Rapid Resolution of Blended or Composite Multigenic Disease in Infants by Whole-Exome Sequencing

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Whole-exome sequencing identified multiple genetic causes in 2 infants with heterogeneous disease. Three gene defects in the first patient explained all symptoms, but manifestations were overlapping (blended phenotype). Two gene defects in the second patient explained nonoverlapping symptoms (composite phenotype). Whole-exome sequencing rapidly and comprehensively resolves heterogeneous genetic disease. *(J Pediatr 2017;182:371-4).*

A variety of disease manifestations in young infants, explainable by combinations of genetic and environmental factors, can hamper an early and complete genetic diagnosis. Whereas targeted analysis of candidate genes might not resolve the complete clinical picture, whole-exome sequencing (WES) is unbiased, allowing identification and evaluation of the majority of the patient's genetic variants. In clinical practice, WES has become the prime test for genetically heterogeneous monogenic disorders, with no straightforward genotype-phenotype correlation and with unknown genes involved. WES also seems preferable to decipher complex disease, which could be caused by genes with overlapping (blended) or discrete (composite) manifestations,[1,2] in young children at an early stage of disease manifestation. This especially could apply to affected children from consanguineous marriages, where the parents potentially share carriership of more genetic diseases than outbred marriages.

Methods

Procedures with human participants were in accordance with the ethical standards of the local medical ethical committee (Maastricht University Medical Center) and the 1964 Declaration of Helsinki. Written informed consent was obtained from the parents of the pediatric patients.

Patient 1, the second child of healthy, consanguineous Moroccan parents (first-degree cousins), was hospitalized from birth on with heterogeneous neurologic symptoms. Her sister was more mildly affected and showed only some of the symptom manifestations. Clinical symptoms of both siblings are depicted in the **Figure**, A and **Table I** (**Table I**; available at www.jpeds.com). Patient 2, a child of healthy, nonconsanguineous parents, was born with multisystemic disease. Clinical manifestations are shown in **Figure**, B and **Table II** (**Table II**; available at www.jpeds.com).

ACY1	Aminoacylase 1
JHF	Juvenile hyaline fibromatosis
PROVEAN	Protein Variation Effect Analyzer
SMALED2	Spinal muscular atrophy, lower extremity-predominant type 2
SNP	Single-nucleotide polymorphism
WES	Whole-exome sequencing

Homozygosity Mapping and WES

Homozygosity mapping was performed by the Human Mapping 250K array (Affymetrix, Santa Clara, California) and Genotyping console 4.0 (Affymetrix). Homozygosity regions were defined by the "Homozygosity mapper," with a cutoff of 5 MB. Exons were captured with SureSelect version 4 exome enrichment kit (Agilent Technologies, Santa Clara, California), including untranslated regions. Sequencing was performed on a HiSeq 2000 platform (Illumina, Inc, San Diego, California), using a 2×100 bp paired end setting. Bcl2fastq 1.8.4 (Illumina) allowed Basecalling and demultiplexing, and Burrows-Wheeler Aligner 0.5.9 (Broad Institute, Cambridge, Massachusetts) was used for read alignment against human reference genome hg19. Duplicate reads were removed by Picard software suite 1.77 (Broad Institute, Cambridge, Massachusetts) and variant calling was performed with GATK 2.1-8 (Broad Institute).

The exome data of patient 1 were filtered for homozygous variants in homozygosity regions. Variants with allele frequencies lower than 1% (dbSNP137, Exome Aggregation Consortium), consisting of nonsynonymous substitutions, indels, nonsense mutations, and splice variants were evaluated. Nonannotated variants were maintained, unless allele frequencies exceeded 5% prevalence in our in-house patient database. Pathogenicity of nonsynonymous missense mutation was estimated by Polymorphism Phenotyping-2 (PolyPhen-2; Harvard, Boston, Massachusetts), Sorting Intolerant From Tolerant (J. Craig Venter Institute, Rockville, Maryland), Protein

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Variation Effect Analyzer (PROVEAN; J. Craig Venter Institute), and MutationTaster (NeuroCure Cluster of Excellence/Berlin Institute of Health, Berlin, Germany). Missense mutations were considered pathogenic if predicted damaging by at least 2 in silico prediction tools. Nonsense, frameshift, and splice-site variations were maintained. WES data of patient 2 were filtered for compound heterozygous, homozygous, and dominant de novo mutations by the use of a comparable strategy. Potential dominant de novo mutations were limited to Online Mendelian Inheritance in Man–reported disease genes with known dominant mutations.

Results

Patient 1: SERAC1, ACY1, and ANTXR2 Mutations

A homozygous in-frame insertion was identified in *SERAC1* (NC_000006.12, NM_032861.3:c.1349_1350insATC), resulting in an additional serine residue (p.Ser450) in exon 13, which has not been reported previously in patients or single-nucleotide polymorphism (SNP) databases. It is highly pathogenic according to PROVEAN (−9.592) and MutationTaster (disease causing). The serine residue located at the site of insertion is conserved perfectly in all vertebrates. Sanger sequencing confirmed a recessive segregation pattern within the family. The sister did not carry this variant.

