THE ROLE OF FLUORESCENCE IN SITU HYBRIDIZATION IN ASSESSING THE CYTOGENETICALLY DIAGNOSIS IN CRYPTICAL MOSAICISM ANEUPLOYDIES

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Abstract

Clinical cytogenetics has evolved into an indispensable diagnostic tool for the identification of chromosome abnormalities. Cytogenetics has become increasingly important for the identification of aneuploidy and unbalanced structural rearrangements in patients that have suggestive phenotype. Although aneuploidy and many chromosomal abnormalities are revealed through cytogenetic studies, conventional cytogenetic analysis peripheral blood lymphocytes cannot reliably detect rearrangements of genomic segments smaller than 5–10 million base pairs (Mb) and might not detect a cryptic mosaicism aneuploidy. The aim of this paper is assessing the role of molecular cytogenetics techniques and in particulary fluorescence in situ hybridization (FISH) in detecting cryptic mosaic aneuploidies in three cases, one case with suspicion of homogeneous 4p deletion and two cases with suspicion of mosaic trisomy 21. Aneuploidy detection using FISH in the interphase nuclei is a proven diagnostic application.

Key words: aneuploidy, karyotype, fluorescence in situ hybridization (FISH).

Introduction

Cytogenetics is an indispensable technique for the identification of aneuploidy and unbalanced structural rearrangements in patients that have suggestive phenotype for a chromosomal syndrome. Although aneuploidy and many chromosomal abnormalities are revealed through cytogenetic studies, conventional cytogenetic analysis from peripheral blood lymphocytes cannot reliably detect rearrangements of genomic segments smaller than 5–10 million base pairs (Mb) and might not detect a cryptic mosaicism aneuploidy. Also, microscopic examination of the chromosomes may not reveal the chromosomal origin of small supernumerary marker chromosomes and may not identify subtle rearrangements of the subtelomeric regions.

The resolution of detecting chromosomal anomalies has been improved by molecular cytogenetic and molecular techniques such as fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA) and comparative genomic hybridization (CGH). FISH was the first molecular cytogenetic technique to overcome the resolution limitations of conventional cytogenetic analysis and also offered a solution for rapid chromosomal detection for common aneuploidies in interphase nuclei. A variety of probe types can be used to detect chromosome rearrangements and aneuploidy. For example, repetitive sequence probes that are unique to each centromere are most commonly used to identify trisomies of the common aneuploidies for chromosomes 18, X and Y. These probes are also often used to identify the chromosomal origin of marker chromosomes. Whole chromosome painting probes may be used to characterize translocations on metaphase and locus specific probes may be helpful for the identification of particular deletion syndromes, based on phenotypical findings (e.g., cardiac defect for Velo-cardio-facial/DiGeorge syndrome) or used for those chromosomes for which unique centromere probes are not available (e.g., chromosomes 13 and 21). However, most FISH assays will reveal only abnormalities from the genomic segments for which the probes have been designed. One exception to this is comparative genomic hybridization (CGH). CGH has the distinct advantage of being able to reveal imbalances across the genome. In CGH, DNA is extracted from a control individual with a known normal karyotype and from an individual with an unknown karyotype or a known abnormal karyotype that requires further investigation. These two DNA specimens are differentially labeled with two different fluorochromes and applied to metaphase chromosomes prepared from a karyotypically normal individual. Discrepancies between the fluorescent intensities along the extent of each chromosome will reveal gains or losses of genomic segments [Levy et al., 1998]. Multiplex ligation-dependent probe amplification (MLPA) was first described in 2002 [Schouten et al., 2002]. The method was designed to detect gene dosage abnormalities in a wide range of diseases by the relative quantification of up to 45 different DNA sequences in one reaction. The results are usually available after 2–3 days.

Material and methods

This work is presenting cytogenetics diagnose of cryptic mosaic aneuploydies in three situations, one case with suspicion of 4p deletion in homogeneous karyotype and two cases with suspicion of mosaic trisomy 21.

First case, a boy, was initially investigated at the age of one (Fig.1) for development milestones delay. He came from a foster home so no data regarding the parents were available.

