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MOLECULAR CHARACTERIZATION OF AN UNUSUAL VARIANT OF THE SHORT ARM OF CHROMOSOME 15 BY FISH-TECHNIQUE

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Summary One of the most frequent translocations involving the long arm of chromosome Y with autosomes is with the short arm of chromosome 15. The regions which are involved in this translocation fluoresce brightly, are highly heteromorphic and thus escape detection. Therefore, these abnormalities could not be fully characterized, especially in cases where parents are not available or paternity is disputed. Results from the employment of the selective staining techniques DA/DAPI and Q-banding have been inconclusive. FISH-technique using whole chromosome painting (WCP) probes should be used to decipher such translocations. We present a case where, even after using a battery of probes, the origin of extra material on chromosome 15p could not be identified though it was not a part of Yq.

Key Words chromosome 15, chromosome Y, FISH, WCP, heteromorphism

Enormous strides have been made towards identifying the chromosomal abnormalities by using a battery of conventional banding techniques (Verma and Babu, 1995). The most routinely used technique is G-banding which differentiates all the human chromosomes into dark and light banded segments with overlapping regions which potentially can mask translocations involving light to light and dark to dark bands. Consequently, other differential or selective staining techniques are utilized to precisely identify the abnormalities in cases where G-banding has been inconclusive. If a Y chromosome is involved in a translocation, then Q-banding becomes the most obvious choice for deciphering the morbidity, since the long arm of chromosome Y fluoresces brightly. In addition, the short arm of all acrocentric chromosomes also display variable brightness. When a translocation occurs involving the Yq constitutive heterochromatin with the short arms of any acrocentric chromosome, it would be a difficult task to rule out whether it is

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an abnormality or a heteromorphism (Verma, 1988). Therefore, parents are requested for cytogenetic evaluation to investigate a possible inherent nature. If the chromosome regions of 15p and Yq constitutive heterochromatin are involved in a translocation, the routinely used technique has been DA/DAPI, as these regions are highly stain specific (Schmid, 1979; Schmid *et al.*, 1983). However, if parents are not available, no definite conclusion can be reached because both of these regions are highly heteromorphic (Babu *et al.*, 1986). The past approaches clearly have vast limitations and caused anxieties, especially in situations where prenatal cases are involved (Spowart, 1979; Hsu, 1994).

The recent availability of chromosome and loci specific probes have alleviated frustration in previous investigations where the FISH-techniques has served as an adjunct in the evaluation of specific chromosome abnormalities, thus, eliminating the need for parental chromosomal analysis in some of these cases.

A four year old male was referred to rule out chromosomal abnormalities because of speech delay. By Q-banding technique the short arm of one chromosome 15 homologue displayed additional genetic material whose morphology was

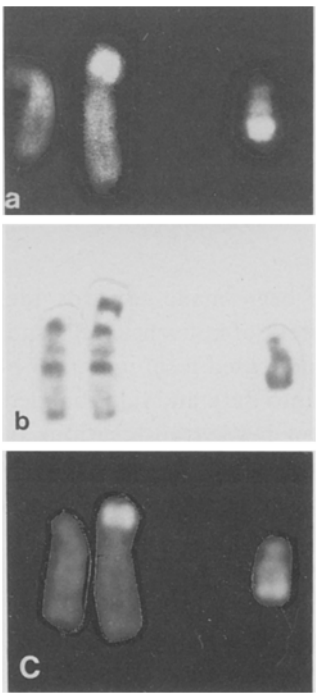

Fig. 1. Partial karyotype of proband. The variant chromosome 15 homologue is on the right. (a) Chromosomes 15 and Y stained by Q-banding technique. (b) Chromosomes 15 and Y stained by G-banding technique. (c) Chromosomes 15 and Y stained by DA/DAPI technique.

not complementary to the cytological satellite chromatin but fluoresced brilliantly by Q-banding (Fig. 1a). The morphological variation was more obscure by G-banding (Fig. 1b). The DA/DAPI stain specific technique which selectively stains the constitutive heterochromatin for chromosomes 1, 9, 15, 16 and Y suggests that the additional material on 15p may either be part of 15p, resulting in an extreme

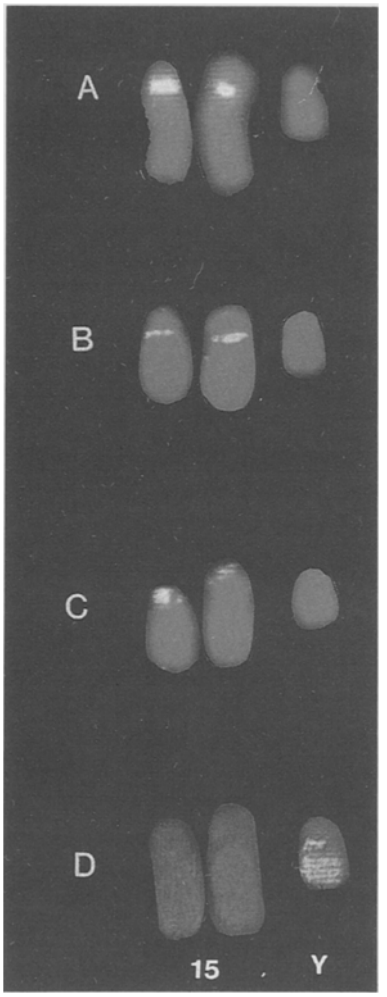

Fig. 2. Partial karyotype of proband's chromosome 15 and Y stained by FISH-technique. The variant chromosome 15 homologue is on the right. (A) Chromosomes 15 hybridized by classical satellite probe (D15Z1). (B) Chromosomes 15 hybridized by alpha satellite 15 probe (D15Z). (C) Chromosomes 15 hybridized by beta satellite probe for a acrocentric chromosomes. (D) WCP paint specific for chromosomes 15 (red) and Y (green) demonstrating that no genetic material exchanged (see text).

