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THE USEFULNESS OF ARRAY COMPARATIVE GENOMIC HYBRIDIZATION IN CLINICAL DIAGNOSTICS OF INTELLECTUAL DISABILITY IN CHILDREN

PRZYPATNOŚĆ METODY PORÓWNAWCZEJ HYBRYDYZACJI GENOMOWEJ DO MIKROMACIERZY W DIAGNOSTYCE KLINICZNEJ NIEPEŁNOSPRAWNOŚCI INTELEKTUALNEJ U DZIECI

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Abstract

Introduction: Intellectual disability (ID)/Developmental delay (DD), which occurs in 1-3% of the population, accounts for a large number of cases regularly seen in genetics clinics. Currently, Array Comparative Genomic Hybridization (array CGH) is recommended by the International Standards for Cytogenomic Arrays (ISCA) Consortium as a first line test in the diagnostics of ID/DD, replacing G-banded chromosome analysis.

The aim: Application of array CGH in clinical diagnostics of developmental delay/intellectual disability in children.

Material and methods: We present the results of 8x60K oligonucleotide array application that was successfully implemented in a cohort of 112 patients with the clinical diagnosis of intellectual disability and accompanying dysmorphic features and/or congenital malformations.

Results: We have identified 37 copy number variants (CNVs) with the size ranging from 40 kb to numerical chromosomal aberrations, including unbalanced translocations and chromosome Y disomy, receiving an overall diagnostic yield of 33%. Known pathogenic changes were identified in 21.4% of the cases. Among patients with pathogenic CNVs identified by array CGH, 41.7% had a previously normal karyotype analysis.

Conclusions: Our studies provide more insights into the benefits derived by using chromosomal microarray analysis and demonstrate the usefulness of array CGH as a first-tier clinical setting test in patients with intellectual disability.

Key words: array CGH, CNVs, intellectual disability/developmental delay

Streszczenie

Wstęp: Niepełnosprawność intelektualna (NI) dotyczy 1-3% populacji i stanowi częsty problem kliniczny w poradniach genetycznych. Obecnie, zgodnie z zaleceniami Międzynarodowego Konsorcjum ds. Standardów w Cytogenetyce (ISCA, International Standards for Cytogenomic Arrays) testem z wyboru w diagnostyce NI powinna być metoda porównawczej hybrydyzacji genomowej do mikromacierzy (array CGH) zamiast analizy prążkowej chromosomów.

Cel pracy: Ocena przydatności porównawczej hybrydyzacji genomowej do mikromacierzy w diagnostyce opóźnienia rozwoju psychoruchowego/niepełnosprawności intelektualnej u dzieci.

*These authors contributed equally to this work.
Materiał i metody: Przedstawiamy wyniki badań diagnostycznych 112 pacjentów z cechami niepełnosprawności intelektualnej ze współistnieniem cech dysmorficznych i/lub wrodzonych wad rozwojowych z zastosowaniem mikromacierzy oligonukleotydowej 8x60K.

 Wyniki: Stwierdzono 37 zmian liczby kopii fragmentów DNA (CNVs, copy number variants) o wielkości w zakresie od 40 kpq do aberracji liczbowych całych chromosomów, a także niezróżnicowanych translokacji chromosomowych i disomii chromosomu Y. Całkowita skuteczność diagnostyczna wynosiła 33%. Znane patogennne CNVs stwierdzone były w 21,4% przypadków. Wśród pacjentów ze stwierdzonymi na mikromacierzy zmianami patogenicznymi, 41,7% miało prawidłowy wynik pierwotnie ocenionego karyotypu.

 Wnioski: Wyniki naszych badań dokumentują przydatność metody array CGH jako metody pierwszego wyboru w diagnostyce klinicznej pacjentów z niepełnosprawnością intelektualną.

