RESEARCH REPORT

Diagnostic Exome Sequencing and Tailored Bioinformatics of the Parents of a Deceased Child with Cobalamin Deficiency Suggests Digenic Inheritance of the *MTR* and *LMBRD1* Genes

Kelly D. Farwell Gonzalez • Xiang Li • Hsiao-Mei Lu •
Hong Lu • Joan E. Pellegrino • Ryan T. Miller •
Wenqi Zeng • Elizabeth C. Chao

Abstract Disorders of cobalamin deficiency are a heterogeneous group of disorders with at least 19 autosomal recessive-associated genes. Familial samples of an infant who died due to presumed cobalamin deficiency were referred for clinical exome sequencing. The patient died before obtaining a blood sample or skin biopsy, autopsy was declined, and DNA yielded from the newborn screening blood spot was insufficient for diagnostic testing. Whole-exome sequencing of the mother, father, and unaffected sister and tailored bioinformatics analysis was applied to search for mutations in underlying disorders with recessive inheritance. This approach identified alterations within two genes, each of which was carried by one parent. The mother carried a missense alteration in the *MTR* gene (c.3518C>T; p.P1173L) which was absent in the father and the sister. The father carried a translational frameshift alteration in the *LMBRD1* gene (c.1056delG; p.L352Lfs*18) which was absent in the mother and present in the heterozygous state in the sister. These mutations in the *MTR* (MIM# 156570) and *LMBRD1* (MIM# 612625) genes have been described in patients with disorders of cobalamin metabolism complementation groups cblG and cblF, respectively. The child's clinical presentation and biochemical results demonstrated overlap with both cblG and cblF. Sanger sequencing using DNA from the infant's blood spot confirmed the inheritance of the two alterations in compound heterozygous form. We present the first example of exome sequencing leading to a diagnosis in the absence of the affected patient. Furthermore, the data support the possibility for potential digenic inheritance associated with cobalamin deficiency.

K.D. Farwell Gonzalez (✉) · X. Li · H.-M. Lu · H. Lu · W. Zeng ·
E.C. Chao
Ambry Genetics, 15 Argonaut,
Aliso Viejo, CA 92656, USA
e-mail: kgonzalez@ambrygen.com

J.E. Pellegrino · R.T. Miller
Department of Pediatrics, SUNY Upstate Medical University,
Syracuse, NY, USA

E.C. Chao
Division of Genetics and Metabolism, Department of Pediatrics,
University of California, Irvine, CA 92697, USA

Introduction

The inborn errors of intracellular cobalamin metabolism are a group of clinically and genetically heterogeneous disorders inherited in an autosomal recessive manner and have been categorized based on complementation analysis and designated *mut* (MIM #251000), *cblA-cblG* (MIM #25110, 277400, 277410, 236270, 277380, 250940) (reviewed in Watkins and Rosenblatt 2011), *CblJ* (MIM #614857) (Coelho et al. 2012), and *CblX* (Yu et al. 2013). Cobalamin deficiency generally leads to an accumulation of methylmalonic acid and homocysteine in the blood and urine stemming from decreased activity of the enzymes methylmalonyl-CoA mutase and methionine synthase (Gulati et al. 1996, 1997; Gailus et al. 2010; Watkins and Rosenblatt 2011). Clinical symptoms generally include hematologic and neurologic defects (Watkins and Rosenblatt 2011). However, even within a single complementation class clinical symptoms

and biochemical profile can be highly variable, which led to the need to distinguish the disorders based on the affected gene (Froese and Gravel 2010). Autosomal recessive mutations within at least 19 genes are associated with disorders of cobalamin metabolism. To our knowledge, there have been no reports of digenic inheritance (Sarafoglou and Hoffman 2009; Froese and Gravel 2010).

The number of patients diagnosed through research and clinical exome sequencing has increased exponentially since the inception of the technology in 2008 and the introduction of the clinical test in 2011 (NIH Health Consensus Conference Statement, 2001; Worthey et al. 2011; Dixon-Salazar et al. 2012; Need et al. 2012; Sailer et al. 2012; Hanchard et al. 2013; Shamseldin et al. 2013). Exome sequencing has been instrumental in discovering the pathogenic etiology in patients in whom traditional molecular methods were uninformative and is uniquely useful in overcoming limitations posed by traditional molecular diagnostic strategies in the identification of oligogenic findings. A recent meta-analysis of digenic inheritance publications points out that despite the capacity of high-throughput sequencing to discover digenic inheritance, strikingly few reports have been published (between 1 and 7 annually), and the rate of discovery has not increased over the last decade (Schaffer 2013). Often complicating the interpretation of genetic variants are factors such as reduced penetrance and variable expressivity, which has been, in part, attributed to a two-locus model (Strauch et al. 2003).

