Short communication

A case report and review of the literature indicate that *HMGA2* should be added as a disease gene for Silver-Russell syndrome

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ABSTRACT

Patients with Silver-Russell syndrome (SRS), a syndromic growth retardation syndrome, usually harbor an epimutation at chromosome 11p15 or a maternal uniparental disomy of chromosome 7. However, to date the genetic cause remains unknown in around 40% of SRS cases, suggesting genetic heterogeneity and involvement of other genes.

We present a 4-year-old female patient with the clinical diagnosis of SRS and negative results in common genetic SRS diagnostics. Whole exome sequencing identified a *de novo* heterozygous 7.3 kb deletion on chromosome 12q14.3 including exon 1 and 2 of *HMGA2*.

HMGA2 encodes an architectural transcription factor and has already been linked to body size variations in various genome-wide association studies and mouse models. Reviewing the literature, we found additional four patients with a phenotype of SRS harboring point mutations or structural variants involving *HMGA2*. We conclude that genetic testing of *HMGA2* should be considered in routine diagnostics in patients with the suspicion of SRS.

1. Introduction

Silver-Russell syndrome (SRS, OMIM #180860) is a syndromic growth disorder that is characterized by prenatal and postnatal growth retardation, relative macrocephaly at birth, body asymmetry and typical facial dysmorphic features (Wakeling et al., 2017). SRS is currently a clinical diagnosis, which can be difficult to make as it varies in severity among affected individuals and many of its features are unspecific (Netchine et al., 2007; Wakeling, 2011). Different scoring systems for SRS exist and recently a task force suggested the Netchine-Harbison clinical scoring system (NH-CSS, see Table 1) as the most practicable for establishing a clinical diagnosis of SRS (Wakeling et al., 2017; Azzi et al., 2015). The most common genetic causes for SRS are an epimutation of the imprinting center region 1 (ICR1) on chromosome 11p15 or

a maternal uniparental disomy of chromosome 7; these pathogenic variants can currently be identified in around 60% of cases with the clinical diagnosis of SRS (Netchine et al., 2007; Abu-Amero et al., 2008; Tümer et al., 2018). The remaining 40% suggest genetic heterogeneity and involvement of other genes. Other than chromosome 11p15 defects, there are only a few chromosomal rearrangements or variants in single genes, *i.e. CDKN1C*, described with SRS phenotype (Wakeling et al., 2017; Brioude et al., 2013), but strong new candidates that find their way in routine molecular diagnostics in SRS are yet missing.

We present a 4-year-old female patient with the clinical diagnosis of SRS in whom we identified a heterozygous *de novo* 7.3 kb deletion of exon 1 and 2 of *HMGA2* by whole exome sequencing (WES). We also review published cases with variants in *HMGA2* and their clinical manifestations with special attention to the SRS phenotype.

Table 1
Clinical and genetic characteristics of our patient and published patients with mutations in HMGA2.

Clinical and genetic features	Patient from this study	Abi Habib et al., 2017		De Crescenzo et al., 2015	Buysse et al., 2009	Ligon et al., 2005
Patient	Pt	*HMGA2* Pt 1	*HMGA2* Pt 2	*HMGA2* Pt	Pt #D0811079	Pt DGAP103
Sex	Female	Female	Male	Female	Male	Male
Mutation in *HMGA2*	7.3kb deletion 12q14.3 (including exon 1&2)	c.193C>T, p.Gln65*	c.189delA, p.Ala64Leufs*102	7 bp deletion (including 3′ AG acceptor site of intron 4)	117–174 kb deletion (including exon 2)	Inv(12) (p11.22q14.3) (breakpoints within intron 3 of *HMGA2*)
Zygosity	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Inheritance	*De novo*	*De novo*	Unknown (father not available)	Inherited by affected mother	Inherited by affected mother	*De novo*
Silver-Russell syndrome *via* NH-CSS (≥4/6)	Yes (4/6)	Yes (5/6)	Yes (5/6)	Yes (5/6)	Score not calculable	No (<4/6)
1) SGA [GAB]: birth length and/or weight (≤−2 SDS)	Yes [39 w]: 44 cm (−3.16)/2220 g (−2.6)	Yes [39 w]: 43.5 cm (−3.9)/2300 g (−2.5)	Yes [37 w]: 40 cm (−4.8)/1720 g (−3.1)	Yes [35 w]: 42.5 cm (−1.3)/1350 g (−2.5)	Yes ["term"]: 45 cm (−2.2 to −3.5)/2400 g (−1.6 to −3.0)	No ["full term"]: 49.5 cm (−1.53 to −1.0)/3267 g (−0.5 to −1.0)
2) Postnatal growth failure: height at 24 + 1 months (≤−2 SDS)	Yes: 88.1 cm at 4 years (−3.5)	Yes: 66 cm at 2 years (−3.4)	Yes: 91.5 cm at 4 years (−3)	Yes: 88 cm at 4 years (−2.9)	Yes: 127.5 cm at 9 years 10 months (−2)	No: 169 cm at 8 years (6.87)
3) Relative macrocephaly at birth	No	Yes	Yes	Yes	N/A	N/A
4) Protruding forehead	Yes	Yes	Yes	Yes	N/A	N/A
5) Body asymmetry	No	No	No	No (not mentioned)	No ("proportionate at 9 years")	No (not mentioned)
6) Feeding difficulties during infancy (feeding tube or cyproheptadine) and/or low BMI (≤−2 SDS at 2 years)	Yes: BMI 12.6 at 4 years (−2.36)	Yes: naso-gastric tube for feeding	Yes: gastric tube for feeding	Yes (no details)	N/A	N/A
Additional phenotype	Triangular face, normal psychomotor development	N/A	N/A	Micrognathia, brachy-clinodactyly V of hands, syndactyly II-III of feet, gastro-esophageal reflux, muscular hypotrophia, café au lait spot, epicanthus, hypertelorism, clitoral hypertrophy	Normal development (despite attention deficit hyperactivity disorder), delayed bone maturation (bone age 8 years and 6 months at time of presentation)	Advanced endochondral bone and dental ages with megaepiphyseal dysplasia, multiple subcutaneous lipomas, persistent thrombocytopenia, arthritis, brachydactyly, stable cerebellar tumor, facial dysmorphism

