The Role of Conventional and Molecular Cytogenetics in the Diagnosis of Microdeletion Syndromes

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ABSTRAK

Kajian ini bertujuan untuk menunjukkan peranan kaedah hibridisasi in situ pendaflor (FISH) dan sitogenetik konvensional (karyotip) pada kes sindrom mikrodelesi yang telah disahkan secara sitogenetik dan klinikal. Sejumlah sembilan kes telah dirujuk dari tahun 2002 hingga 2004 di Unit Sitogenetik, Hospital Universiti Kebangsaan Malaysia (HUKM). Kes-kes tersebut terdiri daripada tiga sindrom Prader-Willi, tiga sindrom DiGeorge, satu sindrom Williams , satu sindrom Miller-Dieker dan satu sindrom Kallmann. Sampel darah kesemua pesakit telah dikultur dan dituai mengikut prosedur standard. Sebanyak 20 serakan metafasa telah dianalisa untuk setiap kes. Analisa FISH dilakukan untuk kesemua kes menggunakan prob komersial (Vysis,USA): SNRPN dan D15S10 untuk sindrom Prader-Willi, LIS1 untuk sindrom Miller-Dieker, ELN untuk sindrom Williams, KAL untuk sindrom Kallmann, TUPLE1 dan D22S75 untuk sindrom DiGeorge. Analisa sitogenetik konvensional menunjukkan kariotip normal untuk kesemua kes kecuali satu yang menunjukkan aberasi kromosom melibatkan kromosom 9 dan 22. Analisa FISH menunjukkan mikrodelesi pada kesemua sembilan kes. Kajian ini menunjukkan dua fakta penting, iaitu hibridisasi in situ pendaflor (FISH) merupakan kaedah yang mesti dilakukan untuk pengecaman sindrom mikrodelesi, manakala kaedah sitogenetik konvensional merupakan kaedah saringan bagi keabnormalan kromosom yang mungkin berkaitan dengan penyakit tersebut.

Kata kunci: sitogenetik, sindrom mikrodelesi, hibridisasi in situ pendaflor (FISH)

ABSTRACT

In this report we demonstrate the role of fluorescence in situ hybridisation (FISH) and conventional cytogenetic methods in clinically and cytogenetically confirmed cases of microdeletion syndromes. A total of nine cases were referred to the Cytopathology and Cytogenetic Unit, Hospital Universiti Kebangsaan Malaysia (HUKM) from 2002 to 2004. They include three Prader-Willi syndrome, three DiGeorge syndrome, one Williams syndrome, one Miller-Dieker syndrome and one Kallmann syndrome. Blood samples from the patients were cultured and harvested following standard procedures. Twenty metaphases were analysed for each of the cases. FISH analysis was carried out for all the

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cases using commercial probes (Vysis, USA): SNRPN and D15S10 for Prader-Willi syndrome, LIS1 for Miller Dieker syndrome, ELN for Williams syndrome, KAL for Kallmann syndrome, TUPLE 1 and D22S75 for DiGeorge syndrome. Conventional cytogenetic analysis revealed normal karyotypes in all but one case with structural abnormality involving chromosomes 9 and 22. FISH analysis showed microdeletions in all of the nine cases studied. This study has accomplished two important findings ie. while the FISH method is mandatory in ruling out microdeletion syndromes, conventional cytogenetics acts as a screening tool in revealing other chromosomal abnormalities that may be involved with the disease.

Keywords: cytogenetics, microdeletion syndromes, fluorescence in situ hybridisation (FISH).

INTRODUCTION

Microdeletion syndromes are genetic syndromes associated with small chromosome deletions which usually involved 1 to 3 million base pairs of DNA (Koerf 2002). Most of the syndromes is a contiguous gene syndrome; a disorder due to deletion of multiple gene loci that are adjacent to one another. The variation in the symptoms of the disease is related to the amount of genetic material lost in the chromosomal deletion. Almost all micro-deletion syndromes involved mental retardation or developmental delay and are usually associated with other abnormalities such as cardiac defects (Mathews 1999).

