Detection for microdeletion syndromes in 427 Thai patients by fluorescence *in situ* hybridization

Lucksamee Malee, Pitichai Phornsarayuth, Veerawat Korkiatsakul, Budsaba Rerkamnuaychoke, Takol Chareonsirisuthigul*

Human Genetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

*Corresponding author: takol.cha@mahidol.ac.th

ABSTRACT

Microdeletion syndromes are a collection of genetic disorders that are associated with very small deletions on certain chromosomes. These disorders may not be detected by conventional karyotyping. However, they can be effectively detected by fluorescence *in situ* hybridization (FISH). Total of 427 Thai patients clinically suspected microdeletion syndrome comprising 306 cases for DiGeorge/ Velocardiofacial syndrome, 67 cases for Williams syndrome and 54 cases for Prader-Willi/Angelman syndromes were detected by FISH. Out of 427 samples, 84 (19.7%) were found to be positive for microdeletion syndromes. Among of these, 41 (48.8%) were DiGeorge/velocardiofacial syndrome, 30 (35.7%) were Williams syndrome and 13 (15.5%) were Prader-Willi/Angelman syndromes. Although there is no specific treatment available for microdeletion syndromes, the use of FISH probes would certainly help with detecting these microdeletion syndromes at an early stage and help to prevent its recurrence in the family through prenatal or preimplantation genetic diagnosis.

Keywords: fluorescence *in situ* hybridization (FISH); microdeletion; DiGeorge/velocardiofacial syndrome; Williams syndrome; Prader-Willi/Angelman syndromes.

INTRODUCTION

Microdeletion syndromes are defined as a group of clinically recognizable disorders. They are characterized by a small (< 5Mb) deletion of a chromosomal segment spanning multiple disease genes, each potentially contributing to the phenotype independently (Shaffer *et al.*, 2001; Weise *et al.*, 2012). Microdeletions are often not detectable by using conventional or high resolution karyotyping (2-5 Mb) (Delach *et al.*, 1994). There are several molecular cytogenetic techniques which can be used to detect microdeletion syndromes. These techniques are fluorescence *in situ* hybridization (FISH) (Sinclair 2002; Sukarova-Angelovska *et al.*, 2007), chromosomal microarray (Dale *et al.*, 2012; Vissers and Stanklewlcz, 2012), multiplex ligation-dependent probe amplification (MLPA) (Jalali *et al.*, 2008), quantitative polymerase chain reaction (Weksberg *et al.* 2005), and Bacs-on-beads (BoBs) technology (García-Herrero *et al.*, 2014). Presently, FISH is the standard diagnostic approach for commonly known microdeletions, such as Williams syndrome (7q11 microdeletion), which occurs in 1 of 7,500 to 1 of 20,000 live births. Prader-Willi/Angelman syndromes (15q11-q13 microdeletion) with prevalence between 1 in 25,000 and 1 in 10,000 live births and DiGeorge syndrome (22q11 microdeletion) with prevalence ranges from 1 in 2000 to 1 in 4000 live births (Vogels and Fryns, 2015). FISH method also allows an increase in the number of solved cases of mental retardation and malformative syndromes. Implementation of this method makes genetic

counselling of the affected families possible. In Thailand, only a few case studies about microdeletion syndromes have been reported. (Dissaneevate *et al.*, 2003). To the authors' knowledge, this study is the first retrospective cohort study to investigate the frequency of microdeletion syndromes; DiGeorge/Velocardiofacial syndrome, Williams syndrome and Prader-Willi andAngelman syndromes in Thailand.

