

Microdeletion Syndromes Detected by FISH – 73 Positive from 374 Cases

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ABSTRACT Fluorescence *in situ* hybridization (FISH) has facilitated the detection of microdeletions seen in Prader-Willi/Angelman (PW/AS), Williams and DiGeorge syndromes. Out of 374 suspected cases tested at Jaslok Hospital in the past 5 years, 73 were positive, including 29 cases of Angelman, 16 of Prader-Willi, 24 of Williams and 4 of DiGeorge syndrome. Male preponderance was seen, mainly in Williams syndrome. The mechanisms causing Prader-Willi and Angelman syndrome include microdeletions, intragenic mutations, uniparental disomy and imprinting defects, though FISH can only detect microdeletions. Metaphase FISH helped to detect 1 case each with deletion of the control (PML) signal and duplication of the critical PW/AS region, which are associated with autism. One suspected case of Prader-Willi syndrome had a Robertsonian translocation t(14;15)(q10;q10) which led to a deletion of a major part of the SNRPN region in 10% cells, resulting in low-grade mosaicism. Another FISH-positive case was due to a reciprocal translocation t(2;15)(q37;q11), where loss of critical genes at the breakpoint on chromosome 15 caused the Prader-Willi phenotype. FISH in a child with an Angelman phenotype showed no microdeletion, though Trisomy 15 was seen in 1 metaphase suggesting uniparental disomy due to trisomy rescue. A known polymorphism in the form of an additional tiny green signal on chromosome 14 was observed in 17 of 284 (6%) cases studied for Prader-Willi/Angelman syndrome. Another inherited polymorphism was seen in 5 cases, where one control signal was very small. Prenatal diagnosis was carried out with normal results, in 12 women with a previously affected child.

INTRODUCTION

Microdeletion syndromes are a collection of genetic disorders that are associated with very small deletions on certain chromosomes, which may not be detected by routine karyotyping. However, these microdeletions can easily be seen by the FISH (fluorescence *in situ* hybridization) technique or other molecular genetic approaches such as array CGH and MPA (Jorde et al. 1999; Shaffer et al. 2007; Cho et al. 2009). DNA FISH probes can be used in metaphase and interphase cells to detect these specific regions of deletion (Ekong et al. 1998). The region deleted is known as typically deleted region (TDR) or critical region. A number of microdeletion syndromes

are associated with distinct clinical features (Baraitser and Winter 1996). There are different microdeletion syndromes such as Prader-Willi/Angelman syndrome, Williams, DiGeorge, Smith-Magenis and Miller-Dieker syndromes. Of these, FISH probes for Prader-Willi/Angelman, Williams and DiGeorge syndromes are currently available in our laboratory. Microdeletions are often characterised by a complex clinical and behavioural phenotype resulting from the imbalance of normal dosage of genes located in that particular chromosomal segment. In PW/AS syndrome there are different molecular mechanisms leading to the loss of maternal or paternal expression of genes at 15q11-13, such as microdeletions, intragenic mutations, uniparental disomy and imprinting defects. There are no intragenic mutations noted in cases of PWS unlike AS where the mutation can occur in the Ubiquitin ligase gene.

Clinical Features

Prader-Willi Syndrome (PWS)

Children with Prader-Willi Syndrome have severe hypotonia, short stature, mental retardation

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dation, obesity with increasing age, hypogonadism, small hands and feet, fair hair and skin. These children have a habit of excessive skin picking. (Robinson et al. 1991; Cassidy et al. 1992).

Angelman Syndrome (AS)

This is also known as Happy Puppet syndrome. The children in general have a happy predisposition with outbursts of laughter often accompanied by frequent hand flapping. These children have mental retardation with microcephaly, jerky movements affecting the trunk and upper limbs, ataxia, unsteady and wide-based gait, wide mouth with constant dribbling and prominent chin (Boyd et al. 1988; Clayton-Smith 1992).

In a majority of cases, both the above clinically different syndromes are caused by the same microdeletion +/- 4 Mb on chromosome 15 at the 15q11-13 region. Because of imprinting, the presence of a microdeletion on the paternal chromosome 15 leads to Prader-Willi syndrome, while a microdeletion on the maternal chromosome 15 causes Angelman syndrome (Wagstaff et al. 1992). Besides microdeletions and imprinting, uniparental disomy and intragenic mutations can also cause the two syndromes (Malcolm et al. 1991).

Williams Syndrome (Williams-Beuren Syndrome) - (WS)

This is caused by a microdeletion on chromosome 7 at the Elastin locus 7q11 (Ewart et al. 1993). The facial features consist of a medial eyebrow flare, a stellate iris pattern, flat nose bridge and long smooth philtrum with widely spaced teeth. The characteristic heart defect is a supravalvular aortic stenosis (Burn 1986; Halladie-Smith and Karas 1988; Jones 1990).