Abbreviations: Pt: Patient, NH-CSS: Netchine-Harbison clinical scoring system, SGA: small for gestational age, GAB: gestational age at birth, SDS: standard deviation score, BMI: body mass index, w: weeks, N/A: not available.

2. Materials and methods

This study was approved by the local Ethics Committee of the Technische Universität München and performed according to the standard of the Helsinki Declaration. After written informed consent, blood samples from the patient and her parents were collected and DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). SNP-Array analysis was done using an Affymetrix CytoScan 750 K SNP array (Affymetrix Inc., Santa Clara, CA, USA). WES of the index patient and the parents was performed using a Sure Select Human All Exon 60 Mb V6 Kit (Agilent, Santa Clara, CA, USA) and a HiSeq4000 (Illumina, San Diego, CA, USA) as previously described (Kremer et al., 2017). Reads were aligned to the UCSC human reference assembly (hg19) with BWA v.0.5.8. More than 98% of the exome was covered at least 20×. Single-nucleotide variants and small insertions and deletions were detected with SAMtools v.0.1.7. Copy number variations were called using the software ExomeDepth and Pindel. Variant prioritization was performed based on an autosomal recessive pattern of inheritance (homozygous or compound heterozygous with a minor allele frequency < 0.1%) as well as an autosomal dominant pattern of inheritance (heterozygous with a minor allele frequency < 0.001% which could not be identified in the parents). Breakpoint-PCR (PCR = polymerase chain reaction) was performed in the patient to confirm the deletion and to determine the exact chromosomal position of the deletion. Primer sequences are available upon request.

3. Results

3.1. Clinical findings

The index patient is a 4-year-old female second child of non-consanguineous parents. Her parents were of normal height (father 180 cm, mother 158 cm) as well as her two siblings (7-year-old brother, 18-month-old sister).

During pregnancy she was diagnosed as small for gestational age and showed growth below the 3rd percentile. When she was born at 39 weeks gestation (38 + 6), her birth weight of 2220 g (−2.6 standard deviation score = SDS), birth length of 44 cm (−3.2 SDS) and head cir-

cumference of 32 cm (−2.0 SDS) were below the 3rd percentile. Although there were no apparent feeding difficulties, her length was still below 3rd percentile on presentation at the age of 4 years with 88.1 cm body height (−3.5 SDS) and 9.8 kg weight (−4.4 SDS). Dysmorphic features observed were a triangular face and a protruding forehead. Clinical examination showed no other pathological findings and her psychomotor development was normal. Likewise, further diagnostics showed no signs of celiac disease or malabsorption, and an endocrinological workup no signs of hypothyroidism or growth hormone deficiency. Radiologic studies revealed delayed bone maturation, with her carpal age corresponding to 2.5 years and her phalangeal age corresponding to 3 years. She fulfilled criteria for clinical diagnosis of SRS according to the NH-CSS (see Table 1).

3.2. Genetic findings

SNP-Array analysis showed no microdeletions > 50 kb or microduplications > 100 kb or regions > 50 Mb with absence of heterozygosity. Methylation-specific multiplex ligation-dependent probe amplification (MLPA) at chromosome 11p15 (ICR1: *H19/IGF2 IG-DMR*) and at chromosome 7 (*MEST* and *GRB10*) showed no genetic correlate for SRS.