To our knowledge, there are no reports of exome sequencing leading to a diagnosis in a patient using only DNA from the affected individual's family members. Herein, we describe this approach and the subsequent postmortem diagnosis of an affected neonate.

Subjects and Methods

DNA from the mother, father, and sister of a patient with cobalamin deficiency were referred to Ambry Genetics (Aliso Viejo, CA) for clinical diagnostic exome sequencing (DES). The patient had previously died at 3.5 months before DNA could be collected. A newborn screening blood spot was available from the patient, providing sufficient DNA for Sanger sequencing confirmation of findings, but not for whole-exome sequencing due to limited DNA quantity and quality. The family was consented for DES and research. The patient was born to non-consanguineous parents after an unremarkable prenatal course. Though B12 levels were not measured, the mother was on a normal diet including meat during pregnancy and breast-feeding. At 27 4/7 weeks gestation, CBC was normal. The patient was apparently healthy for the first 2.5 months. Newborn screening was normal. Specifically,

propionylcarnitine (C3) level at birth was 1.43 μmol/L (normal <7.0 μmol/L), propionylcarnitine/acetylcarnitine (C3/C2) was 0.06 μmol/L (normal <0.20 μmol/L), methylmalonylcarnitine (C4-DC) was 0.10 μmol/L (normal <1.00 μmol/L), and methionine was 0.08 μmol/L (normal <1.25 μmol/L). At 2.5 months, he had recurring apnea episodes and was diagnosed with reflux. He then presented with seizures 10 days later. MRI showed brain atrophy with volume decrease and sulcal enlargement. EEG was consistent with diffuse encephalopathy. He had microcephaly with height and weight at the 50% tile. Ammonia, lactate, and pyruvate were normal. Total homocysteine was elevated at 125 μmol/L (normal 4.0–15.4 μmol/L). Urine organic acids showed mild elevation of MMA (8, normal 0–5) and hydantoin-5-propionate. Urine amino acids revealed homocystinuria (204 μM/gCR) and plasma amino acids showed low methionine (3, normal 10–60). Serum MMA was 0.19 nmol/mL. Vitamin B12 was low at 173 pg/mL (211–946), but mom's level was normal. RBC folate was 580 ng/mL. CSF amino acids showed normal methionine levels, and homocystine was not detected. CSF neurotransmitter studies were normal (5-methylterahydrofolate in CSF was 59 nmol/L). Blood smear was normal without megaloblastic anemia or cytopenia. He was placed on daily hydroxocobalamin IM injections, betaine, B6, folate, and carnitine. His homocysteine fell from 125.2 to 30.5 to 18.6. His methionine increased from 3 to 12 in the same period. RBC folate changed from 508 to 503. The patient was ventilated for nearly 2 weeks and failed extubation due to continued respiratory distress with paradoxical episodes of respiratory pauses. He subsequently died at 3.5 months.

Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from each parent and the sister. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) (Gnirke et al. 2009). Briefly, each DNA sample was sheared, blunt-end repaired, and adaptor-ligated using indexed adapters. Using solution-based hybridization with oligonucleotide probes, the coding exons and neighboring intronic sequence of the genome were enriched and the nontargeted sequences were washed away. The enriched exome libraries were then applied to the solid surface flow cell for clonal amplification and sequencing using paired-end, 100 bp cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA). Each sample was sequenced sufficiently to yield greater than 90% of the targeted region with at least 10x base-pair coverage and greater than 85% of targeted bases with quality score of Q30 or higher, which translates to an expected base-calling error rate of 1:1,000 or an expected base-calling accuracy of 99.9%.

