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Identification of a 21q22 Duplication in a Silver–Russell Syndrome Patient Further Narrows Down the Down Syndrome Critical Region

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Several duplications of chromosome 21q helped to narrow down the Down syndrome (DS) critical region (DSCR) to chromosomal band 21q22 with an approximate length of 5.4 Mb. Recently, it has been suggested that the facial gestalt of DS has been linked to the distal part of the DSCR whereas the proximal region harboring *DSCR1/RCAN* and *DSCAM* should be associated with the cardiac abnormalities. Here, we report on a patient with Silver–Russell syndrome (SRS) and a paternally inherited 0.46 Mb duplication in 21q22 affecting the *KCNE1* and *DSCR1/RCAN* genes. The identification of an involvement of *KCNE1* was interesting because it encodes the β-subunit of the KvLQT1 channel as the slow component of the cardiac delayed rectifier K^+ current. Since duplication of the *KCNQ1* gene encoding the α-subunit of the same channel was reported recently in another SRS patient, we screened both genes for mutations in a cohort of SRS patients without detecting pathologic variants. We presume that the duplication of the two functionally linked genes in different patients with the same disorder is a coincidental finding. However, the lack of DS typical clinical features in our case allows us to further narrow down the DSCR in 21q22. We conclude that *DSCR1/RCAN* is not sufficient for generating phenotypic features associated with DS but our observation does not contradict a possible role for *DSCR1/RCAN* in mediating *DYRK1A*-based effects. © 2010 Wiley-Liss, Inc.

Key words: Down syndrome; *DSCR1/RCAN*; *KCNE1*; *KCNQ1*; CNV; SNP array; Silver–Russell syndrome

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INTRODUCTION

Down syndrome (DS) is typically associated with trisomy of the whole chromosome 21, but cytogenetic and molecular genetic studies in patients with partial duplications of chromosome 21 have narrowed down the DS critical region (DSCR) to 21q22 with an approximate length of 5.4 Mb [Delabar et al., 1993; Arron et al., 2006]. Recently, Ronan et al. [2007] reported on a familial 4.3 Mb duplication in 21q22: the duplication carriers in this family had the typical facial features of DS but no cardiac defects. The duplication in this family included the *DYRK1A* gene but not *DSCR1/RCAN*. The authors suggested that the facial gestalt of DS is linked to the distal part of the DSCR whereas the proximal region harboring *DSCR1/RCAN* and *DSCAM* should be associated with the cardiac abnormalities. Furthermore, increased DSCR1/RCAN expression has been associated with a broad cancer protection in DS [Baek et al., 2009].

The growth restriction disease Silver–Russell syndrome (SRS) has little in common with DS. SRS is a clinically and genetically heterogeneous syndrome. It is mainly characterized by intrauterine and postnatal growth restriction (<2nd centile) and a triangular face with a prominent forehead. Asymmetry of body and limbs, clinodactyly of the fifth digit and macrocephaly are additional

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common clinical signs [for review: Abu-Amero et al., 2008]. While about 10% of patients have a maternal uniparental disomy of chromosome 7 (UPD(7)mat), >38% carry a methylation defect in the telomeric imprinted region on chromosome 11p15 (ICR1) [for review: Eggermann et al., 2008]. Thus hypomethylation of the ICR1 represents the major epigenetic mutation in SRS whereas a mutation of the centromeric 11p15 imprinted region (ICR2) regulating the expression of the potassium channel subunit *KCNQ1* has only described once as a duplication restricted to the ICR2 [Schönherr et al., 2007]. This mutation in the ICR2 consisted of a maternally inherited 1 Mb duplication and included the *KCNQ1* gene.

By screening a SRS patient for cryptic chromosomal imbalances by single-nucleotide polymorphism oligonucleotide arrays (SNP array) we identified a paternally inherited duplication in 21q22 including *KCNE1* and *DSCR1/RCAN*. The patient did neither exhibit cardiac abnormalities nor other DS features. We therefore assume that duplication of *DSCR1/RCAN* does not contribute to the etiology of DS without concominant copy number gain of additional genes [particularly *DYRK1A*; Arron et al., 2006]. Since the functionally linked genes—*KCNE1* and *KNCQ1*—were both affected by duplications in patients with the same disorder we screened these two genes in a cohort of SRS patients but excluded these genes to be involved in the etiology of the disease.

