Journal of Genomes and Exomes

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CASE REPORT

# A Systematic Approach to Implementing Monogenic Genomic Medicine: Genotype-Driven Diagnosis of Genetic Diseases

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**Abstract:** Genomic medicine is an emerging paradigm for disease diagnosis and management that incorporates individual genome sequence information based on and identified by next-generation sequencing. Here we report on the initial experience in implementing genomic medicine for inherited diseases in a large children's hospital. In two families, next-generation sequencing identified molecular diagnoses that had not been disclosed by years of traditional diagnostic tests. Two sisters with progressive ataxia were found to have a mutation in aprataxin gene (APTX c.717G > A, p.Trp239X) and were treated with oral Coenzyme Q10. Two brothers with intellectual disability, dysmorphic features, doughy skin, and truncal obesity were found to have autosomal recessive cutis laxa caused by mutations in pyrroline-5-carboxylate reductase, type 1 (PYCR1 c.120_121delCA). Pediatric genomic medicine appears to enable early diagnosis of inherited diseases that feature clinical or genetic heterogeneity and it may allow for targeted treatment. We discuss several bottlenecks to improving care though genomic medicine, as well as potential solutions.

**Keywords:** genetic diagnosis, mendelian disease, genetics, genomics, inborn error of metabolism, genotype-phenotype relationship, next-generation sequencing, exome sequencing

*Journal of Genomes and Exomes* 2012:1 15–24

doi: 10.4137/JGE.S10091

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## Introduction

Genomic medicine is an innovative and structured approach to disease diagnosis and management that prominently features next-generation sequencing (NGS)-based genome information.[1,2] Of 7,137 monogenic diseases, the molecular basis is known for 3,554, impacting all pediatric specialties.[3] Many of these diseases are tractable for implementation of genomic medicine, in accordance with recommendations for genetic testing.[4,5] Genomic medicine is starting to challenge existing clinical paradigms by dramatically expanding the number of differential diagnoses tested simultaneously, potentially accelerating molecular diagnosis, increasing rates of ascertainment, guiding treatment decisions, and minimizing time-to-genetic counseling.[2] Genomic medicine, while playing a role in adult-onset disorders, is especially relevant in pediatrics, since most monogenic diseases present during childhood. Collectively, they account for approximately 17% of pediatric hospitalizations and an even greater proportion of healthcare costs.[6–9]

As a result, clinicians are starting to evaluate the efficacy of genomic medicine in individual patients and families. One report involved fraternal twins with dopa-responsive dystonia (*DRD*) in whom a molecular diagnosis had not been made.[10] NGS revealed mutations in sepiapterin reductase (Mendelian inheritance in man (MIM) 612716), resulting in additional treatment with 5-hydroxytryptamine and serotonin reuptake inhibitors, which ultimately led to further clinical improvement. Further support for genome-informed treatment involved the case of an infant with fulminant pancolitis, in whom extensive testing failed to disclose a molecular diagnosis.[11] NGS and confirmatory studies established a diagnosis of X-linked lymphoproliferative syndrome (*XLP*2; MIM 300635). Risk of lymphohistiocytosis, a complication of *XLP*2, prompted prophylactic hematopoietic progenitor cell transplant, with resolution of colitis.

While holding exceptional promise for early diagnosis and management of monogenic disorders, significant hurdles remain to broad adoption of genomic medicine.[2] Here we describe the experience of two cases at our institution which offer clinical "lessons" about the use of next generation sequencing to improve pediatric care.

## Patient Presentations

### Case 1

In Case 1, two Caucasian sisters with non-consanguineous parents presented with progressive ataxia.

CMH000001 was delivered at 38 weeks gestation following a pregnancy complicated by pre-eclampsia. She was a healthy infant, with normal growth parameters. She met developmental milestones in the first three years of life, but developed insidious truncal instability and sudden, unexplained movements. Movements became increasingly ataxic. By age 6 years, her wide-based stance, hypotonia, slow unsteady gait, and fatigue prompted use of a walker. She developed scanning speech and dysarthria. Cognitive skills were unaffected (full-scale IQ, 91). Brain magnetic resonance imaging (MRI) at age 6 years revealed increased signal at the right orbital gyrus, diffuse cerebellar atrophy, and a normal pons and basal ganglia. Spectroscopy was normal, without lactic acid elevation. Brain MRI 15 months later showed progression of cerebellar atrophy (Fig. 1). She developed prominent dysmetria and chorea of the upper extremities. Deep tendon reflexes were absent with down-going plantar reflexes. No muscular atrophy

