A Boy with an LCR3/4-Flanked 10q22.3q23.2 Microdeletion and Uncommon Phenotypic Features

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Balciuniene et al. [2007] reported 2 patients with a heterozygous 10q22q23 deletion flanked by low copy repeats (LCRs), designated LCR3 and LCR4. Low copy repeats are known to be hotspots for genomic rearrangements. Since this first report, 13 further cases of such recurrent deletions with breakpoints within LCR3 and LCR4 have been published [Alliman et al., 2010; Reddy et al., 2011; Singh et al., 2011; van Bon et al., 2011]; these are summarized in table 1.

LCR3/4-flanked 10q22.3q23.2 deletions present a recurrent genomic disorder with a well-defined genotype, deleted segments ranging from 7.2 to 7.5 Mbp in size. The common phenotype of the reported patients with such deletions included facial dysmorphic features, such as hypertelorism, up- or downslanting palpebral fissures and flat nasal bridge, developmental delay of varying degrees most notable in language acquisition as well as congenital heart defects (CHD), high-arched palate and club feet in some of the patients. Some of the genes within the deleted region, e.g. NRG3, GRID1, BMPR1A, GLUD1, have been discussed as putative candidate genes associated with the phenotype, especially regarding the neuropsychological development [Balciuniene et al., 2007; van Bon et al., 2011]. In this paper, we focus on the role of BMPR1A (bone morphogenetic protein receptor, type
### Table 1. Summary of published cases with 10q22.3q23.2 deletion

<table>
<thead>
<tr>
<th>Case</th>
<th>Breakpoints</th>
<th>Deletion size</th>
<th>Inheritance</th>
<th>Gender</th>
<th>Age of evaluation</th>
<th>Language development</th>
<th>CHD</th>
<th>Club foot</th>
<th>Palate dysmorphism</th>
<th>Other clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balkiuniene et al., 2007, Case 1</td>
<td>81.62 – 89.211 (89.140)</td>
<td>7.5 Mbp</td>
<td>maternal</td>
<td>male</td>
<td>3 1/2 y</td>
<td>delayed, evaluation by use of Preschool Language Scale, ed 3</td>
<td></td>
<td></td>
<td></td>
<td>mild dysmorphic features, autism</td>
</tr>
<tr>
<td>Balkiuniene et al., 2007, Case 2</td>
<td>81.63 – 89.250 (89.140)</td>
<td>7.5 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>1 1/2 y</td>
<td>mild developmental delay, language development not explicitly mentioned</td>
<td></td>
<td></td>
<td></td>
<td>white forelock, retrocerebellar cyst, small cerebellum</td>
</tr>
<tr>
<td>Alliman et al., 2010, Case 1</td>
<td>81.682,644 – 88.931,994</td>
<td>7.25 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>27/12 y</td>
<td>delayed receptive language</td>
<td>PDA</td>
<td>high-arched</td>
<td>hypertelorism, upslanting palpebral fissures, micrognathia</td>
<td>autism, arachnodactyly, joint hyperextensibility</td>
</tr>
<tr>
<td>Alliman et al., 2010, Case 2</td>
<td>81.682,644 – 88.931,994</td>
<td>7.25 Mbp</td>
<td>de novo</td>
<td>female</td>
<td>17 1/12 y</td>
<td>speech impairment in articulation and verbal expression with an oral-motor component; at age 7 y 2 mo, additional evaluation showed delays in expression, and receptive and auditory language processing</td>
<td></td>
<td>high-arched</td>
<td>downslanting palpebral fissures, prognathism</td>
<td>ADHD</td>
</tr>
<tr>
<td>Alliman et al., 2010, Case 3</td>
<td>81.682,644 – 88.931,994</td>
<td>7.25 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>8 days</td>
<td>patient was too young to be assessed</td>
<td>bilateral</td>
<td></td>
<td>hypertelorism, mild epicanthal folds, mild micrognathia, low-set and posteriorly rotated ears</td>
<td></td>
</tr>
<tr>
<td>Alliman et al., 2010, Case 4</td>
<td>81.682,644 – 88.931,994</td>
<td>7.25 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>18/12 y</td>
<td>evaluation at the age of 17 mo showed severe receptive and expressive delays</td>
<td>high-arched</td>
<td></td>
<td>downslanting palpebral fissures, posteriorly rotated ears, small mouth, frontal bossing</td>
<td></td>
</tr>
<tr>
<td>van Bon et al., 2011, Patient 1</td>
<td>81.6 – 88.9 – 89.1</td>
<td>7.2 Mbp</td>
<td>de novo</td>
<td>female</td>
<td>22 y</td>
<td>spoke her first words at the age of 1 y, attended special school for children with mild cognitive impairment from 6 y of age, learned to read and write</td>
<td></td>
<td></td>
<td></td>
<td>hypertelorism, low-set ears, anteverted nares, flat nasal bridge, large mouth, telecanthus, ptosis</td>
</tr>
<tr>
<td>van Bon et al., 2011, Patient 2</td>
<td>81.6 – 88.7 – 89.1</td>
<td>7.2 Mbp</td>
<td>unknown</td>
<td>female</td>
<td>2 1/2 y</td>
<td>spoke only 7 words at 2 y of age</td>
<td>ASD, VSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Bon et al., 2011, Patient 3</td>
<td>81.4 – 89.1 – 89.3</td>
<td>7.7 Mbp, a 722 kb gain in 2q36.3q36.3</td>
<td>de novo</td>
<td>male</td>
<td>37/12 y</td>
<td>at 44 mo of age, scored at 28 mo for language development using the Denver Developmental test</td>
<td></td>
<td>high-arched</td>
<td>hypertelorism, flat nasal bridge, epicanthal folds, dolichocephaly, malformed teeth</td>
<td>epilepsy, Chiari I malformation</td>
</tr>
<tr>
<td>van Bon et al., 2011, Patient 4</td>
<td>81.2 – 81.6 – 88.6/89.1</td>
<td>7.7 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>12 y</td>
<td>at 20 mo of age, expressive language consisted of only one 2-syllable word; at the age of 12 y special education necessary</td>
<td>tricuspid and pulmonic regurgitation</td>
<td></td>
<td></td>
<td>hypertelorism, almond-shaped eyes, aggressive behavior, 47,XXY, 9 café au lait spots, pectus excavatum, kyphoscoliosis, radioulnar synostosis</td>
</tr>
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Table 1 (continued)

