What can we learn from old microdeletion syndromes using Array-CGH screening?

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Conflict of interest

All authors have no conflicts of interest to declare.

Abstract

Most microdeletion syndromes identified before the implementation of array-CGH were presumed to be well-defined clinical entities. However, the introduction of whole-genome screening led not only to the description of new syndromes, but also to the recognition of a broader spectrum of features for well-known syndromes. Here, we report on 10 patients presenting with mental retardation associated with atypical features not suggestive of a known microdeletion and a normal standard karyotype. Array-CGH analyses revealed 5 microdeletions in the DiGeorge region, 3 microdeletions in the Williams-Beuren region and 2 microdeletions in the Smith-Magenis region. Reevaluation in these patients confirmed that the diagnosis remained difficult on clinical grounds and emphasized that well-known genomic disorders can have a phenotype that is heterogeneous and more variable than originally thought. The widespread use of array-CGH shows that such patients may be more readily achieved on the basis of genotype rather than phenotype.

Keywords: array-CGH, microdeletion syndromes, modifier genes, variable phenotype

Introduction

Segmental aneusomy is a common cause of mental retardation (MR) and advances in technologies allow the routine identification of submicroscopic imbalances in a large number of patients. In the last couple of years, array-CGH screening of patients with MR and/or multiple congenital
anomalies, in whom the karyotype was normal, has led not only to the identification of new microdeletion and microduplication syndromes, but also to the recognition of a broader spectrum of features for previously described syndromes (1). Parallel to these studies, genome-wide screening of patients with MR using array-CGH has also identified regions of the chromosome with recurrent rearrangements for which phenotypic features were subsequently analysed. This genotype-first approach led to the description of new submicroscopic chromosomal imbalances such as the 1q21.1 deletion (2), the 1q41q42 deletion (3), the 15q13.3 deletion (4) and the 16p11.2 deletion (5). These recurrent rearrangements which elude syndromic classification and clinical diagnosis have become a challenge. Here we report on 10 patients presenting with atypical phenotypes associated with well-known microdeletion syndromes.

Methods
Patients with well-known microdeletions and atypical phenotypes were collected between July 2008 and July 2009 in 4 different French Genetic centers (Lille, Dijon, Caen and Bondy). After clinical examination by a geneticist and standard mental retardation evaluations including karyotype and \textit{FMRI} molecular analysis, around 1200 patients benefited from array-CGH analyses on three platforms (Lille, Nancy and Dijon). This number did not include patients in whom a classical microdeletional syndrome was suspected on the basis of medical history or clinical examination, therefore diagnosed by FISH. Either the Human Genome Microarray CGH 44K, 105K, 180K or the 244K from Agilent® was used for array-CGH analyses according to the manufacturer’s protocol (Agilent Technologies, Santa Clara, CA). Data were processed with Feature Extraction (v. 9.1) software and the results were analysed with cgh analytics (v. 4.0) software (Agilent®). Ten of the 1200 patients were selected presenting with a well known microdeletion syndrome and atypical features were selected. Informed consent was obtained from each patient. Copy number changes identified by microarray analyses were confirmed by FISH using specific probes (Vysis) or quantitative q-PCR. Parental studies were performed using the same probes.
Results

Clinical features

The clinical features of the patients are summarized in Table 2. An atypical phenotype was found in all patients, with absence of clinical features that would be suggestive of the diagnosis (clinical history, malformations, dysmorphism, behaviour), but also on the basis of an atypical features (bilateral cleft-hands for patient 1, polydactyly, hypogonadotropic hypogonadism associated with hyposmia suggestive of Kallmann De Morsier syndrome for patient 2, isolated hypogonadism for patient 3, predominant skin manifestations (verruceous hamartoma and angioma) for patient 4, short stature with radiological features suggestive of hypochondroplasia for patient 6, severe to profound mental retardation for patient 9 and pulmonary stenosis with edema suggestive of Noonan syndrome for patient 10).

Array-CGH

Array-CGH studies revealed five classical 22q11.21 deletions, three classical 7q11.23 deletions and two 17p11.2 deletions with one smaller than the classical deletion (Table I). The sizes of all these deletions, which involve the candidate region of each syndrome, have already been described. Patients 1, 2 and 3 had a de novo 22q11.21 deletion (2.8, 2.4 and 2.5 Mb respectively) and patient 4 had a 3.1 Mb deletion inherited from her asymptomatic mother. Patient 5 had a smaller deletion (2 Mb) but the segregation could not be verified since his father had died. Patients 6, 7 and 8 had a de novo classical 7q11.23 deletion of 1.4 Mb. Patient 8 also had a 15q13.3 duplication of 1.7 Mb including the CHRNA7 gene inherited from her asymptomatic mother. Patients 9 and 10 had a de novo 17p11.2 deletion (2.5 Mb and 3.2 Mb respectively). The presence or absence of genes at the breakpoints can be found in Table 1.

