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Abstract

Investigations of apparently balanced chromosomal rearrangements in patients with abnormal phenotype by molecular cytogenetics tools, especially by array CGH, revealed a proportion of unsuspected imbalances. It was estimated recently that 40 % of apparently balanced *de novo* translocations with abnormal phenotype were associated with cryptic deletion.

We explored 47 unrelated mental retardation patients carrying an apparently balanced chromosomal rearrangement with high-resolution oligonucleotides arrays. We included 33 *de novo* cases (21 translocations, 7 inversions and 5 complex chromosomal rearrangements (CCR)) and 14 inherited cases (7 translocations, 5 inversions and 2 CCR).

Twenty of the 47 cases (42.6 %) carried a cryptic deletion ranging from 60 kb to 15.37 Mb. It concerned 16/33 *de novo* rearrangements (8/21 translocations, 4/7 inversions and 4/5 CCR) and 4/14 inherited rearrangements (1/7 translocations, 2/5 inversions and 1/2 CCR). The proportion of imbalances was not statistically different between *de novo* and inherited cases.

Our results support that about 40 % apparently balanced chromosomal rearrangements with abnormal phenotype are in fact imbalanced and that these rearrangements should be systematically investigated by array CGH independently of their *de novo* or inherited character.

1. Introduction

It is estimated that standard karyotype displayed chromosome aberrations in 3 to 15 % of patients affected with mental retardation [24,29,30]. Most of them are unbalanced by either numerical (trisomy, monosomy) or structural (deletion, duplication…) anomalies. However, apparently balanced structural rearrangements are present in 0.6 % of mentally retarded patients [24]. About 6 % of *de novo* apparently balanced translocations [32] and 23 % of apparently balanced complex chromosomal rearrangements (CCR) defined by three or more breakpoints [21] are associated with an abnormal phenotype. These balanced rearrangements were demonstrated to be responsible for the phenotype by different mechanisms such as gene disruption at the breakpoints [13,14], position effect [8,16,17] or disturbance of parental imprinting [6]. Development of Fluorescent *In Situ* Hybridization (FISH) or Comparative Genomic Hybridization (CGH) on chromosomes showed that a proportion of these rearrangements was in fact associated with cryptic imbalances [1,3,12,15,18,23]. More recently, array CGH technology [28] has been used to investigate apparently balanced translocations associated with an abnormal phenotype and has found cryptic deletion in about 30 to 50 % of them [2,4,10].

Here, we explored 47 patients presenting with mental retardation and carrying an apparently balanced chromosomal rearrangement with high-resolution oligonucleotide array in order to detect cryptic imbalances accounting for the phenotype. These rearrangements were either *de novo* (33 patients) or inherited (14 patients) and included reciprocal translocations, peri- and paracentric inversions or CCR. We estimated their frequency and defined the place of this technology in a diagnostic setting.

2. Material and methods

2.1. Patients

Forty-seven patients (26 males and 21 females) with mental retardation and/or multiple congenital malformations have been included in the study after fully informed consent was obtained. All patients had been assessed by a clinical geneticist. Phenotype range was very wide and included variable degree of mental retardation with or without malformations and facial dysmorphism. For all of them, standard karyotype (RHG, 500 bands) showed an apparently balanced rearrangement including 28 reciprocal translocations, 12 inversions and 7 CCR. Thirty-three balanced rearrangements occurred *de novo* (21 translocations, 7 inversions and 5 CCR) and 14 were inherited from a healthy parent (7 translocations, 5 inversions and 2 CCR) (Table 1). There was no other evident cause for their phenotype. Patients' karyotypes are summed up in Table 1. Parents' blood sample was required to assess if the imbalances detected by array CGH were *de novo* or inherited.

2.2. Array CGH

Agilent® oligonucleotide arrays were used according to the manufacturer instruction (Agilent Human Genome CGH Microarray kit 244A® and 44K®). The overall median probe spacing was 43 kb for 44K array and 8.9 kb for 244A array. Thirty eight patients DNA were analyzed with a 44,000 oligonucleotides array and 9 patients DNA with a 244,000 oligonucleotides array (#1, #2, #3, #29, #30, #31, #32, #41, #42). Patient's DNA as well as a reference DNA were digested with RsaI and AluI. Each digested DNA product was labelled by random priming using either Cy5-dUTP or Cy3-dUTP. After columns-purification, probes were denaturated and pre-annealed with 50 μg of human Cot-1 DNA (Invitrogen®, California). Hybridization was performed at 65°C during 40 hours. After washing, the array was scanned and analyzed with Feature Extraction® 9.1 software. Control DNA consisted

either of a sex-matched pool of genomic DNA commercially available (Promega®, USA) or of two other patients DNA, according to the loop model [22]. Results were interpreted with CGH analytics® 4.5 software by two investigators. A copy number variation was considered if at least 3 contiguous oligonucleotides presented an abnormal log ratio (> + 0,66 or < -0,75). Results were compared to data recorded in the database of genomic variants.