Some of the common microdeletion syndromes are Prader-Willi syndrome, Angelman syndrome, Williams syndrome, DiGeorge syndrome, Miller-Dieker syndrome, Kallmann syndrome and Smith-Magenis syndrome. Williams syndrome is the result of a commonly deleted region (approximately 1.5Mb) of 7q11.23 which contains the Elastin (ELN) gene locus (Ewart 1993, Korenberg et al 2000). Miller-Dieker syndrome involves a deletion of 17p13.3 locus which contains the lissencephaly gene (LIS1) (Ledbetter et al 1992). Prader-Willi syndrome is a contiguous gene disorder resulting from deletion of the 15q11-q13 region (Butler et al 1986). DiGeorge syndrome is caused by a large deletion of 22q13 (Greenberg et al 1988). Kallmann syndrome is an X-linked disorder associated with a microdeletion within Xp22.3 of chromosome X.

Many of these syndromes have long been recognized by medical geneticists, but have no further laboratory tests for confirmation. This is due to the fact that the microdeletions involved in these syndromes are generally beyond the resolution of routine conventional cytogenetics ie. karyotyping (Callan et al 1992).

The ability of fluorescence in situ hybridisation (FISH) to detect much smaller chromosomal abnormalities than can be detected with karyotyping has already been well documented (Ward et al 1999). FISH relies upon the presence or absence of a fluorescent signal to identify chromosomes rather than a specific banding pattern. It allows the surveillance of more cells and requires a much smaller sample than karyotyping.

This study was carried out to demonstrate the role of conventional cytogenetics (karyotyping) and FISH methods in the diagnosis of microdeletion syndromes.

MATERIALS AND METHODS

This is a retrospective study carried out by the Cytogenetics Unit, Hospital Universiti Kebangsaan Malaysia (HUKM). A total of nine cases of microdeletion syndromes

were investigated from year 2002-2004. They include three cases of Prader-Willi syndrome, three cases of DiGeorge syndrome, one case of Miller-Dieker syndrome, one case of Williams syndrome and one case of Kallmann syndrome. Chromosomal analysis of each patient was carried out using both conventional and FISH methods. Chromosomes were prepared from peripheral blood lymphocyte cultures following standard procedures (Brown and Lawce 1997). GTG banding patterns (G band chromosomes treated with trypsin and stained with Giemsa) of metaphases were analysed. A total of 20 metaphase spreads were analysed in each case. The karyotype was described in accordance with the International System for Human Cytogenetic Nomenclature (ISCN) (Mitelman 1995).

For FISH analysis, the locus specific probe kit (Vysis, USA) used are as listed:

(a) LSI Prader-Willi/Angelman region probe (D15S10) spectrum orange / CEP 15(D15Z1) spectrum green / PML spectrum orange and probe (SNRPN) spectrum orange/CEP 15 (D15Z1) spectrum green/PML spectrum orange;
(b) LSI DiGeorge N25(D22S75) region probe spectrum orange / ARSA (22q13.3) spectrum green control probe and TUPLE 1 spectrum orange / ARSA (22q13.3) spectrum green control probe;
(c) LSI LIS1 (Miller-Dieker) probe spectrum orange / RARA spectrum green.
(d) LSI Williams syndrome (elastin gene) region probe spectrum orange / D7S486, D7S522 spectrum green control probe; and
(e) LSI Kallmann (KAL) region probe spectrum orange / CEPX spectrum green control probe.

A minimum of 10 metaphases were analysed after selection for a positive signal at the control locus. Presence of microdeletion was indicated by absence of fluorescent signal of the specific locus on the respective chromosomes.

RESULTS

Of the total of nine cases studied, eight showed normal karyotype; five cases with 46,XX and three cases with 46,XY. FISH analysis done on the cases (cases 1 to 8) revealed deletion in the locus/loci responsible for the syndromes, therefore confirm the clinical diagnosis (table 1).