Słowa kluczowe: porównawcza hybrydyzacja genomowa do mikromacierzy, zmiany liczby kopii fragmentów DNA, niepełnosprawność intelektualna/opóźnienie rozwoju psychoruchowego

INTRODUCTION

Intellectual disability (ID)/developmental delay (DD) is a disorder characterized by significantly impaired cognitive functioning and deficits in two or more adaptive behaviors. It affects 1-3% of all children (1, 2). There are many causes of intellectual disability and genetic factors are considered to be the main cause in about half of the patients with severe ID, and in about 15% of patients with mild ID (3). Nowadays patients with intellectual disability, dysmorphic features and/or congenital malformations are diagnosed using chromosomal microarray analysis (CMA). Array CGH has been reported to be useful in detecting causative genomic imbalances in patients with unexplained ID and previously normal conventional karyotype (4). Several studies reported that the average diagnostic yield is about additional 10% higher than in the classical G-banded karyotype (4-7). The review of 33 studies based on 21,698 patients tested by means of the chromosomal microarray analysis revealed that the average diagnostic yield with the use of different types of arrays reached 15-20% (7). CMA makes it possible to detect a high scale of genetic variations but the clinical significance of these variants may be difficult to interpret, especially for the regions of the genome that are not well characterized. In general we classified CNVs into four categories: 1) pathogenic (CNVs including well-known syndromes, de novo variants containing haploinsufficient genes or chromosomal changes greater than 5 Mb), 2) potentially pathogenic (de novo CNVs not previously reported in patients with ID or containing genes that may contribute to ID/DD phenotypes), 3) variants of unknown significance (VOUS) (CNVs inherited from normal parent but containing genes of known function) and 4) CNVs unrelated to the patient's phenotype. The copy number variants of unknown pathogenicity are still a significant group among all detected CNVs, further studies are therefore required for a better understanding of the importance of specific CNVs in the etiology of ID/ DD.

Here we present our results of the array CGH application in a cohort of 112 patients with ID/DD, dysmorphic features and/or congenital malformations and emphasize the usefulness of the whole genome array CGH in the clinical diagnostics of these neurodevelopmental disorders.

MATERIALS AND METHODS

Patients

All 112 patients presenting with intellectual disability/developmental delay accompanied with dysmorphic features and/or congenital malformations were referred to the Department of Medical Genetics at the Institute of Mother and Child. Probands were examined by a clinical geneticist. Informed consents approved by the institutional review board for the Bioethics Commission at the Institute of Mother and Child were obtained in all cases.

Forty patients had normal G-banded karyotype analysis at the 550 band resolution. Moreover, for six patients the Fragile X syndrome was excluded. Eleven patients were previously analyzed using the subtelomeric MLPA test and two by means of the microdeletion MLPA test. Clinical descriptions of patients with detected CNVs are summarized in Tables 1-3.

DNA Isolation

DNA was extracted from whole blood using the Puregene DNA Blood Kit (Gentra, Minneapolis, MN) or Prepito DNA Cyto Pure Kit (PerkinElmer) according to the manufacturer’s instructions.

Array Comparative Genomic Hybridization (array CGH)

Array CGH was performed using 60K microarrays: 8x60K from Agilent Technologies (Agilent 60K, 021924) or 8x60K from Oxford Gene Technology (CytoSure ISCA v2).

DNA digestion, labeling and hybridization were performed following the manufacturer’s instructions.
The usefulness of array comparative genomic hybridization in clinical diagnostics of intellectual disability in children 309