MATERIALS AND METHODS

Sample collection and fluorescence *in situ* hybridization (FISH)

Blood samples from 427 patients at Ramathibodi hospital between January 2010 and October 2014 were used to rule out microdeletion syndromes. The study protocol was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine at Ramathibodi Hospital, Mahidol University (MURA 2014/687). All samples were processed by standard cytogenetic procedures (Moorehead *et al.*, 1960). Approximately 20 μL of cell suspension was used to prepare the slide and was then aged at 60°C for 2 hours. FISH was carried out using Vysis probes kit (Abbott: U.S.A.), following manufacturer's recommendations. Briefly, co-denaturation of the specific probes with the test sample at 73°C for 5 minutes, followed by overnight hybridization at 37°C was performed. After hybridization, slides were washed and dehydrated in ethanol series before being mounted with the counter-stain DAPII (Abbott: U.S.A.). Metaphase spreads were visualized and captured by an automated fluorescent microscope (Zeiss, Germany). A minimum of 20 metaphases were analyzed for detection of microdeletions in each case.
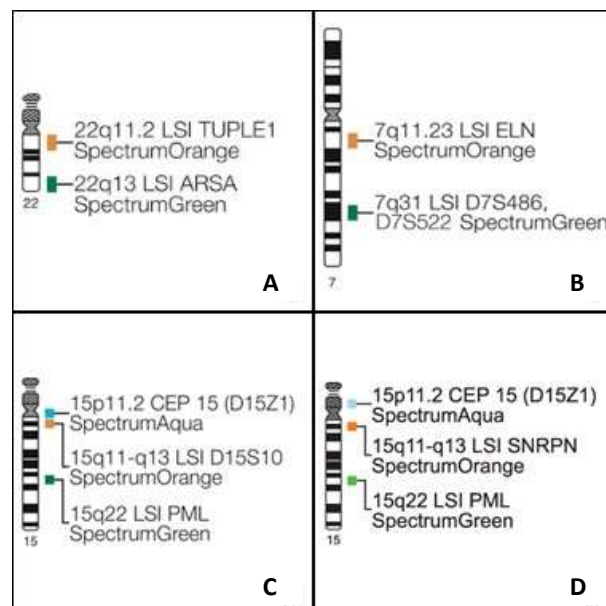
FISH Probes and interpretation

For DiGeorge and Williams syndromes, the control signals were labeled in green by FITC (G)

and the critical region in orange by Cy3 (O) (Abbott: U.S.A.). A normal cell should have the 2G2O pattern whereas a cell with the deletion should have the 2G1O pattern (Figure 1a and b). The SNRPN and D15S10 probe for detection of Prader-Willi/Angelman syndromes (Belloso *et al.*, 2005) had two internal controls, a proximal aqua (A) signal (CEP 15) and a green signal (PML) towards the distal end. The critical regions (SNRPN and D15S10) have an orange signal and lay between the two control signals on chromosome 15 (Abbott, U.S.A.). With this probe, a normal cell should have 2A2G2O signal pattern whereas a cell with the Prader-Willi/Angelman microdeletion should have the 2A2G1O signal pattern (Figure 1c and d). FISH signals on metaphases should be checked with the control probe to avoid false positive results due to deletion of the control orange signal.

RESULTS

Four hundred and twenty seven cases (238 males and 189 females) clinically suggested to have microdeletion syndromes were analyzed in this study by FISH technique. The results demonstrated that among the 427 cases, 306 patients were suspected for DiGeorge/velocardiofacial syndrome, 67 patients for William Syndrome, and 54 patients for Prader-Willi/Angelman syndromes. Overall diagnostic yield from FISH results were 84 (19.7%) positive cases including 41 (48.8%) DiGeorge/velocardiofacial syndrome (Figure 2a), 30 (35.7%) Williams syndrome (Figure 2b) and 13 (15.5%) Prader-Willi/Angelman syndromes (Figure 2c and d) (Table1). In this study, the percentage of suspected cases for each syndrome were 44.78% for Williams syndrome, 24.07% for Prader-Willi/Angelman syndromes and 13.40% for DiGeorge/velocardiofacial syndrome. Moreover, two cases of particular interest were identified as described below.

