ABSTRACT

Detection of subtelomeric rearrangements using multiplex ligation-dependent probe amplification (MLPA) is becoming a routine procedure for investigating children presenting with developmental delay and multiple congenital anomalies (MCA). These patients are often (but not always) referred after conventional karyotyping has given a normal result and other causes of mental retardation have been ruled out (e.g. Fragile X).

Of the most recent 250 samples processed, 20 were found to have deletions/duplications by MLPA. The majority of these have been referred due to developmental delay but the remainder are from patients with known abnormal karyotypes. Unlike FISH telomere screens, the MLPA subtelomeric kits (P036BC & P070) include probes mapped to the proximal q arms of the acrocentric chromosomes.

INTRODUCTION

MLPA has become routine in many labs
- Ligation-dependent PCR-based assay using standard lab equipment
- Medium-throughput and inexpensive
- Probes available for many syndromes (≈250 kits commercially available), with the design of others easy (www.mlpa.com)
- Protocol as described by kit manufacturer
- Require for good quality DNA (e.g. spin column)

Subtelomeres involved in ≈5-10% of idiopathic MR
- Alternative screening methods are time consuming and expensive
- Previously, a bias in ascertainment due to pre-selected patient populations existed (most severe tend to be selected)
- Less phenotypically defined patient populations can now be routinely screened (i.e. going beyond de Vries criteria)
- Effective screening programme

Subtelomeric MLPA as an adjunct to karyotyping (Figure 1)
- After a normal karyotype, subtelomeric MLPA may be requested
- Building up a chronomorphological origins of structurally abnormal chromosomes (e.g. der and mar)
- Useful pre-screen prior to any FISH or aCGH

CASE 1

Abnormal female karyotype observed in CVS indicating presence of extra structurally abnormal chromosomes (ESAC)
- While chromosome paint showed ESAC derived from chromosome 22 material
- FISH analysis confirmed chromosome 22 (de novo) with both centromeres and 22q11.2 material
- MLPA subtelomeric screen confirmed chromosome 22 duplication (see Figure 3). However, MLPA screen also indicated 4q deletion of maternal origin (see Figure 4)

Cell Eye Syndrome and 22q11.2 duplication
- Cell Eye Syndrome caused by duplication of 22q11.2 region
- Characterised by its coloboma plus auric atresia, microphthalmia, heart and renal malformations and normal to moderate mental retardation
- Full spectrum of clinical features from marginally affected to all symptoms with lethal outcome
- Small supernumerary chromosome present with 2nd decondensed centromere, bisatellited, representing an ide(22)q(21.1)
- Variability of duplicated/bisatellited segment in different patients with no correlation between length and severity of clinical features

Developmental delay and 4q deletion
- 4q terminal deletions associated with a distinctive phenotype dependent on deletion size
- Evidence indicates 4q deletions are present in phenotypically normal individuals and in those with developmental delay
- Clinical features can be present: small hands and feet, dysmorphic skull, 5th digit anomalies and aggressive behavior
- MLPA in addition to karyotyping
- Due to the occurrence rate of the 4q deletion, the management of future pregnancies is important. This information was only obtained after MLPA analysis due to the identification of an ESAC

DISCUSSION/CONCLUSIONS

MLPA routine in many labs
- The lack of additional specialist equipment required to perform MLPA has ensured that this technique is now becoming more widely accepted as a routine part of the diagnostic work-up for measuring copy number changes
- The level of uptake has fuelled the exponential increase in kits now available and ensures a vast number of diseases and syndromes are being investigated this way

Subtelomeric MLPA kits useful when presented with idiopathic MR, DD or autism
- Our data indicates 6–8% of patients screened for subtelomeric imbalances are detected by these kits
- We further find a prevalence of chromosome 15 anomalies but this may be due to a bias in ascertainment

REFERENCES


SUMMARY

ABSTRACT

Here we present a case study that identified an imbalance in 15q11-q13 in a patient with a suspected 1p deletion presenting with global developmental delay. This technique also proves useful when presented with complex prenatal karyotyping. Here we demonstrate the use of MLPA for identifying the origin of marker chromosomes with subsequent confirmation by single specific FISH probes. This approach avoids using multiple FISH assays, thus proving more efficient and cost effective.

With subtelomeric MLPA now a mainstream, medium-throughput technique, the case studies illustrate the usefulness of these kits for purposes other than the management of patients with developmental delay due to subtelomeric imbalances.

Here we present a retrospective analysis of 250 blood referrals requesting subtelomeric analysis by MLPA
- 20 % of patients (~8%)
- 24 chromosome rearrangements (Table 1) with an equal number of increases and decreases in gene dosage observed
- Almost a ratio of 2:1:1

Subtelomeric MLPA also useful as an adjunct to karyotyping
- Case studies presented demonstrate the valuable information that can be derived from the subtelomeric MLPA kits P036BC and P070
- Knowing the location of breakpoints may aid in the clinical management of the patient and affect outcomes as the phenotype may be affected by the size of the alteration (e.g. see Case 1 & 2)
- Case 1 also shows the ability of MLPA to detect cryptic aberrations that may otherwise go unnoticed

Bridging the gap
- The breakpoint differences between molecular genetics and conventional cytogenetics has led to a bias in the diagnosis of diseases concentrated at the ends of the size-spectrum
- MLPA (and other techniques such as aCGH) allow for the gap to be bridged enabling genetic contributions to disease in this mid-range to be detected. This thus syndromes with previously non-apparent genetic component may now find themselves falling in this middle class of mutation