Table I. CNVs clinically relevant for ID/DD.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Locus/Inheritance</th>
<th>Size [Mb]</th>
<th>MIM/Genes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wiek (lata)</td>
<td>Locus/Pochodenie</td>
<td>Wielkość [Mb]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>f</td>
<td>8</td>
<td>del 1p36.32 pat</td>
<td>1.96</td>
<td>607872</td>
<td>Moderate/severe ID, absent speech, behavioral abnormalities (hyperactivity, aggressivity), growth retardation, hypotonia, dysmorphic features (hypertelorism, prominent forehead, up-slanted palpebral fissures, small nose, small chin), frequent respiratory tract infections</td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>26</td>
<td>del 1q21.1 mat</td>
<td>1.15</td>
<td>612474</td>
<td>Mild ID, positive familial history of ID (son with borderline ID and heart defect, brother with mild ID)</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>5</td>
<td>del 2q37.1q37.3 dn</td>
<td>7.9</td>
<td>600430</td>
<td>Delayed psychomotor and speech development, episodes of febrile seizures, abnormal EEG, dysmorphic features, dislocation and dysplasia of right hip joint</td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>4</td>
<td>dup 7q11.22 dn</td>
<td>0.28</td>
<td>AUTS2</td>
<td>DD, delayed speech, spectrum of atypical autism symptoms, discreet dysmorphic features</td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td>3</td>
<td>del 7q11.23</td>
<td>1.6</td>
<td>613729</td>
<td>DD, microcephaly, dysmorphic features (bilateral epicanthus, broad nasal bridge, sacral dimple), growth retardation, history of infantile seizures</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>7 months</td>
<td>dup 8p23.3p21.3 dn</td>
<td>19</td>
<td>12</td>
<td>DD, hypotonia, anisocoria, mild dysmorphic features, overweight</td>
</tr>
<tr>
<td>7</td>
<td>f</td>
<td>8</td>
<td>del 9p24.3p24.1</td>
<td>5.8</td>
<td>158170</td>
<td>DD, mild ID, hypotonia, bilateral spastic paresis, dysmorphic features, additional nipple</td>
</tr>
<tr>
<td>8</td>
<td>f</td>
<td>13</td>
<td>del 12p12.3p12.1 dn</td>
<td>6.26</td>
<td>-</td>
<td>DD, moderate ID, delayed speech, microcephaly, discreet dysmorphic features (micrognathia, open mouth), clinical symptoms of Duchenne's syndrome (elevated transaminases and CPK, microscopic abnormalities in muscle biopsy), progressive motor impairment</td>
</tr>
<tr>
<td>9</td>
<td>f</td>
<td>3</td>
<td>dup 14q11.2q21.1 dn</td>
<td>18.6</td>
<td>-</td>
<td>DD, hypotonia, cleft palate, failure to thrive, dysmorphic features (microcephaly, epicanthus, up-slanting palpebral fissures, broad philtrum, low-set ears, clinodactyly IV toes, sacral dimple), hypothyroidism</td>
</tr>
</tbody>
</table>
Table I. Cont.