FISH studies
Discussion

Our study emphasizes the considerable phenotype diversity associated with haploinsufficiency of 22q11.21, 7q11.23 and 17p11.2 regions. Phenotype variability is already well known in DiGeorge syndrome (MIM ID #188400), which usually includes cardiac malformations, craniofacial, limb and digit anomalies, hypoplasia or aplasia of the thymus with associated deficiency of T cells, hypocalcemia with hypoplasia or aplasia of the parathyroids, language disorders, and increased vulnerability to psychiatric diseases. Here we report on 5 patients in whom the diagnosis of classical 22q11.21 deletion was made at 15, 27, 14, 3 and 38 years of age respectively. None of them had cardiac malformation or hypocalcemia. Patient 1 presented with cleft-hands which have never been described in this syndrome. Skeletal anomalies are reported in more than 20% of 22q11.21 deletion cases and frequently include polydactyly, syndactyly and clubfoot (6, 7). Patient 2 presented with hypogonadotropic hypogonadism associated with hyposmia suggesting Kallmann’s syndrome. Patients 3, 4 and 5 were referred to the genetics department for moderate mental retardation but no cardinal features of DiGeorge syndrome. Although 90% of the affected individuals shared approximately the same 3 Mb deletion (between LCR A and B), their phenotype was highly variable since more than 180 features have been associated with this deletion, some of them being uncommon (iris and retinal coloboma, ear tags or pits, polymicrogyria, diaphragmatic hernia and hirschsprung’s megacolon) (8, 9). Phenotype expression has not been shown to be related to the deletion size and furthermore the phenotype can be widely different even within a single family, indicating the involvement of additional modifiers (10). The 22q11.21 deletion in patient 4 was also identified in her mother, who presented language delay and mild learning difficulties in infancy (verbal intellectual quotient = 67, performance intellectual quotient = 72). The mother also suffered from recurrent otitis leading to conductive deafness on the right side. She had no known history of
congenital cardiac heart defect nor hypocalcemia. Physical examination revealed minor dysmorphic features comprising a long face and prominent median maxillary incisors. This example illustrates the intrafamilial variability associated with such microdeletion syndromes.

Three patients with a classical 7q11.23 deletion (1.41-1.88 Mb) are described in our report. Two of them were adults and none presented with the cardinal features of Williams-Beuren syndrome (MIM ID #194050) such as the distinctive facial dysmorphism, arterial stenosis, high sociability and empathy for others. Patient 6 (31 years old) had a short stature with short limbs at first suggesting hypochondroplasia. The adult phenotype of Williams-Beuren syndrome is less well-known. Ferrero et al [2007] (11) investigated 22 Williams-Beuren Syndrome patients from 1 day to 39 years old and showed that the mean age at diagnosis was 5.4 years, being 1 year when there was a heart defect and 10.7 years in cases of mental retardation without patent cardiac anomaly. While many of the clinical features during childhood have been well characterized, studies concerning the adult population are small. The mean adult height is 159 cm for men (for 6 boys at 19 years) and 147 cm for women (for 8 girls at 19 years) (12) and in our report the patient’s’ height was 142 cm. Of note, failure to thrive and a slim build in childhood often gives way to relative obesity in adults, with a characteristic “Hottentot” pattern of fat distribution, particularly in women. With age the face becomes narrower, facial features are coarser, and a loss of subcutaneous tissue may lead to an emaciated appearance. These patients also present a protuberant hyoid bone (13). Patient 8 had a 15q13.3 duplication associated with the 7q11.23 deletion. This duplication (1.7-1.8 Mb), inherited from her asymptomatic mother, includes the $CHRNA7$ gene. This anomaly has been associated with incomplete penetrance for mild to moderate developmental delay or mental retardation and neuropsychiatric abnormalities including autism spectrum disorders (14, 15). This duplication might contribute to the patient’s phenotype.