MATERIALS AND METHODS

Patient

Patient SR12 was ascertained by the University Children's Hospital in Tübingen because of features of SRS. The patient was born small for gestational age of 42 weeks with a weight of 2,600 g (−2.83 SDS), a length of 48 cm (−2.11 SDS) and with a relative macrocephaly having a head circumference of 36 cm (+0.40 SDS). Target height was 176.5 cm (father's height 172 cm, mother's height 168 cm). Asymmetry of his body was evident. His face was suggestive but not absolute typical for SRS with a triangular form and abnormal ears. In addition, clinodactyly of the fifth digit was noted. At the age of 12.2 years, he was started on high-dosed recombinant human growth hormone (dose 68 µg/kg/day). At that time, his height was 127.8 cm (−3.22 SDS), weight 29.4 kg (−1.88 SDS), and head circumference 49 cm (−3.31 SDS). Bone age was 11.5 years. Puberty started at the age of 13 years and was suppressed for 2 years by using an LHRH-agonist. Nevertheless, the patient reached a final height of only 156 cm (measured at the age of 22 years). He has finished secondary school ("Realschulabschluå"). Special additional features were: congenital hip dysplasia and luxation as well as scoliosis. There was no cardiac defect.

Hypomethylation of the ICR1 in 11p15 and UPD(7)mat were excluded [Eggermann et al., 2007]. Conventional cytogenetic analysis revealed a normal male karyotype.

Screening for *KCNE1* and *KCNQ1* mutations was performed in a further 10 patients with SRS, also belonging to the aforementioned study [Eggermann et al., 2007]. The diagnosis of SRS was based on the following criteria: intrauterine growth retardation (birth weight or length below the 3rd centile), lack of postnatal catch-up growth, and at least two of the following criteria: typical face, relative macrocephaly, and skeletal asymmetry. In all patients, UPD(7)mat)mat and ICR1 hypomethylation had been excluded before. For further characterization of novel variants we used DNA of >100 healthy individuals. The study was approved by the ethical committee of the University Hospital Aachen.

DNA Studies

Genomic DNA of the patient and his parents was isolated from peripheral lymphocyte cells by a simple salting-out procedure.

The whole family was initially investigated by micorarray typing. The used 500 K array (Affymetrix, High Wycombe, UK) consists of two arrays, the *Nsp*I kit and the *Sty*I kit which together include >500,000 SNPs. DNA was processed according to the manufacturers instructions. Hybridization and washing was performed according to the manufacturer's manual. Arrays were recorded using an Affymetrix GeneChip®Scanner 3000 7G. Data processing including quality assessment was performed using the "R" statistical framework (http://www.r-project.org) with dedicated extensions from the "aroma.affymetrix" project [Bengtsson et al., 2008]. Copy number segmentation results were visualized using tools, developed for the Progenetix project [http://www.progenetix.net; Baudis and Cleary, 2001]. For final copy number profile generation, the data from the *Nsp*I array was omitted due to prominent probe level noise. The duplication in SR12 was validated with Affymetrix Genotyping 6 arrays as well as by analysis of 21q22 microsatellite and by quantitative PCR (qPCR). To exclude point mutations in the *DSCR1/RCAN* gene which might inactivate the third supernumerary gene copy and thereby functionally cover the trisomy, we sequenced the coding exons of this gene in patient SR12. Additionally, we analyzed the promotor and coding regions of *KCNE1* (5 exons) and *KNCQ1* (15 exons) in 10 further SRS patients. These fragments were amplified by PCR and PCR products were directly sequenced by using the BigDyeTerminatorCycleSequencing System (Applied Biosystems, Weiterstadt, Germany). Samples were electrophoresed on an automatic ABI3130 sequencing system (Applied Biosystems). Information on primers and PCR conditions can be requested from the authors.

For rapid genotyping of novel variants in both genes restriction assays were established. The respective assays are available on request.

RESULTS AND DISCUSSION

By whole-genome analysis using a high-density SNP array system we identified a 0.46 Mb duplication in 21q22 in a SRS patient and his healthy father (Fig. 1). Indeed, the duplicated segment in our patients partially overlaps with an already known copy number variation (CNV) (34534). The CNV 34543 has recently been reported once in 1,200 control samples but further information on the carrier was scarce [Zogopoulos et al., 2007]. Despite the overlap with a known CNV with unknown significance, the identification of a 21q22 duplication in our patient was interesting because (a) it affects the *KCNE1* gene, and (b) it includes the *DSCR1/RCAN* gene as a major candidate gene for DS.

