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Original article

Additional cryptic CNVs in mentally retarded patients with apparently balanced karyotypes

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ABSTRACT

Apparently balanced chromosome abnormalities are occasionally associated with mental retardation (MR). These balanced rearrangements may disrupt genes. However, the phenotype may also be caused by small abnormalities present at the breakpoints or elsewhere in the genome. Conventional karyotyping is not instrumental for detecting small abnormalities because it only identifies genomic imbalances larger than 5–10 Mb. In contrast, high-resolution whole-genome arrays enable the detection of submicroscopic abnormalities in patients with apparently balanced rearrangements.

Here, we report on the whole-genome analysis of 13 MR patients with previously detected balanced chromosomal abnormalities, five *de novo*, four inherited, and four of unknown inheritance, using Single Nucleotide Polymorphism (SNP) arrays. In all the cases, the patient had an abnormal phenotype. In one familial case and one unknown inheritance case, one of the parents had a phenotype which appeared identical to the patient's phenotype. Additional copy number variants (CNVs) were identified in eight patients. Three patients contained CNVs adjacent to one or either breakpoints. One of these patients showed four and two deletions near the breakpoints of a *de novo* pericentric inversion. In five patients we identified CNVs on chromosomes unrelated to the previously observed genomic imbalance.

These data demonstrate that high-resolution array screening and conventional karyotyping is necessary to tie complex karyotypes to phenotypes of MR patients.

1. Introduction

Recent advances in molecular cytogenetic technologies provide a resolution that exceeds that of conventional karyotyping and increased the detection of aberrations from 5% to approximately 17% in patients with mental retardation (MR) [13,16,17,19,23,28,31,32,34–36].

A disadvantage of the array technique is the incapability to detect balanced structural abnormalities such as translocations and inversions. Balanced rearrangements have a prevalence of at least 1:500 and in approximately 6% of antenatal patients with a balanced rearrangement an abnormal phenotype is found [20,37]. The abnormal phenotype of these patients can be explained by (1) breakpoint regions directly disrupting genes or transcription regulatory regions [21], (2) indirectly by submicroscopic copy number variants (CNVs) near one or both of the breakpoints [4], (3) the rearrangement hosts 'cryptic' complex chromosomal rearrangements (CCRs) [24], (4) submicroscopic CNVs unrelated to the translocation or inversion [3,6,18], or (5) another unidentified genetic or environmental factor.

Reports of single patients or small series of patients with apparently balanced aberrations have identified unexpected complexity and instability of the human genome [3,6,18,24]. Some studies investigated the difference between additional CNVs in carriers of *de novo* 'balanced' reciprocal translocations and CCRs with normal and abnormal phenotypes [1,11]. In approximately 35% of the phenotypically abnormal patients additional candidate disease-causing CNVs were identified, mostly occurring around the breakpoints of the translocations. In the phenotypically normal cohort no additional genomic CNVs were identified. Sismani and colleagues studied 12 MR patients both with *de novo* and familial apparently balanced translocations for the presence of cryptic CNVs [33]. Two *de novo* and one familial case had additional abnormalities. Recently, Schluth-Bolard and colleagues analyzed 47 MR patients with *de novo* and familial apparently balanced chromosomal rearrangements [30]. All familial rearrangements were

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inherited from phenotypically normal parents. Imbalances were detected in 16 *de novo* cases (48.5%) and in 4 inherited cases (28.6%).

We report on 13 patients carrying an apparently balanced translocation or inversion detected with conventional karyotyping. High-resolution Single Nucleotide Polymorphism (SNP) array analysis was performed to search for cryptic CNVs. In eight patients additional CNVs were detected. Herein we focus on the interpretation of the detected CNV in relation to the phenotypes of the patients.

2. Materials and methods

2.1. Patients

This study included 13 patients with MR, with or without multiple congenital malformations, and an apparently balanced translocation or inversion observed with conventional karyotyping (five *de novo*, four inherited, and four of unknown inheritance). A summary of the clinical and cytogenetic data of all patients is shown in Table 1. Karyotyping on GTG-banded chromosomes from cultured lymphocytes of the patient was performed according to standard techniques. The study was approved by the Leiden University Medical Center Clinical Research Ethics Board, conforming to Dutch law and the World Medical association Declaration of Helsinki.