We report 2 patients with a 17p11.2 deletion both including the $RAI1$ gene. Most patients with Smith-Magenis syndrome (SMS, MIM ID #182290) have the same interstitial genomic deletion of approximately 3.5 Mb at chromosome 17p11.2 comprising 20 expressed genes (16, 17).
Despite a common deletion size, the only consistent features among these patients are sleep disturbances, low adaptive functioning, and mental retardation (18). Sleep disturbance has been reported in 75-100% of SMS cases and is one of the earliest diagnostic indicators of SMS, typically in the first year of life (19). Behavioural issues are among the unique characteristic features of SMS; self-injurious behaviour, including head banging, skin pricking, and wrist biting as early as 15-18 months of age (20). Smaller or larger deletions were seen in around 12% and 10% of patients with SMS respectively (18). Genotype-phenotype studies of the patients with SMS showed no phenotype difference between deletions of 1.5–9 Mb in size (21, 22). The RAI1 gene is probably responsible for the majority of the SMS features, but other deleted genes in the 17p11.2 region may modify the overall phenotype. Patient 9 had a shorter deletion of at least 2.57 Mb including RAI1. He presented with severe mental retardation and dysmorphic features which did not evoke SMS, possibly because the first examination was performed in adulthood. His sleep disturbances were not mentioned in light of the other more severe behavioural manifestations. The poor familial context probably worsened the mental retardation. Patient 10 presented with pulmonary artery stenosis and neck and limb oedema that first suggested Noonan syndrome and were not suggestive of SMS. Sleep disturbance only appeared after the diagnosis and retrospectively, pulmonary artery stenosis was found to be a rare feature of SMS (19).

Clinical diagnosis of these 10 patients was difficult for different reasons: i) the presence of atypical features suggesting another diagnosis (patients 1, 2, 6, 9 and 10); ii) adult age at first clinical examination in the majority of patients (patients 2, 5, 6, 8, and 9); iii) the influence of environmental factors (patient 6 and 9) or other genetic abnormalities (patient 8). Other possibilities may account for phenotype variability such as the association with polymorphic variations, epigenetic phenomena, modifier genes, miRNA, expression of regulatory variation among genes, environmental modifiers, and the unmasking of recessive variants residing on the single remaining allele. The presence or absence of genes at the breakpoints may also likely contribute to the variable phenotype (23). Interest in modifier genes is growing because of their ability to modulate the
expression of monogenic or multigenic traits or diseases (24). Arrays are powerful tools that can permit to diagnose well-known microdeletion syndromes that cannot be diagnosed clinically, but also detect several copy number anomalies in the same patient that might explain the atypical phenotype. As an example, patient 8 also had a 15q13.3 duplication, and recent series concluded that this duplication was of unknown clinical significance (25). This advantage can be put forward in the recent discussions proposing a novel diagnostic approach to all MR patients by first analyzing every patient with an SNP-array or CGH-array instead of conventional karyotyping (26). The other main advantage is a much higher diagnostic yield (15%-20%) for genetic testing of individuals with unexplained developmental delay or multiple congenital abnormalities than a G-banded karyotype (approximately 3%, excluding Down syndrome and other recognizable chromosomal syndromes), primarily because of its higher sensitivity for submicroscopic deletions and duplications (26).

In conclusion, the widespread use of array-CGH, has revealed a degree of a phenotype diversity that complicates genetic diagnosis and counseling. As these reports accumulate, it is becoming increasingly clear that modifiers play an important role in the variable expressivity associated with imbalances of some genome regions.

References


3. Shaffer LG, Theisen A, Bejjani BA, et al. The discovery of microdeletion syndromes in the post-


Fig. 1. **ABC**: Patient 1

A: Cleft-right hand post-surgery. The right hand presented with a cleft between the 3rd and the 4th finger. The 4th finger is absent and the 5th is hypoplastic with camptodactyly. **B**: Cleft left hand post-surgery. There is an irreducible camptodactyly of the 4th finger whereas the camptodactyly of the third one is reducible and slightly hypoplastic. There was originally a fifth finger poorly implemented which was surgically removed. **C**: Radiography of the right hand.