Figure 1 Ideogram demonstrating regions detected by Vysis probes (Abbott: U.S.A.) of (a) DiGeorge/velocardiofacial syndrome, (b) Williams syndrome, (c) Prader-Willi/Angelman syndromes (D15S10) and (d) Prader-Willi/Angelman syndromes (*SNRPN*).
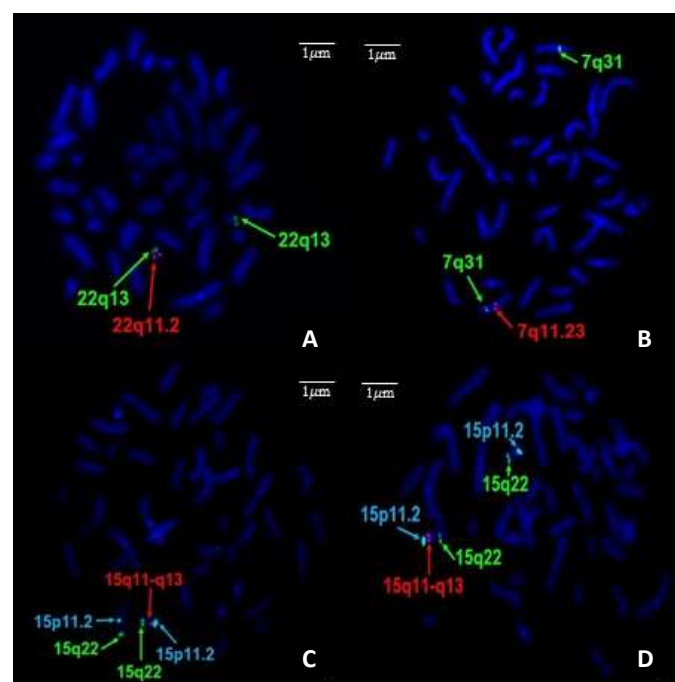
Figure 2 The metaphases FISH showing deletion hybridization pattern of (a) DiGeorge/velocardiofacial syndrome, (b) Williams syndrome, (c) Prader-Willi/Angelman syndromes (D15S10) and (d) Prader-Willi/Angelman syndromes (*SNRPN*). For DiGeorge and Williams syndromes, the control signals were labeled in green by FITC (G) and the critical region in orange by Cy3 (O). The SNRPN and D15S10 probe for detection of Prader-Willi /Angelman syndromes (Belloso *et al.*, 2005) had two internal controls, a proximal aqua (A) signal (CEP 15) and a green signal (PML) towards the distal end. The critical regions (SNRPN and D15S10) have an orange signal and lay between the two control signals on chromosome 15.

Table 1 Detection of most common microdeletion syndromes at Ramathibodi Hospital between January 2010 and October 2014 and Jaslok Hospital (Madon *et al*., 2010) by FISH

|		Syndromes			
		DGS/VCF	WS	PDW/AS	Total
Suspected cases	This study	306	67	54	427
	Madon *et al*., 2010	23	67	284	374
Positive case	This study	41(48.8%)	30(35.7%)	13(15.5%)	84 (100%)
	Madon *et al*., 2010	4(5.5%)	24(32.9%)	45(61.6%)	73 (100%)
Positive Males	This study	19	18	10	47
	Madon *et al*., 2010	3	16	27	46
Positive females	This study	22	12	3	37
	Madon *et al*., 2010	1	8	18	27
% Positive out of suspected cases for each syndrome *	This study	13.40	44.78	24.07	19.67
	Madon *et al*., 2010	17.39	35.82	15.86	19.52

DiGeorge/velocardiofacial (DGS/VCF), Williams (WS) and Prader-Willi/Angelman syndromes (PWS/AS).
* % positive out of suspected cases for each syndrome = (Positive case / Suspected cases) x 100.

Case 1: A male infant suspected to have DiGeorge/velocardiofacial syndrome. He had clinical diagnosis with congenital diaphragmatic hernia and hypoplastic transverse aortic arch. Conventional cytogenetic study has found normal karyotype. FISH analysis on metaphase showed normal patterns, but interphase showed duplication of the orange (TUPLE1) signal and the third orange signal was visible (Figure 3a, b). The FISH result suggested that the patient had 22q11 duplication.