TABLE 1: Results of Conventional and FISH analysis using locus specific probe of the nine cases studied

Case	Diagnosis	Results	
		Conventional	FISH
1	PWS	46,XX	46,XX.ish del(15)(q11.2q11.2) (SNRPN-,D15S10-)
2	PWS	46,XX	46,XX.ish del(15)(q11.2q11.2) (SNRPN-,D15S10-)
3	PWS	46,XX	46,XX.ish del(15)(q11.2q11.2) (SNRPN-,D15S10-)
4	MDS	46,XX	46,XX.ish del(17)(p13.3;p13.3)(LIS1-)
5	WS	46,XY	46,XY.ish del(7)(q11.23q11.23)(ELN-)
6	KS	46,XY	46,XY.ish del(X)(p22.3p22.3)(KAL-)
7	DGS	46,XY	46,XY.ish del(22)(q11.2q11.2) (TUPLE1-,D22S75-)
8	DGS	46,XX	46,XX.ish del(22)(q11.2q11.2) (TUPLE1-)
9	DGS	45,XX, -22,t(9;22) (p23;q11.2)	45,XX, -22,t(9;22)(p23;q11.2). ishdel(22)(q11.2q11.2) (TUPLE1-,D22S75-)

PWS, Prader-Willi syndrome; MDS, Miller-Dieker syndrome; WS, Williams syndrome; KS, Kallmann syndrome ; DGS, DiGeorge syndrome

Case number 9 (DGS) showed translocation of the whole of the long arm of chromosome 22 onto the short arm of chromosome 9 (figure 1). Further analysis

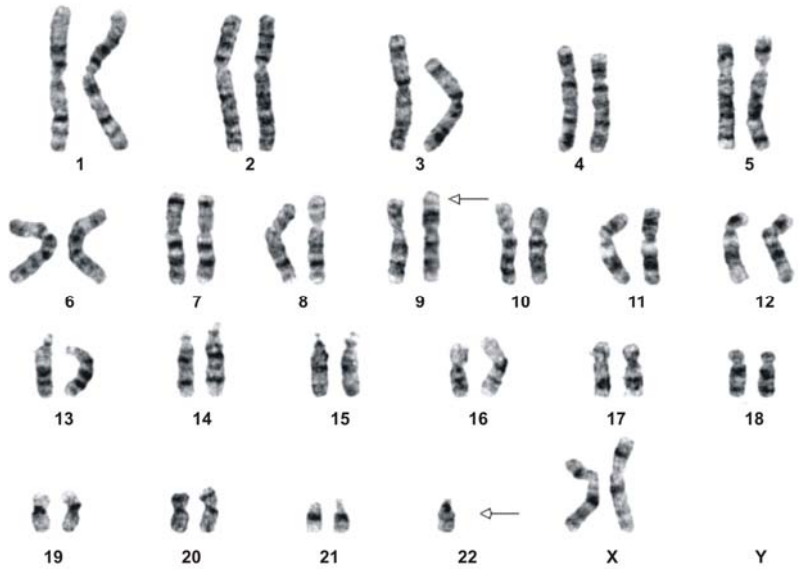

FIGURE 1: Giemsa-banded chromosomes showing the karyotype of the patient with DiGeorge syndrome (case 9). The whole of the long arm of chromosome 22 is translocated onto the short arm of chromosome 9, resulting in monosomy of chromosome 22 (bottom arrow).

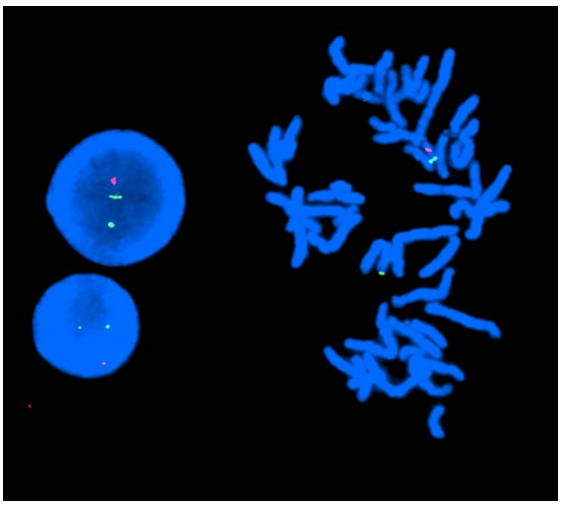

FIGURE 2: Interphase and metaphase spreads hybridised with LSI DiGeorge TUPLE 1 region probe. Absence of the red signal on one of the chromosome 22 indicates deletion of the locus at 22q11.2.

