Constitutional Haploinsufficiency of Tumor Suppressor Genes in Mentally Retarded Patients With Microdeletions in 17p13.1

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Key Words
Array CGH · Mental retardation · Microdeletion · TP53 · Tumor suppressor genes

Abstract
Chromosome microdeletions or duplications are detected in 10–20% of patients with mental impairment and normal karyotypes. A few cases have been reported of mental impairment with microdeletions comprising tumor suppressor genes. By array-CGH we detected 4 mentally impaired individuals carrying de novo microdeletions sharing an overlapping segment of ~180 kb in 17p13.1. This segment encompasses 18 genes, including 3 involved in cancer, namely KCTD11/REN, DLG4/PSD95, and GPS2. Furthermore, in 2 of the patients, the deletions also included TP53, the most frequently inactivated gene in human cancers. The 3 tumor suppressor genes KCTD11, DLG4, and GPS2, in addition to the GABARAP gene, have a known or suspected function in neuronal development and are candidates for causing mental impairment in our patients. Among our 4 patients with deletions in 17p13.1, 3 were part of a Brazilian cohort of 300 mentally retarded individuals, suggesting that this segment may be particularly prone to rearrangements and appears to be an important cause (~1%) of mental retardation. Further, the constitutive deletion of tumor suppressor genes in these patients, particularly TP53, probably confers a significantly increased lifetime risk for cancer and warrants careful oncological surveillance of these patients. Constitutional chromosome deletions containing tumor suppressor genes in patients with mental impairment or congenital abnormalities may represent an important mechanism linking abnormal phenotypes with increased risks of cancer. Germline mutations in highly penetrant tumor suppressor genes are rare events that, when not lethal before adulthood, may be associated with hereditary cancers. This is the case for mutations involving the genes TP53, BRCA1, and BRCA2. Chromosome microdeletion is an alternative mechanism for the constitutional loss of function of tumor suppressor genes. This has been described in a few microdeletion syndromes associated with the oc-
currence of tumors, such as the WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation syndrome, MIM 194072), caused by deletions in 11p13 encompassing the WT1 gene. Another example is the NF1 microdeletion syndrome, affecting 5–20% of patients with neurofibromatosis (MIM 162200).

Since the introduction of array-CGH in routine diagnosis, it has been shown that 10–20% of patients with idiopathic mental retardation and/or congenital abnormalities show submicroscopic chromosome imbalances [Shaw-Smith et al., 2004; Rosenberg et al., 2006]. Here we report 4 patients with syndromic mental impairment and chromosomal microdeletions in 17p13.1 of variable size encompassing several tumor suppressor genes, including TP53. Although acquired deletions of this chromosomal segment have been reported in tumors [De Smaele et al., 2004; Ferretti et al., 2005], constitutional deletions have not been described and their long term consequences are undetermined to date.

Patients and Methods

Patients

The patients presenting with mental retardation, additional clinical signs, and normal G-banded karyotypes were selected for chromosome submicroscopic investigation by array-CGH. The research protocol was approved by the ethics committees of the institutions. Informed consent for publishing results and photos were obtained from the patients’ legal guardians. Data on the patients were deposited in the DECIPHER database (Database of Chromosomal Imbalances and Phenotype in Humans using Ensembl Resources, https://decipher.sanger.ac.uk), and the corresponding DECIPHER number is given. Patient 1 was studied at the Wessex Regional Genetics Laboratory (WRGL), UK, and patients 2, 3, and 4 were part of a sample of 300 Brazilian patients with syndromic mental retardation and normal karyotypes investigated by a 1 Mb BAC array-CGH at the University of São Paulo.

The clinical and familial data on the patients are described below. For patients 2 and 4, records of measurements at birth were not available. Figure 1 shows the facial features of the patients.

Patient 1 (DECIPHER 2346). A girl born with 3,650 g, following a pregnancy complicated by decreased fetal movements. Bilateral hip dysplasia was noted and required splinting, but surgery was not required. By 6 weeks of age unusual eye movements were present that subsequently improved, but a squint appeared to be secondary to an inability to focus; the retinal examination was normal. By the age of 8 months she had poor head control, and by 2 years of age she was sitting up without support. At 3 years of age she could not walk or speak; dysmorphology examination showed brachycephaly, mild ptosis, and a full nasal tip. A spinal tail was noted and she wore a brace for mild scoliosis. She had generally low tone and her reflexes were difficult to elicit. X rays of the spine revealed 4 extra distal sacral vertebrae. Brain MRI detected white matter changes (bilateral but not symmetrical patchy areas of signal, most prominent in the peritrigonal regions). At 5 years her height was 100 cm, weight 14.5 kg, and head circumference 49 cm, which are all between the 3rd and 9th centiles.