Initial data processing and base calling, including extraction of cluster intensities, was done using RTA

1.12.4 (HiSeq Control Software 1.4.5). Sequence quality filtering script was executed with the Illumina CASAVA software (ver 1.8.2, Illumina, Hayward, CA). Data yield (Mbases), %PF (pass filter), # of reads, % of raw clusters per lane, and quality scores were examined in Demultiplex_Stats.htm file. The sequence data were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA and PINDEL (Ye et al. 2009). Exons plus at least two bases into the 5′ and 3′ ends of all the introns were analyzed. Data analysis focused on nonsense variants, small insertions and deletions, canonical splice site alterations, or non-synonymous missense alterations. The human gene mutation database (HGMD) (Strauch et al. 2003), the single nucleotide polymorphism database (dbSNP) (Sherry et al. 2001), 1,000 Genomes (1000, Genomes Project Consortium, 2010), HapMap data (International HapMap C 2003), and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. The filtering pipeline protects all variants annotated within the HGMD (Stenson et al. 2009) and/or the online Mendelian inheritance in man (OMIM) databases. Stepwise filtering included the removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based on family history and possible inheritance models. Data are annotated with the ambry variant analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency (ESP and 1,000 genomes), and predicted functional impact (including PolyPhen (Gitiaux et al. 2013) and SIFT (Grohmann et al. 2004) in silico prediction tools). Each candidate mutation was assessed by a molecular geneticist to identify the most likely causative mutation(s). Multiple sequence alignments were viewed using IGV (integrative genomics viewer) software (Robinson et al. 2011).

Nonstandard bioinformatics filtering was performed in order to search for gene alterations which may have been transmitted to the deceased son. Specifically, analysis was performed to search for mutations in recessive genes carried by both the mother and father and not present in the homozygous or compound heterozygous state in the sister, as well as for X-linked recessive genes carried by mother. This analysis included all captured genes, including clinically novel genes. Additionally, a manual review of the known cobalamin deficiency genes was performed including the following genes: *ABCD4*, *AMN*, *CD320*, *CUBN*, *FTCD*, *GIF*, *HCFC1*, *LMBRD1*, *MCEE*, *MMAA*, *MMAB*, *MMACHC*, *MMADHC*, *MTHFR*, *MTR*, *MTRR*, *MUT*, *PCFT*, *TCN1*, and *TCN2*.

Identified candidate alterations were confirmed using automated fluorescence dideoxy sequencing. Co-segregation analysis was performed using each available family member.
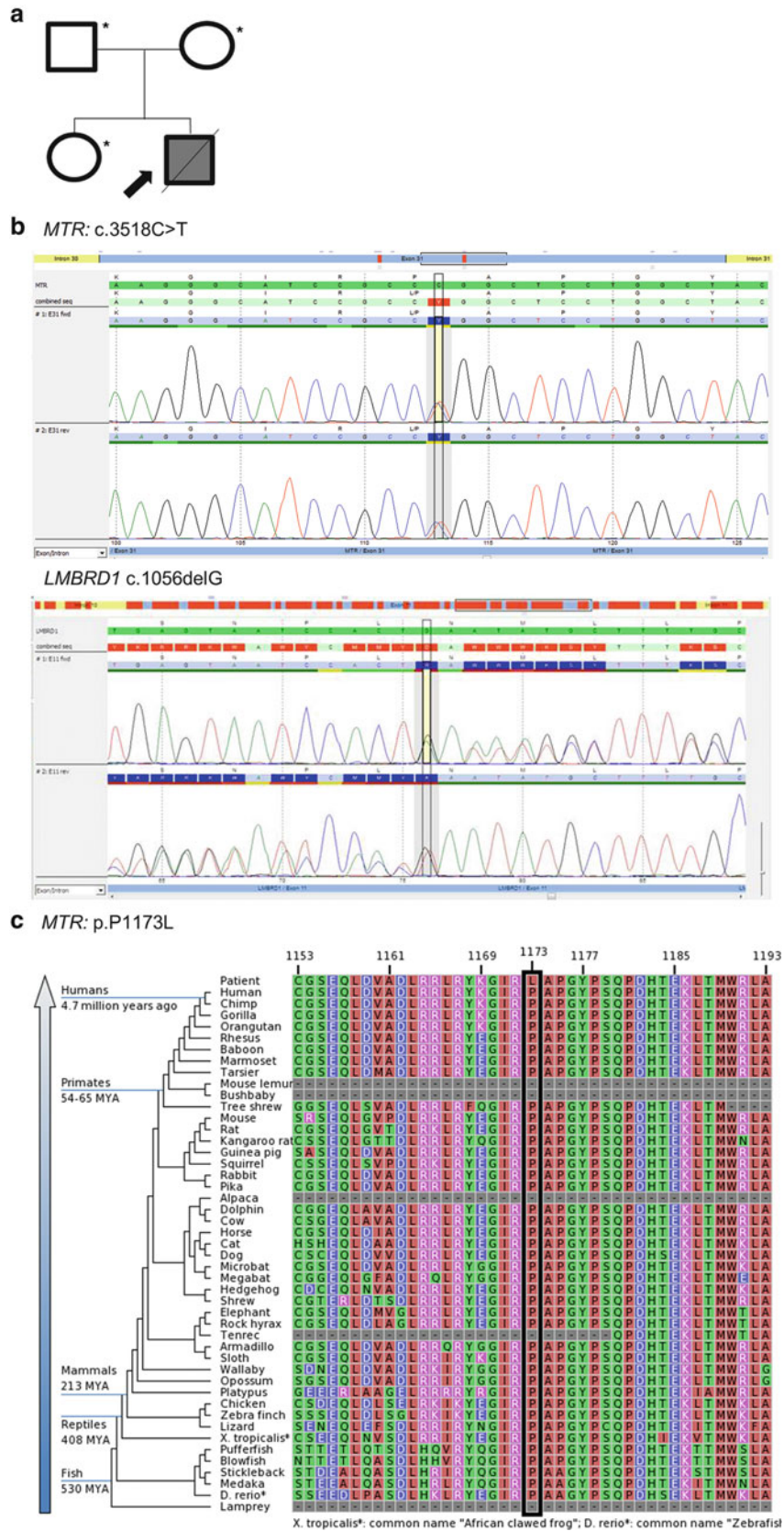
Amplification primers were designed using PrimerZ (Guenther et al. 2009). PCR primers were tagged with established sequencing primers on the 5′ end. Sequencing was performed on an ABI3730 (Life Technologies, Carlsbad, CA) using standard procedures.