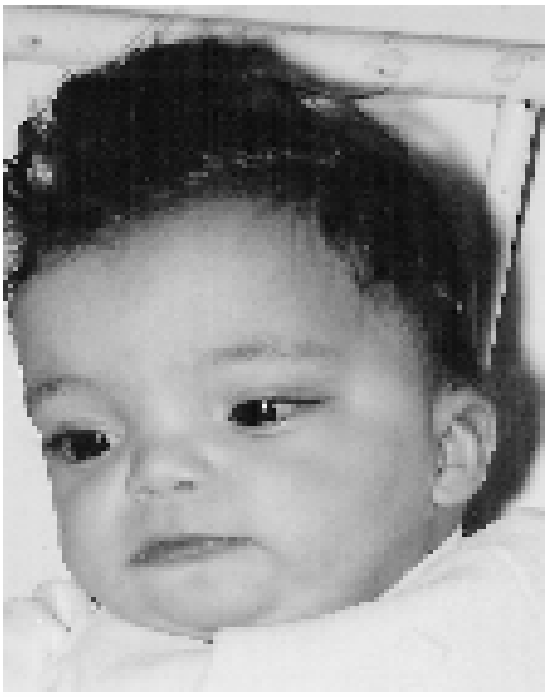

Fig. 1. Facial appearance at age of one (patient 1).

The birth weight was 1500 g and length was 44 cm, the newborn presenting severe growth retardation. Upon clinical examination facial features were suggestive for Wolf-Hirschhorn syndrome: high forehead with a prominent metopic suture, high frontal hairline, prominent glabellum, hypertelorism, epicanthic folds, sclera with a blue tint, broad nasal root, prominent philtrum, thin upper lip, down turned mouth low-set ears. Cytogenetic analysis from peripheral blood lymphocytes was performed. Metaphase slides were prepared from peripheral blood cultures chromosome analysis was performed according to routine methods and GTG-banding technique. All 30 metaphases examined revealed a deletion of short arm of chromosome 4 (4p16.3del). The boy was reevaluated at the age of 4 for severe mental retardation and a change in phenotypical appearance that was suggesting a Williams syndrome: small upturned nose, long philtrum (upper lip length), wide mouth, small chin, and puffiness around the eyes (Fig.2).

Fig. 2. Facial appearance at age of four (patient 1)

The cytogenetic analysis was performed again and surprisingly from the total number of 30 cells counted 28 showed a deletion 4p and 2 euploid cells were found. FISH analysis was available and it was performed for interphase cells as well as in metaphase cells from cultured lymphocytes from peripheral blood using Vysis Wolf-Hirschhorn Region Probe LSI WHS Spectrum Orange (4p16.3) and CEP 4 Spectrum Green on one slide and Vysis

Williams Region Probe (7q11.23 LSI ELN Spectrum Orange and 7q31 D7S486, D7S522 Spectrum Green) on another slide because of the Williams syndrome phenotype. 200 cells were counted in interphase nuclei on the first slide and mosaicism for del 4p16.3 was found (35% normal cells)(Fig. 3, Fig. 4).

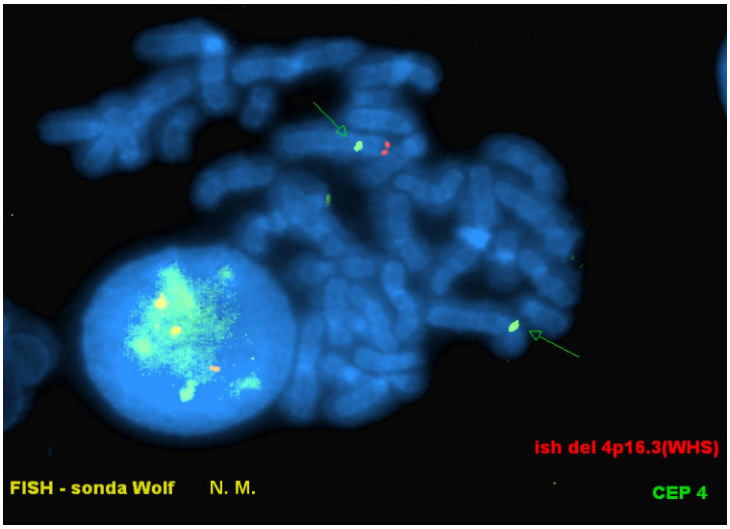

Fig. 3. Metaphase spread containing normalchromosomes 4 with the presence of theLSI WHS (4p16.3) Spectrum Orange and the CEP 4 Spectrum Green signals.