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<tr>
<td>van Bon et al., 2011, Patient 5</td>
<td>81,81/81.6</td>
<td>89.14</td>
<td>maternal</td>
<td>male</td>
<td>5 y</td>
<td>first words at the age of 2 y; at 4 1/2 y Schilting test for language production indicated a clear language/speech delay; at 5 y produced 3-word sentences</td>
<td>–</td>
<td>bilateral</td>
<td>hypotelorism, posteriorly rotated ears, flat nasal bridge, broad nasal base</td>
<td>shawl scrotum size evaluation, foot dysmorphism, cleft palate</td>
<td>right-sided club foot, broad nose, everted lower lip vermilion border</td>
</tr>
<tr>
<td>Reddy et al., 2011, Case 2</td>
<td>81,437,039</td>
<td>89,108,131</td>
<td>81,541,288</td>
<td>81,634,060</td>
<td>89,068,181</td>
<td>7.46 Mbp</td>
<td>de novo</td>
<td>female</td>
<td>5 1/2 y</td>
<td>onset not delayed, lacked fluency; Griffith Mental Developmental assessment performed at 5.5 y scored language skills equivalent to 4–4.5 y</td>
<td>–</td>
</tr>
<tr>
<td>Singh et al., 2011, Case Report</td>
<td>81,643,451</td>
<td>88,947,473</td>
<td>7.3 Mbp</td>
<td>de novo</td>
<td>male</td>
<td>age-appropriate development of productive and receptive language, evaluated via SETK</td>
<td>–</td>
<td>–</td>
<td>VSD = ventricular septal defect, PFO = persistent foramen ovale, VSD = ventricular septal defect; y = years.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ADHD = Attention deficit-hyperactivity disorder; ASD = atrial septal defect; CHD = congenital heart disease; mo = months; PDA = patent ductus arteriosus; PFO = persistent foramen ovale; SETK = ‘Sprachentwicklungstest für zweijährige Kinder’ (Language development test for German children at age of 2 years); VSD = ventricular septal defect; y = years.
Genetic Diagnostics