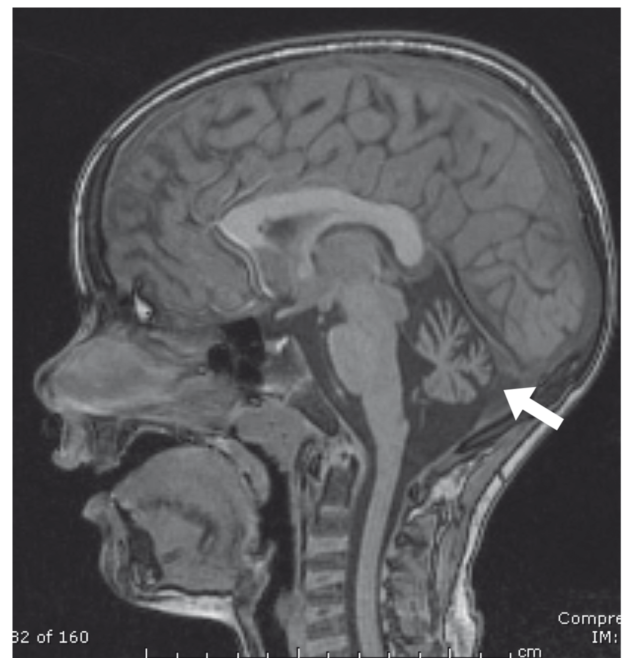


**Figure 1.** Brain MRI of proband CMH000001. An MRI taken at 8 years of age in patient CMH000001 demonstrated marked cerebellar atrophy, as denoted by the arrow.

or fasciculations were observed. An ophthalmologic exam was normal. CMH000001 had no pigmentary or vascular abnormalities of the skin. By age 8, she required the use of a wheelchair.

CMH000002, a younger full sister, was delivered at 38-weeks gestation by elective Caesarean section. She was a healthy newborn with normal growth parameters, but had mild early developmental delay and hypotonia. She first sat independently at age 8 months and walked at 19 months. She said “mama” by 10 months, but subsequent language development was delayed. By age 22 months she spoke only three intelligible words. Truncal ataxia and choreiform movements of the upper extremities developed during the first two years of life. Ophthalmologic exam was normal, and she had no dermatologic findings. She was dysarthric, had hypotonia without muscular atrophy or fasciculations, chorea of the proximal upper extremities, decreased deep tendon reflexes, negative Romberg sign, and an ataxic gait. A brain MRI at age 22 months was unremarkable, whereas three years later, diffuse cerebellar atrophy was noted.

## Case 2

In Case 2, two Kurdish brothers with consanguineous parents (first cousins, Fig. 2) presented with cognitive disability, dysmorphic features, lax skin, and truncal obesity.

CMH000006 was the product of a 37 week gestation pregnancy. Birth weight was less than 1 kg. Left hip dysplasia, bilateral cryptorchidism, and loose abdominal skin were recognized in infancy. Hearing was normal and abdominal ultrasound revealed mild distal ureteral dilation. Dysmorphic features included macrocephaly, frontal bossing, low set ears, cutaneous laxity, xeroderma along the dorsum of the hands and fingers, bilateral 2–3 toe syndactyly, thoracic scoliosis, and hyperextensible joints. Development was delayed and he required special education services. He developed upper extremity tremor and epilepsy by age 6. An electroencephalogram and brain MRI were normal. Insulin resistance was diagnosed at age 11 years. Despite dietary modifications and exercise, truncal obesity progressed.

CMH000007, a full brother, was born at 39 weeks gestation with bilateral hip dysplasia, cryptorchidism and cutaneous laxity. Birth weight, length, and head circumference were less than 3%. He was hypotonic and development was delayed. His growth accelerated during the first year of life and by 14 months his weight was at the 5th percentile, length the 40th percentile, and occipitofrontal