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender</th>
<th>Age</th>
<th>karyotype</th>
<th>karyotype description</th>
<th>IQ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>f</td>
<td>6</td>
<td>del 15q13.3 pat</td>
<td>DD, severe ID, absent speech, behavioral problems (hyperactivity, aggressivity), spectrum of autistic features, dysmorphic features, case of ID in patient’s family</td>
<td>1.65</td>
<td>612001</td>
</tr>
<tr>
<td>11</td>
<td>m</td>
<td>8</td>
<td>del 15q13.3q14 dn</td>
<td>DD, mild ID, speech delay, cryptorchidism, dysmorphic features (large, prominent ears, prognatism), stereotypic movements</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>m</td>
<td>2</td>
<td>del 15q21.2q22.31 dn</td>
<td>DD, enlarged brain ventricles, craniosenosis, brachycephaly, high forehead, additional nipples</td>
<td>9.36</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>m</td>
<td>7</td>
<td>dup 16p13.11p12.3 pat</td>
<td>Mild ID, delayed psychomotor and speech development, stereotypic movements, dysmorphic features, high pain threshold</td>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>m</td>
<td>13</td>
<td>del 16p11.2 mat</td>
<td>Mild ID, speech delay, hyperactivity, overweight, excess of subcutaneous fat tissue, history of infantile seizures, positive familial history (ID in patient’s sister)</td>
<td>0.45</td>
<td>611913</td>
</tr>
<tr>
<td>15</td>
<td>m</td>
<td>9</td>
<td>dup 16p11.2 dn</td>
<td>Mild ID, IUGR, attention deficit disorder, hyperactivity, discreet dysmorphic features, brain malformation (subependymal heterotopy), abnormal EEG</td>
<td>0.65</td>
<td>614671</td>
</tr>
<tr>
<td>16</td>
<td>f</td>
<td>5</td>
<td>del 17q21.31 dn</td>
<td>DD, moderate ID, delayed speech, autistic spectrum features, hypotonia, IUGR, postnatal growth retardation, dysmorphic features (high forehead, bulbous nose, open mouth, long philtrum), pleasant personality</td>
<td>0.43</td>
<td>610443</td>
</tr>
<tr>
<td>17</td>
<td>m</td>
<td>10</td>
<td>dup 19p13.2</td>
<td>Moderate ID, absent speech, stereotypic hand movements, hypotonia, autistic spectrum disorder</td>
<td>2.8</td>
<td>NFIX</td>
</tr>
<tr>
<td>18</td>
<td>f</td>
<td>3</td>
<td>dup 22q11.21 mat</td>
<td>DD, IUGR, agenesis of corpus callosum, unilateral choanal atresia, cleft of uvula, only one central incisor, single capillary haemangioma of skin, dysmorphic features (asymmetric crying, hypotelorism, down turned corners of the mouth)</td>
<td>2.47</td>
<td>608363</td>
</tr>
<tr>
<td>19</td>
<td>f</td>
<td>6</td>
<td>dup 22q12.3q13.33 dn</td>
<td>DD, severe ID, absent speech, behavior abnormalities (autoagression, stereotypic movements), epilepsy, IUGR, postnatal growth retardation, trigonocephaly, clinodactyly V&lt;sup&gt;+&lt;/sup&gt; finger, dysmorphic features (hypertelorism, wide and flat nasal bridge, low set ears, short philtrum, high palate, sparse eyebrows &amp; eyelashes), nevus flammeus on the back, dry skin</td>
<td>14.6</td>
<td>606232</td>
</tr>
</tbody>
</table>
### Table I. Cont.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Locus/Inheritance</th>
<th>Size [Mb]</th>
<th>Genes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>m</td>
<td>5</td>
<td>dup Xp22.31 dn, dup Yp11.32q12 dn</td>
<td>0.04 57.8</td>
<td>-</td>
<td>Mild ID, delayed speech, Asperger syndrome, prematurity, symptoms of hemiparesis (cerebral palsy features)</td>
</tr>
<tr>
<td>21</td>
<td>f</td>
<td>6</td>
<td>del Xq13.2q28 dn, dup 5q13.2q35.3 dn</td>
<td>82 110</td>
<td>-</td>
<td>Mild ID, delayed speech development, prematurity, IUGR, postnatal short stature, microcephaly, discreet dysmorphic features</td>
</tr>
<tr>
<td>22</td>
<td>m</td>
<td>3</td>
<td>dup Xq28 mat</td>
<td>0.17 300260</td>
<td></td>
<td>DD, absent speech, hypotonia, joint laxity, recurrent inner ear infections, dysmorphic features (high forehead, long face, full lower lip)</td>
</tr>
<tr>
<td>23</td>
<td>f</td>
<td>2</td>
<td>mosaic monosomy of X chromosome</td>
<td>155</td>
<td>-</td>
<td>DD, hypotonia, dysmorphic features (short, broad neck, prominent forehead, scarce hair), hyperphenylalaninemia</td>
</tr>
<tr>
<td>24</td>
<td>m</td>
<td>2</td>
<td>der(Y)ins(Y;10) (q11.23 q24.31) dn</td>
<td>33</td>
<td>-</td>
<td>DD, postnatal microcephaly, cleft palate, ectopic testis, pectus carinatum, dysmorphic features (bilateral epicantus, broad nasal bridge, high forehead)</td>
</tr>
</tbody>
</table>

f – female/żeńska, m – male/mężsk
ID – intellectual disability/niepełnosprawność intelektualna, DD – developmental delay/opóźnienie rozwoju psychoruchowego
IUGR – intrauterine growth restriction/wewntrzmaciczne zahamowanie wzrostu, EEG – electroencephalography/elektroencefalografia
dn – de novo, mat – maternal/matczynie, pat – paternal/ojcowskie

### Table II. CNVs potentially pathogenic for ID/DD.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Locus/Inheritance</th>
<th>Size [Mb]</th>
<th>Genes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>f</td>
<td>3</td>
<td>dup 18p11.23 p11.22 dn</td>
<td>0.55</td>
<td>RAB12, KIAA0802</td>
<td>DD, hypotonia, cleft palate, failure to thrive, dysmorphic features (microcephaly, epicantus, up-slanting palpebral fissures, broad philtrum, low-set ears, clinodactyly IV toes, sacral dimple), hypothyroidism</td>
</tr>
<tr>
<td>25</td>
<td>f</td>
<td>4</td>
<td>del 18q22.3 dn</td>
<td>0.13</td>
<td>ZNF407</td>
<td>Severe DD, absent speech, epilepsy, dysmorphic features (ptosis, open mouth, hypertelorism, camptodactyly of V&lt;sup&gt;th&lt;/sup&gt; finger)</td>
</tr>
<tr>
<td>26</td>
<td>m</td>
<td>3</td>
<td>dup 20q13.2q13.31</td>
<td>0.72</td>
<td>TFAP2C, AURKA</td>
<td>DD, seizures, dysmorphic features (hypotelorism, prominent ears, earlobe malformation)</td>
</tr>
</tbody>
</table>

