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Short report

Using a combination of MLPA kits to detect chromosomal imbalances in patients with multiple congenital anomalies and mental retardation is a valuable choice for developing countries

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ABSTRACT

Conventional karyotyping detects anomalies in 3–15% of patients with multiple congenital anomalies and mental retardation (MCA/MR). Whole-genome array screening (WGAS) has been consistently suggested as the first choice diagnostic test for this group of patients, but it is very costly for large-scale use in developing countries. We evaluated the use of a combination of Multiplex Ligation-dependent Probe Amplification (MLPA) kits to increase the detection rate of chromosomal abnormalities in MCA/MR patients. We screened 261 MCA/MR patients with two subtelomeric and one microdeletion kits. This would theoretically detect up to 70% of all submicroscopic abnormalities. Additionally we scored the de Vries score for 209 patients in an effort to find a suitable cut-off for MLPA screening. Our results reveal that chromosomal abnormalities were present in 87 (33.3%) patients, but only 57 (21.8%) were considered causative. Karyotyping detected 15 abnormalities (6.9%), while MLPA identified 54 (20.7%). Our combined MLPA screening raised the total detection number of pathogenic imbalances more than three times when compared to conventional karyotyping. We also show that using the de Vries score as a cut-off for this screening would only be suitable under financial restrictions. A decision analytic model was constructed with three possible strategies: karyotype, karyotype + MLPA and karyotype + WGAS. Karyotype + MLPA strategy detected anomalies in 19.8% of cases which account for 76.45% of the expected yield for karyotype + WGAS. Incremental Cost Effectiveness Ratio (ICER) of MLPA is three times lower than that of WGAS, which means that, for the same costs, we have three additional diagnoses with MLPA but only one with WGAS. We list all causative alterations found, including rare findings, such as reciprocal duplications of regions deleted in Sotos and Williams–Beuren syndromes. We also describe imbalances that were considered polymorphisms or rare variants, such as the new SNP that confounded the analysis of the 22q13.3 deletion syndrome.

1. Introduction

Multiple congenital anomalies and mental retardation (MCA/MR) comprise a large, heterogeneous group of diseases that affect approximately 3% of newborns. The ability of conventional karyotyping to detect chromosomal anomalies in newborns varies considerably, ranging from 3 to 15% depending on patient selection and the inclusion of Down syndrome in the cohort [1,2]. Using

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molecular biological techniques such as fluorescent in situ hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA) and whole-genome array screening (WGAS) almost doubles the detection rate of chromosomal abnormalities in MCA/MR [3–7]. WGAS alone detects 99% of all chromosomal alterations, and recent reports have suggested that WGAS should replace conventional karyotyping as the first diagnostic test used to detect chromosomal abnormalities in patients with MCA/MR [2,8–10]. WGAS is more costly than traditional karyotyping, followed by targeted FISH, but is an attractive alternative because of its increased clinical effectiveness [11]. The Brazilian government has included genetic testing in social medical services and the compulsory package of insurance companies, but the high cost of these techniques has made implementation difficult in Brazil and other developing countries. Unfortunately, to identify subtelomeric imbalances and microdeletions, FISH is also prohibitively expensive. Therefore, the low cost of MLPA makes it a viable alternative that would substantially increase the identification of chromosomal alterations.

The aim of this project was to determine the ability of a combination of MLPA kits to detect chromosomal abnormalities such as submicroscopic microdeletions and subtelomeric imbalances in a group of MCA/MR patients. Additionally, we wanted to test the feasibility of this approach for use in routine diagnosis.

2. Material and methods

2.1. Patients

This was a prospective study including 261 patients with multiple congenital malformations with or without mental retardation (MCA/MR). Clinical geneticists evaluated all patients prior to referral to the study. Common aneuploidies, such as trisomies 21, 13, 18 and Turner syndrome, were not included. The first 209 patients were assessed for the presence of prenatal growth retardation, postnatal growth abnormalities, facial and non-facial dysmorphisms, congenital abnormalities and familial occurrence of mental retardation and were given a de Vries score (a check list for subtelomeric imbalances) [12]. The de Vries score was used as an abnormality evaluation, not as criteria for inclusion or exclusion in molecular screening. The presence and severity of mental retardation could not be determined for all cases because many patients were neonates or younger than three years old at the time of referral.

2.2. Methods

All patients were screened with three MLPA kits: two for subtelomeric imbalances (P036 and P070) and one for the most common microdeletion syndromes (P064), which include deletion 22q11.21, Williams, Prader–Willi/Angelman, Smith–Magenis, Miller–Dieker and deletion 1p36 (MRC-Holland, Amsterdam, The Netherlands). When needed, confirmatory testing was performed with kit P356, which is also able to detect microdeletion syndromes in chromosome 22. The details of regions detected by each kit can be found at www.mlpa.com. Chromosomal analysis by GTG-banding was performed by independent offsite cytogenetic laboratories.