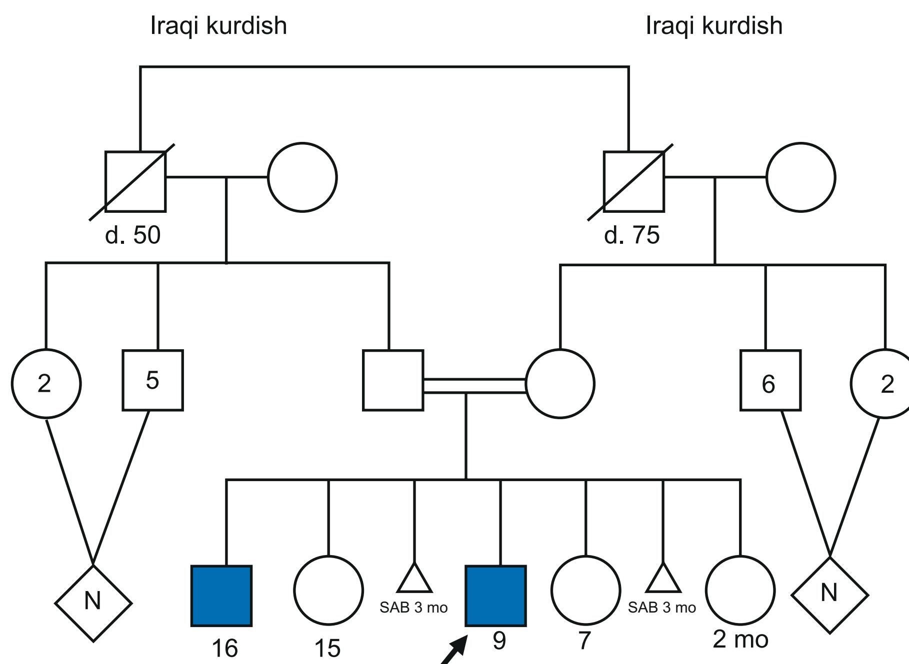


**Figure 2.** Pedigree of Family 2.
**Notes:** The proband, CMH000006, is indicated by an arrow. CMH000007 is also indicated in blue. There is known consanguinity in the family; the parents are related as first cousins.

circumference was at the 70th percentile. By age 6, they were in the 92nd percentile, >99th percentile, and >98th percentile, respectively. Physical examination was notable for frontal bossing, low set ears, facial asymmetry, gynecomastia with inverted nipples, pannus without organomegaly, hyperextensible joints, inversion of ankles with flat plantar arch, bilateral 2–3 toe syndactyly, tremor, hypotonia, and soft, doughy skin (Fig. 3). Computed brain tomography revealed agenesis of the corpus callosum and colpocephaly.

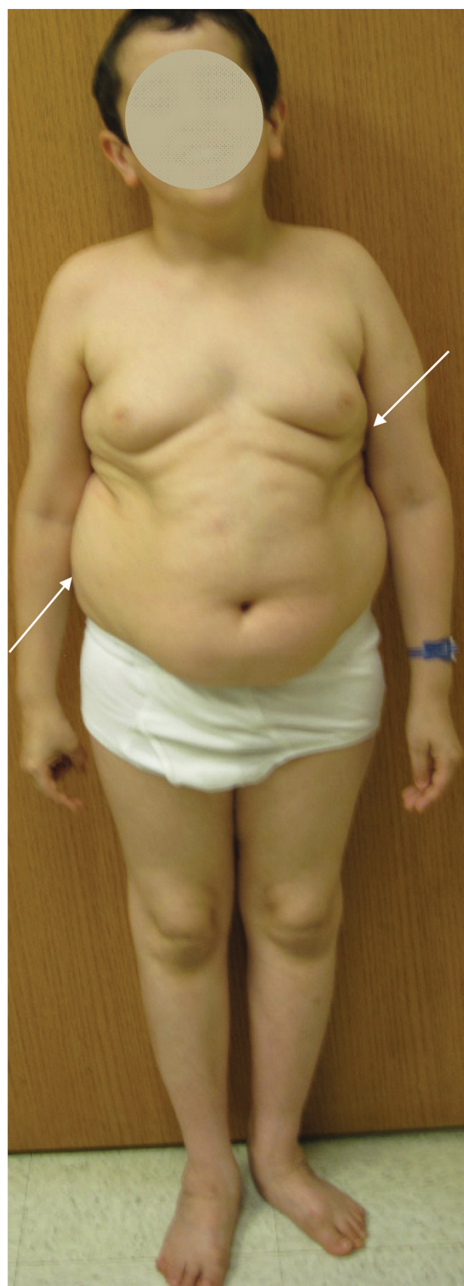

**Figure 3.** Clinical features of proband CMH000006.
**Notes:** Arrows demonstrate areas of abnormal fat deposition. Informed consent to reproduce a photograph of the patient was obtained.