DiGeorge Syndrome (Velocardiofacial Syndrome or CATCH 22) - (DS)

The chromosomal microdeletion is at 22q11. The clinical features include hypoplasia, hypoparathyroidism and cardiac malformations. Dysmorphic features include hypertelorism, low-set ears and micrognathia. The most common cardiac defects include interrupted aortic arch, often with VSD (Ventricular Septal Defect) and a persistent

truncus arteriosus (Driscoll et al. 1992; Laena-Cox et al. 1996; Scambler et al. 1992). It is also called CATCH 22 because it describes the abnormal findings of cardiac anomalies, abnormal facies, thymic hypoplasia, cleft palate and hypocalcemia due to a deletion on chromosome 22.

FISH Probes and Interpretation

Microdeletion probes have control signals on the same chromosome, which can clearly be seen on metaphases. For Williams and DiGeorge syndromes, the control signals are labeled in green (G) and the critical region with orange (O) in the present study. Therefore a normal cell will show a 2G2O pattern whereas a cell with the deletion will show a 2G1O pattern (Fig. 1a, b). The SNRPN probe for detection of PW/AS has got two internal controls, a large proximal green signal (CEP 15) and a smaller orange signal (PML) towards the distal end. The critical region (SNRPN) has a small orange signal and lies between the two control signals on chromosome 15, adjacent to the green signal. With this probe, normal cells show a 2G4O signal pattern whereas cells with the microdeletion show a 2G3O signal pattern (Baumer et al. 1999) (Fig. 1c, d). FISH signals on metaphases should be checked with this probe to avoid false positive results due to deletion of the control orange signal. The D15S11 probe for PW/AS is used to double check such cases as it has only 1 internal control region in green.

MATERIAL AND METHODS

Over the past 5 years, a total of 374 blood samples of patients referred by pediatricians across the country were tested for microdeletions at Jaslok Hospital. PHA stimulated 72 hour whole blood cultures were set up to obtain metaphases using standard techniques. Fixed WBC pellets of blood cultures were also accepted from other laboratories. FISH was carried out using Vysis (Abbott) probes by codenaturation of the probe with the test sample at 73°C for 5 minutes, followed by overnight hybridization at 37°C. After washing as per the manufacturer's protocol, the slides were mounted in the counter-stain DAPI and observed under a Zeiss fluorescent microscope. The images were captured and processed with Metasystems *isis* software. About 100-200

interphase nuclei and 5-10 metaphases were usually analyzed for detection of microdeletions by FISH. Currently, the following Vysis (Abbott) microdeletion probes are used in our laboratory:

PWS/AS- LSI SNRPN (orange) / PML (orange)/ CEP 15 (green) dual color DNA probe and LSID15S11(orange)/CEP 15 (green) probe

WS - LSI ELN (orange)/ LSID7S486, D7S522 (green) dual color DNA probe

DS - LSITUPLE 1 (orange)/ LSIARSA (green) dual color DNA probe

RESULTS

Out of 374 samples tested, 73(20%) were found to be positive for various microdeletions (Table 1). Among the 73 positive cases, 29(40%) had Angelman syndrome, 16(22%) had Prader-Willi syndrome, 24(33%) had Williams syndrome and 4(5%) had DiGeorge syndrome. There was a male preponderance in DiGeorge syndrome (3/4 cases). Out of the suspected cases tested syndrome-wise, the percentage of positive cases detected by FISH was 36% (24/67) for Williams syndrome, 18% (29 of 163) for Angelman, 17% (4/23) for DiGeorge and 13% (16/121) for Prader-Willi syndrome. A few interesting cases are described below.

Case 1 (BF219): A male child suspected to have Prader-Willi syndrome was incidentally found to have a Robertsonian translocation, while observing FISH signals on metaphases with the SNRPN probe. This was later confirmed to be t(14;15)(q10;q10) on karyotyping. FISH showed that of the 2 green (control) signals at 15p11.2, the one on the normal 15 was of the regular size

and the other on the translocated chromosome was very small in 90% cells (Fig. 2a,b). In 10% cells, the green signal and the orange signal (critical region) adjoining it on the translocated chromosome were barely visible, probably because of a partial deletion of this region due to the translocation. This suggested low-grade mosaicism for the microdeletion (Table 2).

Case 2 (BF438): An 8 year old male child with a Prader-Willi phenotype was found to have a reciprocal translocation t(2;15)(q37;q11) on karyotyping in another laboratory. FISH in our laboratory showed the presence of the microdeletion in 95% cells, though this was caused by loss of genes in the critical SNRPN region where the breakpoint on chromosome 15 was located. The control CEP 15 green signal was missing in all cells, because of the translocation (Fig. 2c,d).