MLPA was carried out according to the manufacturer's instructions. Initially, electrophoresis and fragment analysis were performed using the Mega-BACE TM 1000 DNA analysis system and Fragment Profiler software version 1.2 (GE Healthcare). Statistical analyses were carried out using excel spreadsheets developed by the National Genetics Reference Laboratories (NGRL, Manchester, UK, www.ngrl.org.uk). During the second phase, electrophoresis was performed using an ABI 3100 Genetic Analyzer, and data were analyzed out using GeneMarker software (Softgenetics, www.softgenetics.com).

Results were considered abnormal when the relative peak height ratio was below 0.75 or above 1.25. Exceptions were made for results obtained with probes known to be variable. When possible, aberrant results were checked by independent reactions using parental samples in the same reaction batch. The DGV database (http://projects.tcag.ca/variation/) was consulted for all abnormal probes to determine whether they were located in regions with known polymorphic copy number variants (CNVs). An abnormal result was considered causative when it was detected by both subtelomeric kits and was not present in parental samples. Inherited subtelomeric alterations were classified as non-causative variants and were not considered pathogenic. When an abnormality was detected using a subtelomeric kit and parental DNA was not available, the result was considered a) causative when additional methodologies confirmed the alteration or b) inconclusive when confirmation could not be performed. For the microdeletion syndrome kit, results were considered causative when more than two probes to the same region produced abnormal results. When abnormal results were obtained with only one probe, the test was repeated with parental samples to exclude the possibility of inherited variants. Parents of patients with deletions at 22q11.21 were checked to determine if they were carriers. Confirmation of the MLPA results by a second independent technique was not performed for all cases due to financial restrictions. The clinical data of all patients were reviewed on the basis of the anomaly found and whenever these data were conflicting further studies were performed (see below).

To clarify the economic aspect of the possible strategies used to diagnose chromosomal rearrangements in MCA/MR, a decision analytic model was constructed using TreeAge Pro 2011 (TreeAge Pro Inc, Williamstown, MA, USA). We designed our model with three possible strategies: karyotype, karyotype + MLPA and karyotype + WGAS. Data from karyotype and karyotype + MLPA strategies were taken from our results.

Costs were initially calculated in local currency, Brazilian real (RS $) and then converted to US dollars (US $) using exchange rate (US $ 1.00 = RS $ 1.673). Effectiveness was calculated as the number of additional diagnoses compared to karyotype.

3. Results and discussion

Currently, WGAS is the preferred technique for the diagnosis of patients with multiple congenital anomalies and mental retardation (MCA/MR) [10,11]. However, the high cost of whole-genome array screening makes it unfeasible for many countries. In this study, we evaluated, the ability of a combination of MLPA kits to detect subtelomeric imbalances and common microdeletion syndromes such as DiGeorge (deletion 22q11.21), deletion 1p36, Williams, Prader–Willi/Angelman, Smith–Magenis and Miller–Dieker syndromes.

Our cohort of patients included 261 individuals (132 males and 129 females). Referral ages varied from three days to 26 years and 3 months, with a mean age of 6 years and 1 month. Karyotype and MLPA analyses revealed chromosomal imbalances in 87 (33.3%) patients (Table 1). Conventional karyotyping detected 17 of the abnormalities (6.5%), while MLPA identified 83 (31.8%). Three abnormalities were detected exclusively by cytogenetics, while 70 imbalances were detected only by MLPA. After analysis of parental samples and CNV, 24 of the imbalances were considered inherited non-causative variants. Additionally, the 47 XYY result found in a newborn male was considered not related to the phenotype. Five imbalances were considered inconclusive. Therefore, in our total

Table 1
Total results for the screening for chromosomal abnormalities with karyotyping and MLPA.

	Combined approach	(%)	MLPA	(%)	Karyotyping	(%)
Total cohort	261	100.0	261	100.0	217	100.0
Total abnormalities	87	33.3	83	31.8	17	7.8
Inherited variants[a]	24	9.2	24	9.2	0	0.0
Not related	1	0.4	1	0.4	1	0.5
Inconclusive	5	1.9	3	1.1	1	0.5
Causative	**57**	**21.8**	**54**	**20.7**	**15**	**6.9**

[a] Known heterochromatin polymorphisms in karyotype were not recorded.

cohort, 57 anomalies were classified as causative of the observed phenotype (21.8%).