## Results

Extensive testing over a period of five years in each case failed to yield a definitive diagnosis (Supplementary Table 1). Genomic DNA was isolated from affected children, exomes (all protein-coding exons) were enriched, and sequence was generated utilizing a HiSeq 2000 instrument and TruSeq v3 reagents (Illumina, Cambridge, UK). Functional consequences of variants were predicted with RUNES[2] and known disease-causal or likely pathogenic variants common to both affected siblings were identified (Table 1).

Sisters CMH000001 and CMH000002 were found to be homozygous for a premature stop codon in aprataxin (*APTX*, dbSNP: rs104894103, HGMD: CM012898, Chr 9:32974493C > T, c.717G > A, p.Trp239X, NHLBI MAF 0.0231). This mutation is known to cause early onset ataxia with oculomotor apraxia 1 and hypoalbuminemia (EAOH, MIM 208920,).[12] Sanger sequencing confirmed homozygosity in the sisters and heterozygosity in both parents (Fig. 4).

Brothers CMH000006 and CMH000007 were found to be homozygous for a novel variant in pyrroline-5-carboxylate reductase, type 1 (*PYCR1* c.120_121delCA). *PYCR1* mutations cause autosomal recessive cutis laxa, type 2 (ARCL2, MIM 219200). This variant is expected to be disease-causing since it results in a frame shift and introduction of a premature stop codon in *PYCR1* (p.Thr41SerfsX23). Sanger sequencing confirmed homozygosity in the brothers and heterozygosity in their father (Fig. 5). The patients' mother was unavailable for sequencing and the patients were subsequently lost to follow up.

## Discussion

These cases, the first, second, sixth and seventh patients enrolled in our genomic medicine program, demonstrate the relative rapidity of exome NGS and confirmatory testing when compared to serial, single, molecular testing for molecular diagnosis in childhood monogenic disorders with substantial genetic heterogeneity. In both cases, years of costly, conventional testing did not disclose a molecular diagnosis and, in one, treatment had been guided by clinical diagnosis. Molecular

**Table 1.** ACMG category 1–3 variants shared by siblings in families 1 and 2.

| | Shared known mutations | Shared known homozygous mutations | Shared known homozygous mutations with MAF < 0.01 | Shared novel variants likely to be disease causing | Shared novel homozygous variants likely to be disease causing | Shared novel homozygous variants likely to be disease causing with MAF < 0.01 |
|---|---|---|---|---|---|---|
| CMH000001 and CMH000002 | 20 | 5 | 1, *APTX* | 528 | 70 | 0 |
| CMH000006 and CMH000007 | 9 | 4 | 0 | 253 | 87 | 1, *PYCR*1 |

**Notes:** Variants were categorized by RUNES.[2] Known mutations are variants that are categorized as ACMG category 1,[31] which have previously been reported in HGMD[28] as disease causing. Novel variants are variants that have not been previously reported in association with disease, ACMG category 2 and 3 variants. Each variant that is called is given a minor allele frequency (MAF) based on cumulative exomes that are analyzed at CMH.

diagnosis enabled specific genetic and prognostic counseling for both families. Importantly, they demonstrate the utility of exome NGS for molecular diagnosis when clinical features do not precisely fit the classical presentation of the disorder. Specifically, truncal obesity and macrocephaly are not documented features of *ARCL2*. Likewise, oculomotor apraxia, hypoalbuminemia and peripheral neuropathy are typically present in *EAOH*, but not in the patients reported herein. Such clinical heterogeneity can obfuscate clinical diagnosis by expert physicians or conventional, targeted testing. Given the novelty of clinical NGS, the extent of clinical heterogeneity in monogenic disorders is not yet known. In addition, monogenic disease presentations often evolve with time, with poor differentiation at presentation, and subsequent development of pathognomonic features. By dramatically expanding the number of differential diagnoses that are tested simultaneously, NGS has the potential for early and comprehensive examination of genotype-phenotype relationships in such cases. To date, our center has enrolled approximately 300 patients, and a molecular diagnosis has been made in approximately 30% of patients.