2.3. Fluorescent *in situ* hybridization (FISH)

FISH analyses on patient metaphases were performed in order to confirm the results of array CGH. Each time a microdeletion was confirmed, FISH was performed on parents' sample in order to check if the genomic imbalance was *de novo* or inherited. BACs were chosen on UCSC and Ensembl databases. FISH using BAC clones was performed as described by Romana *et al* [24]. DNA was fluoresceine or rhodamine labeled by nick-translation. Probes were coprecipitated with human Cot-1 DNA (Invitrogen®, California) and then resuspended in hybridization buffer (50 % formamide). After denaturation, overnight hybridization and post-hybridization wash, slides were DAPI counterstained and were read using a fluorescent microscope equipped with a CCD camera. All details about BAC clones used are available on request.

2.4. Quantitative PCR (qPCR)

When FISH was not possible, the confirmation of allelic imbalance in the target chromosome region was analyzed using SYBR Green I based quantitative real time PCR with Light-Cycler® (Roche Diagnostics, GmbH, Mannheim, Germany). The protocol involved amplification of a target gene and a reference gene (*ADORA2B, HGNC:264*) (primers sequences available on request). Quantitative PCR protocol was conducted in triplicate in a 20 µL final volume containing 10 µL of SYBR PCR Master Mix (2X) (Qiagen®, GmbH, Hilden,

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Germany), 1.5 µM of each primer and 10 ng of genomic DNA. The thermal cycling conditions comprised a 15 min polymerase activation at 95°C followed by 35 cycles of 15 s at 95°C, 30 s at 60°C and 15s at 72°C. Experiments need to have a coefficient of variation for triplicate samples inferior to 0.1. PCR efficiency is calculated as followed: $10^{-1/slope}$. Efficiency of qPCR is equal to 100% when $10^{-1/slope} = 2$ meaning that quantity of DNA is multiplied by 2 at each cycle of the PCR. We consider that PCR efficiency do be superior to 98% to assure good results.

A melting curve step was used to examine each sample for purity and specificity and the size of the amplicons was checked by electrophoresis. The calculation is based on the Ct values (minimal number of PCR cycles necessary to detect a fluorescence issued by the SYBR Green I) obtained by the Light Cycler software. A series of 5-fold dilutions of human genomic DNA corresponding to 0.5 to 8 ng/µL was included in each experiments in order to generate an external standard curve (Ct = A x log [Concentration DNA] + B) that allowed to estimate DNA concentration for each sample. Relative copy number is then calculated as the [target gene]/[reference gene] ratio.

3. Results

Overall, array CGH demonstrated cryptic imbalances in 20 of the 47 patients (42.6%). It consisted of 21 deletions ranging from 60 kb to 15.37 Mb. Nine of the 21 imbalances were located in regions not involved in the balanced rearrangement. Two of these cryptic imbalances were inherited from a healthy parent. The proportion of imbalances detected in *de novo* (16/33, 48.5 %) and in inherited rearrangements (4/14, 28.6%) was not statistically different (p<0.34). Results and phenotypes of these patients are described in detail in Table 2.

De novo rearrangements

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Microdeletions were detected in 16 of the 33 *de novo* structural rearrangements (48.5%). They ranged from 60 kb to 15.37 Mb and were all *de novo*. Nine of them were at distance from initial breakpoints. One patient (#42) presented two deletions.

Reciprocal translocations: 38.1 % (8/21) of apparently balanced translocations showed an associated deletion ranging from 60 kb to 15.37 Mb. Three of them were located at distance from translocation breakpoints (#1, #22, #27).

Inversions: Four of the seven (57.1 %) *de novo* inversions were imbalanced. Microdeletions ranged from 1.06 Mb to 8.87 Mb. Three of them were located on a different chromosome than the chromosome carrying the inversion (#29, #34, #36).