Several months subsequent to exome sequencing, we were notified of the availability of Guthrie blood spot card from the patient and isolated gDNA for genotyping to confirm the presence of identified mutations.

Results

Exome sequencing of the mother, father, and sister resulted in an average of 16.7 Gb of sequence per sample (Fig. 1a; Table 1). Mean coverage of captured regions was 141x per sample, with 89% covered with at least 10x coverage and an average of 89% base call quality of Q30 or greater. Stepwise filtering for the removal of SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and synonymous variants resulted in ~111,000 variants per sample (Table 2). Nonstandard bioinformatics filtering was performed in order to search for gene alterations which may have been transmitted to the deceased neonate but not to his unaffected sister. Five alterations met these criteria. Four were within two genes with described autosomal recessive inheritance and the fifth by the mother on the X chromosome. Of these three genes none related to the patient's phenotype. Therefore, all of the autosomal recessive genes with alterations carried by the father (58) and mother (51) were analyzed for relation to the patient's phenotype. Among these, two notable genes (two alterations) with potential clinical relevance were identified: (1) a missense alteration in the *MTR* gene (c.3518C>T; p.P1173L) present in the mother and (2) a single nucleotide deletion predicting a translational frameshift alteration in the *LMBRD1* gene (c.1056delG; p.L352Lfs*18) present in the heterozygous state in the father. Automated fluorescence dideoxy sequencing using DNA from the infant's blood spot confirmed the inheritance of each of the two alterations in heterozygous state (Fig. 1b). Furthermore, familial co-segregation analysis revealed that the *MTR* alteration was absent in the father and sister and the *LMBRD1* alteration was absent in the mother and present in the heterozygous state in the sister (Table 3).

Special consideration was given to the known cobalamin metabolism genes which had almost complete base-pair coverage at ≥10x (>99%), with exceptions noted in some exons in *AMN*, *CD320*, *FTDC*, *MMAB*, and *HCFC1* (Supplementary Table 1). No candidate alterations were identified, other than the described *MTR* and *LMBRD1* alterations.