WES identified a heterozygous 7257 bp deletion on chromosome 12q14.3 including exon 1 and 2 of *HMGA2*. The deletion could not be identified in leucocyte derived DNA from both parents. PCR with primers spanning the deletion and consecutive sequencing confirmed the deletion and provided the exact breakpoints: Chr12:g.66,217,407_66,224,664del (see Fig. 1).

4. Discussion

After ruling out the known genetic causes of SRS, we used WES to investigate the genetic cause in a patient with clinical diagnosis of SRS. Sequence analysis identified a *de novo* 7.3 kb deletion including exon 1 and 2 of *HMGA2* (see Fig. 1).

Of note, the array analysis performed failed to identify the deletion in *HMGA2*, which was later identified by WES, implying superiority of WES techniques over array analyses in detecting smaller microdeletions.

HMGA2 is one of the four members of the "high mobility group A" (HMGA) protein family that function as architectural transcription fac-
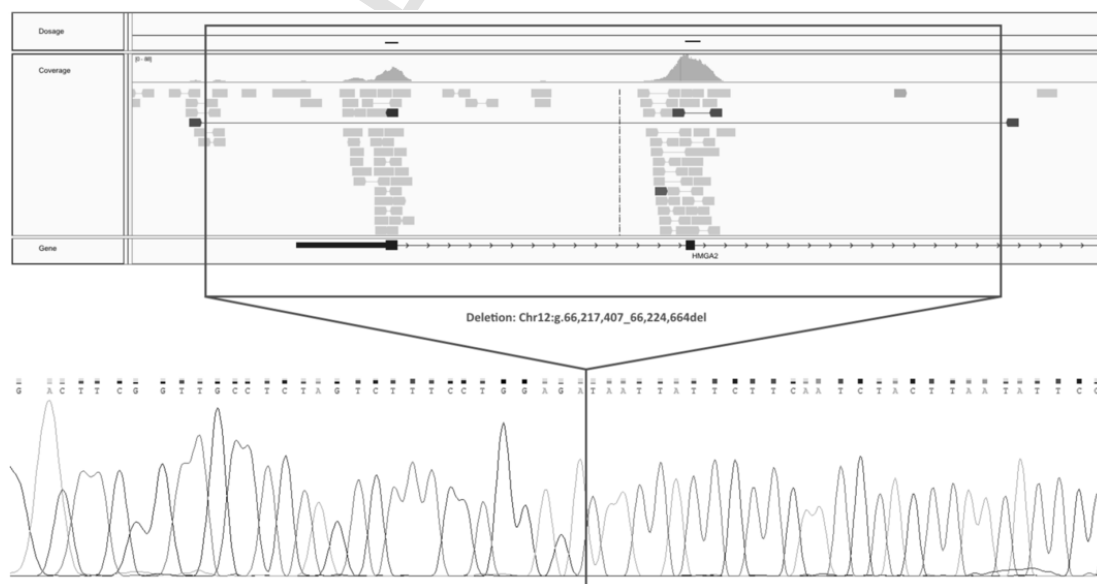

Fig. 1. IGV (integrative genomics viewer) view of deletion from our patient and chromatogram from Sanger sequencing of the breakpoint PCR fragment: IGV window divided in three parts showing the DNA dosage (with deletions below and duplications above the normal dosage line), the coverage and the gene with untranslated regions & exons (black boxes) and introns (line with arrows); deletion marked with a rectangle and linked to its position within the chromatogram's sequence which was created with primers spanning the deletion.

tors, and is encoded by *HMGA2*. The three other members are HMGA1a, HMGA1b and HMGA1c, and result from alternative splicing of one single gene, *HMGA1*. HMGA proteins contain three domains, called AT-hooks, that can bind to the minor groove of AT-rich DNA-sequences, and regulate the transcriptional activity of several genes. They don't have transcriptional activity themselves, but interact with the transcription machinery and alter DNA conformation. They are mainly expressed during embryonic development and regulate diverse biological processes like cell growth, proliferation, differentiation and death (Cleynen and Van de Ven, 2008). It has been shown that HMGA2 regulates the expression of the potent fetal growth factor *IGF2* linking the gene to the phenotype of growth restriction (Abi Habib et al., 2017).