Complex chromosomal rearrangements: Four of the five *de novo* CCR were imbalanced (80 %) with deletions ranging from 690 kb to 4.9 Mb. Case #42 showed two deletions, one on a chromosome 2 at distance from the breakpoint and one on a chromosome 6 at the breakpoint.

Inherited rearrangements

Cryptic rearrangements were detected in 4 of the 14 inherited structural rearrangements (28.6%). These were only deletions ranging from 80 kb to 3.64 Mb. Two of them were inherited from a healthy parent (#9, #31). Two of them were at distance from initial breakpoints (#9, #31).

Reciprocal translocations: One of the seven (14.3 %) apparently balanced translocations showed a 1.21 Mb deletion at distance from the breakpoints (#9). It was inherited from the healthy father who also carries the balanced translocation.

Inversions: Two of the five (40 %) inversions were imbalanced. Deletion size was respectively of 80 kb and 1.23 Mb. One of these imbalances was located on a different

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chromosome than the chromosome carrying the inversion and was inherited from the father who also transmitted the inversion (#31).

Complex chromosomal rearrangements: One of the two CCR (50 %) presented a 3.64 Mb *de novo* deletion.

Results according to the array resolution

Thirty-eight cases were studied with 44K array. It displayed 15 microdeletions ranging from 300 kb to 15.37 Mb (mean = 4.24 Mb). Nine cases were investigated with a 244K array (#1, #2, #3, #29, #30, #31, #32, #41, #42). Six microdeletions in five patients were found ranging from 60 kb to 8.87 Mb (mean: 3.16 Mb). In two cases (#1, #31) in whom the deletion was of small size (less than 100 kb), it could not be concluded if the deletion accounted for the phenotype really.

4. Discussion

Development of more and more accurate molecular cytogenetics techniques such as FISH, CGH on chromosomes and array CGH allowed to dissect apparently balanced chromosomal rearrangements in patients with abnormal phenotype. These techniques displayed the complexity of reciprocal translocations that could in fact involve up to 5 chromosomes in a combination of translocations, insertions and inversions [1,3,10,23]. They also showed presence of cryptic imbalances either at the chromosomal breakpoint or at distance [1,3,10,12,15,18,23]. Characterization by FISH of 40 apparently balanced chromosomal rearrangements from the Developmental Genome Anatomy Project revealed imbalances in 37 % of them (15/40 cases) [12]. Array CGH is the most recent technology used to characterize this type of rearrangements and has the advantage over FISH approach to investigate the entire genome and not only breakpoint regions.

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Cryptic imbalances in apparently balanced *de novo* rearrangements

Beside several isolated case reports [5,11], larger studies using array CGH estimated the proportion of imbalances in apparently balanced *de novo* translocations (Table 3) [2,4,10]. Gribble *et al*. studied 10 patients using a BAC/PAC array and found 5/10 imbalances from 1.2 to 6.2 Mb [10]. De Gregori *et al* investigated 59 patients including *de novo* reciprocal translocations and CCR. They showed i) that 40 % of apparently balanced translocations with abnormal phenotype are associated with a cryptic deletion, ii) that 18 % of them are in fact more complex and iii) that almost all CCR with abnormal phenotype are imbalanced [4]. More recently, Baptista *et al* found four imbalances in 14 patients (28.6 %) [2]. In the present study, we found cryptic deletion in 48.5 % of cases (16/33) of apparently balanced *de novo* rearrangements associated with an abnormal phenotype including 8 translocations, 4 inversions and 4 CCR. Twenty-seven percent of them revealed more complex as initially thought. In 15 of these cases, the phenotype could be related to the cryptic imbalance according to its *de novo* nature, its size and its gene content [19]. For example, case #43 showed a *de novo* 690 kb deletion involving the *TWIST* gene that accounted for his Saethre-Chotzen phenotype and was described elsewhere [26]. These results are consistent with the previous studies leading us to conclude that about 40 % of apparently balanced *de novo* rearrangements with abnormal phenotype are associated with a cryptic imbalance. The yield of array CGH in MCA/MR patients with normal standard karyotype is about 10 to 17 % [20,27]. So it seems that genomic imbalances are more likely to be found in MCA/MR patients with structural chromosome rearrangements than in patients without. Interestingly, no case of our cohort had copy-number gains, which is also consistent with the previous studies [2,4,10]. This could be the fact of a particular mechanism generating preferentially loss of material. But gains of material could also be associated with milder phenotype or a different phenotype than MCA/MR and may not have been recruited. Finally, microdeletions unrelated

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to the breakpoints involve about 20 % of cases [4]. However, their significance is still unclear. They may either be part of a complex rearrangement involving multiple breakpoints and fusions at different part of the genome or be associated to the balanced rearrangement fortuitously.