a

b *MTR:* c.3518C>T

LMBRD1 c.1056delG

c *MTR:* p.P1173L

X. tropicalis*: common name "African clawed frog"; D. rerio*: common name "Zebrafish"

Table 1 HiSeq sequencing run metrics

	Father	Mother	Sister	Average
Yield (Gb)	14.722	17.446	18.036	**16.735**
Quality				
% of >= Q30 bases (PF)	88.715	88.945	88.995	**88.89**
Mean quality score (PF)	35.115	35.195	35.205	**35.17**
Base coverage				
%Base_1x (%)	94.03	94.24	94.05	**94.11**
%Base_4x (%)	91.75	92.21	92.07	**92.01**
%Base_5x (%)	90.72	91.31	91.18	**91.07**
%Base_10x (%)	89.31	90.05	89.96	**89.77**
%Base_20x (%)	85.22	86.44	86.43	**86.03**
%Base_50x (%)	73.65	76.95	77.26	**75.95**
%Base_100x (%)	50.60	58.85	59.74	**56.40**
Mean_coverage (%)	121.46	148.73	153.09	**141.09**

The bold values signify the average of the three individual's (father, mother, sister), values

Table 2 Bioinformatics stepwise variant filtering

Stepwise filtering[a]	Father	Mother	Sister	Average
No. of variants in coding regions[b]	110,636	108,825	112,270	**110,577**
No. post-removal of intergenic and 3′/5′ UTR variants	82,300	81,626	83,470	**82,465**
No. post-removal of non-splice-related intronic[c] variants	21,855	21,963	22,319	**22,046**
No. post-removal of synonymous variants	11,631	11,622	11,874	**11,709**
(A) Autosomal recessive[d]	2 genes (4 alterations)			
No. of genes in (A) related to phenotype	0 genes (0 alterations)			
(B) X-linked recessive[e]	1 gene (1 alteration)			
No. of genes in (B) related to phenotype	0 genes (0 alterations)			
(C) Autosomal recessive carrier variants[f]	58	51	N/A	**N/A**
No. of genes in (C) related to phenotype	2 genes (2 alterations)			

The bold values signify the average of the three individual's (father, mother, sister), values

[a] Stepwise filtering protects variants annotated within the human gene mutation database (HGMD) and/or the online Mendelian inheritance in man (OMIM) databases

[b] Variants refer to single nucleotide alterations, insertions, deletions, and indels with at least 10x base-pair coverage

[c] Intronic refers to >3 bp into the introns

[d] Mother and father carrier, daughter negative or carrier

[e] Mother carrier

[f] Mother and father

Fig. 1 Pedigree and *MTR* c.3518C>T (p.P1173L) and *LMBRD1* c.1056delG (p.L352fsX18) mutations. (**a**) Familial pedigree. *Shaded shapes* indicate affected individuals. *Asterisk* (*) indicates whole-exome sequencing performed. (**b**) An electropherogram of the *MTR* c.3518C>T (p.P1173L) and *LMBRD1* c.1056delG (p.L352fsX18) alterations in the proband. (**c**) Sequence conservation plots at the *MTR* p.P1173L mutated site amino acid position across different species

Table 3 Familial co-segregation analysis results

Gene(s)	Protein	RefSeq id	Alteration	Mother	Father	Sister	Proband
LMBRD1	LMBR1 domain containing 1	NM_018368	c.1056del (p.L352fsX18)	−/−	+/−	+/−	+/−
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	NM_000254	c.3518C>T (p.P1173L)	+/−	−/−	−/−	+/−

The P1173L (c.3518C>T) alteration is located in exon 31 of the *MTR* gene (NM_000254) (OMIM), is predicted to be deleterious by PolyPhen (Adzhubei et al. 2010) and SIFT (Ng and Henikoff 2006) in silico analyses, and is located at highly conserved amino acid position (Fig. 1c). Based on data from the NHLBI Exome Sequencing Project (ESP), the T-allele has an overall frequency of approximately 0.04% (4/10,744) total alleles.

The c.1056delG (p.L352Lfs*18) alteration is located in exon 11 of the *LMBRD1* gene and results from the deletion of 1 nucleotide, causing a translational frameshift with a predicted alternate stop codon.

Discussion

Herein, whole-exome sequencing and nonstandard bio-informatics analysis of the parents and sister of a deceased infant with cobalamin deficiency identified alterations within well-described genes associated with cobalamin deficiency, of which each parent was a carrier. Sanger sequencing using DNA from the infant's blood spot confirmed the presence of both alterations in compound heterozygous form. To our knowledge, these results represent both the first example of the molecular diagnosis in the absence of the affected patient as well as the first example of digenic inheritance associated with cobalamin deficiency.