2.2. SNP arrays

DNA was extracted from whole blood by a Gentra Puregene DNA purification Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. The Affymetrix GeneChip Human Mapping 262 K *NspI*, 238 K *StyI* arrays (Affymetrix, Santa Clara, CA, USA) and Illumina HumanHap300, Human CNV370 BeadChips (Illumina Inc., San Diego, CA, USA) were performed following the manufacturers' instructions and data was analyzed as described previously [17]. Table 1 shows which SNP array platform was used for each patient.

2.3. Evaluation and validation of CNVs

Detected CNVs were evaluated as described previously [17]. The potentially pathogenic CNVs were confirmed with Fluorescence In Situ Hybridization (FISH) analysis or another type of SNP array using an independent DNA sample. If parents were available, segregation analysis was performed by FISH or SNP array analysis. FISH analysis was carried out by standard procedures as described previously [9]. BAC clones mapping to the unbalanced chromosome regions were selected based on their physical location within the affected region (http//:www.ensembl.org, Ensembl release 54 – May 2009, Genome build NCBI36).

All potentially pathogenic CNVs were assessed with Ensembl (Ensembl release 54 – May 2009, Genome build NCBI36) and DECIPHER (https://decipher.sanger.ac.uk) for gene content and patients with similar CNVs respectively. Finally, data of all patients with (potentially) pathogenic CNVs was added to the DECIPHER database.

3. Results

SNP array analysis demonstrated 16 additional submicroscopic CNVs in eight of the 13 patients (61.5%); five out of the five *de novo*, one out of the four familial and two out of the four unknown

Table 1
Cytogenetic, clinical and SNP array data of all patients.

ID	Structural aberration	Phenotype	SNP array	Chromosome	Starting SNP	Ending SNP	Size
CNV near/at breakpoint(s)							
1	inv(5)(q22q31.3) *dn*	Mental retardation[a]	*NspI* and 317 K	4x del 5q14.3, *dn*	rs4920853	rs1862233	59 kb
					rs16902356	rs10051603	83 kb
					rs7715840	rs1062035	320 kb
					rs357509	rs304151	638 kb
				2x del 5q33.3, *dn*	rs6556381	rs9637861	130 kb
					rs6884239	rs3846687	159 kb
2	t(11;12)(q13.3;p12.3), inv(12)(p12.3p13.1) n/a	Mental retardation, psychiatric problems	*NspI*	del 12p12.3p12.3, n/a	rs7306438	rs1865936	2.51 Mb
3	inv(7)(q31.3q34) *dn*	Mental retardation, anxiety disorder, autistic behavior	*NspI*	del 7q21.11q21.3, *dn*	rs2373207	rs17166393	8.29 Mb
CNV on unrelated chromosome(s)							
4	inv(6)(p21.3q15) n/a	Mental retardation, obesity	*NspI* and 370 K	del 18q21.31q21.31, n/a	rs4940582	rs4940754	877 kb
				del 18q21.32q21.33, n/a	rs1877055	rs588677	2.6 Mb
5	t(3;18)(p14.2;q23) mat	Mental retardation[a]	317 K	del 22q11.22q11.23, n/a	rs38114997	rs6003620	630 kb
6	t(2;6)(q37.1;q13) *dn*	Mental retardation[a]	317 K	del 13q12.3q12.3, *dn*	rs1023166	rs7332696	1.24 Mb
7	t(2;6)(q24.1;p24.3) *dn*	Developmental delay[a]	*NspI*	dup 1p32.3p32.2, *dn*	rs563403	rs6670302	2.78 Mb
CNV at or near the breakpoint plus additional, unrelated CNV							
8	t(12;14)(q21.3;q32.1) *dn*	Dysmorhpic features[a]	*NspI*	del 3p12.3p12.3, *dn*	rs7622824	rs11920974	1.15 Mb
				del 4q28.3q31.23, *dn*	rs1519335	rs6838916	10.11 Mb
				del 12q21.31q21.33, *dn*	rs1994104	rs7133204	6.88 Mb
No additional CNV							
9	t(1:4), pat	Psychomotor retardation	*NspI*	Balanced			
10	t(2;9)(q14.2;q32), mat	Mild developmental delay	*NspI*	Balanced			
11	t(12;13)(p11.2;q31), n/a	Severe speech delay, mild developmental delay, pectus carinatum, high and thin palatum	317 K	Balanced			
12	t(9;13)(p22;q14.3), pat	'Psuedo'achondroplasia	317 K	Balanced			
13	t(4;10)(q21.1;q25.2), n/a	Short stature	*StyI*	Balanced			

dn, de novo; n/a, (one of the) parents not available; mat, maternally inherited; pat, paternally inherited.
[a] Extended clinical information provided in results.