f – female/żeńska, m – male/mężsk
DD – developmental delay/opóźnienie rozwoju psychoruchowego
dn – de novo, mat – maternal/matczynie, pat – paternal/ojcowskie
Table III. Variants of Unknown Clinical Significance (VOUS) or CNVs unrelated to patient’s phenotype.
_Tabela III. Aberracje chromosomowe o nieznanym znaczeniu klinicznym lub niezwiązane z fenotytem pacjenta._

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Locus/Inheritance</th>
<th>Size [Mb]</th>
<th>Genes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

**Variants of Unknown Clinical Significance**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Locus</th>
<th>Size</th>
<th>Gene</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>m</td>
<td>5</td>
<td>dup 2q14.3 mat</td>
<td>0.99</td>
<td>CNTNAP5</td>
<td>Mild ID, delayed speech, Asperger syndrome, prematurity, symptoms of hemiparesis (cerebral palsy features)</td>
</tr>
<tr>
<td>27</td>
<td>f</td>
<td>2</td>
<td>dup 3p26.3 pat</td>
<td>0.05</td>
<td>CHL1</td>
<td>DD in significant degree, hypotonia, astigmatism, strabismus, abnormal EEG</td>
</tr>
<tr>
<td>19</td>
<td>f</td>
<td>6</td>
<td>dup 3q29 pat</td>
<td>0.45</td>
<td>KIAA0226</td>
<td>DD, severe ID, absent speech, behavior abnormalities (autogression, stereotypic movements), epilepsy, IUGR, postnatal growth retardation, trigonoccephaly, clinodactylyof Vth finger, dysmorphic features (hypertelorism, wide and flat nasal bridge, low set ears, short philtrum, high palate, sparse eyebrows &amp; eyelashes), nevus flammeus on the back, dry skin</td>
</tr>
<tr>
<td>28</td>
<td>m</td>
<td>3</td>
<td>del 6p21.2 mat</td>
<td>0.25</td>
<td>BTBD9</td>
<td>Severe DD, postnatal growth retardation, microcephaly, epilepsy, pyramidal-extra-pyramidal symptoms (abnormal muscle tone, axial hypotonia/ limbs spasticity), signs of ischemic encephalopathy in brain MRI</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>5</td>
<td>dup Xq22.1q22.2 pat</td>
<td>0.26</td>
<td>WBP5</td>
<td>Delayed psychomotor and speech development, episodes of febrile seizures, abnormal EEG, dysmorphic features, dislocation and dysplasia of right hip joint</td>
</tr>
</tbody>
</table>

**CNVs unrelated to patient’s phenotype**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Locus</th>
<th>Size</th>
<th>Gene</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>m</td>
<td>15</td>
<td>del 6q26 mat</td>
<td>0.40</td>
<td>PARK2</td>
<td>DD, moderate ID, seizures, arrhythmias, disproportionate short stature (short trunk, long limbs), hypothyroidism, combined pituitary hormone deficiency, joint laxity</td>
</tr>
<tr>
<td>30</td>
<td>m</td>
<td>6</td>
<td>del 17p12 pat</td>
<td>1.33</td>
<td>PMP22</td>
<td>DD, mild/moderate ID, pre- &amp; postnatal growth retardation, microcephaly, seizures, cryptorchidism, dysmorphic features (upper-slanling, narrow palpebral fissures, ptosis, clinodactyly of V finger), gastro-esophageal reflux, hyperactivity</td>
</tr>
</tbody>
</table>

_f – female/żeńska, m – male/mężczyzna_

_ID – intellectual disability/niepełnosprawność intelektualna, DD – developmental delay/opóźnienie rozwoju psychoruchowego, IUGR – intrauterine growth restriction/wewnętrznicze zahamowanie wzrostu_

_EEG – electroencephalography/elektroencefalografia, MRI – magnetic resonance imaging/obrazowanie metodą rezonansu magnetycznego_

_dn – de novo, mat – maternal/matczynne, pat – paternal/ojcowskie_
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Scanned images were quantified using Agilent Feature Extraction software (v10.0). The customized IMiD-web2py software and Cytosure (OGT) software were used for chromosomal microarray analysis. All the genomic coordinates are based on the March 2006 assembly of the reference genome (NCBI136/hg18).