Mutations in the *MTR* and *LMBRD1* genes have been described in patients with disorders of cobalamin metabolism. Specifically, *MTR* gene alterations are associated with homocystinuria/hyperhomocysteinemia, and *LMBRD1* gene alterations are observed in patients with methylmalonic acidemia/aciduria and hyperhomocysteinemia/homocystinuria (Watkins and Rosenblatt 2011). c.3518C>T (p.P1173L) is the most common alteration observed in the *MTR* gene, with a frequency of about 40% (16/38 chromosomes) of patients with cblG deficiency (Watkins et al. 2002). *LMBRD1* c.1056delG (p.L352Lfs*18) 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 et al. 2009, 2011).

The identified alterations are likely founder mutations among individuals of European ancestry, consistent with the family's reported ancestry (Watkins et al. 2002; Rutsch et al. 2011). *MTR* and *LMBRD1* are two of the several proteins essential to cobalamin metabolism (reviewed in Sarafoglou and Hoffman 2009; Watkins and Rosenblatt 2011). *MTR* is a cobalamin-dependent enzyme that catalyzes a methyl transfer reaction from methyltetrahydrofolate to homocysteine to form methionine and tetrahyrofolate (Gulati et al. 1997). Mutations in the *MTR* gene lead to the cblG complementation class of the cobalamin metabolism disorders (Gulati et al. 1996; Watkins et al. 2002). Patients with cblG typically present in the first year of life with elevated homocysteine, decreased methionine, seizures, and cerebral atrophy (Sarafoglou and Hoffman 2009). Other clinical findings in patients with cblG include decreased S-adenosylmethionine, lethargy, feeding difficulties, vomiting, abnormal tonus, mental retardation, failure to thrive, blindness, ataxia, delayed myelination, and megaloblastic anemia (Sarafoglou and Hoffman 2009). *LMBRD1* is a lysosomal membrane protein thought to be involved in lysosomal export of cobalamin in which alteration leads to the cblF complementation class of the cobalamin metabolism disorders (Rutsch et al. 2009, 2011; Gailus et al. 2010). Cells from cblF patients accumulate large amounts of free cobalamin within the lysosomes, but there is a deficiency of both cobalamin coenzyme derivatives and decreased activity of MMA mutase and methionine sythetase (reviewed in Watkins and Rosenblatt, 2011). Immortalized fibroblasts from patients with cblF show restored cobalamin coenzyme synthesis and function upon transfection with an *LMBRD1* wild-type complementary DNA (cDNA) construct (Rutsch et al. 2011). Typical clinical findings in patients with cblF are evident by the first year of life and include increased homocystine and MMA, encephalopathy, poor head growth, decreased cobalamin levels, mental retardation, ataxia, and megaloblastic anemia and/or pancytopenia (Sarafoglou and Hoffman 2009).

The proband's clinical phenotype did not fit any of the well-characterized cobalamin metabolism disorders but had overlapping features. His elevated homocysteine and low methionine are seen in both cblF and cblG, while the elevated MMA and low cobalamin levels are seen with cblF but not typically with cblG. He did not have the megaloblastic anemia that is observed in both cblF and

cblG. However, the phenotype produced from digenic inheritance is often distinct from that observed in either of the contributing genes (e.g., Helwig et al. 1995; Van Goethem et al. 2003; Yang et al. 2009). In fact, a recent review on digenic inheritance cautions medical geneticists to consider digenic inheritance as a possible mechanism for patients with novel phenotypes (Schaffer 2013). Further, the clinical features observed among patients with *LMBRD1* are widely variable (Froese and Gravel 2010; Watkins and Rosenblatt 2011), and specifically patients with the c.1056delG alteration in the compound heterozygous or homozygous state show considerable phenotypic variability ranging from death in infancy to asymptomatic long-term survival (Rutsch et al. 2009).

The unaffected mother, unaffected father, and unaffected sister do not carry both the *MTR* and *LMBRD1* alterations which would have ruled out the possibility for a digenic affect. 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. Gross deletions and duplications have not been reported in *MTR*, but a single report of a 6,785 bp deletion of exon 2 has been reported in *LMBRD1* (Miousse et al. 2011). A gross deletion in *LMBRD1* would either have occurred *de novo* in the proband or be inherited from a parent (most likely the mother). The mother and father's next-gen sequencing data show comparable 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). Due to insufficient DNA isolated from the blood spot of the deceased proband we are unable to entirely exclude this possibility. Whole- or partial-gene deletions including exon 11 of *LMBRD1* or exon 31 or *MTR* are excluded on the basis of heterozygosity of the mutation call.