The GTG-bands of the proband's chromosomes showed a normal male karyotype 46,XY. Upon this a SNP array analysis was performed, which revealed a 7,304-kb deletion on one homolog of chromosome 10, as the deleted chromosome displays no hybridization signal for the probe RP11-479O17 (green) representing the deleted region, whereas the hybridization signal of flanking probe RP11-322M19 (red) is marked on both homologs. The deleted chromosome 10 was verified by FISH analysis with informative BAC probes. More than 100 interphase nuclei and additional 23 metaphases were analyzed in the deleted region. The signals of the BAC from the deleted region consistently showed a single signal, whereas the flanking BAC had 2 signals in each nuclei. Therefore, a clinically significant mosaicism in the patient could be ruled out. Neither of the parents was carrier of the deletion.

In a recent publication, Nowakowska et al. [2012] showed that approximately 2.1% of the apparently de novo interstitial copy number variations are the result of a submicroscopic insertion in one of the parents. To exclude such parental insertion, which, if present, would change the recurrence risk for further family members, FISH was performed. No parental insertion was detected (not shown).
**Methods**

**Cytogenetics**
Chromosomes were prepared from PHA stimulated peripheral blood lymphocytes of the index patient as well as the parents following a standard procedure, and standard karyotyping was performed based on GTG-banding at a level of approximately 450 bands.

**Molecular Karyotyping**
In order to identify cryptic chromosomal changes, a genome-wide SNP array was performed using the HumanCytoSNP-12v2.1 BeadChip Kit (Illumina Inc., San Diego, Calif., USA). This array contains 300,000 markers distributed with an average interSNP distance of around 10 kb. Briefly, genomic DNA was prepared from peripheral blood following the standard salt extraction method, and 200 ng of genomic DNA from the index patient and parent were hybridized to the BeadChip in an Infinium® HD Assay according to the manufacturer’s protocol. After hybridization, the BeadChip was scanned with the Illumina BeadArray Reader, and the data were analyzed by examining signal intensity (log R ratio) and allelic composition (BAF) with GenomeStudio v2010.1 and cnvPartition v3.1.6 software. A minimum of a 5-probe cut-off value was used to define a copy number change. The call rates of the samples were larger than 99.0%.

**FISH Analysis**
BAC clones from the 10q22.3q23.2 region were selected from the Ensembl genome browser site (http://www.ensembl.org/) and ordered from the Children’s Hospital Oakland Research Institute (http://bacpac.chori.org/). DNA was extracted by alkaline lysis and labeled by nick translation with Fluorescein-12-dUTP (Roche) or Tetramethyl-Rhodamine-5-dUTP (Roche). After hybridization, washing and counterstaining chromosomes were analyzed with a Zeiss AxioImager microscope. Image acquisition and analysis were performed using a CCD camera and FISHView 2.0 software (Applied Spectral Imaging). At least 20 metaphases and additional 100 interphase nuclei were evaluated per BAC probe.

**Discussion**

One phenotypic feature present in almost all of the published cases of LCR3/4-flanked 10q22.3q23.2 deletion that have been published is a developmental delay of varying degrees, most prominently in language and speech. However, the latter claim must be put into perspective with the fact that the majority of patients in previous studies were not evaluated by standardized tests. Most available information seems to reflect parental attitudes or occasional clinical evaluation. The current patient was examined by a standardized language test, and in contrast to other patients, he showed a normal development of receptive and expressive language at the age of 2 years. Thus, this is a rare case of a patient with an LCR3/4-flanked 10q22.3q23.2 deletion, who shows no speech/language impairment upon objective evaluation. In view of the considerable variance in speech/language delay ranging from mild to severe in the majority of published patients (table 1), the normal development of language acquisition and production of the index patient may be the result of an even broader phenotypic expressivity of the underlying chromosomal microdeletion. Otherwise, it is possible that the test was not sensitive enough to detect a mild deficit or that speech/language delay may manifest at a later age. Therefore, further examinations with standardized language tests are planned at the age of 4 and 5 years.