To verify the copy number variants identified by array CGH, we used MLPA, fluorescence in situ hybridization (FISH) or karyotyping. When available, blood samples were obtained from the patients’ parents, array CGH or FISH analysis were performed to investigate the inheritance of the CNVs.

Multiplex Ligation-dependent Probe Amplification (MLPA)

MLPA experiments were performed according to the manufacturer’s instruction with the kit P245, P036 or P297 (MRC Holland) in the 2720 thermal cycler (Applied Biosystems, Foster City, CA). Information regarding the probe sequence and ligation sites can be obtained at www.mlpa.com. Probes were analyzed using the ABI3100 sequencer with the size standard Gene Scan 500 Rox (Applied Biosystems). Gene Marker v8.1 software (Softgenetics) was used to conduct data analysis.

Fluorescent In Situ Hybridization (FISH)

Confirmatory FISH analyses were performed in phytohemagglutinin-stimulated peripheral blood lymphocytes using standard procedures with the bacterial artificial chromosome (BAC) clones specific for the aberrations regions (9).

Karyotype analysis

GTG banding analysis with 550-band resolution was performed according to the standard protocol in peripheral blood lymphocytes.

RESULTS

We identified 37 CNVs in 30 out of the 112 patients studied. All the CNVs detected were confirmed by MLPA, FISH or karyotyping. We classified these CNVs into four groups: pathogenic, potentially pathogenic, variants of unknown significance and unrelated to patient's phenotypes (Tables I-III).

Among 27 CNVs from the first group, seven were greater than 5 Mb in size. In patient 8, with previously reported balanced translocation t(X;3)(p22.13;p13), additional large aberration on chromosome 12 has been identified. Other large aberrations were the unbalanced products of translocations: t(8;9)(p23.3;p24), t(X;5)(q13.2;q13.2), t(Y;10)(q11.23;q24.31) and also disomy of the Y chromosome, and mosaic monosomy of chromosome X. Moreover, this group includes nine known recurrent rearrangements which arose at hotspots: microdeletions 1p36, 1q21.1, 7q11.23, 15q13.3, 16p11.2, 17q21.31 and microduplications 16p11.2, 22q11.2 and Xq28. We also identified different-size non-recurrent duplications: 7q11.22, 9p13.2 and 16p13.11p12.3 (Table I).

The second group consists of three potentially pathogenic CNVs: rare de novo deletion at 18q22.3, as well as de novo duplication at 18p11.23p11.22 and 20q13.13.2q13.31 duplication of unknown inheritance (Table II).

In the last group we classified three deletions and four duplications of unknown clinical significance or unrelated to the patient’s phenotypes (Table III).

Normal karyotypes were previously reported in 15 patients out of 30 with identified chromosomal imbalances. Among these 15 probands there are 10 cases with pathogenic CNVs (41.7%), including four patients with aberrations exceeding 5Mb. Moreover, among 30 patients with detected chromosomal changes, the subtelomeric MLPA test and microdeletion MLPA test were also normal for seven and one patients, respectively.

DISCUSSION

The use of microarray technology provides an opportunity for an accurate molecular characterization and better genotype-phenotype correlation of the identified potentially disease-related CNVs. To date, the patient cohorts studied by array CGH consisted of individuals with neurodevelopmental disorders and in many cases enabled the identification of causative genome regions and genes (10-15).

To determine the pathogenic role of the identified CNVs, we considered their type (deletion or duplication) and size, gene content, inheritance pattern, and available information from clinical and genetic databases. In general, we divided the detected CNVs into four groups: known published, well-recognized genomic imbalances or large changes that we classified as clinically relevant for ID (Table I), rare copy number variants that could be novel and potentially causative for ID (Table II) and finally, variants of unknown clinical significance and unrelated to the patient’s phenotypes (Table III).

Our analyses revealed CNVs in 30 out of the 112 patients (26.8%). Pathogenic changes for intellectual disability were found in 24 patients (Table I), yielding a 21.4% detection rate, which is in good correlation with previously reported studies (16-17). Within this group there were nine CNVs covering known microdeletion/microduplication syndromes (Table I).