Although a digenic inheritance pattern has not been previously reported in a patient with cobalamin deficiency, it is well described in a growing number of inherited conditions including nonsyndromic hearing loss, Bardet–Biedl syndrome, retinitis pigmentosa, polycystic kidney disease, and facioscapulohumeral muscular dystrophy type 2 (Vockley 2011; Schaffer 2013). Common to syndromes in which digenic inheritance has been described are variable expressivity, reduced penetrance, pleiotrpy, and genetic heterogeneity (reviewed in Vockley 2011), all of which are observed in cobalamin deficiency disorders. The data herein suggest that digenic inheritance may represent a novel molecular mechanism for disorders of cobalamin deficiency. It should be noted, however, that we could not completely rule out an alteration occurring in a dominant *de novo* manner, a novel gene alteration, or alterations not detectable by exome sequencing (including copy number variation). Biochemical analysis and *in vitro* transfection studies are needed 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.

Compliance with Ethics Guidelines

Synopsis

In this report, we simultaneously demonstrate the power of new molecular technologies to diagnose a deceased patient while uncovering the first potential example of digenic inheritance associated with cobalamin deficiency.

Conflict of Interest

Kelly Gonzalez, Xiang Li, Hsiao-Mei Lu, Hong Lu, Elizabeth Chao, and Wenqi Zeng are employed and receive a salary from Ambry Genetics. Exome sequencing is among the commercially available tests. Joan Pellegrino and Ryan Miller declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for which identifying information is included in this article.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Details of the Contributions of Individual Authors

Kelly Gonzalez was involved in the study's conception and design, analysis and interpretation of data, and drafting the article and is the guarantor for the manuscript. Xiang Li, Hsiao-Mei Lu, and Hong Lu were involved in analysis and interpretation of data and critical review and revision of drafts for important intellectual content. Joan Pellegrino and Ryan Miller were involved in drafting the article, analysis

and interpretation of data, and critical review and revision of drafts for important intellectual content. Elizabeth Chao and Wenqi Zeng were involved in the study's conception and design, analysis and interpretation of data, and critical review and revision of drafts for important intellectual content.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

ESP [Internet]: Exome variant server, NHLBI GO exome sequencing project (ESP), Seattle, WA. http://evs.gs.washington.edu/EVS/. Accessed Feb 2013.

OMIM [Internet]: Online Mendelian inheritance in man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD. World Wide Web. http://omim.org/. Accessed 2 June 2013.

RefSeq: NCBI (2002) The NCBI handbook. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda (MD). Chapter 18, The reference sequence (RefSeq) project.

References

1000 Genomes Project Consortium, Abecasis GR, Altshuler DL, Auton A et al (2010) A map of human genome variation from population-scale sequencing. Nature 467(7319): 1061–1073

Adzhubei IA, Schmidt S, Peshkin L et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4): 248–249

Coelho D, Kim JC, Miousse IR et al (2012) Mutations in ABCD4 cause a new inborn error of vitamin B12 metabolism. Nat Genet 44(10):1152–1155

Dixon-Salazar TJ, Silhavy JL, Udpa N et al (2012) Exome sequencing can improve diagnosis and alter patient management. Sci Trans Med 4(138):138ra178

Froese DS, Gravel RA (2010) Genetic disorders of vitamin B(1)(2) metabolism: eight complementation groups–eight genes. Expert Rev Mol Med 12:e37

Gailus S, Hohne W, Gasnier B, Nurnberg P, Fowler B, Rutsch F (2010) Insights into lysosomal cobalamin trafficking: lessons learned from cblF disease. J Mol Med 88(5):459–466

Gitiaux C, Bergounioux J, Magen M et al (2013) Diaphragmatic weakness with progressive sensory and motor polyneuropathy: case report of a neonatal IGHMBP2-related neuropathy. J Child Neurol 28(6):787–790

Gnirke A, Melnikov A, Maguire J et al (2009) Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotech 27(2):182–189

Grohmann K, Rossoll W, Kobsar I et al (2004) Characterization of Ighmbp2 in motor neurons and implications for the pathomechanism in a mouse model of human spinal muscular atrophy

with respiratory distress type 1 (SMARD1). Hum Mol Genet 13 (18):2031–2042

Guenther UP, Handoko L, Laggerbauer B et al (2009) IGHMBP2 is a ribosome-associated helicase inactive in the neuromuscular disorder distal SMA type 1 (DSMA1). Hum Mol Genet 18(7): 1288–1300