Conventional karyotyping was performed in 217 patients and detected 15 causative alterations (6.9%). Using MLPA to screen patients raised the total detection number of pathogenic chromosomal imbalances to 57 (21.8%), which is three times the number detected by karyotyping.

3.1. Causative abnormalities

All 57 causative anomalies are listed in Table 2. Only three alterations detected by conventional karyotyping were not detected by MLPA: two *de novo* apparently balanced translocations in patients 1 and 2 and an additional segment in chromosome 21 of patient 3. As expected, MLPA was important for the characterization of derivative and marker chromosomes not identified by conventional cytogenetics. This is illustrated by the two cases of cat eye syndrome (patients 6 and 7), where the signal of both probes to 22"p" (22q11.21) indicated four copies of the region, suggesting that the marker chromosome was an inverse duplication (22). We also found that other chromosomes were involved in the imbalances of patients 8, 9, 12 and 13. However, we did not identify the extra segment present on the short arm of chromosome 21 in patient 3.

Regarding only patients with normal karyotype, submicroscopic subtelomeric imbalances were found in 19 patients (7.3%); 11 of them were pure terminal or cryptic deletions, and the rest were balanced translocations. The incidence of submicroscopic subtelomeric imbalances in our sample did not differ significantly from other studies [13]. The incidence of common microdeletion and microduplication syndromes identified was slightly higher than that of previous studies (24/261; 9.2%), which illustrates that submicroscopic imbalances may be responsible for a large portion of chromosomal abnormalities in MCA/MR patients [1,14,15]. Deletion 22q11.21 was the most frequent abnormality found (12/261–4.6%), representing 21.1% of all alterations. In similar studies with different selection criteria for patients, detection of the 22q11.21 deletion varied from 0.5% to 2.4% [1,14,15]; in comparison, the rate in our study is surprisingly high. Interestingly, prior to

Table 2
Causative abnormalities found in the 261 patients screened.

Patient	Karyotype	MLPA kit P036	MLPA kit P070	MLPA kit P064
1	46, XX, t(11q;13q)	nrl	nrl	nrl
2	46, XX, t(1;2)(p36.2;q35), t(6;18)(p11.2;p11.2)	nrl	nrl	nrl
3	46, XX, add(21q10)	nrl	nrl	nrl
4,5	45, X	del Xp/del Xq	del Xp/del Xq	nrl
6,7	47, XY, + mar	dup 22"p"[a]	dup 22"p"[a]	nrl
8	6, XX, dup 3pter	del 3p/dup 7q	del 3p/dup 7q	nrl
9	46, XY, der(3)	del 3p/dup 11q	del 3p/dup 11q	nrl
10	46, XY, del 4p15	del 4p	del 4p	nrl
11	46, XX, del 4qter	del 4q	del 4q	nrl
12	46, XX, dup 4qter	del 4q/dup 10q	del 4q/dup 10q	not performed
13	46, XY, der(18q+)	del 18q/dup 4q	del 18q/dup 4q	not performed
14	45, X [10]/46, X, + mar [20]	del Xp/del Xq	del Xp/del Xq	nrl
15	47,XX,+der(9)	dup 9p	dup 9p	nrl
16	46, XX	del 2p	del 2p	nrl
17,18	46, XX or 46, XY	del 3p	del 3p	nrl
19	46, XX	del 4p/dup18p/del 4q[b]	del 4p/dup18p	not performed
20,21	46, XX	del 4p	del 4p	nrl
22	46, XX	del 5p	del 5p	nrl
23	46, XX	del 1q	del 1q	nrl
24	46, XX	del 3q[c]	nrl	nrl
25	46, XX	del 4q/dup 2p	del 4q/dup 2p	nrl
26	46, XX	del 6q/dup 14q	del 6q/dup 14q	nrl
27	not available	del 10p/dup 9q	del 10p/dup 9q	nrl
28,29	46, XX	del 10q	del 10q	nrl
30	46, XY	del 18q/dup 8q	del 18q/dup 8q	nrl
31,32	46, XX or 46, XY	del 18q/dup 3q	del 18q/dup 3q	nrl
33	not available	del 22q	del 22q	nrl
34	46, XY	dup 1p/del 22q	dup 1p/del 22q	dup 6 probes at 1p36[d]
35	not available	not performed	del 1p	del 7 probes at 1p36
36,37	46, XX[e]	del 15"p"[a]	del 15"p"[a]	del 5 probes at 15q11 (Prader/Angelman Syndrome)
38–49	46, XX or 46, XY[e]	nrl	nrl	del 6 probes in 22q11.23 (DiGeorge Syndrome)
50–52	46, XX[e]	nrl	nrl	del 6 probes in 7q11.23 (Williams Syndrome)
53,54	46, XY[e]	nrl	nrl	del 5 probes at 17p11.2 (Smith–Magenis Syndrome)
55	46, XY	nrl	nrl	del 7 probes in 17p13.3 (Miller–Dieker Syndrome)
56	not available	nrl	nrl	dup 3 probes in 5q35.3 (*NSD1 gene*)
57	not available	nrl	nrl	dup 5 probes in 7q11.23 (Williams Syndrome region)