Case 2: Amniotic fluid from a 37-year old woman who had clinical diagnosis with her fetus, which had cystic hygroma. Cytogenetic analysis found two marker chromosomes (47,X,+mar1,+mar2). Results from FISH with specific probes for DiGeorge/velocardiofacial and Prader-Willi/Angelman syndromes showed normal patterns for DiGeorge/velocardiofacial syndrome. However, the probe for Prader-Willi/Angelman syndromes showed 2 signals, each marker chromosome on 15p11.2, whereas the critical region probe (15q11-q13) showed normal patterns. Thus, the fetus was not diagnosed with DiGeorge/velocardiofacial syndrome or Prader-Willi/Angelman syndromes (Figure 4a, b).
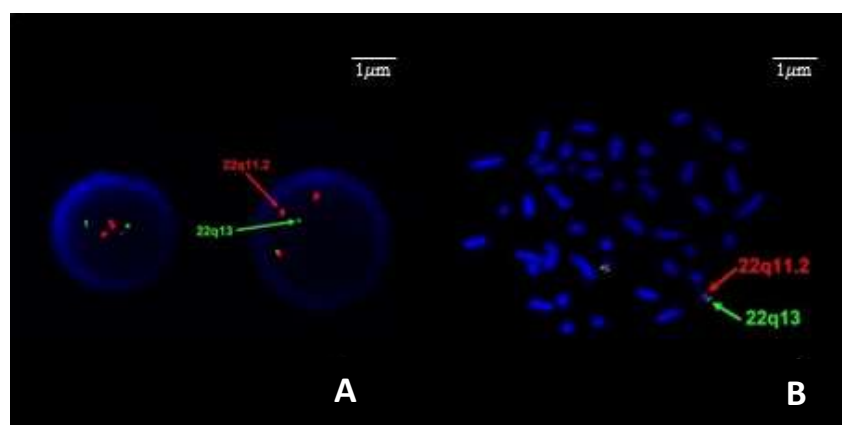
Figure 3 Case 1: FISH from (a) Interphase cells showing an extra orange signal (2G3R) and (b) Metaphase cells showing normal signal pattern (2G2R). The control signals were labeled in green by FITC (G) and the critical region in orange by Cy5 (O).
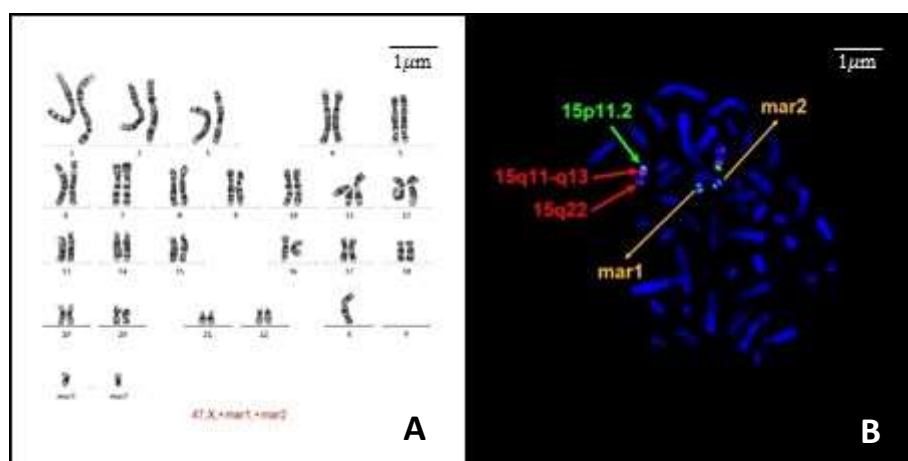
Figure 4 Case 2: (a) Karyotype showing 47,X,+mar1,+mar2 and (b) FISH from a metaphase cell showing two signals of each marker chromosome 47,X,+mar1,+mar2. Ish idic(15)(p11.2)(D15Z1++),idic(15)(p11.2)(D15Z1++).