In one case (Patient 18) the maternally inherited duplication of 22q11.21 was detected. The phenotype of 22q11.2 microduplication is known to be extremely variable ranging from normal or mild learning disability to multiple congenital defects (18). Many families in which patients inherited the duplication from almost phenotypically healthy parents (as it is in the case of Patient 18), or asymptomatic parents, have been reported (19, 20). The basis of phenotype variability of 22q11.2 microduplication remains to be elucidated. Imbalances inherited from phenotypically normal parents may contribute to the phenotype through variable penetrance or expressivity, or both, through epigenetic effects, environmental factors or stochastic events during fetal development (21, 22).

Those factors could also be responsible for the occurrence of the characteristic phenotype in Patient 10 with the deletion of 15q13.3 inherited from a phenotypically normal father. Only ~ 25% of 15q13.3 microdeletions
occur de novo and the rest are inherited (23). It is not possible to reliably predict the phenotype based on the laboratory finding of 15q13.3 microdeletion. The variability of phenotypic expression observed in Patient 10 and his father can be explained by the heterogeneity of the 15q13.3 microdeletion clinical feature spectrum.

The phenotypic spectrum of 16p11.2 aberrations (Patient 14 and Patient 15) includes the autism spectrum disorders (ASDs), ID and/or possibly other primary psychiatric disorders, but a normal outcome is also possible (24, 25). The latter appears more likely for duplications than for deletions. Deletions can also be associated with non-specific major or minor dysmorphism (26). As pointed out by Rosenfeld, the ultimate phenotype of the child is probably affected by his/her genetic background and other environmental factors, the vast majority of which are unknown and therefore cannot be tested (27).

Microdeletion of 17q21.31 in Patient 16 occurred de novo, that is in concordance with the fact that none of the reported cases of the Koolen-De Vries syndrome were inherited from a normal parent. It is known that the syndrome is caused by the haploinsufficiency of KANSL1 (MIM 612452) (28), resulting in a clearly recognizable clinical phenotype of mental retardation, hypotonia, and characteristic face (long face, high forehead, large, prominent ears, upward-slanting palpebral fissures, epicanthal folds, bulbous nasal tip, pear-shaped nose, and long, slender features). Other features including cardiac septal defects, seizures, and cryptorchidism can also be present (29), which is with good concordance with the phenotype of our patient.

Patient 5 with DD, dysmorphic features and history of infantile seizures carries a heterozygous microdeletion distally adjacent to the Williams-Beuren syndrome region on chromosome 7q11.23 encompassing two haploinsufficient genes: HIP1 (601767) and YWHAG (605356). The clinical features presented in our patient are in good correlation with the already presented cases (30). Ramocki claimed that the deletion of Huntington-interacting protein 1 (HIP1) and tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein gamma (YWHAG) is causative for intellectual disabilities, epilepsy and neurobehavioral problems (30).

In another case (Patient 4 with DD, spectrum of atypical autism symptoms, speech delay, microcephaly), we identified a de novo duplication of exon 6 of the AUTS2 gene. The function of this gene is still unknown, but recent studies show that the gene is expressed in developing mouse brain, and may have a critical role in the development of cortical regions (31, 32). It was found that many individuals with ASDs, intellectual disability or developmental delay have distinct heterozygous structural variants disrupting the AUTS2 region (33-36). Beunders presented a cohort of patients with AUTS2 exonic deletions whose phenotypes correlate with our patient’s clinical features (37). He revealed intellectual disability in ~ 95% of the patients and additionally microcephaly in ~ 70% of probands with AUTS2 deletions. We claim that the disruption of AUTS2 by single exon duplication contributes to the patient’s phenotype. Our result is supported by Ben-David who found truncating duplications of AUTS2 likely to be the cause of monoallelic expression, leading to ID, epilepsy and autism (38).