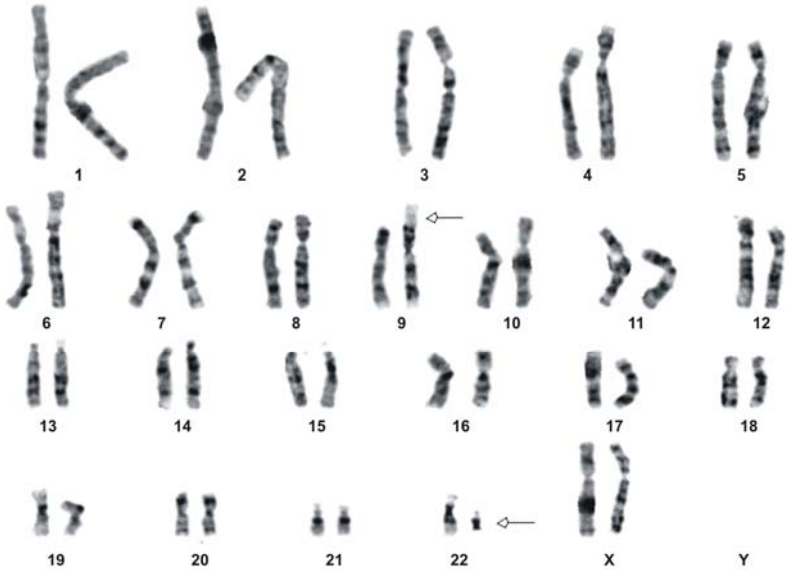

FIGURE 3: Giemsa-banded chromosomes showing karyotype of patient's (Case 9) mother with translocation between the long arm of chromosome 22 and the short arm of chromosome 9 (arrows).

done on this case using FISH showed deletion on the locus responsible for DGS (figure 2).

Both of the parent's blood were also obtained and analysed to rule out familial DGS. The karyotype of the father showed normal chromosomal constitution (46,XY) whilst the mother's karyotype showed structural abnormality involving chromosomes 9 and 22 (figure 3). FISH analysis using probe for DiGeorge (TUPLE1 and D22S75) showed no microdeletions involved in the mother; indicating that she is a carrier.

DISCUSSION

Cytogenetic techniques have improved over the years and have made it possible for microdeletion syndromes to be described. Most of the microdeletion syndromes involve multiple genes and can be considered as contiguous gene deletion syndromes e.g. William syndrome in which the deletion involves the elastin gene and a protein kinase LIMK1, and Prader-Willi and Angelman syndromes which involved many genes in the 15q11-q13 region (Korenberg et al 2000, Butler 1986).

In the present study, all of the cases showed normal karyotype by conventional cytogenetic analysis, except for one case, which showed structural chromosomal abnormality involving chromosomes 9 and 22 (case 9). However, microdeletion was revealed in all of the cases studied by FISH analysis. The reason for the discrepancy in the results between conventional and FISH analysis seen in this study is that the latter method is able to detect deletions at a greater resolution. In a study done on Prader-Willi and Angelman syndromes, it has been reported that even high-resolution cytogenetics (a resolution higher than 550 bands) cannot detect the deletions involved (Smith et al 1995).

The application of FISH technique in the diagnosis of microdeletion syndromes has been shown to be extremely useful in many previous studies (Smith et al 1995,

Demetrick 1999) as well as in the present study. FISH reveals microdeletion in the locus/loci responsible for the syndromes in eight cases (case 1-8) studied which otherwise shows normal karyotype. However, due to some cases of microdeletion syndromes which are caused by mechanisms other than chromosome deletion, conventional cytogenetics should always be applied along with FISH. As clearly demonstrated in case 9 from our study, the chromosomal abnormality was detected by the conventional method, while FISH identified microdeletion in the region involved with DiGeorge syndrome ie. 22q11.2. Another study has shown that about 5% of Prader-Willi syndrome and Angelman syndrome involved rearrangements of chromosome 15. For this reason, FISH cannot be the sole technique without conventional cytogenetics (Donaldson et al 1994).

