LETTER TO JMG

Epigenetic mutations in 11p15 in Silver-Russell syndrome are restricted to the telomeric imprinting domain

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Introduction: Silver-Russell syndrome (SRS; also know as Russell-Silver syndrome) is a heterogeneous syndrome which is characterised by severe intrauterine and postnatal growth retardation and typical dysmorphic features. Recently, the first SRS patients with (epi)genetic mutations in 11p15 affecting the telomeric imprinting domain have been identified. Interestingly, opposite mutations are associated with Beckwith-Wiedemann syndrome (BWS). However, the general significance of epigenetic mutations in 11p15 for the aetiology of SRS remained unclear.

Methods: We screened a cohort of 51 SRS patients for epimutations in ICR1 and KCNQ1OT1 by methylation sensitive Southern blot analyses.

Results: ICR1 demethylation could be observed in 16 of the 51 SRS patients, corresponding to a frequency of approximately 31%. Changes in methylation at the KCNQ1OT1 locus were not detected.

Discussion: Combining these data with those on maternal duplications in 11p15, nearly 35% of SRS cases are associated with detectable (epi)genetic disturbances in 11p15. We now have to also consider a general involvement of 11p15 alterations in growth retarded patients with only minor or without further dysmorphic features. SRS and BWS may now be regarded as two diseases caused by opposite (epi)genetic disturbances of the same chromosomal region displaying opposite clinical pictures.

Silver-Russell syndrome (SRS; also known as Russell-Silver syndrome) is a heterogeneous syndrome which is mainly characterised by severe intrauterine and postnatal growth retardation (<3rd percentile). The typical craniofacial features include a prominent forehead, a triangular face, hemihypotrophy, and clinodactyly V. So far, little is known about the causes of the disease: several reports on SRS families as well as on chromosomal disturbances point to a genetic background (for a review see Hitchins *et al*[1]). In 7–10% of SRS patients, a maternal uniparental disomy of chromosome 7 (UPD7) can be detected.

Recently, six growth retarded patients with duplications of maternal 11p15 were reported; four of these patients showed SRS(-like) features (for a review see Eggermann *et al*[2]). These cases led to the hypothesis that (epi)genetic alterations in 11p15 opposite to those in Beckwith-Wiedemann syndrome (BWS) are involved in the aetiology of SRS.[2] In fact, the first SRS patients with epigenetic mutations in the telomeric imprinting domain of 11p15 have recently been identified: Gicquel *et al*[3] reported an epimutation consisting of a (partial) loss of paternal methylation at the *H19-IGF2* imprinting centre (ICR1), at the *H19* promoter, and at the *IGF2* DMR2 in five out of nine patients with the classic SRS phenotype. Methylation of KvDMR1 in *KCNQ1OT1* was normal in this cohort. However, the general significance of epigenetic mutations for the aetiology of SRS remained unclear due to the small number of patients investigated by Gicquel *et al*.[3]

METHODS

We screened a cohort of 51 SRS patients for epimutations in ICR1 and *KCNQ1OT1* using methylation sensitive Southern blot analyses as reported previously.[3 4] Total genomic DNA was digested overnight with *Rsa*I and *Hpa*II for ICR1 and with *Bam*HI and *Not*I for KvDMR1 in *KCNQ1OT1*. Samples were electrophoresed on 1.2% or 0.7% agarose gels, blotted, and hybridised with digoxigenin labelled PCR products for ICR1 or *KCNQ1OT1*.[3 4] Methylation index was achieved by densitometry of autoradiographs using a GelDoc2000 system (BioRad, Munich, Germany). In these patients, maternal UPD7 and maternal duplications of 11p15 had been previously excluded. All patients showed severe intrauterine and postnatal growth retardation (<3rd percentile) and at least three further signs typical for SRS according to Wollmann *et al*.[5]

RESULTS

ICR1 demethylation with an methylation index ranging from 0.20 to 0.37 could be observed in 16 out of 51 SRS patients, corresponding to a frequency of approximately 31%. ICR1 demethylation was excluded in two further patients with maternal UPD7. Changes in methylation at the *KCNQ1OT1* locus were not detected in the 51 patients. More than 80% of the patients with ICR1 demethylation showed body asymmetry together with other clinical features, thus supporting a postzygotic origin and mosaicism of the disturbance as suggested by Gicquel *et al*.[3] Assuming this formation mechanism, there may be even more epigenetic alterations, which remain undetected, in the telomeric imprinting region than reported here.