[a] The 13, 14, 15, 21 and 22 p probes target the q arm, close to centromere.
[b] SNP in probe site, inherited.
[c] *de novo* 1.6-Mb interstitial deletion at band 3q29 detected by Affymetrix 100 K SNP array.
[d] Breakpoint between probes located at FLJ10782 and *TP73*.
[e] For some patients no karyotyping is available because MLPA was the first choice of testing.

molecular testing, six of our patients had no clinical suspicion of the 22q11.21 deletion. The young age of the patients combined with the diverse clinical expressivity of the 22q11.21 deletion syndrome may have contributed to this underdiagnosis.

Reciprocal duplications of regions deleted in Sotos and Williams–Beuren syndromes were found in patients 56 and 57, respectively, reinforcing the results of Kirchhoff et al. [14], which found that these microduplications may not be as rare as reported by Shaffer et al. [15], where only eight duplications of the Williams–Beuren region and one of the Sotos syndrome region were found in 8789 patients.

Using two kits instead of one to screen for subtelomeric imbalances is important to confirm the results from extremely polymorphic regions. Special care should be taken in cases where only one kit finds an MLPA abnormality, which happened quite frequently in this study. Most single detection imbalances were inherited and classified as non-causative variants (see below). *De novo* subtelomeric anomalies found with only one kit were confirmed by other techniques, such as WGAS. Deletion of the region detected by the 3qter probe, BDH (kit P036), in patient 24 was confirmed by Affymetrix 100 K SNP array. The Affymetrix genome screening detected a *de novo* 1.6 Mb interstitial deletion at band 3q29, which indicated that the breakpoint of this deletion was between the two subtelomeric MLPA probes used.

3.2. Inherited non-causative variants

We found inherited variants in twenty-four patients (Table 3). The copy number of specific variations in the genome can influence clinical symptoms. Additionally, the presence of the same abnormality in a patient and his/her healthy parent may be a result of incomplete penetrance or imprinting mechanisms. Moreover, recessive disorders may be revealed by the loss of one allele and the mutation of the other. However, in this study, we considered subtelomeric abnormalities inherited from healthy parents as non-causative.

When abnormal results were detected, the genomic region corresponding to each probe was examined for the presence of CNVs. Determining that probes were located at CNVs was important but not sufficient to determine whether the results were associated with the patient's phenotype. MLPA imbalances, particularly deletions, should be interpreted carefully. Firstly, a deletion detected by MLPA may be a result of hemizygosity or the presence of a SNP in the probe hybridization site. Secondly, the extent of the imbalance detected by subtelomeric MLPA is not known. Therefore, we cannot assume that alterations found in patients completely correspond to known alterations in the CNV database. All probes listed in Table 3, with the exception of 12p *RBBP2* (kit P070), are located at known CNVs. However, as described below, at least two of the alterations found were due to a SNP in the probe site and not to deletion of the corresponding CNV.

The 4q deletion found using older versions of kit P036 detected in both heterozygous and homozygous states in our samples were due to a SNP in the probe site (MRC-Holland information sheet). Probes for 2p and 4p in kit P070 have been described as variable by the manufacturer. Moreover, we detected several alterations that had never been reported as polymorphic by MLPA users, including a 16p deletion, a 12p deletion and duplication, a 22q deletion and a 5p duplication. Each of these new alterations was inherited from healthy parents. Therefore, the presence of parental samples was extremely important for interpretation of the data. Unlike previous studies [3], we did not have problems obtaining parental samples, which were collected at the same time as the patient's sample.

We were not able to determine the mechanisms underlying all the above mentioned variants. It is reasonable to think that duplications are due to CNVs, but deletions can be caused either by CNVs or SNPs at the probe sites.