Case 3 (BF198): A 3 year old male child with a phenotype of Angelman syndrome did not show the microdeletion by FISH. However, Trisomy 15 was clearly seen in 1 metaphase by FISH. Therefore, this was probably a case of AS due to uniparental disomy (UPD) caused by trisomy rescue, resulting in loss of the maternal homologue and paternal disomy (Fig. 2e).

Case 4 (BF300): In a child with autism, FISH analysis on metaphases with the SNRPN probe showed partial deletion of the distal control orange (PML) signal, where the fourth orange was barely visible (Fig. 2f).

In another child, duplication/amplification of the critical PW/AS region was seen in some cells, instead of a deletion. A known polymorphism in the form of an extra small green signal on chromo-

Table 1: Distribution of positive cases

Syndrome	Suspected cases	Positive cases	Positive females	Positive males	%+ve out of total (73) positive cases	% +ve out of suspected cases for each syndrome
Angelman	163	29	12	17	40	18
Prader-Willi	121	16	6	10	22	13
Williams	67	24	8	16	33	36
DiGeorge	23	4	1	3	5	17
Total	374	73	27	46	100	20

Table 2: Variation in signals seen using the SNRPN probe in Case 1

Locus	Green CEP 15		Orange PML/SNRPN	No. of cells	Interpretation
Signals per cell	1 big	1 small	4	180	No deletion of the SNRPN region (90%)
	1 big	1 small	3+1(small)	20	Partial deletion of the SNRPN region (10%)

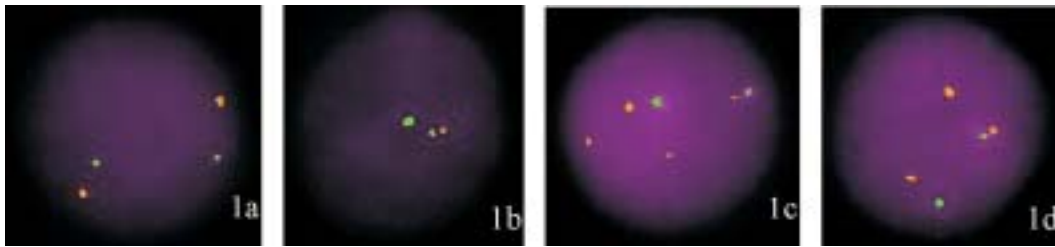


Fig. 1. FISH on interphase cells using probes with a single control signal (a,b) and 2 control signals (c,d). 1a: 2G2O (Normal); 1b: 2G1O (Deletion); 1c: 2G4O (Normal); 1d: 2G3O (Deletion).

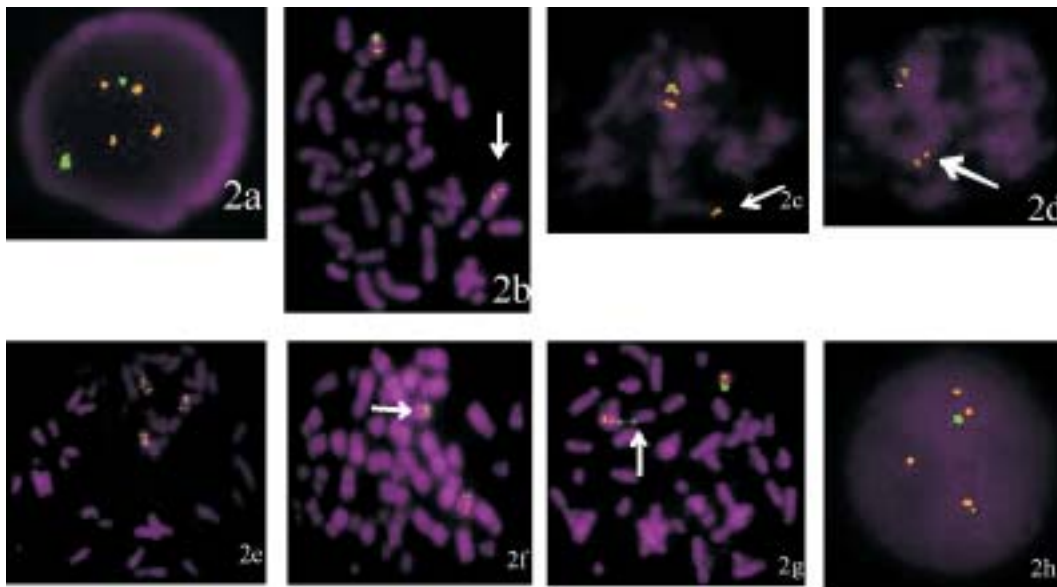


Fig. 2 a,b: Case 1. Arrow indicates t(14;15) with a partially deleted (small) green signal.
2 c,d: Case 2. Arrow indicates t(2;15) with a del. of CEP 15 & SNRPN (2c) /deletion CEP 15 only (2d).
2e: Case 3. Trisomy 15 w/o SNRPN deletion. UPD.
2f: Case 4. Deletion of distal PML signal in autism.
2g: Additional small green signal on chr. 14.
2h: Variation (inherited) in size of CEP 15 signal.