Gulati S, Baker P, Li YN et al (1996) Defects in human methionine synthase in cblG patients. Hum Mol Genet 5(12):1859–1865

Gulati S, Chen Z, Brody LC, Rosenblatt DS, Banerjee R (1997) Defects in auxiliary redox proteins lead to functional methionine synthase deficiency. J Biol Chem 272(31):19171–19175

Hanchard NA, Murdock DR, Magoulas PL et al (2013) Exploring the utility of whole-exome sequencing as a diagnostic tool in a child with atypical episodic muscle weakness. Clin Genet 83(5): 457–461

Helwig U, Imai K, Schmahl W et al (1995) Interaction between undulated and patch leads to an extreme form of spina bifida in double-mutant mice. Nat Genet 11(1):60–63

International HapMap C (2003) The international HapMap project. Nature 426(6968):789–796

Miousse IR, Watkins D, Rosenblatt DS (2011) Novel splice site mutations and a large deletion in three patients with the cblF inborn error of vitamin B12 metabolism. Mol Genet Metabol 102 (4):505–507

National Institutes of Health Consensus Development Panel (2001) National institutes of health consensus development conference statement: phenylketonuria: screening and management, October 16–18, 2000. Pediatrics 108(4):972–982

Need AC, Shashi V, Hitomi Y et al (2012) Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49(6):353–361

Ng PC, Henikoff S (2006) Predicting the effects of amino acid substitutions on protein function. Ann Rev Genom Hum Genet 7:61–80

Robinson JT, Thorvaldsdottir H, Winckler W et al (2011) Integrative genomics viewer. Nat Biotech 29(1):24–26

Rutsch F, Gailus S, Miousse IR et al (2009) Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B12 metabolism. Nat Genet 41(2):234–239

Rutsch F, Gailus S, Suormala T, Fowler B (2011) LMBRD1: the gene for the cblF defect of vitamin B(1)(2) metabolism. J Inherit Metab Dis 34(1):121–126

Sailer A, Scholz SW, Gibbs JR et al (2012) Exome sequencing in an SCA14 family demonstrates its utility in diagnosing heterogeneous diseases. Neurology 79(2):127–131

Sarafoglou K, Hoffman G (2009) Pediatric endocrinology and inborn errors of metabolism. McGraw-Hill Medical, New York

Schaffer AA (2013) Digenic inheritance in medical genetics. J Med Genet 50(10):641–652

Shamseldin HE, Swaid A, Alkuraya FS (2013) Lifting the lid on unborn lethal Mendelian phenotypes through exome sequencing. Genet Med 15(4):307–309

Sherry ST, Ward MH, Kholodov M et al (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29(1): 308–311

Stenson PD, Mort M, Ball EV et al (2009) The human gene mutation database: 2008 update. Genome Med 1(1):13

Strauch K, Fimmers R, Baur MP, Wienker TF (2003) How to model a complex trait. 2. Analysis with two disease loci. Hum Hered 56(4):200–211

Van Goethem G, Lofgren A, Dermaut B, Ceuterick C, Martin JJ, Van Broeckhoven C (2003) Digenic progressive external ophthalmoplegia in a sporadic patient: recessive mutations in POLG and C10orf2/Twinkle. Hum Mutat 22(2):175–176

Vockley J (2011) Digenic inheritance. In: Book digenic inheritance. Wiley, Chichester

Watkins D, Rosenblatt DS (2011) Inborn errors of cobalamin absorption and metabolism. Am J Med Genet C: Semin Med Genet 157(1):33–44

Watkins D, Ru M, Hwang HY et al (2002) Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. Am J Hum Genet 71(1):143–153

Worthey EA, Mayer AN, Syverson GD et al (2011) Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 13(3):255–262

Yang T, Gurrola JG 2nd, Wu H et al (2009) Mutations of KCNJ10 together with mutations of SLC26A4 cause digenic nonsyndromic hearing loss associated with enlarged vestibular aqueduct syndrome. Am J Hum Genet 84(5):651–657

Ye K, Schulz MH, Long Q, Apweiler R, Ning Z (2009) Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. Bioinformatics 25(21):2865–2871

Yu HC, Sloan JL, Scharer G et al (2013) An X-linked cobalamin disorder caused by mutations in transcriptional coregulator HCFC1. Am J Hum Genet 93(3):506–514