The two deletions detected by the 22q *ARSA* probe (kit P070) in patients 86 and 87 were additionally studied. This probe is located in the region deleted in 22q13.3 syndrome (Phelan–McDermid syndrome), an important and well-known mental retardation syndrome. The two patients, however, did not match the clinical symptoms of the syndrome. In both cases, the relative peak height for the *ARSA* probe was between 0.75 and 0.70 and was therefore considered abnormal. The relative peak height of the paternal sample for patient 86 was in the same range. The parental results for patient 86 were considered normal (the maternal result was 0.95, and paternal result was 0.78). We suspect the presence of a SNP in the probe site because the peak height for the same probe in patients 33 and 34, who also had deletions at the 22qter probe site, was approximately 0.5 (Fig. 1). Follow-up analysis with kit P356 did not produce any abnormal results for the 17 probes to the 22q13.3 region for patient 86 or 87 or their parents. Furthermore, the results for the positive control (patient 33) were clearly abnormal (Fig. 1). Sequence analysis of the ARSA probe site in patients 86 and 87 and their respective fathers showed the presence of an undescribed variant NT_011526.7:g.651808C > G (Fig. 1), which explained the lower signals detected by the P070 reactions. Although the ARSA probe in kit P070 targets the same region as the ARSA probe in kit P356, the hybridization site length of the latter is

Table 3
Non causative, false positive, inconclusive or polymorphic abnormalities found in 261 patients with multiple congenital anomalies and mental retardation.

Patient	Karyotype	MLPA P036[a]	MLPA P070[a]	MLPA P064	Conclusion
58	46, XYY	dup Xp/dup Xq/dup Yq11	dup Xp/dup Xq/dup Yq11	nrl	non-causative
59	46, XY	del 7p	nrl	nrl	inconclusive
60	45, XX, der(13;13)[b]	nrl	nrl	nrl	inconclusive
61	46, XY	del 14q	del 14q	nrl	inconclusive
62	46, XY	nrl	del 2p	nrl	inconclusive
63	46, XY	nrl	dup Xq	nrl	inconclusive
64	46, XX	dup 16q	dup 16q/del 15q	nrl	inherited variant
65	46, XY	dup 16q	dup 16q/del 4p	nrl	inherited variant
66	46, XX	dup 12p	dup 12p	nrl	inherited variant
67	46, XY	del 12p	nrl	nrl	inherited variant
68	46, XX	del 4q/del 12p	nrl	nrl	inherited variant
69–77	46, XX or 46, XY	del 16p	nrl	nrl	inherited variant
78–83	46, XX or 46, XY	del 4q	nrl	nrl	inherited variant
84,85	46, XX	dup 5p	nrl	nrl	inherited variant
86,87	46, XX or 46, XY	nrl	del 22q	nrl	inherited variant
88	46, XX	nrl	dup 17q	nrl	inherited variant

[a] With the exception of the X and Y probes and for the 12p *RBBP2* probe (kit P070) all other probes here in listed are located at known CNVs.
[b] Parents refused further studies, such as isodisomy.