Patient 2 (DECIPHER 2173). A boy, the second child of a healthy non-consanguineous couple; his elder and younger sisters were clinically normal. He was born at term after an uneventful pregnancy, and was cyanotic and hypotonic. His cry was weak and he could not be breast-fed. Milestones were delayed: he held up his head at about 5 months of age and walked at 3 6/12 years. When examined at 8 8/12 years, mental deficiency appeared to be profound; he was hyperactive and interactions were practically absent. He appeared to listen to music and to be happy when touched. Speech was absent. His head circumference was 50 cm (2nd centile), height 126 cm (25th centile), and weight 25.5 kg (50th centile). On examination, he had turriccephaly with narrow forehead, a low frontal hairline, epicanthic folds, telecanthus, narrow palpebral fissures, a bulbous nose, narrow high palate, posteriorly rotated dysplastic ears, and long slender fingers.

Patient 3 (DECIPHER 2009). A boy, the second child of a healthy non-consanguineous couple; his older sister was clinically normal. He was born at term after an uneventful pregnancy, and was cyanotic and hypotonic. His cry was weak and he could not be breast-fed. Milestones were delayed: he held up his head at about 5 months of age and walked at 3 6/12 years. When examined at 8 8/12 years, mental deficiency appeared to be profound; he was hyperactive and interactions were practically absent. He appeared to listen to music and to be happy when touched. Speech was absent. His head circumference was 50 cm (2nd centile), height 126 cm (25th centile), and weight 25.5 kg (50th centile). On examination, he had turriccephaly with narrow forehead, a low frontal hairline, epicantthic folds, telecanthus, narrow palpebral fissures, a bulbous nose, narrow high palate, posteriorly rotated dysplastic ears, and long slender fingers.

Patient 4 (DECIPHER 2345). A boy, the second child of a healthy non-consanguineous couple; his older sister was clinically normal. He was born at term after an uneventful pregnancy, and was cyanotic and hypotonic. His cry was weak and he could not be breast-fed. Milestones were delayed: he held up his head at about 5 months of age and walked at 3 6/12 years. When examined at 8 8/12 years, mental deficiency appeared to be profound; he was hyperactive and interactions were practically absent. He appeared to listen to music and to be happy when touched. Speech was absent. His head circumference was 50 cm (2nd centile), height 126 cm (25th centile), and weight 25.5 kg (50th centile). On examination, he had turriccephaly with narrow forehead, a low frontal hairline, epicantthic folds, telecanthus, narrow palpebral fissures, a bulbous nose, narrow high palate, posteriorly rotated dysplastic ears, and long slender fingers.

Fig. 1. Facial features of patients carrying deletions in 17p13.1. a Patient 1, 3 years of age. b Patient 2, 8 years of age. c Patient 3, 6 years of age. d Patient 4, 18 years of age.
Table 1. Genomic mapping of 17p13 deletions and additional chromosomal alterations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genomic position of the 17p13 deletion (kb)</th>
<th>Size of the 17p13 deletion (kb)</th>
<th>Additional cytogenetic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7054.4–7341.0</td>
<td>287</td>
<td>– Mother with deletion in 17p12 (14014.3–15479.4 kb)</td>
</tr>
<tr>
<td>2</td>
<td>6507.6–7567.6</td>
<td>1060</td>
<td>Maternally inherited duplication in 20p12.1 (8670.1–9349.9 kb)</td>
</tr>
<tr>
<td>3</td>
<td>5405.1–8133.5</td>
<td>2728</td>
<td>Maternally inherited deletion in 3q29 (19760.4–19859.2 kb)</td>
</tr>
<tr>
<td>4</td>
<td>2796.6–7232.7 (50% mosaic)</td>
<td>4436</td>
<td></td>
</tr>
</tbody>
</table>

Results

Deletions in chromosome 17p13.1 were identified by array-CGH in four patients (table 1; fig. 2). All the deletions were shown to be de novo (parents of patient 1 were investigated by MLPA using primers specific to TNKI, and those of patients 2, 3, and 4, were analyzed by FISH). Although the array profile of patient 4 clearly revealed the deletion in 17p13.1, its log2 ratio value was higher than the values obtained for the deletions in the two other Brazilian patients investigated in simultaneous hybridization experiments (fig. 2). In fact, this deletion was present in a mosaic state in about 50% of blood cells, as revealed by FISH (fig. 3a). The deletions mapped to chromosome 17p13.2→p13.1 covering a 5.3 Mb region, as estimated by array-CGH (fig. 4a). The breakpoints were unique for each of the 4 deletions. However, there was an overlapping segment of approximately 180 kb in 17p13.1, encompassing 18 genes (fig. 4b).
Patient 4 carried an additional deletion in chromosome 3q29, which was maternally inherited (fig. 3b). The mother of patient 2 was found to carry the recurrent deletion of \( PMP22 \) in 17p12, lying approximately 6.4 Mb proximal to the 17p deletion in her son.

Table 1 summarizes the genomic positions of the detected chromosomal alterations.

**Discussion**

We described 4 patients with differently sized deletions in 17p13.1. Copy number variations of segments within this region have neither been detected among our in-house controls nor described in the Database of Genomic Variants (DGV [http://projects.tcag.ca/variation/]). The phenotypes of the patients were variable, which might be partially explained by the different genes involved in the specific deletions. In spite of this, all patients presented with severe to profound mental retardation and absent or very poor speech. A small head and poor growth were also common features.