Approximately 90%-96% of DGS patients show a microdeletion of the 22q11.2 region (Driscoll et al 1992). Two of three patients with DGS in the present study have normal karyotypes by chromosome analysis, except in one case (case 9) where a deletion in the 22q11.2 region was suspected. By using FISH technique with DiGeorge critical region probe, all three patients were confirmed as having a 22q11.2 deletion, thus establishing DGS in these patients (100% concordance).

In 70% of PWS patients, an interstitial deletion of the (15)(q11q13) region derived from the paternal chromosome is a characteristic feature (Ledbetter et al. 1981). About 30% of PWS cases are due to maternal uniparental disomy (Nicholls 1993). FISH analysis with PWS region probe confirmed the deletion in all of the three cases in this study (table 1).

The phenotype of patients with Williams syndrome (WS) becomes variable with advancing age which makes diagnosis more challenging. However, since complete deletion of one elastin allele on chromosome 7 at band 7q11.23 in WS patients has been demonstrated, diagnosis is now easier. While chromosome analysis showed normal karyotype in our single case of WS, a deletion of elastin locus was identified by FISH analysis.

There is one case of Miller-Dieker syndrome (MDS) and another of Kallman syndrome which were also confirmed by FISH in this study. The main indicators of MDS are severe brain damage with microcephaly and severe mental as well as psychomotor retardation. The gene responsible for the X-linked form of Kallmann syndrome, KAL1, encodes a protein, anosmin, which plays a key role in the migration of gonadotropin-releasing hormone (GnRH) neurons and olfactory nerves to the hypothalamus. Although mental or intellectual disturbance was described (Kallmann et al 1944), analyses of the genotype-phenotype relationship showed that Kallmann syndrome patients with mental disorders have large deletions on Xp22.3 that extends beyond the KAL1 locus (Nagata et al 2000). In contrast, almost all patients with mutations restricted to the KAL1 locus are free of mental disturbance.

Although the microdeletion syndromes mentioned above have distinctive clinical features, they tend to have in common the characteristics that they are usually sporadic but occasionally are transmitted in families as dominant traits. Most of the microdeletions occur spontaneously but individuals who have a deletion, if they are able to have children, have a 50% chance of transmitting the deletion to any child. In the absence of a parental deletion, there is a low risk of recurrence as germ line mosaicism is rare.

In view of the structural chromosomal abnormality from case 9, the findings from both the conventional and FISH analysis suggest that the patient has a familial DiGeorge syndrome; the syndrome has been transmitted from the mother. Genetic counseling was advised for the couple since they have a high risk of having

another abnormal child and recurrent abortions.

The fact that FISH is a more powerful method compared to conventional karyotyping has been demonstrated in this study as well as other studies (Ward et al 1999, Demetrick 1999). Besides being rapid, sensitive and simple, FISH technique can also be performed on interphase cells hence interphase FISH was introduced. It can be applied for haematological malignancies, for example in the identification of *bcr/abl* gene fusion in chronic myeloid leukemia (CML) and prenatal test for detection of common trisomies of chromosomes 13,18,21, as well as X and Y chromosomes (Cianciulli et al 2004, Hogge et al 1996).

Despite all the advantages mentioned above, there are two disadvantages of FISH; it will only provide information about the probe being tested, other aberrations will not be detected and the probes used are relatively expensive. However, due to the increased accuracy and time saved compared with the more laborious high-resolution cytogenetic analysis, FISH may be more cost effective than the conventional method for detection of deletions eventhough the probes are relatively expensive.

CONCLUSION

In conclusion, the present study has proven that combined FISH and conventional cytogenetics complement each other in the diagnosis of microdeletion syndromes. It is important for confirmation of the diagnosis, genetic counseling, and facilitation of prenatal testing.

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