**D-E**: Patient 5 at the age of 38 years showing the strabismus and the prominent ears.

**F**: Patient 4 at the age of 3 years showing hypertelorism, thin upper lip and a flat philtrum.

**G**: Patient 8 at the age of 18 years showing triangular face with a flat philtrum, a large nose and a wide mouth with a thick lower lip.
<table>
<thead>
<tr>
<th>Patients</th>
<th>Microdeletions</th>
<th>Age (years)</th>
<th>Phenotype</th>
<th>Last measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22q11.21 deletion</td>
<td>15</td>
<td>MR, bilateral cleft-hands (Fig. 1A-B-C), seizures, left hemiparesis with a right cerebral hemiatrophy, bilateral epicanthus, upslantling palpebral fissures, thick lower lip and prominent ears. Normal cardiac and renal ultrasounds, no recurrent infections.</td>
<td>H: 156 cm (5-10th centile) W: 65 kg (75-90th centile) OFC: 53 cm (5-10th centile)</td>
</tr>
<tr>
<td>2</td>
<td>22q11.21 deletion</td>
<td>27</td>
<td>Moderate MR, speech delay, hypogonadotropic hypogonadism, polydactyly, hypoplasia, bilateral cryptorchidism, hypotelorism, downslanting palpebral fissures, prominent nose. No recurrent infections. Normal pituitary MRI, cardiac and renal ultrasounds. A Kallmann de Morsier syndrome was suggested but studies of the KAL1, FGFR1, PROK2, PROKR2 and FGF8 genes were normal.</td>
<td>H: 174 cm (50th centile) W: 72.5 kg (75th centile) OFC: 55.5 cm (16th centile)</td>
</tr>
<tr>
<td>3</td>
<td>22q11.21 deletion</td>
<td>14</td>
<td>Moderate MR, speech delay, triangular face, horizontal eyebrows, short and up-slanted palpebral fissures, tubular nose, small mouth and prognathia. Hypogonadism with delayed puberty and micropenis. Normal cardiac ultrasound.</td>
<td>H: 160 cm (50th centile) W: 65 kg (97th centile) OFC: 56 cm (50th centile)</td>
</tr>
<tr>
<td>4</td>
<td>22q11.21 deletion</td>
<td>3</td>
<td>Moderate MR, speech delay, hypertelorism, thin upper lip and flat philtrum (Fig. 1F), verrucous hamartoma, tuberous angiomata of the neck. Normal cardiac and abdominal ultrasounds. Severe neonatal hypotonia, walked at 30 months of age, tooth eruption and hair growth delay, hydrocephaly with arachnoid cysts, MR, aggressive behaviour with psychopathic traits, large mouth, prominent ears, strabismus (Fig. 1D-E), short hands, elbows and knees contractures, abdominal stretch marks. Normal cardiac ultrasound.</td>
<td>H: 171 cm (20-30th centile) W: 88 kg (&gt;97th centile) OFC: 60.3 cm (97th centile)</td>
</tr>
<tr>
<td>5</td>
<td>7q11.23 deletion</td>
<td>31</td>
<td>Motor milestones and speech delay, moderate MR, short stature with stocky with short limbs and narrow lumbar canal suggestive of hypochondroplasia, wide mouth, thick nose, abnormal behaviour with anxiety and logorrhea. Normal cardiac and abdominal ultrasounds. Studies of the FGFR3 gene were normal.</td>
<td>H: 142 cm (&lt;3rd centile) W: 68.6 kg (&gt;97th centile)</td>
</tr>
<tr>
<td>6</td>
<td>7q11.23 deletion</td>
<td>6</td>
<td>Moderate MR, mild dysmorphic features including a wide mouth, no history of hypercalcemia, normal social behaviour. Absence of heart defect.</td>
<td></td>
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<tr>
<td>7</td>
<td>7q11.23 deletion</td>
<td>18</td>
<td>Moderate MR with speech delay, triangular face with flat philtrum, large nose and wide mouth with a thick lower lip (Fig. 1G), myopia, strabismus and astigmatism, normal social behaviour. Normal cardiac ultrasound.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17p11.2 deletion</td>
<td>37</td>
<td>Severe to profound MR, no speech, self-injurious, walk with help, brachycephaly, prognathism with a wide mouth and macroglossia, dental anomalies, short and tubular fingers with 2-3 syndactyly and scoliosis.</td>
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</tr>
<tr>
<td>9</td>
<td>17p11.2 deletion</td>
<td>3</td>
<td>Pulmonary stenosis, axial hypotonia, short thick neck with excess skin, cleft palate, abnormal helix, edema of the extremities, scrotal hypoplasia with unilateral cryptorchidy and buried penis, normal behaviour. No associated visceral malformations. Suspicion of Noonan syndrome. A mutation of the PTPN11 gene was excluded. Recent sleep disturbances.</td>
<td>H: 157 cm (1st centile) W: 49 kg (&lt;3rd centile) OFC: 53 cm (5-10th centile)</td>
</tr>
</tbody>
</table>

MR: mental retardation; H: height; W: weight; OFC: occipito-frontal-circumference
<table>
<thead>
<tr>
<th>Patients</th>
<th>Chromosomal anomaly</th>
<th>Inheritance</th>
<th>Array resolution</th>
<th>Molecular position*</th>
<th>Minimal size (bp)</th>
<th>Proximal breakpoint MIM ID</th>
<th>Distal breakpoint MIM ID</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>22q11.21 deletion</td>
<td>De novo</td>
<td>105K</td>
<td>17,274,835-20,128,755</td>
<td>2,853,920</td>
<td>DGCR6</td>
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</table>

bp : base pair; * Ensembl genome browser, hg18