Targeted Next Generation Sequencing Approach in Patients Referred for Silver-Russell Syndrome Testing Increases the Mutation Detection Rate and Provides Decisive Information for Clinical Management

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Objective To investigate the contribution of differential diagnoses to the mutation spectrum of patients referred for Silver-Russell syndrome (SRS) testing.

Study design Forty-seven patients referred for molecular testing for SRS were examined after exclusion of one of the SRS-associated alterations. After clinical classification, a targeted next generation sequencing approach comprising 25 genes associated with other diagnoses or postulated as SRS candidate genes was performed.

Results By applying the Netchine-Harbison clinical scoring system, indication for molecular testing for SRS was confirmed in 15 out of 47 patients. In 4 out of these 15 patients, disease-causing variants were found in genes associated with other diagnoses. These patients carried mutations associated with Bloom syndrome, Mulibrey nanism, KBG syndrome, or IGF1R-associated short stature. We could not detect any pathogenic mutation in patients with a negative clinical score.

Conclusions Some of the differential diagnoses detected in the cohort presented here have a major impact on clinical management. Therefore, we emphasize that the molecular defects associated with these clinical pictures should be excluded before the clinical diagnosis “SRS” is made. Finally, we could show that a broad molecular approach including the differential diagnoses of SRS increases the detection rate. (J Pediatr 2017; ■■■■■■■).
diagnostic workup of SRS. Furthermore, single case reports have proven significant clinical overlap between various differential diagnoses and SRS (for review Wakeling et al). The most important differential diagnoses for SRS include cancer predisposition syndromes as Bloom syndrome, Nijmegen break-age syndrome, and Mulibrey nanism, growth retardation because of mutations in IGF1 or IGFIR, as well as Microcephalic Osteodysplastic Primordial Dwarfism II, Meier-Gorlin syndrome, 3M syndrome, Short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay syndrome, Floating Harbor syndrome, and Intrauterine growth restriction (IUGR), Metaphyseal dysplasia, Adrenal hypoplasia congenita, and GENitourinary abnormalities syndrome.

With next generation sequencing (NGS), a further step toward a comprehensive analysis of patients with growth retardation and NH-CSS features has been reached. As we assumed that a significant number of patients referred for molecular testing for SRS without a molecular diagnosis after standard testing is actually affected by one of the many differential diagnoses, we set up a NGS-based approach to analyze the currently known genes for differential diagnosis of SRS.

Methods

The cohort consisted of 47 patients with IUGR and/or postnatal growth retardation, referred for SRS routine diagnostic testing. Whenever possible, clinical scoring for SRS was carried out with the NH-CSS. Postnatal growth was examined at varying ages because biometric data at the same ages were not available for all. Therefore, the criterion “protruding forehead” was scored using childhood pictures in patients 1 and 2 who were examined above the age of 3 years. SDS were calculated by using Ped(z) according to German and Swiss epidemiologic data.

In the routine diagnostic workup, upd(7)mat and hypomethylation of H19/IGF2 IG-DMR had been excluded for all patients. aberrant methylation/uniparental disomy of the imprinting loci PLAGL1:alt-TSS-DMR in 6q24, IGF2R:Int2-DMR in 6q25, MEG3:TSS-DMR and MEG3/DLK1:IG-DMR in 14q32, SNURF:TSS-DMR in 15q11, GNAS-AS1:TSS-DMR and GNAS-XL:Ex1-DMR in 20q13 as well as upd(16)mat and pathogenic submicroscopic copy number variations (by SNP6.0 or Cytoscan Array; Affymetrix, Wycombe, United Kingdom) were excluded in nearly all patients. The study was approved by the Ethical Review Board of the University Hospital Aachen (Germany).

DNA from peripheral blood samples was isolated either by a conventional salting out method or with the Qiagen Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations.

A NGS panel was designed covering 25 genes, which either have been reported to be associated with differential diagnostic disorders of SRS or which have been suggested as SRS candidate genes (Table I; available at www.jpeds.com). The library enrichment was performed by using a custom enrichment kit designed with the Illumina DesignStudio (Illumina, Inc, San Diego, California) in 46 patients or, in 1 case, the Nextera Rapid Capture Exome (FC-140-1083; Illumina, Inc) according to the manufacturer’s protocols.

Sequencing of the custom enrichment library was performed on a MiSeq platform with 2 × 151 paired end reads and v2 chemistry. Sequencing data were aligned to the hg19 reference genome and variant call format files and binary sequence alignment/map files were generated by the Illumina MiSeq Reporter with standard software settings.

The whole exome enrichment library was sequenced on a NextSeq500 platform with 2 × 151 paired end reads and NextSeq high output v2 chemistry. Fastq files were locally generated from sequencing raw data (BCL2fastq2). Using Illumina BWA Enrichment workflow (v 2.1.0, reference genome: hg19) binary sequence alignment/map files and variant call format files were generated. On average, a coverage of ×10 was reached in 97.8% of regions of interest in case of the targeted NGS assay. For the whole exome sequencing approach ×10 coverage was obtained in 98.9% of regions of interest. Variants located in regions that had a low coverage as well as variants that did not pass the defaults filter variables provided by Illumina were not considered.

Variant annotation was performed using the Illumina Variant Studio (v 2.2.1). For variant filtering, the following variables were chosen: variants with a minor allele frequency >1% in the 1000 genomes project database (April 2012 phase 1 call set), in the Exome Variant Server (NHBLI GO Exome Sequencing Project, Seattle, Washington (evs.gs.washington.edu/EVS, accessed November 2012), or the Exome Aggregation Consortium database (Cambridge, Massachusetts (http://exac.broadinstitute.org, accessed August 2016), and synonymous variants were excluded. All remaining insertion, deletion, start loss, stop loss, stop gain, splicing as well as missense variants scored as “damaging” by PolyPhen and “deleterious” by SIFT in Variant Studio were investigated and rated with the American College of Medical Genetics guidelines. For the further estimation of pathogenicity of missense variants Alamut 2.7.2, SIFT, PolyPhen-2, and MutationTaster (all accessed August 2016) were applied. However, it must be considered that the accuracy of these in-silico prediction programs is less than 80%.

Confirmation and segregation of pathogenic and likely pathogenic variants detected in the NGS approaches were performed by Sanger sequencing on an ABI3130 Sequencer (Applied Biosystems, Waltham, Massachusetts) in the patients and their families.

In patients carrying homozygous mutations, CytoScan HD Array data (Affymetrix, Santa Clara, California) were reanalyzed for the detection of regions with loss of heterozygosity to exclude a compound heterozygosity for a point mutation and a larger deletion.
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