Somatic mutations in *HMGA2* have been identified in various tumors such as uterine leiomyoma, gastric cancer or in pediatric lipoma (Lagana et al., 2017; Zhu et al., 2017; Dadone et al., 2015). In addition, genome-wide association studies identified common germline variants in *HMGA2* to be associated with height in various populations in both children and adults (Weedon et al., 2007; Weedon et al., 2008; Lettre et al., 2008; Wood et al., 2014). Following the idea of an allelic series, rare variants in *HMGA2* might have a larger effect on body size. This is supported by the observation that patients with the 12q14 microdeletion syndrome show, among other features, significant short stature or even SRS phenotype when *HMGA2* is included (Mari et al., 2009; Lynch et al., 2011, Heldt et al., 2018). Furthermore, it was shown that a knockout of *Hmga2* in mice results in the pygmy phenotype, which is characterized by pre- and postnatal growth retardation and remarkable decrease in adipose tissue (Zhou et al., 1995). Heterozygous mice show a milder pygmy phenotype suggesting a dosage effect (Zhou et al., 1995).

Reviewing the literature, we found four additional patients with point mutations as well as structural variants in *HMGA2* (see Fig. 2), whose phenotype is characterized by short stature (Abi Habib et al., 2017; De Crescenzo et al., 2015; Buysse et al., 2009;). Clinical and genetic characteristics of these patients are summarized in Table 1. Applying the NH-CSS to these four patients, three of them fulfilled the criteria for SRS. The fourth patient, described as patient #D0811079 and as having idiopathic short stature by Buysse et al., might also meet the

criteria, but the clinical description lacks information to calculate the score (Buysse et al., 2009).

However, another patient, "DGAP103" published by Ligon et al. (2005), with a *de novo* pericentric inversion of chromosome 12, leading to a *HMGA2* truncation, showed a contrary phenotype, characterized by extreme somatic overgrowth and multiple lipomas (see Table 1). Accordingly, transgenic mice carrying a comparable *Hmga2* truncation, containing only the three AT-hook domains, show a similar phenotype of somatic overgrowth and lipomatosis (Battista et al., 1999). Moreover, chromosomal rearrangements involving *HMGA2* have repeatedly been described in adipocytic and benign mesenchymal tumors, and NIH3T3 cells transform neoplastically when they are transfected with a truncated or a chimeric *HMGA2* gene, either carrying the three DNA-binding domains alone or combined with a fusion partner (Dadone et al., 2015; Schoenmakers et al., 1995; Ashar et al., 1995; Fedele et al., 1998). We therefore assume that patient "DGAP103" shows his overgrowth phenotype because his variant leads to a gain of function.

5. Conclusion

Taking into account all published patients with genetic *HMGA2* alterations and the present case, we conclude that germline loss-of-function variants in *HMGA2* cause SRS. We therefore suggest that genetic testing of *HMGA2* should be considered in routine SRS diagnostics after exclusion of the epigenetic defect of 11p15.5 or maternal disomy of chromosome 7 and should be added to growth retardation disorder panels.

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Conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
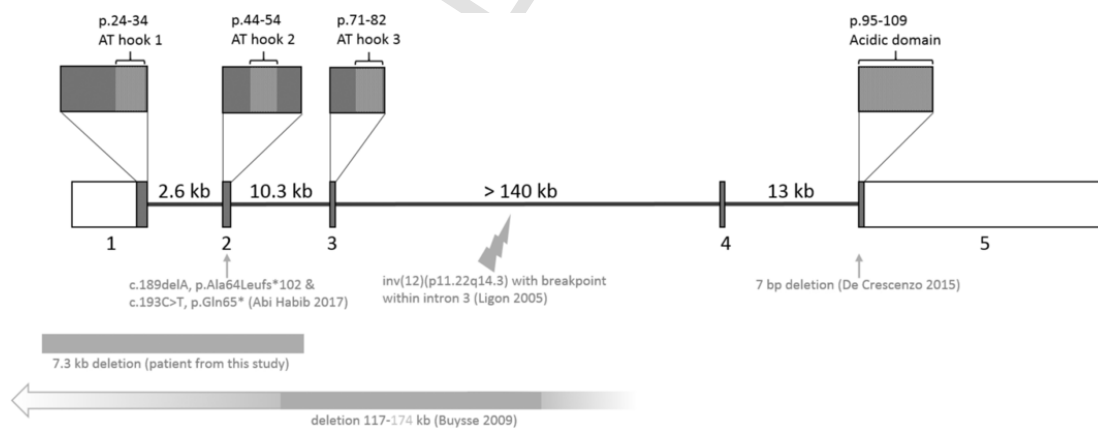

Fig. 2. *HMGA2* and its functional domains, adapted from Cleynen and Van de Ven (2008) and UniProt (http://www.uniprot.org/uniprot/P52926): exons (numbered boxes), introns & genomic sequence (lines), 5′ and 3′ untranslated regions (open boxes), coding sequences (dark grey boxes), sections with functional domains (AT hooks 1–3, acidic domain) are enlarged to show their positions (white dotted areas). Published human germline variants of *HMGA2* and their positions (grey).

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