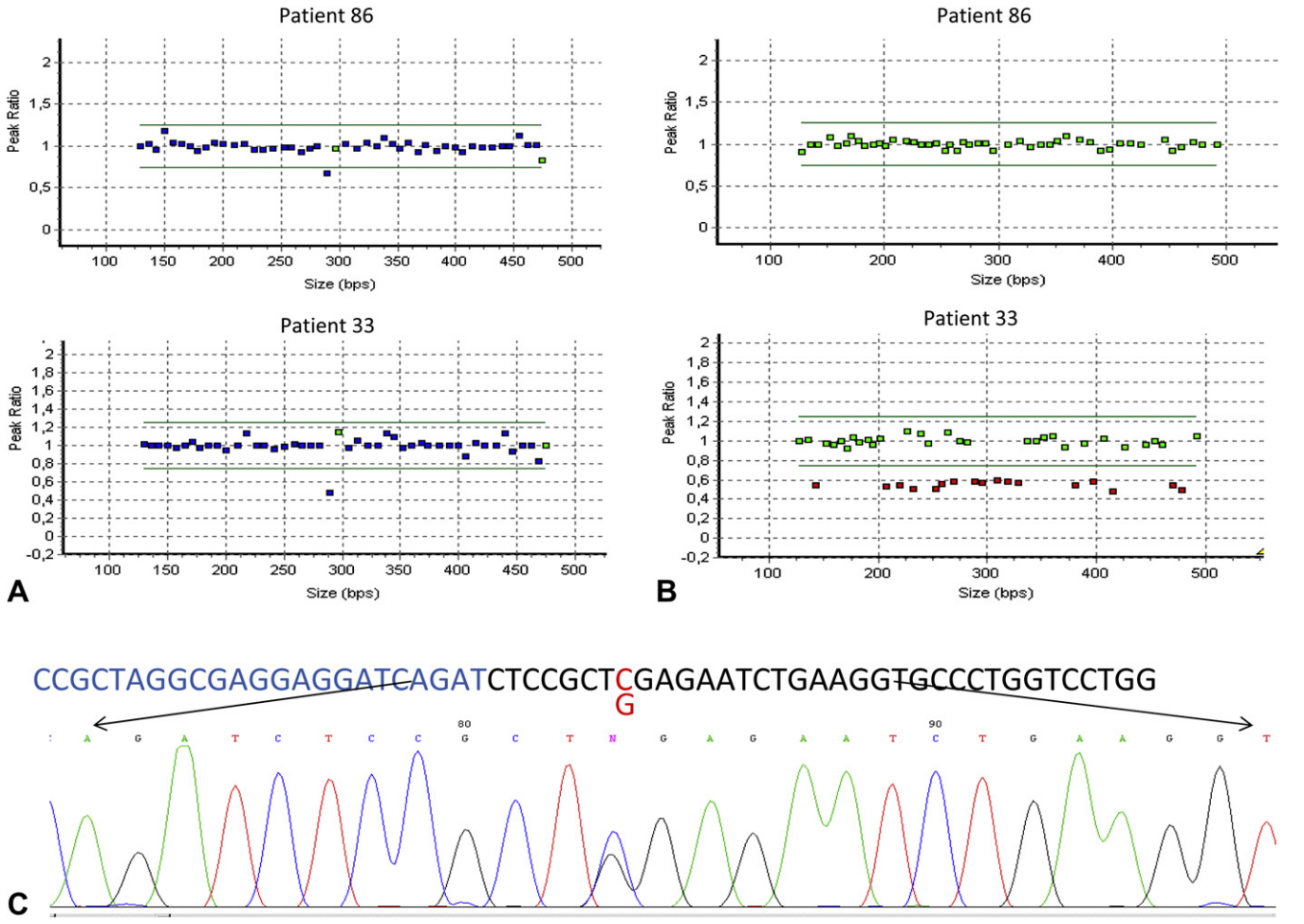

Fig. 1. A, MLPA analyses, with subtelomeric kit P070, showing abnormal results for the ARSA probe at 22q13.3 in patient 86 (peak ratio 0,70) and patient 33 (peak ratio 0,5). B. Follow-up analysis with 22q kit P356 shows normal results for patient 86 and the deletion of all 17 probes to the 22q13.3 region for patient 32. C. Sequence analysis of the ARSA probe site in patient 86 depicting the exact position of the C to G change in probe sequence (above).

longer and may hybridize better during the MLPA reaction, which may explain why normal results were obtained for all individuals that were tested with kit P356 (MRC-Holland, personal communication).

Had we used the 0.70 and 1.30 threshold values for normal MLPA results we would not have considered the above findings abnormal. After this study we have a better knowledge of our population's variants and may consider broadening the threshold values to decrease false positive results.

3.3. *Inconclusive findings*

Five results were considered inconclusive. A Robertsonian translocation between chromosomes 13 in patient 60 does not explain her clinical findings. Further studies, such as a test for isodisomy, were offered but were refused. We also considered three imbalances detected by MLPA to be inconclusive because we could not prove they were *de novo* without parental samples (patients 61–63). Both subtelomeric kits detected a 14q imbalance for patient 61, which suggests a deletion rather than a SNP in the target region of each probe. The two probes target *MTA1*, which is mapped at a CNV that has already been described as being deleted in healthy controls. Patient 63 has a duplication that maps to pseudoautosomal region Xqter/Yq12. Surprisingly, a duplication was detected by the probe specific for *VAMP7* from kit P070, which maps to the genomic position 57,680 kb (build 36.3), but was not detected by the *VAMP7* probe from kit P036 that maps 4 kb proximal to the probe from kit P070. An InDel located at Yq12 (57,670,868–57,671,032 bp) is duplicated in some controls from the DGV database. It is possible that the *VAMP7* probe from kit P070 maps inside this InDel. We could not determine whether results obtained using the *VAMP7* probe caused disease without also checking the status of the parents. Patient 62 had a deletion of 2p that was detected by the *ACP1* probe from kit P070, which was intriguing because the deletion detected by the P070 kit probe is only 2 kb distal to the site detected by the normal *ACP1* probe from kit P036. Duplications of the entire *ACP1* gene are described for normal controls in DGV, but deletions have not previously been described. On the other hand, a deletion of *ACP1* has been found in several healthy controls in a Dutch population using the *ACP1* probe from kit P036 (MRC-Holland information sheet). In the case of patient 62, we know the mother was not a carrier of the deletion, but we were unable to obtain a paternal sample for testing.