Cryptic imbalances in apparently balanced inherited rearrangements

Four out of 14 inherited cases of chromosomal rearrangements were imbalanced: one translocation, 2 inversions and 1 CCR (cases #9, #31, #40 and #45). In two cases the cryptic imbalance occurred *de novo* and could be causally related to the phenotype of the patients. In two other cases (#9 and #31) the cryptic imbalance was inherited from the healthy parent who also transmitted the balanced rearrangement. So it cannot be concluded if these deletions were new benign variations not reported in databases yet or if they contributed to the phenotype in a complex manner like variable expression, unmasking of recessive mutation on the other allele, disturbance of parental imprinting or combination of multiple genetic defects. So, we believe that array CGH is also useful to explore patients with an abnormal phenotype carrying an inherited chromosomal rearrangement.

Diagnostic yield according to array resolution

Diagnostic yield of 244K arrays was not statistically different from 44K arrays in the present study. Although 244K array identified deletions less than 100 kb in two cases (cases #1 and #31), it was not possible to conclude about their pathological significance. Indeed, these deletions included a single gene, neither referenced in databases of genomic variants nor morbid databases (OMIM). Investigations to confirm the role of these genes in the phenotype of patients are time-consuming and go beyond the means of a diagnostic laboratory. The smallest deletion detected by 44K was 300 kb in this series (case #22). Moreover, it has

already been demonstrated that 44K array detected as much as pathological imbalances than 244K array but detected less copy number polymorphisms [7]. So, the use of 44K arrays seems compatible in a high-throughput diagnostic setting since it provides a good diagnostic yield and avoids too many false positive cases and time-consuming verifications.

Conclusion

In conclusion, we studied 47 cases of MCA/MR patients presenting an apparently balanced chromosomal rearrangement either *de novo* or inherited by array CGH. Genomic imbalances were identified in 48.5 % of *de novo* cases and 28.6 % of inherited cases. These results support previous studies showing that 40 % of patients with MCA/MR and an apparently balanced translocation carry a cryptic imbalance that can account for the phenotype. We suggest that the management of MCA/MR patients with an apparently balanced chromosome rearrangement should include a systematic investigation by array CGH, whatever the type of rearrangements (translocation, inversion or CCR) and whatever the inheritance (*de novo* or familial). If array CGH fails to detect any imbalance, breakpoints should be investigated to look for position effect [8,16,17] or gene disruption [13,14] that occurs in 35% to 50% of balanced rearrangements [2,9]. Of course, a fortuitous association between a balanced rearrangement and MCA/MR of another etiology cannot be excluded. In prenatal diagnosis, array CGH should be proposed to fetuses presented an apparently balanced *de novo* chromosomal rearrangement associated to malformations according to the literature [4].

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WEB RESSOURCES

Database of Genomic Variants: http://projects.tcag.ca/variation/

UCSC Genome Bioinformatics: http://genome.ucsc.edu/

Ensembl: http://www.ensembl.org/index.html

OMIM: http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim

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Legends to Tables

Table 1: Conventional karyotypes of the 47 patients

Table 2: Array CGH results in patients carrying an imbalance and corresponding phenotype