The *ANTXR2* gene contained a homozygous missense mutation (NG_015987.1, NM_001145794.1:c.1142A>G). The p.(Tyr381Cys) substitution affects an evolutionary conserved amino acid position and was reported previously as a disease-causing mutation (Human Gene Mutation Database: CM033747).[3] The parents were heterozygous, and both the index patient and sister were homozygous for the mutation.

A third homozygous mutation was identified in *ACY1* (NG_012036.1, NM_000666.2:c.811G>A), changing a nonpolar alanine to polar threonine (p.[Ala271Thr]) in a conserved M20 dimerization domain (Pfam07687). The variant has an allele frequency of ~0.0017% (Exome Aggregation Consortium database). Polyphen2 (0.979, probably damaging) and MutationTaster (disease causing) classified the mutation as pathogenic (the variant, rs765511074, is of uncertain significance according to ClinVar [National Institutes of Health, Bethesda, Maryland]). Segregation testing revealed that both parents and the sister were heterozygous for the mutation.

Patient 2: HPS1 and BICD2 Mutations

Patient 2 was compound heterozygous for 2 *HPS1* mutations, p.(Arg173*) and p.(Gln397Serfs*2) (NG_009646.1, NM_000195.4:c.[517C>T];[1189delC]). The nonsense mutation causes an early stop codon, implying a loss of 527 amino acids or, more likely, nonsense-mediated decay of the mRNA. p.(Arg173*) has not been reported in patients or annotated in SNP databases. The p.(Gln397Serfs*2) mutation results in a frameshift with an early stop codon, most likely causing nonsense-mediated mRNA decay. The mutation has been reported as disease causing (Human Gene Mutation Database: CD982692).[4] Both parents were heterozygous mutation carriers.

A second gene defect was located in *BICD2* (NG_033908.1, NM_001003800.1:c.539A>C). The dominant missense mutation affects a conserved amino acid position (p.[Asp180Ala]) and has highly damaging properties according to MutationTaster (disease causing), PROVEAN (−7.6), Sorting Intolerant From Tolerant (deleterious: 0.03), and PolyPhen2 (0.980, probably damaging). Several pathogenic heterozygous *BICD2* mutations have been reported (both autosomal dominant and de novo); however, this specific mutation has not been reported previously in patients or SNP databases. Sanger sequencing and nonpaternity testing validated both parents as noncarriers, confirming a de novo introduction of the dominant *BICD2* mutation.

Discussion

Clinical symptoms of patient 1 were compared with other patients, having mutations in similar genes (**Table I**). Similar to the *SERAC1* cases, patient 1 showed Leigh syndrome–related disease manifestations, with characteristic magnetic resonance imaging and neurologic and muscular impairments.[5] Involvement of 3-methylglutaconic aciduria with sensorineural deafness and encephalopathy typically is explained by *SERAC1* mutations, underlying 3-methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like disease syndrome. Most of the reported symptoms of 3-methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like disease syndrome were present in patient 1. *ANTXR2* gene defects underlie juvenile hyaline fibromatosis (JHF) or infantile systemic hyalinosis, with clinical similarity involving subcutaneous skin nodules, gingival hypertrophy, and joint contractures. Similar to 2 reported Moroccan siblings with JHF,[3] both affected siblings were homozygous for the p.(Tyr381Cys) mutation. *ACY1* mutations have been reported to cause ACY1 deficiency. Urinary metabolite screening of patient 1 provided a specific profile of accumulating N-acetylated amino acids, characteristic for ACY1 deficiency. Comparable with other cases of *ACY1*, patient 1 suffered from pronounced neurologic symptoms with hypotonia, spasticity, and growth delay.[6,7] Although several patient reports highlight a role for ACY1 deficiency in disease manifestation,[6] the clinical significance of the enzyme deficiency also has been questioned.[8,9]

Symptoms deriving from the 3 individual gene defects showed overlap in patient 1 (**Figure**, A). Because all neurologic features could be explained from the *SERAC1* defect, it is uncertain whether ACY1 deficiency contributed to disease manifestation or its severity. Yet, based on the available data, a possible role cannot be excluded. If parents would opt for prenatal diagnosis in a subsequent pregnancy, ACY1 cannot be ignored, and mutation presence needs to be tested. Discussions on the clinical impact of a genetic defect can cause dilemmas in a prenatal diagnosis context, as is the case for JHF, in which symptom manifestation might be relatively mild. Preimplantation genetic diagnosis and selecting mutation-free embryos can be a good alternative, but selection for 3 or more diseases requires a trophectoderm biopsy, or new single-cell