The last inconclusive case was patient 59, with a deletion of the region detected by the 7pter CENTA1 probe (kit P036). This deletion was not confirmed by Agilent 44 K oligo array. Parental samples did not show the same MLPA abnormality. Due to the low quality of the original sample and parental refusal of a new collection, we repurificate patient's DNA before MLPA and array testing. It is possible that our inconsistent results were due to the DNA quality.

We were not able to sequence the probe site for this patient to check if he has a SNP at the probe site. Therefore, this result was also considered inconclusive.

3.4. de Vries score

A de Vries score was collected for 209 patients. The mean score for all samples was 4.8. The mean de Vries score for patients with causative abnormalities and for patients with normal/inconclusive/non-causative abnormalities was 5.0 and 4.7, respectively. Patients with karyotype abnormalities had a higher mean de Vries score of 5.5 compared to 4.8 in the group with submicroscopic imbalances. Likewise, patients with subtelomeric abnormalities had a higher mean de Vries score of 5.3 compared to 4.4 for patients with interstitial microdeletions or microduplications. The differences in mean scores may be reflective of the original purpose of the de Vries score, which was to define a check list for the study of submicroscopic subtelomeric imbalances. Table 4 shows the number and percentage of patients within each de Vries score. It was not beneficial to use a cut-off value as a deciding factor for whether to include a patient in the screening. We detected abnormalities in 22.7% of patients with a de Vries score of up to 6, with a substantial increase of detected abnormalities in patients with a de Vries score of 7 or higher. However, with a cut-off of 7, we would have been screening significantly fewer patients and excluding almost 80% of the detected chromosomal abnormalities. Taking financial restrictions into account, we would suggest using MLPA in patients with a de Vries score of at least 4, which would detect 83% of abnormalities.

3.5. Decision analytic model

In our economic analysis we only considered 217 cases that had both karyotype and MLPA results since it is impossible to determine if karyotype could identify the anomaly when only MLPA was performed. Our purpose was to compare karyotype, a routine and less expensive strategy, with a suggested strategy karyotype + MLPA and with karyotype + WGAS.

We calculated tests costs including only reagents and import taxes for a private laboratory setting. Based on that, karyotype cost was US$ 14.94, MLPA, US$ 235.67 (for the three kits combined), sequencing, US$ 97.43 and WGAS, US$ 1141.88.

Our results, shown in Fig. 2, provided probabilities to diagnose (Dx) the anomaly when only karyotype was performed (15 Dx/217 cases−0.069) and when both karyotype + MLPA were performed (42 Dx/217 cases−0.194). Moreover, we added on our model, all additional tests we used after karyotype + MLPA. We performed WGAS in two cases and sequenced two other cases. Only one case was diagnosed after WGAS. Even though WGAS and sequencing did not contribute to solve all investigated cases after karyotype + MLPA, we included these data to precisely calculate all costs involved in this strategy. To compare with WGAS strategy, we used our data on detection rate for chromosome aberrations, 6.9% and the data from a review of 29 array-based studies of unselected MCA/MR patients that yield ∼19% pathogenic aberrations [2]. It was establish therefore that the probability of diagnosis with karyotype + WGAS was 6.9% + 19% = 25.9% (0.259). The karyotyping + MLPA strategy detected anomalies in 19.8% of cases which account for 76.45% of the expected detecting yield for karyotype + WGAS (25.9%).

In clinical practice in Brazil, karyotype is the current diagnostic test used to identify chromosomal abnormalities. So, we calculated Incremental Cost Effectiveness Ratio (ICER) to compare the strategies karyotype + MLPA and karyotype + WGAS with karyotype. ICER gives us the incremental cost necessary to provide one more diagnosis. Our analysis shows that when MLPA (plus all confirmatory tests, such as the two WGAS and sequencing) is added to karyotype there is an incremental cost of US$ 19.11/diagnosis (ICER = 19.11). When WGAS is performed, the incremental cost raises up to US$ 60.13/diagnosis (ICER = 60.13), which means that costs necessary to obtain one more diagnosis are more than three times higher when using WGAS when compared to the combined MLPA strategy.

For the costs of the combined MLPA strategy we used the number of reactions needed to diagnose one patient that was 5.6 reactions, which includes three MLPA kits, parental samples, negative controls and repetition due to technical failure (results not shown). On the other hand, costs for WGAS were calculated for a single Affymetrix 100 K SNP array testing, not including parental testing or technical failure.

In total, 44 patients had no conventional cytogenetic analyses due to clinical suspicion of a microdeletion syndrome or other reasons. We believe our overall results would not have changed significantly had karyotyping been performed for all patients. We do not expect a large number of anomalies in the patients with normal MLPA results and no available karyotype. The chances of patients having an interstitial imbalance, a balanced translocation or mosaicism is approximately 15% [2].