Table 3: Review of the literature and comparison with the present study

Table 1: Conventional karyotypes of the 47 patients

Case	Karyotype	Inheritance	Array resolution
1	46,XY,t(1;18)(q11.1;q12.1)	dn	244K
2	46,XX,t(1;18)(p36;q21)	dn	244K
3	46,XY,t(4;22)(q21;q12)	dn	244K
4	46,XY,t(3;5)(q24;q21)	pat	44K
5	46,XX,t(10;11)(p14;p15)	dn	44K
6	46,XY,t(4;11)(q27;q22;3)	dn	44K
7	46,XY,t(12;14)(q21;q31)	dn	44K
8	46,XY,t(7;10)(q3?5;q25?)	dn	44K
9	46,XY,t(2;6)(q36;q26)	pat	44K
10	46,XY,t(2;12)(p16;q14)	dn	44K
11	46,X,t(Y;2)(q12;p24)	dn	44K
12	46,XX,t(8;18)(q21;q22)	dn	44K
13	46,XX,t(5;14)(q34;q31)	dn	44K
14	46,XX,t(4;11)(q3?2;q25)	dn	44K
15	46,XY,t(1;4)(q43;q22)	mat	44K
16	46,XY,t(2;9)(q37.2;p23)	pat	44K
17	46,XY,t(2;9)(q32;q13)	dn	44K
18	46,XY,t(5;12)(q34;q23)	dn	44K
19	46,XY,t(7;14)(p14;q21)	mat	44K
20	46,XX,t(2;5)(p22;q12)	dn	44K
21	46,XY,t(9;12)(p23;q21)	dn	44K
22	46,XX,t(2;18)(p15;q21)	dn	44K
23	46,XX,t(1;18)(p31;q12.3)	mat	44K
24	46,XX,t(1;11)(q12;q13)	mat	44K
25	46,XX,t(1;14)(q31;q12)	dn	44K
26	46,XY,t(2;8)(q22;q24.2)	dn	44K
27	46,XY,t(7;12)(p11;p11)	dn	44K
28	46,XY,t(1;6)(q4?1;q1?4)	dn	44K
29	46,XX,inv(8)(p22q12.2)	dn	244K
30	46,XY,inv(11)(p15q13)	mat	244K
31	46,XY,inv(4)(p13q22)	pat	244K
32	46,XX,inv(8)(q21q24.2)	pat	244K
33	46,XX,inv(1)(q42q44)	dn	44K
34	46,XX,inv(4)(p16q32)	dn	44K
35	46,XX,inv(13)(q12.13q34)	dn	44K
36	46,XY,inv(7)(p13q21)	dn	44K
37	46,XY,inv(2)(p13q13)	dn	44K
38	46,X,inv(X)(p21.1q21.1)	dn	44K
39	46,XY,inv(7)(p14q21.1)	mat	44K
40	46,XX,inv(1)(p21q13)	mat	44K
41	46,XY,t(1;7;11)(p35;q33;q12)	dn	244K
42	46,XX,t(2;3;6)(q21;q22;p26.1)	dn	244K
43	46,XY,t(2;7)(p24;p21),ins(7)(p21.3q21.3q22)	dn	44K
44	46,XY,inv(5)(p14q23)t(1;inv(5))(p21;q23)	pat	44K
45	46,XX,ins(7;4)(q31;q27q32)	mat	44K
46	46,XX,t(2;5;10)(2pter→2q22::2q34→2qter; 5pter→5q21::2q22→2q33::10p14→10pter;10qter →10p14::2q33→2q34::5q21→5qter)	dn	44K

Left margin vertical labels: TRANSLOCATIONS (cases 1–28), INVERSIONS (cases 29–40), CCR (cases 45–46)

47	46,XX,t(1;2;9)(1pter→1q31::9p12→9pter; 2pter→2q24::1q41→1qter;9qter→9p12: :1q31→1q41::2q24→2qter),t(11;14)(q11;q23)	dn	44K

dn: *de novo* ; pat: paternal; mat: maternal

Table 2: Array CGH results in patients carrying an imbalance and corresponding phenotype