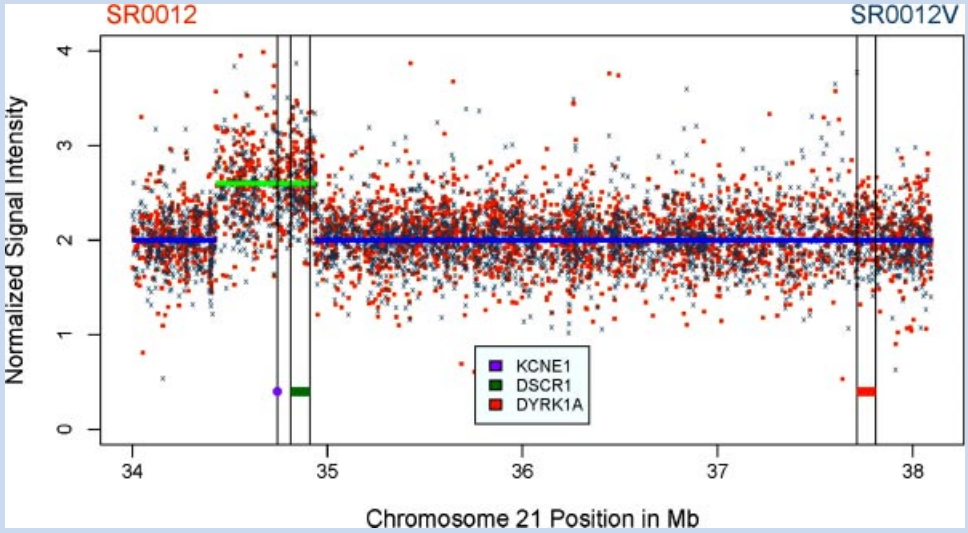

FIG. 1. Local Affymetrix Genotyping 6 signal distribution pattern and segmentation result in patient SR12 (SR0012, orange data) and his father (SR0012V, steelblue data). In both samples a duplication in the DSCR can be observed, affecting the whole *KCNE1* and *DSCR1/RCAN* coding regions. In contrast, *DYRK1A* lays ~2.5 Mb distal of the duplication. Only the genes discussed in this article are shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(a) The identification of a duplication affecting *KCNE1* is remarkable because it encodes the β-subunit of the KvLQT1 channel as the slow component of the cardiac delayed rectifier K^+ current. We recently described the duplication of the gene for the α-subunit of the same channel in another patient with SRS [Schönherr et al., 2007]: the α-subunit of KvLQT1 is encoded by *KCNQ1* localized within the ICR1 region in 11p15. The 11p15 duplication in our case additionally included the *CDKN1C* gene. While point mutations in the *KCNQ1* and *KCNE1* genes result in long or short QT syndromes, an association to other diseases has not yet been reported.

Nevertheless we decided to screen the coding regions of both genes for point mutations: Mutation analysis of *KCNE1* and *KCNQ1* in 10 patients with SRS only revealed three variants which were also detected in healthy controls or reported in the databases. Despite the small number of screened patients we think it to be highly unlikely that both the *KCNE1* and *KCNQ1* genes are involved in the etiology of SRS and presume that the duplication of the two functionally linked genes in different patients with the same disorder is a coincidental finding.

(b) For the first time we were able to show a paternally inherited duplication involving the *DSCR1* gene, heretofore considered part of the minimum region required for DS. The lack of clinical features associated with DS allows us to narrow down further the DSCR in 21q22. After exclusion of mutations in the coding region in our patient we conclude that *DSCR1/RCAN* is not sufficient for generating phenotypic features associated with DS. Additionally, although genes proximal to *DSCR1/RCAN* may contribute to the complex phenotypic changes associated with trisomy 21/DS, this region can be excluded from the DS minimal region. Our case furthermore supports the results from expression studies revealing that expression of *DSCR1/RCAN* is likely compensated and thus does not contribute to the DS phenotype [Olson et al., 2007]. However, our observations do not contradict a possible role for *DSCR1/RCAN* in mediating *DYRK1A*-based effects [Arron et al., 2006] while Korbel et al. [2009] recently argued against a synergy of the two genes.

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