Although confirmatory tests using other technologies such as FISH or WGAS are valuable, in most cases, MLPA results in combination with karyotyping and/or revision of clinical findings were sufficient for diagnosis.

MLPA is easy to perform and produces good, reliable results. We had estimated that we could detect 55–72.5% of all submicroscopic abnormalities found in MCA/MR with MLPA. Subtelomeric imbalances account for 30–40% of the total chromosomal abnormalities, and interstitial microdeletions and microduplications represent 50–65%. Half of these are located in regions of the most common microdeletion syndromes listed above, and 3% are consistent with complex rearrangements or mosaicism [1,2,15]. By screening for imbalances in all subtelomeres and regions with the most common microdeletion syndromes, we can detect all subtelomeric imbalances (30–40%) and half of the interstitial microdeletions and

Table 4
Sensitivity and specificity of the de Vries score for the first 209 patients screened.

de Vries Score	Total	Number of patients with causative anomalies	% patients with alteration within the score group (specificity)	% patients with alteration within abnormality group (sensitivity)
≥0	209	47	225	100.0
≥1	208	47	22.6	100.0
≥2	205	46	22.4	97.9
≥3	194	44	22.7	93.6
≥4	161	39	24.2	83.0
≥5	113	27	23.9	57.4
≥6	68	14	20.6	29.8
≥7	32	10	31.3	21.3
≥8	18	6	33.3	12.8
≥9	4	2	50.0	4.3
≥10	4	2	50.0	4.3

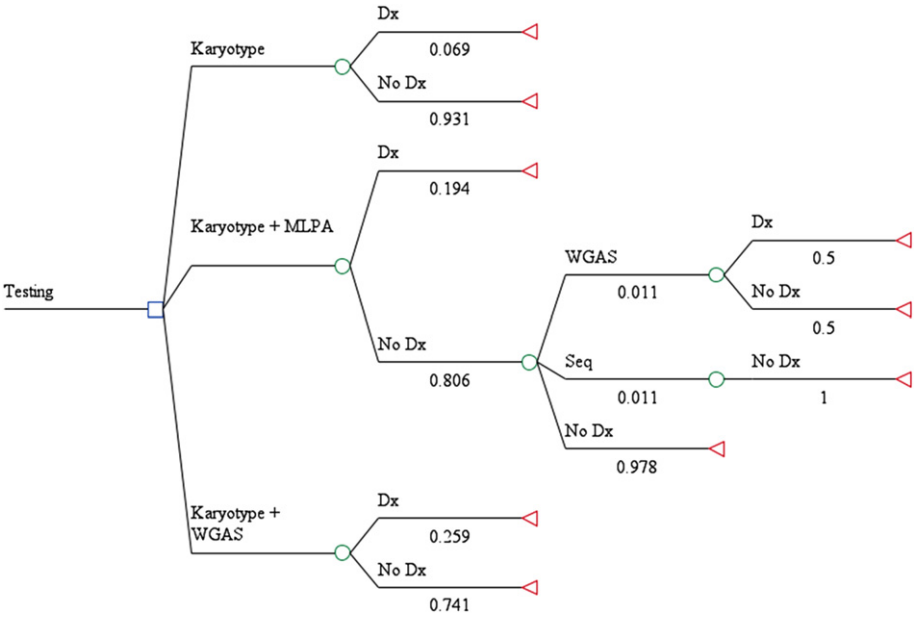

Fig. 2. Decision Analytic Model. Open square represents a decision node, open circle a transition probability, open triangle a terminal node. Dx, diagnosis. Se, sequencing. Each terminal node has a correlated cost and effectiveness. Costs were calculated depending on tests performed in each branch until terminal node and effectiveness were determined as additional diagnoses compared to karyotype every 100 cases.

microduplications (25–32.5%) in patients with MCA/MR. Indeed, our decision analytic model showed that we were able to detect 76.45% of the total chromosomal abnormalities expected (karyotype + WGAS).

Economic models are important to help health professionals to take decisions based on available strategies. The mathematical analysis together with socio economic characteristics of the country is fundamental when a new strategy is considered to be taken, especially in developing countries where resources are limited.

In conclusion, the combination of three MLPA kits to screen for the most frequent submicroscopic imbalances is a valuable resource for the detection of abnormalities in MCA/MR patients. MLPA is an effective alternative when WGAS is unavailable and may increase genetic diagnosis of chromosomal imbalances up to four times. We recommend the analysis of parental samples whenever available to minimize false positive results. Careful analysis should be carried out for each patient when no other confirmatory test can be provided. MLPA is more efficient when patient and parental samples are collected at the same time and when there is close communication between the clinic and laboratory.

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