Additionally, in the group of pathogenic CNVs for ID we detected deletion at 12p12 in Patient 8. This probe carries apparently balanced translocation t(X;3) (p22.13;p13) and was addressed for aCGH screening because the imbalance within the breakpoints was suspected. It has previously been reported that 29-60% of cytogenetically apparently balanced translocations in patients with abnormal phenotype have de novo cryptic imbalances in one or both breakpoints (39-41), which shows the instability of the human genome, when translocation occurs (42). However, De Gregori presented that 40% of the patients with apparently balanced translocations had at least one deletion not always in the breakpoints, suggesting that cryptic deletions are mostly responsible for the phenotypic effect (43). In our patient with balanced translocation t(X;3), aCGH did not reveal any aberrations within the breakpoints, whereas CMA identified a de novo ~ 6 Mb deletion within the chromosome 12p12 which encompasses many genes including SOX5. This gene encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of central nervous system differentiation. Recently, deletions of this gene have been described in patients with neurodevelopmental disability and dysmorphic features (44). The size of the deletion reported in our patient as well as the gene content indicates its pathogenicity in ID.

The second group of CNVs consists of three potentially pathogenic changes for intellectual disability (Table II). A rare de novo deletion of the third exon of ZNF407 (member of the zinc finger protein family) found in Patient 25 is possibly responsible for severe DD, epilepsy and absent speech. This type of zinc finger is one of the most common DNA binding motifs existing in the mammalian nucleus. Its binding properties depend on the amino acid sequence that links individual fingers, the number of finger structures, and the overall structure. Therefore, it is possible that attenuation of ZNF407 due to either mutations or reduction of its expression by deletion/duplication of a part of the gene can result in potential haploinsufficiency that may be involved in the ID/autistic phenotype (45). To this group we also classified two other CNVs: de novo 18p11.23p11.22 duplication (Patient 10), including genes of unclear function and the 20q13.2q13.31 duplication (Patient 26) of unknown inheritance including haploinsufficient genes.

The copy number variants in the third group were classified as variants of unknown significance (VOUS) for the intellectual disability/developmental delay. Moreover, in the last group we included CNVs unrelated to the patient’s phenotype (Table III). All the patients in these two groups inherited the chromosomal changes from an apparently normal parent. Two CNVs found in Patient 29 and 30 could be associated with late-onset diseases. In the first patient (Patient 29), a deletion of exons 7-9 of the PARK2 gene at 6q26 was revealed. Exon rearrangements and point mutations are common in PARK2, the most important causative gene of early-onset Parkinson
disease (EOPD) with onset age ≤40 years. Although no Parkinson symptoms are yet visible in the patient’s mother (aged 51), the Parkinson symptoms could still not be visible. The study of Kim suggests that a significant portion of EOPD patients with apparent contiguous multi-exon deletions may actually be compound heterozygous for two different adjacent exon deletions. He presented that over 80% of the patients with contiguous multi-exon deletions were diagnosed as compound heterozygotes (46).

The second case (Patient 30) is the deletion at 17p12 encompassing the PMP22 (MIM 601097) gene. Peripheral myelin protein 22 encodes a 22-kD protein that comprises 2 to 5% of myelin in the peripheral nervous system. Level of PMP22 refers to Charcot-Marie-Tooth type-1A (CMT1A) with trisomy of PMP22, hereditary neuropathy with liability to pressure palsies (HNPP) with heterozygous deletion of PMP22, and CMT1E with point mutations of PMP22 (47, 48). Up to 84% of HNPP cases are due to the 1.5-Mb deletion at 17p11.2p12 containing the PMP22 gene. Since deletion is present in our patient, we suspected that if not in the child, at least in the 37-year-old father some of the phenotypic features characteristic for the HNPP could be present. So far the patient’s father does not present any of the features of HNPP, which may be due to the complexity of the disease and to the fact that the age range of the first attack extends from two to 70 years (49–51).

The detection of copy number variants not related to the patient’s phenotype raises ethical considerations. This is the case for many conditions, concerning genetic susceptibility to disease with later onset in adulthood, but also may interfere with appropriate medical management and could reveal a risk to other family members or future offspring. CMA will likely become a first-tier test in clinical genetic diagnostics, which means that ethical considerations will arise more often (7).

CONCLUSIONS

Our study demonstrates that array comparative genomic hybridization enables a more effective detection of clinically significant chromosomal imbalances in patients with intellectual disability than conventional cytogenetic methods. It also points out the ethical issues that need to be considered in genetic counseling concerning variants not related to the patient’s phenotype but known to be associated with late-onset diseases.

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