Eighteen genes map to the deleted segment common to all 4 patients (fig. 4), and 4 of them appear as candidates for causing mental impairment, namely \( KCTD11/REN \) (potassium channel tetramerization domain-containing protein 11, MIM 609848), \( DLG4/PSD95 \) (postsynaptic density protein 95, MIM 602887), \( GPS2 \) (G protein pathway suppressor 2), and \( GABARAP \) (GABA-A receptor-associated protein). \( KCTD11 \) is a developmentally regulated gene that promotes neuronal differentiation [Gallo et al., 2002], \( DLG4 \) is a major protein found in virtually all mature excitatory glutamatergic synapses in the brain [Beique and Andrade, 2003], \( GPS2 \) was shown in mice to modulate transactivation of genes involved in brain morphogenesis [Zhang et al., 2008], and \( GABARAP \) mediates inhibitory neurotransmission [Weiergraber et al., 2008]. These neurodevelopmental genes may well play a role in the mental impairment of our patients.

A further 17p gene known to be disease-associated is deleted in patient 4, the cholinergic receptor, nicotinic, epsilon polypeptide gene (\( CHRN\); MIM 100725). It is one of the genes responsible for postsynaptic congenital myasthenic syndrome. The generalized muscle atrophy and weakness of patient 4 may be related to this; however, the phenotype-genotype correlation in the patient is quite complex, both because his 17p deletion is in mosaic form and is combined with a 3q29 deletion [Ballif et al., 2008].

The 17p13.1 deletions have a further challenging aspect for genetic counseling, as several of the deleted genes are known or suspected to have a role in tumorigenesis. The deletion of this chromosomal segment is the most frequent genetic lesion in medulloblastomas, and \( KCTD11 \) was found to be either deleted or down-regulated in the majority of these tumors [Di Marcotullio et
**Fig. 3.** Chromosomal alterations detected in patient 4. **a** Mosaic deletion at 17p13.1, as revealed by FISH (~50% of metaphases of cultured lymphocytes): metaphase with the fluorescent signal of BAC RP11-144K9 on both chromosomes 17 and a nucleus with a single signal (arrows). **b** Deletion at 3q29 present in the patient and his mother. Left side: array-CGH profile in the patient; right side: FISH on a metaphase from the patient’s mother, showing the red fluorescent signal of BAC GS-56-H22 on only one chromosome 3 (arrows).
Krepischi-Santos et al.

Cytogenet Genome Res 2009;125:1–7

6

KCTD11 is known to induce apoptosis and growth arrest and is considered a tumor suppressor gene [Beique and Andrade, 2003]. DLG4, although less well characterized, is likely a tumor suppressor gene implicated in the development of HPV-associated cancers [Handa et al., 2007]. Furthermore and possibly with higher clinical impact, two of the patients (patients 2 and 3) have a deletion of the TP53 tumor suppressor gene. Germline mutations in TP53 are associated with the Li-Fraumeni syndrome (LFS, MIM 151623), a complex autosomal dominant syndrome characterized by familial predisposition to multiple early-onset cancers [Malkin, 2001; Nichols et al., 2001]. In LFS, penetrance is about 50 and 90% by the ages of 30 and 70 years, respectively, and probably higher in cases of full loss of TP53 [Olivier et al., 2003]. A recent publication reports the genome-wide profile of germline copy number variations (CNVs) in LFS families [Shlien et al., 2008]. This study showed that the number of CNVs was strikingly enriched in patients with TP53 mutations. In this cohort, however, CNVs are likely a consequence of impairment of apoptosis caused by TP53 loss-of-function mutations. As a complicating factor, the GPS2 gene, also deleted in all our patients, modulates TP53 activity and contributes to apoptosis [Peng et al., 2001].

Constitutional chromosome deletions containing tumor suppressor genes in patients with mental impairment or congenital abnormalities may represent an important mechanism linking abnormal phenotypes with increased risks of cancer. In fact, several works show an association between childhood neoplasia and congenital abnormalities [Mehes et al., 1987; Altmann et al., 1998; Agha et al., 2005]. A recent publication [Bjorge et al., 2008] comprising over 5 million children and their families from Sweden and Norway showed that children with birth defects have a 1.6–1.7-fold higher risk of developing cancer in childhood and early adulthood than the control population, and this risk seems to be over 5 times higher when the patient presents multiple congenital abnormalities.

Considering the relatively young age of our 4 patients, the fact that no tumor has so far been reported is within expectation. Because no cases of constitutional deletion
of KCTD11 or DLG4 have been reported, no empirical risk estimates of cancer can be inferred for these conditions. However, for what we know of the deleted genes, it is likely that our patients have a significantly increased risk for cancer. The identification of potential tumor suppressor genes within microdeletions brings an unusual aspect in genetic counseling for mental impairment. We provided the families of our patients with information concerning cancer risks as well as referral to oncological services for proper follow-up. Besides the clinical implications, follow-up of these patients will contribute to better cancer risk estimates.

We detected deletions on 17p13.1 in 3 out of 300 (1%) mentally impaired Brazilian patients, suggesting that such deletions represent a major cause of mental retardation in addition to likely conferring a significant risk to cancer. Long-term oncological surveillance is necessary.

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References