polymorphism or due to a t(Y;15) (Fig. 1c). This variant was not present in the mother and the father is unknown. The obvious questions one will ask are: Is it a t(Y;15), which is one of the most common abnormalities involving acrocentric chromosomes (Fryns *et al.*, 1985) or is it a heteromorphism displaying a large brilliant satellite (15p)? Employing whole chromosome paint (WCP) specific probes for chromosomes 15 and Y, which paints the 15q, Yp and Yq arms respectively, it was revealed that this additional material on 15p was not a part of the Y chromosome because it was not painted by the Y WCP probe. Since the 15 WCP does not hybridize with 15p, the extra material on the variant could not have its origin attributed to chromosome 15. Consequently, we decided to characterize the additional material by using chromosome 15 specific classical satellite (D15Z1), alpha satellite (D15Z) and acrocentric beta satellite probes (Oncor, Gaithersberg, MD). Recently, it has been suggested that the short arms of acrocentric chromosomes contain distinctly different DNA families that are arranged in a specific pattern. This arrangement, starting from the centromeric region and ending at the telomeres, is: alpha-satellite, satellite III, beta-satellite, ribosomal DNA, beta-satellite, cytological satellite and telomeric DNA (Gravholt *et al.*, 1992; Greig and Willard, 1992). The extra material on the short arm of the variant apparently does not belong to the DNA family categories of chromosome 15 classical satellite, chromosome 15 alpha satellite or the beta acrosomic satellite DNA. This is due to lack of a hybridization signal of the corresponding probes in the location between the proximal beta satellite and classical satellite DNA regions. The conventional banding techniques: Q, G and DA/DAPI suggests that this extra material is AT-rich. Some variants of acrocentric chromosomes are due to tandemly repeated ribosomal RNA genes and their associated spacer regions (Hillis *et al.*, 1991). This region was not duplicated in our case leaving us perplexed (Fig. 2). It is quite enigmatic that FISH-technique was not completely helpful either (Verma *et al.*, 1994, 1995). The inheritance of its origin could not be deciphered because the father is unknown. The mechanisms of evolution of such heteromorphic markers remains obscure and its clinical consequences are inconclusive at present. Perhaps, another kind of a so-called "rare variant" has emerged whose discovery is fortuitous or it may very well be a simple heteromorphism with very large cytological satellite fluorescing brilliantly.

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REFERENCES

Babu KA, Macera MJ, Verma RS (1986): Classification of intensity heteromorphisms of human chromosome 15p by DA/DAPI technique. Hum Genet **73**: 298–300

Fryns JP, Kleczkowska A, Van den Berghe H (1985): Clinical manifestations of Y/autosome translocations in man. In: Sandberg AA (ed). The Y chromosome, Part B: Clinical aspects

of Y chromosome abnormalities. Alan R Liss, New York, pp 213–243
Gravholt CH, Friedrich U, Caprani M, Jorgersen AL (1992): Breakpoints in Robertsonian translocations are localized to satellite III DNA by fluorescence *in-situ* hybridization. Genomics **14**: 924–930
Greig GM, Willard HF (1992): Beta satellite DNA: Characterization and localization of two subfamilies from the distal and proximal short arms of the human acrocentric chromosome. Genomics **12**: 573–580
Hillis DM, Moritz C, Porter CA, Baker RJ (1991): Evidence for biased gene conservation in concerted evolution of ribosomal DNA. Science **251**: 308–310
Hsu LYF (1994): Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. Am J Med Genet **53**: 108–140
Schmid M (1979): Demonstration of Y/Autosomal translocations using Distamycin A. Hum Genet **53**: 107–109
Schmid M, Schmidtke J, Kruse K, Tolksdorf M (1983): Characterization of a Y/15 translocation by banding methods, distamycin A treatment of lymphocytes and DNA restriction endonuclease analysis. Clin Genet **24**: 234–239
Spowart G (1979): Reassessment of presumed Y/22 and Y/15 translocations in man using a new technique. Cytogenet Cell Genet **23**: 90–94
Verma RS (1988): Heterochromatin: Molecular and structural aspects. Cambridge University Press, New York
Verma RS, Luke S, Conte RA (1994): FISH technique: what's all the fuss about? GATA **11**: 106–109
Verma RS, Babu A (1995): Human chromosomes: Principles and techniques. McGraw-Hill, New York
Verma RS, Batish SD, Ramesh KH, Gogineni SK (1995): Are we FISHing in troubled waters? Cell Mol Biol Res **41**: 81–84