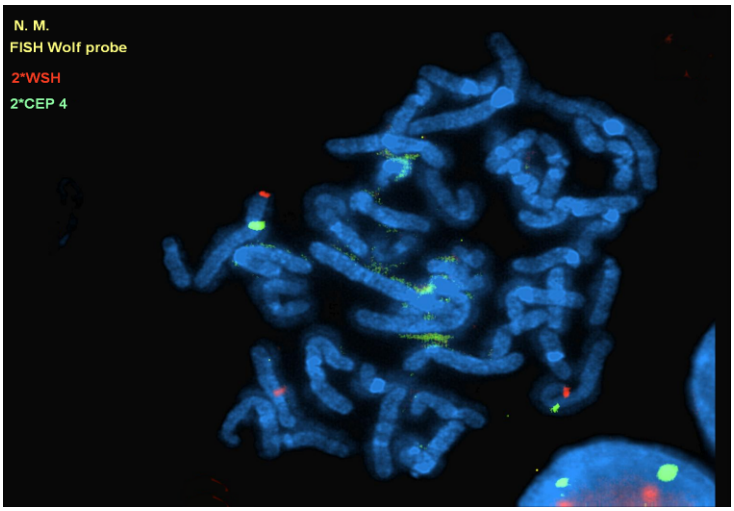

Fig. 4. Metaphase spread containing one chromosome 4 with the CEP 4 Spectrum Green but without the LSI WHS Spectrum Orange signal.

The second slide turned out to be negative for Williams syndrome as both orange and green signals were present on both chromosomes 7 in all metaphases and interphase cells examined (Fig. 5).

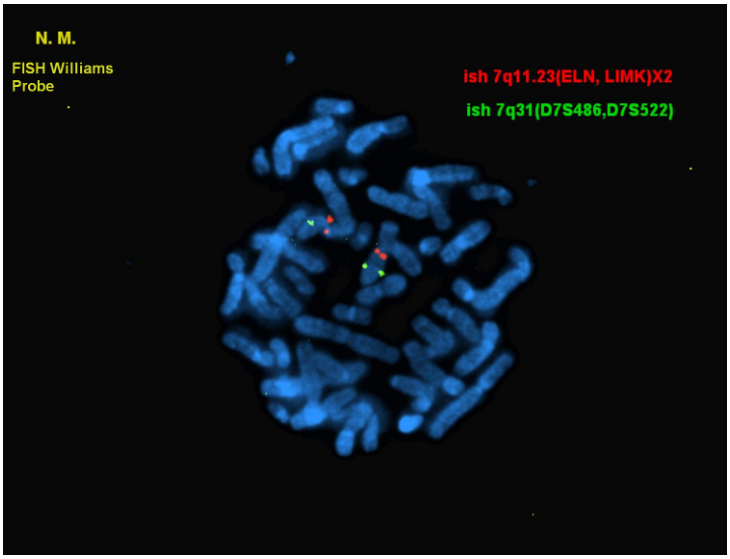

Fig. 5. LSI ELN Metaphase spread without deletion for Williams syndrome.

The other two cases evaluated were both trisomy 21 patients, newborns, that had a typical phenotype for Down syndrome and the clinical examination did not raise any diagnose difficulty. The cytogenetics analysis was performed to confirm the clinical diagnosis and the conventional method from peripheral blood lymphocytes revealed in each case metaphases with normal karyotype. Taking in account that in literature the proportion of cryptical mosaicism was reported and the fact that mosaic trisomy 21 patients have a better later development we considered as necessary to reevaluate these cases by performing a molecular cytogenetics technique. FISH was carried out using Vysis LSI 21 Spectrum Orange probe on interphase nuclei and metaphase cells from cultured lymphocytes from peripheral blood from the two newborns (Fig. 6, Fig.7). The possibility of mosaicism was unfortunately ruled out, because in all nuclei and metaphases examined in both patients three signals for chromosome 21 were present.