a) de novo rearrangement group

	Case	Karyotype	Rearranged region	Inheritance	Size Mb	Phenotype
T R A N S L O C	1*	46,XY,t(1;18)(q11.1;q12.1)	del(14)(q32.2q32.2)	dn	0.06	Moderate mental retardation, macrosomia, leukodystrophy, hexadactyly
	3	46,XY,t(4;22)(q21;q12)	del(4)(q21.22q21.22)	dn	0.77	Moderate mental retardation, facial dysmorphism
	8	46,XY,t(7;10)(q3?5;q25?)	del(7)(q35q36.1)	dn	5.13	Severe mental retardation, autistic troubles, seizures, facial dysmorphism
	17	46,XY,t(2;9)(q32;q13)	del(9)(q21.13q21.31)	dn	6.49	Mild mental retardation, speech delay, facial dysmorphism, hirsutism
	21	46,XY,t(9;12)(p23;q21)	del(9)(p24.2p23)	dn	11.61	Severe mental retardation, speech delay, aggressivity, muscular hypotonia, hydronephrosis, cryptorchidism, diabetes
	22*	46,XX,t(2;18)(p15;q21)	del(2)(p21p21)	dn	0.30	Mental retardation, speech delay, hyperactivity, facial dysmorphism, cleft lip and palate, congenital cardiac defect, syndactyly
	25	46,XX,t(1;14)(q31;q12)	del(1)(q25.2q31.2)	dn	15.37	Moderate mental retardation, hypotonia, growth retardation, facial dysmorphism, trigonocephaly
	27*	46,XY,t(7;12)(p11;p11)	del(2)(q33.1q33.1)	dn	2.02	Severe mental retardation, autistic troubles, growth retardation, facial dysmorphism, microcephaly, dental anomalies
I N V	29*	46,XX,inv(8)(p22q12.2)	del(1)(q24.1q24.2)	dn	8.87	Mild mental retardation, seizures, growth retardation, facial dysmorphism
	34*	46,XX,inv(4)(p16q32)	del(13)(q12.3q13.1)	dn	3.52	Moderate mental retardation, speech delay, obesity, facial dysmorphism, camptodactyly
	35	46,XX,inv(13)(q12.13q34)	del(13)(q12.3q13.1)	dn	3.85	Severe mental retardation, microcephaly, muscular hypertonia, growth retardation, liver steatosis
	36*	46,XY,inv(7)(p13q21)	del(14)(q22.1q22.1)	dn	1.06	Mild mental retardation, spasticity, poor motor coordination
C C R	42*	46,XX,t(2;3;6)(q21;q22;p26.1)	del(2)q34q34)	dn	4.3	Moderate mental retardation , seizures, facial dysmorphism, fingers hyperlaxity
			del(6q25.1q25.2)	dn	4.9	
	43	46,XY,t(2;7)(p24;p21),ins(7)(p21.3q21.3q22)	del(7)(p21.3p21.3)	dn	0.69	Mild mental retardation, craniosynostosis, syndactyly (Saethre-Chotzen syndrome), cryptorchidism, kidney hypoplasia
	46	46,XX,t(2;5;10)	del(2)(q33.3q33.3)	dn	3.64	Facial dysmorphism, congenital cardiac defect, brain malforamtion, hepatosplenomegaly, cryptorchidism
	47	46,XX,t(1;2;9),t(11;14)(q11;q23)	del(1)(q23.3q24.2)	dn	3.57	Mental retardation, muscular hypotonia, growth retardation, facial dysmorphism, corpus callosum hypoplasia, renal hypoplasia, anal anteposition, sacrococcygeal dimple

b) inherited rearrangement group

Case	Karyotype	Rearranged region	Inheritance	Size Mb	Phenotype
9*	46,XY,t(2;6)(q36;q26)pat	del(6)(q24.2q24.3)	pat	1.21	Autistic troubles
31*	46,XY,inv(4)(p13q22)pat	del(15)(q21.3q21.3)	pat	0.08	Mild mental retardation, seizures, growth retardation, facial dysmophism, brachymesophalangia
40	46,XX,inv(1)(p21q13)mat	del(1)(p21.2p21.2)	dn	1.23	Severe mental retardation, microcephaly, facial dysmorphism
45	46,XX,ins(7;4)(q31;q27q32)mat	del(4)(q31.3q32.1)	dn	4.03	Moderate mental retardation, seizures, scoliosis

Transloc: translocation; Inv: inversion: CCR: complex chromosomal rearrangement; dn: de novo; pat: paternal; mat: maternal; Mb: megabases
Patients carrying a deletion unrelated to the balanced rearrangement breakpoint are marked by an asterix.

Table 3: Review of the litterature and comparison with the present study

	Gribble et al.[10]	De Gregori et al.[4]	Baptista et al.[2]	Present study	
Array	BAC/PAC	Oligonucleotides	BAC/PAC	Oligonucleotides	
Resolution	3,500 clones	44K/244K	30,000 clones	44K/244K	
Rearrangements	t/CCR	t/CCR	t	t/**inv**/CCR	
de novo /inherited	*de novo*	*de novo*	*de novo*	*de novo*	inherited
Number of patients	10	59	14	33	14
Rate imbalance	50% (5/10)	45.7% (24/59)	28.6 %(4/14)	48.5 % (16/33)	28.6% (4/14)
Imbalance size (Mb)	2.2-6.2	0.5-8.4	0.17-2.5	0.06-15.37	0.08-3.64
Imbalance at distance from breakpoint	60% (3/5)	41.6% (10/24)	25% (1/4)	56.2% (9/16)	50% (2/4)

t: translocation; CCR: complex chromosomal rearrangement; inv: inversion