DISCUSSION

Our data show that ICR1 demethylation is indeed an important, if not the most important, genetic disturbance in SRS. Combining these data with those on maternal duplications in 11p1,[2] nearly 35% SRS cases are associated with detectable (epi)genetic disturbances in 11p15 (table 1). Considering that 7–10% of SRS patients show maternal UPD7 and chromosomal rearrangements affecting 7p11.2–p13,

Abbreviations: BWS, Beckwith-Wiedemann syndrome; GH, growth hormone; SRS, Silver-Russell syndrome; UPD7, uniparental disomy of chromosome 7

Table 1 Comparison of genetic alterations and their frequencies in BWS and SRS

Type of mutation	BWS*		SRS†	
Uniparental disomy of 11p15	Paternal	10–20%	Maternal	– (0/46)[2]
Structural chromosomal rearrangements of 11p15	Paternal duplications	1%	Maternal duplications	4% (2/46)[2]
	Inversions/translocations	1%		
In telomeric 11p15 imprinting domain	Hypermethylation of *H19*	2%	Hypomethylation of ICR1	31–55%‡,[3] (16/51, 5/9)
	Loss of imprinting of *IGF2*	25–50%		
In centromeric 11p15 imprinting domain	Mutations in *CDKN1C*	5–10% sporadic 25% aut. dominant	Mutations in *CDKN1C*	–[6]
	Hypomethylation of KvDMR1	50%	Hypermethylation of KvDMR1	–‡[3]
Others			Maternal UPD7/duplications in 7p	10%
	Unknown	10–20%	Unknown	45–69%

*Frequencies are derived from Weksberg *et al*[8]; †superscript numbers in the last column are references; ‡present study.

a genetic alteration with probable functional significance can now be diagnosed in more than 45% of SRS cases. As a consequence, the diagnostic algorithm for SRS should comprise conventional cytogenetic analyses, ICR1 methylation analyses, and a search for maternal UPD7.

In contrast to BWS, mutations in the centromeric imprinting domain of 11p15 may be neglected in the aetiology of SRS (table 1). We found no epimutations of KvDMR1 in any of the 51 patients, nor were mutations in the transcribed sequences of *CDKN1C* or *KCNQ1OT1* detected.[6 7] This is in agreement with the results of Gicquel *et al*[3] who excluded changes in methylation of KvDMR1 in their cohort of nine patients.

The surprising data on the significance of 11p15 disturbances in SRS shed more light on the aetiology of the disease, but also raise a lot of questions.

If we assume that the growth retardation is caused by the defective expression of *IGF2* as a factor of the *IGF/IGF1R* axis, several genes in the cascade may be disrupted resulting in similar phenotypes. Indeed, chromosomal aberrations observed in SRS affect the regions 7p11.2–p14 as well as 15q26 which harbour the genes *IGFBP1*, *IGFBP3*, *GRB10*, and *IGF1R*, but mutations in these genes have previously been excluded in SRS patients (for a review see Hitchins *et al*[1]). Since body asymmetry is not restricted to SRS patients with proven 11p15 epimutations, mosaicism for undetected epigenetic alterations or chromosomal aberrations is conceivable and needs further evaluation.

Interestingly, the clinical picture in patients with epigenetic mutations in the telomeric imprinting domain in 11p15 is more consistent with SRS than that in patients with maternal duplications of the same region; among the six duplication carriers reported so far, only four showed SRS features (for a review see Eggermann *et al*[2]). Thus many patients with milder symptoms are likely to remain undiagnosed. Consequently, we now have to also consider a general involvement of 11p15 alterations in growth retarded patients with only minor or without further dysmorphic features.

CONCLUSIONS

With the identification of a major genetic disruption in SRS, individual and more directed therapy is conceivable. Until recently, it was nearly impossible to define the genetic and functional subgroups of SRS, and thus treatment of the growth retardation was undirected. The response of SRS patients to growth hormone (GH) treatment is highly variable, with approximately half of the patients showing rapid catch-up growth. The potential for increasing final height using early GH treatment is still being assessed. Interestingly, one of the patients treated successfully is a carrier of a maternal duplication of 11p15.[2] It will be interesting to see whether this is a isolated case or whether SRS patients with 11p15 mutations or other 11p15 disturbances in general benefit from GH treatment in comparison with other SRS subgroups.

Similar to Prader-Willi and Angelman syndrome, SRS and BWS may now be regarded as two diseases caused by opposite (epi)genetic disturbances of the same chromosomal region. Apart from this exciting genetic background, it is in particular interesting that the two imprinting syndromes also display opposite clinical pictures.

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