some 14 was observed in 17 of 284 (6%) cases studied for Prader-Willi/ Angelman syndrome (Fig. 2g). Another rare polymorphism was seen in 5 cases, where one green signal was of the regular size while the other was much smaller (Fig. 2h). On studying some parents, it was observed that these are normal polymorphisms inherited from one of the parents. Prenatal diagnosis was carried out with normal results, in 12 women with a previously affected child.

DISCUSSION

Apparently balanced Robertsonian translo-

cations can cause phenotypic abnormalities in 3-4% cases (Groupe de Cytogeneticiens Francias 1989). A de novo microdeletion at 14q32 on an inherited 45,XX,t(dic)(14;21)(p11;p11) translocation was reported, implicating that the translocation was responsible for the subsequent de novo structural anomaly (Bonthron et al. 1993). Case 1 in the present study showed a microdeletion close to the breakpoint of the Robertsonian translocation t(14;15)(q10;q10) in 10% cells, suggesting that this was also a secondary event, though it could even have been due to a variable breakpoint in the adjacent SNRPN (15q11.2) region. Both uniparental

disomy and a small de novo deletion on an inherited t(6;15) were observed in one family, causing Prader-Willi syndrome in one cousin and Angelman syndrome in another cousin (Smeets et al. 1992). Case 2 in the present study had a different reciprocal translocation t(2;15), resulting in the Prader-Willi phenotype. Uniparental disomy (UPD) occurs in 24% of PWS patients as compared to 3-5% of AS and is most likely to be due to trisomy 15 rescue, suggested by observation of trisomy 15 mosaicism in patients with unusual PWS manifestations. If the cause is uniparental disomy, it will not be detected by FISH analysis as was seen in Case 3 in the present study. Imprinting defects are found in 2% of the AS cases and in less than 1% of the PWS cases (Vogels and Fryns 2004). Absence of all or a part of the PML gene, such as a 1 megabase deletion in 15q22-q23 was identified in a patient with autism, developmental delay and mild dysmorphism (Smith et al. 2000), similar to Case 4 in our study. This could have been mistaken as a positive case of PW/AS if only interphase cells were scored. The polymorphism with an extra small green signal at the centromeric region on one homologue of chromosome 14 is present in 10-15% cases (Vysis SNRPN probe pamphlet), and was seen in 6% cases in our study from the Indian population.

An Indian study of chromosome 22 microdeletions in isolated congenital heart disease (Gawde et al. 2006) showed the microdeletion in 6/105 (5.71%) patients. In the present study, chromosome 22 microdeletions were seen in 4/23 (17%) cases.

Duplications as compared to deletions produce less serious complications (Thomas et al. 2006). In approximately 1% cases of autism, duplication of the 15q11-13 region has been reported (Peters et al 2004; Battaglia 2005; Koochek et al. 2006). In the present study, duplication/amplification of the critical PW/AS region was seen in some cells in one patient. Reciprocal duplication in a case of Williams syndrome was shown to be associated with severe delay in expressive speech (Somerville et al. 2005).

Recently, preimplantation genetic diagnosis (PGD) using FISH on 1-2 blastomeres biopsied from embryos obtained by IVF-ICSI has been successfully used to detect a microdeletion in women predisposed to cancer demonstrating that FISH-based PGD is a straightforward approach

to detect microdeletions in single blastomeres (Vanneste et al. 2009). The facility of FISH-based PGD is available in our Department at Jaslok Hospital.

CONCLUSION

Although there is no specific treatment available so far for microdeletion syndromes, early diagnosis with the use of FISH probes, accurate interpretation and genetic counseling would certainly help detect these microdeletion syndromes at an early stage and help prevent its recurrence in the family through prenatal diagnosis or PGD.

RECOMMENDATIONS

Utmost care and expertise is required while performing FISH analysis and interpreting results. It is always better to analyze both interphase as well as metaphase cells. If FISH for PW/AS using the SNRPN probe is carried out only on interphase cells, there is a possibility of getting a false positive result, if there is a partial deletion of the distal 15q region (PML) where the control orange signal is situated. On metaphases, the orange signal of the critical region is clearly seen adjacent to the green control signal. In doubtful cases, FISH can be repeated using a probe such as D15S11, which has only one internal control.

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