

Search for Subtelomeric Imbalances by Multiplex Ligation-Dependent Probe Amplification in Silver–Russell Syndrome

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Chromosomal aberrations are typically associated with primordial growth retardation, psychomotoric constrictions, and dysmorphisms. Since these features may be present in patients with Silver–Russell syndrome (SRS) and chromosomal disturbances are also detected in a subgroup of SRS patients, we screened a cohort of 45 SRS patients for cryptic subtelomeric imbalances. Submicroscopic deletions/duplications in the telomere regions are meanwhile well known to cause a broad spectrum of conspicuous phenotypes, characterized by mental retardation and multiple further congenital anomalies. We hypothesize that SRS might represent at the mild end of the broad phenotypic range of subtelomeric imbalances. Screening of the patients was performed by multiplex ligation-dependent probe amplification (MLPA), a technique that has already been shown to be effective and reliable for measuring copy numbers. We excluded pathogenetically relevant copy number variations in the subtelomeres in our SRS patient cohort, but one patient carried an apathogenic polymorphic Yq deletion. It can therefore be concluded that this type of chromosomal aberration does not belong to the genetic causes of SRS and it is not necessary to include this test in the diagnostic algorithm of the disease.

Introduction

INTRAUTERINE AND POSTNATAL GROWTH RETARDATION (IUGR/PNGR) are typical hallmarks for different chromosomal aberrations, ranging from aneuploidies and structural aberrations to submicroscopic chromosomal imbalances. In the latter group, deletions/duplications affecting the subtelomeric regions were in the focus of interest within the last years due to the high content of genes located within these loci. Indeed, screening for cryptic subtelomeric aberrations in cohorts of mentally retarded (MR) patients with unspecific multiple congenital anomalies (MCA) revealed that imbalances in the subtelomeric regions are relatively common, accounting for 3–7% (for review: Balikova *et al.*, 2007).

Apart from these data on MR patients, little is known on the contribution of cryptic subtelomeric imbalances to other clinical features, for example, growth retardation. Indeed, several growth-retarded patients with few or no further clinical signs were diagnosed to be carriers of (cryptic) subtelomeric duplications (Fisher *et al.*, 2002; Eggermann *et al.*, 2005). Among these cases, some were diagnosed as having Silver–Russell syndrome (SRS). SRS (RSS; OMIM 180860) describes a uniform malformation growth retardation syndrome. Apart from the obligate pre- and postnatal growth restriction (<P3), the disease includes a relative macrocephaly, a small triangular face, asymmetry, clinodactyly V, and other less constant fea-

tures. The psychomotoric abilities of SRS patients are controversially discussed: in a sibling-controlled study, Noeker and Wollmann (2004) observed a moderate cognitive impairment in SRS patients.

In addition to several chromosomal disturbances, currently two major (epi)mutations have been described in SRS: while ~10% of patients carry a maternal uniparental disomy of chromosome 7 (UPD7), >35% show a hypomethylation at the imprinting center region 1 (ICR1) in 11p15 regulating the expression of *H19* and *IGF2* (for review: Schönherr *et al.*, 2007). The functional relevance of these disturbances is unclear at the moment; in case of the 11p15 epimutation, it can be speculated that it is a secondary result of another, so far unknown, genetic or environmental factor (for review: Mackay *et al.*, 2006).

The identification of (cryptic) chromosomal aberrations in growth retardation patients, some with SRS features (Fisher *et al.*, 2002; Eggermann *et al.*, 2005), prompted us to speculate whether subtelomeric imbalances might contribute to the genetic causes of the disease. We hypothesized that the SRS features IUGR/PNGR, mild psychomotoric restriction (if present at all), and dysmorphisms represent the mild end of a broad range of clinical features detectable in carriers of subtelomeric rearrangements.

We therefore investigated SRS patients with and without 11p15 epimutations systematically for subtelomeric deletions/

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duplications. These imbalances were assessed by the use of two multiplex ligation-dependent probe amplification (MLPA) assays, the effectiveness and reliability of which has been demonstrated in several MR/MCA cohorts (Rooms *et al.*, 2004; Balikova *et al.*, 2007).

Study Population

The studied population consisted of 45 patients with SRS ascertained as part of ongoing molecular investigations on SRS (Eggermann *et al.*, 2006). The diagnosis of SRS was based on the following criteria: intrauterine growth retardation (birth weight or length below the third percentile), lack of postnatal catch-up growth, and at least two of the following criteria: typical face, relative macrocephaly, and skeletal asymmetry. Maternal uniparental disomy of chromosome 7 had been excluded before. The cohort included a subpopulation of 11 carriers of an ICR1 hypomethylation. The study was approved by the ethics committee of the University Hospital, RWTH Aachen. For validation of the technique, we additionally tested carriers of different chromosomal numeric and structural aberrations, including two with 11p15 duplications (Eggermann *et al.*, 2005) and material of a trisomy-13 abortion.

Materials and Methods

Genomic DNA was extracted from peripheral blood by a simple salting out procedure. MLPA was carried out using the SALSA P036 and P070 kits (MRC-Holland, Amsterdam, The Netherlands). One hundred nanograms of DNA was used; the MLPA reaction was performed according to the manufacturer's protocol. One microliter of PCR products was analyzed by capillary electrophoresis on an ABI Prism 3130 genetic analyzer (Applied Biosystems, Darmstadt, Germany), and quantitative data were extracted by the ABI Genemapper software (Applied Biosystems). Raw data were analyzed using the NGRL-Manchester analysis spreadsheets (<http://www.ngrl.org.uk/Manchester>).

Results and Discussion

As a first step to check the reliability and robustness of subtelomere MLPA, we tested DNA of carriers of different chromosomal aberrations, among them two SRS patients with 11p15 duplications (Eggermann *et al.*, 2005). In all these cases the respective imbalance could be confirmed. Among the 45 SRS patients, including 11 individuals carrying an 11p15 hypomethylation, no abnormalities were detected, but a terminal Yq deletion was found.

Several subtelomeric aberrations have meanwhile been described to be polymorphisms without phenotypic consequences, among them Yq terminal deletions (Ravnan *et al.*, 2006; Balikova *et al.*, 2007). The Yq subtelomeric deletion in the patient was inherited from the phenotypically normal father, and it may therefore be regarded as benign.

Despite the numerous reports of chromosomal findings in patients with SRS features, we could not establish a relevant role of subtelomeric imbalances in the etiology of SRS. From the diagnostic point of view, we therefore suggest that genetic testing for SRS should include conventional chromo-

somal analysis after exclusion of 11p15 hypomethylation and maternal UPD7, but subtelomeric aberrations may be neglected. The most prominent chromosomal disturbances known in SRS are those affecting the regions 7p11–7p13 and 11p15. While the chromosome 7 aberrations would escape the subtelomeric screening and are detectable by conventional karyotyping, only one of the 11p15 duplication cases would have been detected exclusively by subtelomere tests but not by conventional cytogenetic analysis due to the small size of the duplication (case SR90 from Eggermann *et al.*, 2005). After exclusion of the major molecular alterations in SRS and microscopic aberrations, we therefore suggest to focus further search for submicroscopic disturbances mainly on the region 11p15: we think that this directed search will help us to identify further cases with cryptic copy number variations in this region, as shown by Schönherr *et al.* (2007). However, one should bear in mind that cryptic imbalances affecting other chromosomal areas than the subtelomeric regions might be present in SRS.

To sum up, subtelomeric deletions/duplications are not considerably involved in SRS despite several evidences such as growth retardation, a mild psychomotoric delay, and dysmorphism.

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