Another phenotypic feature present in all patients is craniofacial dysmorphism, most commonly hypertelorism (8/14, 57%), flat/broad nasal bridge (6/14, 43%), upslanting or downslanting palpebral fissures (5/14, 36%), and high-arched palate (4/14, 29%). Other rare features are micrognathia, low-set or posteriorly-rotated ears, and epicanthal folds; the patient presented here is the first one exhibiting a median cleft palate. In a recent publication, Saito et al. [2012] presented a conditional knockout mouse model, generated by expressing a dominant negative BMPR1A protein (dnBMPR1A) in neural-crest-derived cells (dnBMPR1A lacks the intracellular kinase domain and, thus, inhibits the BMPR1A-mediated signaling pathway). The mutant mice exhibited either facial fusion defects such as a cleft face and cleft palate, or facial dysmorphism corresponding to hypertelorism and flat nasal bridge in humans. An incomplete expansion of neural-crest-derived mesenchymal cells due to extensive apoptosis was found in the mutant embryos. In addition, 50% of the mutant mice with a facial cleft also showed heart defects. The patient reported here also showed a small, VSD and persistent foramen ovale. Thus, the craniofacial dysmorphic features and the heart septal defects observed in the patients with LCR3/4-flanked 10q22.3q23.2 deletion may be partly caused by a reduction of BMPR1A-mediated signaling.

The BMPR1A gene is also associated with the JPS that is characterized by the development of hamartomatous polyps in the gastrointestinal tract. Heterozygous point mutations or partial deletions of BMPR1A are found in approximately 20% of patient with JPS [Larsen et al., 2011]. Among patients with 10q22q23 microdeletions, JPS has been observed only in those patients having both genes BMPR1A and PTEN deleted. PTEN is a gene associated with Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome involved in the deletion, suggesting that the contiguous deletion of both BMPR1A and PTEN is required in order for polyposis to manifest [Delnatte et
al., 2006; Dahdaleh et al., 2012]. PETN is telomeric to BMPR1A as well as to LCR4, so it is not involved in the LCR3/4-flanked 10q22.3q23.2 deletion; none of the patients with such deletions were reported to have JPS, supporting the supposition that the contiguous deletion of both BMPR1A and PETN is needed for the development of the polyposis phenotype. Unfortunately, the number of described patients is very small, and in rare cases, patients with mutations in BMPR1A showed Bannayan-Riley-Ruvalcaba syndrome-like features [Zhou et al., 2001]. Therefore, an accurate genotype-phenotype correlation is not possible. In patients with JPS, the risk of gastrointestinal cancer is increased even though the majority of polyps are benign. Our patient did not show any JPS symptoms at the time of evaluation. Nevertheless, he was considered as a patient of risk for JPS, and baseline screening according to Larsen et al. [2011], including a complete blood count, colonoscopy and upper gastrointestinal endoscopy beginning at 15 years of age or at initial symptoms such as gastrointestinal bleeding was recommended.

Five of the 14 patients (36%), including the one presented here, had CHD, most commonly VSD. Breckpot et al. [2012] published a case report on a boy with VSD, short stature and facial dysmorphism, who had an intragenic BMPR1A deletion. Reviewing literature for cases with distal chromosome 10q deletions and using computed gene prioritization, the authors showed that BMPR1A is the best candidate gene for CHD in patients with 10q22q23 deletions. Saito et al. [2012] also evaluated heart morphology of the mutant animals and showed that some of those with a facial cleft exhibited a ventricular septum defect as well. Thus, deletions of BMPR1A may contribute to various phenotypes. Detailed phenotypic characterization of patients with BMPR1A deletions and other mutations is warranted, to further delineate the role of BMPR1A in the clinical presentation of the LCR3/4-flanked 10q22.1q23.2 deletion syndrome and in human embryonic development in general.

In conclusion, the phenotype of the LCR3/4-flanked 10q22.1q23.2 deletion varies significantly, especially in speech and language development, ranging from severe delay to age-appropriate development as in the patient presented here. Thus, the prognosis of infants with an ascertained genotype regarding their language development should be given with caution. Long-term follow-up studies of affected patients are needed to further delineate the natural history of this rare disorder.

Acknowledgement
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References