DISCUSSION

In this study, we investigated the frequency of microdeletion syndromes; DiGeorge/Velocardiofacial syndrome, Williams syndrome, and Prader-Willi/Angelman syndromes in 427 Thai patients by FISH. The most common microdeletion syndrome identified in this study was Williams syndrome at about 35.82%. Likewise, in a previous study of the Indian people by Madon *et al*. (Table 1). The positive cases were primarily in male. In our study, the most common clinically suspected microdeletion was DiGeorge/velocardiofacial syndrome, which is in contrast to Prader-Willi/Angelman syndromes in Indian patients (Madon *et al*., 2010).

DiGeorge syndrome (DS) is caused by chromosomal microdeletion is at 22q11. 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. In Case 1, clinical features overlapped with the DiGeorge/velocardiofacial syndrome. However, FISH results showed 22q11 duplication. Ensenauer *et al*. demonstrated that an average of 90% of interphase cells could be detected to have three TUPLE1 probe signals whereas the microduplication ranged from 0% to 100% in metaphase cells (Ensenauer *et al*., 2003).

In Case 2, results from analysis of two marker chromosomes by FISH using DiGeorge/velocardiofacial and Prader-Willi/Angelman syndromes probe showed no microdeletion. The two markers were isodicentric15 (idic15) (Battaglia, 2008) and were not involved in Prader-Willi/Angelman critical region. Therefore, the fetus was not diagnosed to have the Prader-Willi/Angelman syndromes. The major cause of cystic hygroma in the fetus is correlated with 45,X chromosomal complement (Turner syndrome) (Thongsong, 2010). In this case, the mother had mosaic karyotype (mos 45,X[5]/46,XX[95]) and the father had normal karyotype. Therefore, the 45,X (Turner syndrome) was maternally inherited with *de novo* mutation of marker chromosome 15.

Prader-Willi/Angelman syndromes are a complex multisystem disorder characterised by a variety of clinical features. Prader-Willi and Angelman syndromes resulted from loss of paternal or maternal expression, respectively, of genes located on the human chromosome 15q11-13 region.

Different molecular mechanisms leading to this loss of expression have been identified, including microdeletions, intragenic mutations, uniparental disomy and imprinting defects. Therefore, some of the children who have Prader-Willi/Angelman syndromes due to the imprinting mutation and uniparental disomy could not be identified by FISH (Cassidy *et al.*, 2012). Methylation of the *SNRPN* gene should be evaluated if suspicion of the syndrome still exists. In addition, careful selection of patients with a specific set of dysmorphic signs is a prerequisite for a successful application of some of the commercial probes (Xu and Chen, 2003).

Since some of the microdeletion syndromes give a wide variety and overlapping of phenotypic signs, clinical diagnosis can be challenging. Application of FISH probes is a prerequisite   for confirmation. If a deletion is found, parental karyotype analysis is also needed in order to assess recurrence risk and provides proper genetic counseling. Preimplantation genetic diagnosis (PGD) using FISH on 1-2 blastomeres biopsied from embryos obtained by IVF-ICSI has been successful in detection of microdeletions in women predisposed to cancer, demonstrating that FISH-based PGD is a straightforward approach to detect microdeletions in single blastomeres (Vanneste *et al.*, 2009).

In conclusion, FISH has been widely used to diagnose microdeletion syndromes. Although there is no specific treatment available 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.

REFERENCES

Battaglia A (2008) The inv dup (15) or idic (25) syndrome (Tetrasomy 15q). Orphanet Journal of Rare Diseases. http://www.ojrd.com/content/3/1/30 (November 2013)

Belloso JM, Caballin MR, Gabau E, Baena N, Vidal R, Villatoro S, Guitart M (2005) Characterization of six marker chromosomes by comparative genomic hybridization. Am J Med Genet 136A: 169–174.

Cassidy SB, Schwartz S, Miller JL, Driscoll DJ (2012) Prader-Willi syndrome. Genet Med 14: 10–26.

Dale RC, Grattan-Smith P, Nicholson M, Peters GB (2012) Microdeletions detected using chromosome microarray in children with suspected genetic movement disorders: a single-centre study. Dev Med Child Neurol 54: 618–623.

