for further testing- something that often becomes more valuable to the family as grieving abates and they confront the possibility of recurrence in a subsequent pregnancy.
- We sequenced the exome of the parents and unaffected sister of an infant with suspected cobalamin metabolism disorder and for whom no appropriate DNA sample was available for exome sequencing.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the parents and unaffected sister. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA). Several months later, we were notified of the availability of Guthrie blood spot card from the patient and extracted DNA in order to perform single site mutation analysis on positive findings.
- Exome data undergoes alignment, base calling, and variant calling. Passing base calls have at least 7x coverage and quality scores of Q20 or higher, which translates to a base call error rate probability of 1:100, or a base call read accuracy of 99%. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. Variants were filtered further based on family history and possible inheritance models. Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database (HGMD; Stenson, 2009)), OMIM, and several other databases).
- A molecular geneticist performed interpretive filtering based on the deleterious nature of the candidate alterations literature search and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype.
- Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.

Figure 1. Family Pedigree

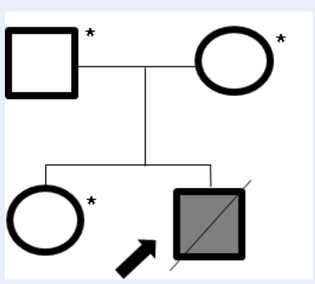
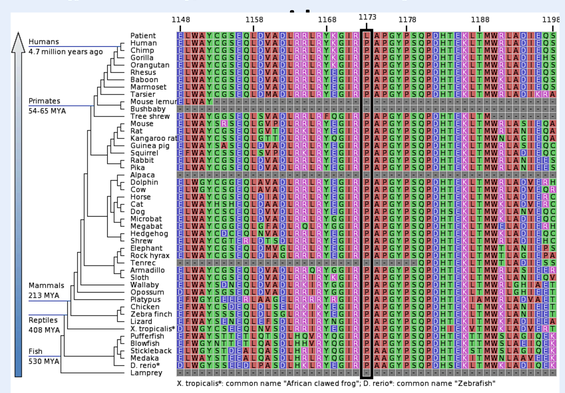


Table 1: Bioinformatics Variant Filtering

Stepwise Variant Filtering[1]		Father	Mother	Sister
No. of variants in coding regions[2]		110,636	108,825	112,270
No.post-removal of intergenic and 3'/5' UTR variants		82,300	81,626	83,470
No. post-removal of non-splice-related intronic[3] variants		21,855	21,963	22,319
No. post-removal of synonymous variants		11,631	11,622	11,874
A)	Autosomal recessive (mother and father carrier, daughter negative or carrier)	2 genes (4 alterations)		
	No. of genes in A) related to phenotype	0 genes (0 alterations)		
B)	X-linked recessive (mother carrier)	1 gene (1 alteration)		
	No. of genes in B) related to phenotype	0 genes (0 alterations)		
C)	Autosomal recessive carrier variants (mother and father):	58	51	N/A
	No. of genes in C) related to phenotype	2 genes (2 alterations)[4]		

[1]Stepwise filtering protects variants annotated within the Human Gene Mutation Database (HGMD) and/or Online Mendelian Inheritance in Man (OMIM).
[2]Variants refers to single nucleotide alterations, insertions, deletions, and indels with at least 10x base pair coverage. [3]Intronic refers to >3 bp into the introns.

Figure 2. Mother is a heterozygous carrier of *MTR* c. 3518C>T (p.P1173L), an evolutionarily conserved amino



RESULTS/ DISCUSSION

- Through custom bioinformatics analysis and family history variant filtering, we identified mutations in two genes in the cobalamin metabolism, *LMBRD1* and *MTR* (**Figure 1, Table 1**).
- Special consideration was given to the known cobalamin metabolism genes. The majority of the exons among these genes were well covered (**Table 2**). No variations were identified, other than in *MTR* and *LMBRD1*.
- *LMBRD1* c.1056delG is the most frequently reported alteration, found to be present in 75% (18/24 chromosomes) of one cohort of 12 patients with cblF deficiency (Rutsch, 2011). Likewise, the c.3518C>T (p.P1173L) alteration identified in the *MTR* gene is the most common alteration, observed at a frequency of about 40% (16/38 chromosomes) of patients with cblG deficiency (Watkins, 2002).
- Both alterations are predicted to cause protein damage: The LMBRD1 alteration, c.1056delG (p.L352fsX18), is a truncating mutation translational frameshift with a predicted alternate stop codon; The *MTR* missense alteration is located at an evolutionarily conserved amino acid (**Figure 2**).
- The patient's clinical history is consistent with a mixed presentation for cblF and cblG. Biochemical studies also demonstrated overlap between these two subtypes with borderline high levels of methylmalonic acid in blood and urine (mixed), decreased plasma methionine (cblG) and homocysteinuria (cblF) (See **Figure 3** for cobalamin metabolic pathway.)
- Both parents are carriers of a single mutation. The mother carriers a well described missense mutation in *MTR* (c.3518C>T; p.P1173L), while both the father and unaffected sister carry the *LMBRD1* mutation (c.1056delG; p.L352fsX18) (**Table 3**).
- Several months later, we were notified of the availability of Guthrie blood spot card from the patient and confirmed the presence of both mutations in the proband by Sanger sequencing (**Table 3**).