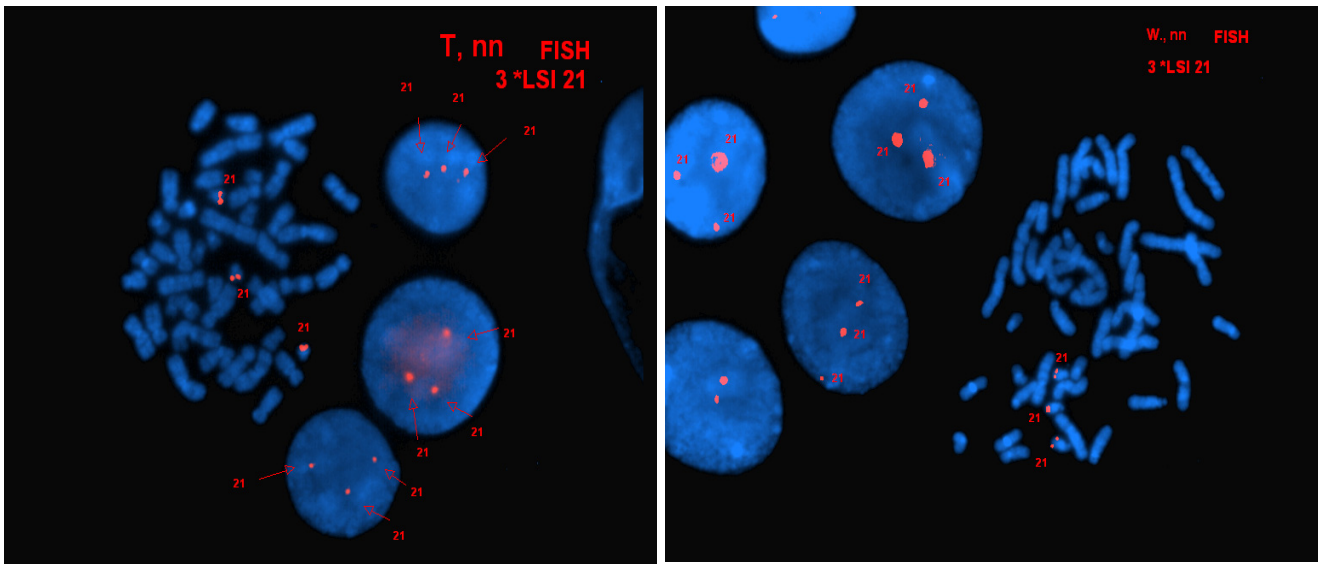

Fig. 6, Fig. 7. LSI 21 Spectrum Orange hybridized showing trisomy 21.

Discussions and conclusions

Previous studies have demonstrated the potential of FISH analysis to detect cryptical mosaicism aneuploydies in patients who have phenotypical dysmorphism for a chromosomal syndrome. To assess the rate of mosaicism, both conventional cytogenetic analysis and FISH analysis should be performed in such cases. Our results demonstrate the importance of using FISH for diagnosing numerical and structural chromosomal anomalies. This study confirmed cryptical mosaicism for one case and declined it for the other two situations.

The development of molecular cytogenetic technologies has increased the ways to detect chromosomal aberrations. The target would be towards the development, selection, and assessment of molecular techniques that will be suitable for use in routine diagnostic settings. For sure, these techniques will have a major contribution in establishing the chromosomal basis of fetal anomalies in a efficient manner that will permit the detection of chromosome abnormalities associated with phenotypical manifestations, allowing for early intervention in the newborn child. However, until these newer techniques become standard of care in some form, these should be considered additional to the standard karyotype.

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