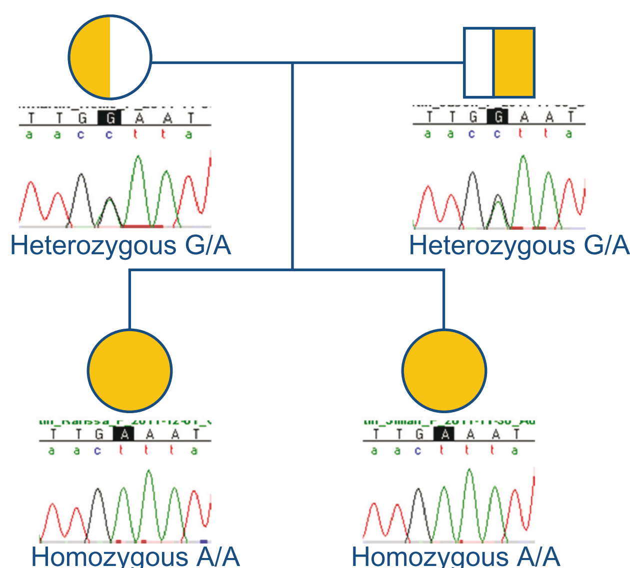


**Figure 4.** *APTX* Variant Confirmation.
**Note:** Sanger sequences showing homozygosity for *APTX* c.717G > A in CMH000001 and CMH000002, and heterozygosity in both parents.

These cases also illustrate that NGS-based molecular diagnosis may affect management. CoQ10 deficiency has been identified in several patients with EAOH, with anecdotal responses to CoQ10 supplementation.[13–15] Dosing and pharmacokinetics of CoQ10 have, however, not been stringently evaluated, exemplifying a challenge to implementation of individualized genomic medicine in orphan diseases, without which improved care will not be realized. Previously, CoQ10 dosing in EAOH and related ubiquinone deficiencies has been arbitrary, ranging from 0.5–5.0 mg/kg/day.[16] Pharmacokinetic investigation of CoQ10 in children is limited to a single dose-ranging study in 16 children with Down Syndrome, in which plasma CoQ10 concentrations were determined following 4 weeks of treatment with a liquid formulation at doses of 1 and 10 mg/kg/day. Considerable inter-individual variability in plasma concentration was found, suggesting that increased dosing was not linearly associated with plasma values.[17] CoQ10 is an unregulated dietary supplement and thus bioavailability and purity are not documented. Furthermore, no clinical tools for objective assessment

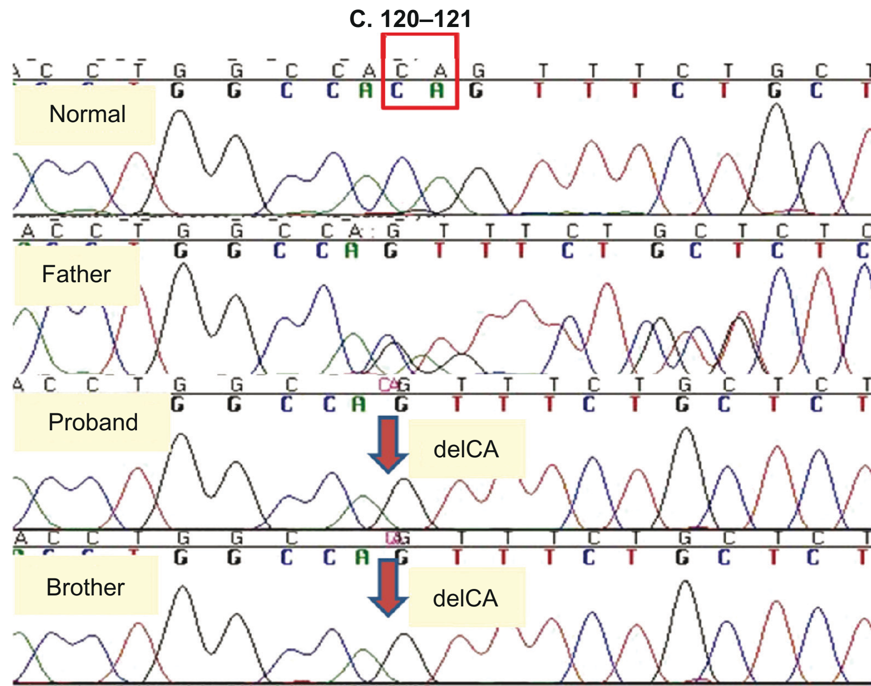


**Figure 5.** PYCR1 Variant Confirmation.
**Note:** Sanger sequencing showing homozygous PYCR1 c.120_121delCA in CMH000006 and CMH000007, heterozygous father, and normal sequence for comparison.