Table 2. Coverage of the Known Cobalamin

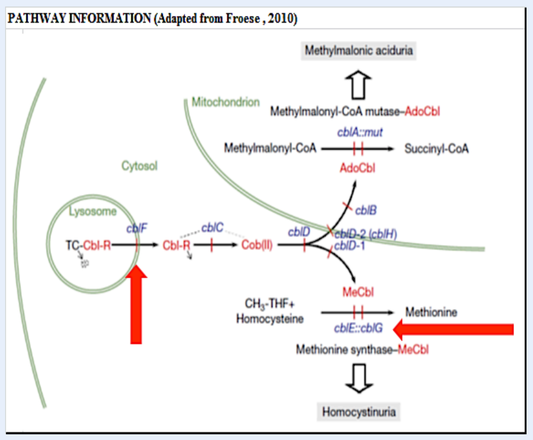
Gene	Gene Coverage (%)[1]
ABCD4	>99%
AMN	~55%[2]
CD320	~80%[3]
CUBN	>99%
FTCD	~77%[4]
GIF	>99%
LMBRD1	>99%
MCEE	>99%
MMAA	>99%
MMAB	~91%[5]
MMACHC	>99%
MMADHC	>99%
MTHFR	>99%
MTR	>99%
MTRR	>99%
MUT	>99%
PCFT	>99%
TCN1	>99%
TCN2	>99%

[1]Gene coverage is based on % of coding base pairs with ≥10x coverage and quality scores of ≥Q30
[2]*AMN* gene low coverage regions include CDS 1, 7, 8, 9, 10, 11
[3]*CD320* gene low coverage regions include CDS 1, 2, 3
[4]*FTDC* gene low coverage regions include CDS 3, 4, 9, 10, 12
[5]*MMAB* gene low coverage regions include CDS 6

Table 3. Sanger sequencing confirmation and co-segregation

GENE(S)	PROTEIN	REFSEQ ID	ALTERATION	MOTHER	FATHER	DAUGHTER	PATIENT
LMBRD1	LMBR1 domain containing 1	NM_018368	c.1056del (p.L352fsX18)	**-/-**	**+/-**	**+/-**	**+/-**
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	NM_000254	c.3518C>T (p.P1173L)	**+/-**	**-/-**	**-/-**	**+/-**

Figure 3. Cobalamin Metabolic Pathway



CONCLUSIONS

- Herein, whole exome sequencing and custom bioinformatics analysis of the parents of a deceased child with cobalamin deficiency revealed that each parent was a carrier of a well-characterized heterozygous mutation within a well-described gene associated with cobalamin deficiency.
- The identified alterations are likely founder mutations among individuals of European ancestry, consistent with the family's reported ancestry herein (Rutsh, 2011, Watkins, 2002).
- The alterations were present in the compound heterozygous state in the proband, reducing, but not eliminating, the likelihood for copy-number variation (CNV) on the corresponding allele of each gene. However, the mother and father's next-gen sequencing data show even coverage within this gene compared with other genes, reducing the likelihood for the presence of deletion within the gene (~120X vs.mean= 134X+/-44.4). Whole gene deletions of either gene including exon 11 of *LMBRD1* or exon 31 or *MTR* are excluded on the basis of heterozygosity of the mutation call.
- Biochemical analysis and *in vitro* studies are ongoing to further define the impact of digenic haploinsufficiency on this metabolic pathway.
- This unique case highlights the power of whole exome sequencing as a diagnostic tool even in the absence of a sample from the affected individual, and suggests a novel mechanism and inheritance pattern for disorders of cobalamin metabolism.

REFERENCES:

- Froese DS, *et al.* (2010) *Exp Rev Mol Med* **12**(e37):1-20.
- Rutsch F, *et al*. (2011) *J Inherit Metab Dis* **34**:121-126.
- Watkins D, *et al.* (2002) *Am J Hum Genet* **71**:143-153.