Delach JA, Rosengren SS, Kaplan L, Greenstein RM, Cassidy SB, Benn PA (1994) Comparison of high resolution chromosome banding and fluorescence *in situ* hybridization (FISH) for the laboratory evaluation of Prader-Willi syndrome and Angelman syndrome. Am J Med Genet 52: 85–91.

Dissaneevate S, Jaruratanasirikul S, Chanvithan P, Janjindamai W (2003) Congenital malformations of newborns at Songklanagarind Hospital. Songkla Med J 21: 267-276.

Ensenauer RE, Adeyinka A, Flynn HC, Michels VV, Lindor NM, Dawson DB, Thorland EC, Lorentz CP, Goldstein JL, McDonald MT, *et al.* (2003) Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. Am J Hum Genet 73: 1027–1040.

García-Herrero S, Campos-Galindo I, Martínez-Conejero JA, Serra V, Olmo I, Lara C, Simón C, Rubio C (2014) BACs-on-Beads technology: A reliable test for rapid detection of aneuploidies and microdeletions in prenatal diagnosis. BioMed Research International 2014: 1–7.

Jalali GR, Vorstman JAS, Errami A, Vijzelaar, Biegel J, Shaikh T, Emanuel BS (2008) Detailed analysis of 22q11.2 with a high density MLPA probe set. Hum Mutat 29: 433–440.

Madon PF, Athalye AS, Sanghavi K, Parikh FR (2010) Microdeletion syndromes detected by FISH – 73 positive from 374 cases. Int J Hum Genet 10: 15–20.

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20: 613–616.

Shaffer LG, Ledbetter DH, Lupski JR (2001) Molecular cytogenetics of contiguous gene syndromes: mechanisms and consequences of gene dosage imbalance. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Vogelstein B, et al., The online metabolic and molecular bases of inherited diseases. 8th ed. McGraw-Hill. New York, pp 1291–1324.

Sinclair A (2002) Genetics 101: cytogenetics and FISH. CMAJ 167: 373–374.

Sukarova-Angelovska E, Piperkova K, Sredovska A, Ilieva G, Kocova M (2007) Implementation of fluorescent *in situ* hybridization (FISH) as a method for detecting microdeletion syndromes - our first experiences. Prilozi 28: 87–97.

Tongsong T (2010) Cystic Hygroma. Obstetrics and Gynecology CMU. http://www.med.cmu.ac.th/dept/obgyn/2011/index.php?option=com_content&view=article&id=63:cystic-hygroma&catid=43&Itemid=401 (November 2010)

Vanneste E, Melotte C, Debrock S, D'Hooghe T, Brems H, Fryns JP, Legius E, Vermeesch JR (2009) Preimplantation genetic diagnosis using fluorescent *in situ* hybridization for cancer predisposition syndromes caused by microdeletion. Hum Reprod 24: 1522–1528

Vissers LE, Stankiewicz P (2012) Microdeletion and microduplication syndromes. Methods Mol Biol 838: 29–75.

Vogels A, Fryns JP (2015) Microdeletions and Molecular Genetics. Atlas of Genetics and Cytogenetics in Oncology and Haematology, http://atlasgeneticsoncology.org/Educ/MicrodeletionID30059ES.html (February 2015)

Weksberg R, Hughes S, Moldovan L, Bassett AS, Chow EW, Squire JA (2005) A method for accurate detection of genomic microdeletions using real-time quantitative PCR. BMC Genomics 6: 180–190.

Weise A, Mrasek K, Klein E, Mulatinho M, Llerena JC Jr, Hardekopf D, Pekova S, Bhatt S, Kosyakova N, Liehr T (2012) Microdeletion and microduplication syndromes. J Histochem Cytochem 60: 346–358.

Xu J, Chen Z (2003) Advances in molecular cytogenetics for the evaluation of mental retardation. Am J Med Genet C Semin Med Genet 117C: 15–24.