of treatment response in *EAOH* have been validated. Consequently, we decided to treat the sisters with 30 mg of oral CoQ10 per day, increasing gradually to 600 mg per day. Prior to CoQ10 treatment, the Brief Ataxia Rating Scale[18] was used to determine baseline ataxia levels. After four months, parents and teachers of both girls reported improved stamina; however, progression of ataxia was found by follow up testing with the Brief Ataxia Rating Scale.[18]

Likewise, biochemical knowledge of *PYCR1*, the gene mutated in Case 2, may potentially lead to innovative treatments. *PYCR1* is part of a mitochondrial NADP/NADPH antioxidant cycle.[19] *PYCR1*-deficient fibroblasts show altered mitochondrial morphology and membrane potential, and increased apoptosis upon oxidative stress.[20,21] These data suggest a possible role for antioxidants in the treatment of ARCL2.

Recent reports indicate that this experience is not atypical.[10,11] For example, NGS suggested folate receptor mutations to be causal in pedigrees with intellectual disability, seizures, and motor deficiencies.[22–24] Likewise, histamine receptor mutations were suggested to be causal in a pedigree with Tourette's syndrome.[25] Thus, there exists a need for paradigms for proceeding from timely molecular diagnoses in such cases to institution of known or potential novel therapies. These are likely to be suggested by expert knowledge of intermediary metabolism and based on repurposing of the existing pharmacopeia. Rational treatment designs, with objective quantitation of response, safety, and tolerability, are also needed.

Implementing genomic medicine into existing medical practices is not without difficulty. Our experience has suggested several factors that assisted in the introduction of genomic medicine at our institution (Box 1). These factors reflect the reality that genomic medicine is being added to an already complex and burdened medical enterprise. They also reflect many physicians lack of expert knowledge of clinical genomics, as well as the wide variety of resources that must be polled for consensus opinions for individual patient treatments.[26] Finally, they reflect an urgent need for medical preparedness to handle the imminent broadening in availability of individual genome sequences.[26] While genomic medicine clearly holds exceptional promise for timely diagnosis and management of monogenic disorders, early protagonists may have underestimated the sociological complexity and depth of resources and patience that will be needed for any broad adoption.

**Box 1. Factors for effective implementation of genomic medicine for monogenic diseases.**

1. A multi-disciplinary team of clinicians, genomicists, bioinformaticians, molecular pathologists, bioethicists, molecular biologists, biochemists and pharmacologists.
2. Consideration of medical geneticists, genetic counselors and molecular pathologists as stakeholders.
3. Pre-existing infrastructure includes CLIA (Clinical Laboratory Improvement Amendments) compliant laboratories, pathology services, IT (information technology) and EMR (electronic medical records).
4. Referring physicians remain clinicians of record.
5. The scope is consultation, clinical NGS, interpretation of diagnosis and prognosis, recommendations of individualized therapies and objective biomarkers, and response monitoring.
6. Co-investigators from various subspecialties are points of contact and assist in program decisions.
7. Clinician education about services, contacts, consent, test indications, included/excluded conditions, limitations, applicability to practice, results format, support.
8. Effective, ongoing communication with the referring physician and affected family.
9. A culture of ongoing assessment of efficacy and outcomes.
10. A standardized framework enables sharing of knowledge.
11. Communication of results and guidance occurs in several increments.

## Concise Methods

### Consent

The study was approved by the Institutional Review Board of Children's Mercy Hospital (CMH). Informed written consent and assent was obtained from all patients and their parents. All research was performed in accordance with the Declaration of Helsinki.

### Exome sequencing

Isolated genomic DNA was also prepared for sequencing using a standard Illumina TruSeq protocol and enriched twice by Illumina TruSeq hybrid selection with more than 340,000 95-mer probes for 20,794 genes, targeting 62 Mb of the human genome. Samples were sequenced on a HiSeq 2000 instrument with TruSeq v3 reagents, yielding 11.85 GB and 12.03 GB of singleton 100 bp reads in samples CMH000001 and CMH000002, and 10.02 GB and 12.34 GB for CMH000006 and CMH000007, respectively. The average quality score for all samples was 32.75. Samples CMH000001 and CMH000002 had 51.09% and 51.84% of reads on target, representing an approximate 25 fold enrichment in each sample. Samples CMH000006 and CMH000007 had 56.58% and 52.19% of reads on target respectively, representing an approximate 27 fold enrichment in each sample.

### Sequence analysis

Basecaling was completed by CASAVA 1.8.2 (Illumina) and Genome Short Nucleotide Alignment Program (GSNAP) performed alignment to the reference nuclear and mitochondrial genome sequences (Hg19 and GRCH37 [NC_012920.1], respectively) and GATK conducted variant identification. Sequence analysis employed base call files (bcl), the compressed binary version of the Sequence Alignment/Map format (bam, a representation of nucleotide sequence alignments) and Variant Call Format (VCF, a format for nucleotide variants). Variant calls were annotated with RUNES,[2] the CMH variant characterization pipeline, which incorporated the VEP,[27] comparisons to NCBI dbSNP, known disease mutations from the Human Gene Mutation Database,[28] and additional in silico prediction of variant consequences using ENSEMBL and UCSC gene annotations.[29,30] RUNES assigned each variant an American College of Medical Genetics (ACMG) pathogenicity category[4,5,31] and an allele frequency derived from CMH's Variant Warehouse database, which holds records 11 million variants detected in CMH sequencing projects since October 2011.

## Acknowledgements

We thank the families for their participation in this study, Melonie Patterson and Lisa Krivohlavek for technical assistance, and Michael Artman and Jennifer Lowry for discussions that contributed to this work.

## Funding

This work was funded by Children's Mercy Hospital, the Marion Merrell Dow Foundation and NIH

grant R01DK091823. A Deo mirificatio, ab amicis auxilium.

## Competing Interests

NAM is a stockholder in Illumina, Inc., manufacturer of sequencing technologies used in this study. LDS is a member of Hunter's Outcome Survey Board and the Shire VPRIV advisory board, and has also received funding from Shire, Biomarin and Genzyme. Other authors disclose no competing interests.

## Author Contributions

Conceptualized and designed the study, drafted the initial manuscript: SK. Designed the data collection instruments, coordinated and supervised data collection: SS. Designed the bioinformatics tools, and coordinated bioinformatics efforts: NM. Coordinated and supervised sample and clinical data collection, provided genetic counseling to the families, obtained consent. AA. Carried out the initial analyses: CS, DD, NAA. Was the physician-of-record: LS. Undertook the exome sequencing: DD. Led the pharmacogenetic work, suggested several of the components of the genomic medicine effort: SL. Reviewed and revised the manuscript: CS, DD, NAA. Critically reviewed the manuscript: NM, AA. Critically reviewed and edited the manuscript: SL, LS. All authors approved the final manuscript as submitted.

## Disclosure and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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## Supplementary Tables

**Table S1.** Negative diagnostic tests performed before exome sequencing.

| Patient | Negative diagnostic tests performed before exome sequencing |
|---|---|
| CMH000001 | Urine organic acids, urine amino acids, brain magnetic resonance imaging (x2), brain magnetic resonance spectroscopy, Basic metabolic panel, pyruvate (x3), plasma ammonia, plasma amino acids, lactate (x2), acylcarnitine profile, copper level, liver function test, thyroid function test, comlete blood count, $\alpha$-fetoprotein, Vitamin E, karyotype,array comparative genomic hybridization (aCGH), electromyogram, molecular analysis (frataxin, ataxia-teleangiectasia mutated, glial fibrillary acid protein, pyruvate carboxylase, common MELAS (mitochondrial encephalomyopathy, lactic acidosis, stroke) and MERRF (mitrochondrial encephalomyopathy, ragged red fibers)) |
| CMH000002 | Metabolic panel, karyotype |
| CMH000006 | Urine organic acids, thyroid function test, karyotype, Fragile X syndrome, *RSK*2 gene sequencing for Coffin-Lowry syndrome, methylation testing for Prader-Willi syndrome |
| CMH000007 | Expanded newborn screening (NeoGen, Perkin Elmer, Foster City), plasma amino acids, acylcarnitine profile, urine oligosaccharides, urine free glycan profile, quantitative urine mucopolysaccharides, lactic acid, pre-albumin, 17-hydroxyprogresterone, desmosterol, lathosterol, 7-dehydrocholesterol, and sterol intermediate panel (for Smith-Lemli-Opitz syndrome), serum transferrin screening for congenital disorders of glycosylation, array Comparative Genomic Hybridization, Fragile X syndrome (FMR1 targeted mutation analysis), Prader Willi syndrome (methylation assay), *VPS*13*B* gene sequencing for Cohen syndrome |