CHAPTER 4

DISCUSSION

In this study, we reported on the results of cytogenetic, subtelomeric FISH analysis and FXS screening in patients with idiopathic mental retardation or autism. We found subtelomeric rearrangements in 6.1% (5/82) of Thai patients with IMR. Conventional G-banding analysis on IMR patients failed to show these rearrangements, while FISH using subtelomeric-specific probes for all chromosome ends could detect the abnormalities. Cryptic subtelomeric rearrangements have been shown to be a significant cause of unexplained mental retardation. Although we found that two subtelomeric deletion cases showed normal parental chromosomes, some studies have found that almost half of their positive cases were familial (Knight et al., 1999; Barker et al., 2002). Therefore, parental blood samples are necessary to clarify the origin of subtelomeric rearrangements. Also, further studies in characterizing chromosome breakpoint and identifying relevant genes that cause IMR are essential for a fuller understanding of mental retardation and clinical management in these patients.

The current techniques used for detecting subtelomeric rearrangement are FISH, using probes localized in the subtelomeric region and polymorphic microsatellite markers localized in the subtelomeric region. However, these techniques cannot detect all chromosomal aberrations. Advanced techniques, such as array-based comparative genomic hybridization (array-CGH), multiplex amplifiable probe hybridization (MAPH), and multiplex ligation dependent probe amplification (MLPA), have been used for detecting submicroscopic chromosomal rearrangement in patients with idiopathic mental retardation (de Vries et al., 2003; Smeets, 2004; White et al., 2004). By these techniques, human genome can be scanned in a single hybridization using multiprobes FISH, and many chromosomal abnormalities have been reported in recent studies (Rodriguez-Revenga et al., 2004; Schoumans et al., 2005). Not only cryptic subtelomeric rearrangements but also other submicroscopic chromosomal abnormalities such as interstitial deletions, inversions and duplications can be found by these techniques.

Several studies have shown that the prevalence of subtelomeric abnormalities in populations with idiopathic MR had variable detection rates (Table 10) (Flint et al., 1995; Knight et al., 1999; de Vries et al., 2003). This variable rate may be correlated with the size of population and inclusion criteria. For this reason, a clinical checklist was developed by de Vries et al. (2001)

in order to facilitate preselection of cases for subtelomeric testing. This checklist included 5 items: (1) family history of MR, (2) prenatal onset growth retardation, (3) postnatal growth abnormalities (either poor or overgrowth), (4) ≥ 2 facial dysmorphic features, and (5) one or more of non-facial dysmorphisms and/or congenital anomalies.

The current study was divided into 2 groups: (1) IMR patients with no MCA, and (2) IMR patients with MCA. The frequency of subtelomeric rearrangements in IMR patients with MCA (4/47 = 8.51%) was higher than IMR with no MCA (1/35 = 2.85%). There was only one abnormal case from the group of IMR patients with no MCA, while the remaining subtelomeric abnormalities were found in 4 patients with MR and MCA. This lends supports to the suggestion that preselection of cases using the above-mentioned checklist, or a similar one, for subtelomeric testing may increase the positive findings of subtelomeric rearrangement in IMR patients (Table 10).

Table 10 Summary of the prevalence of cryptic subtelomeric rearrangements in IMR patients.

Reference	Selection criteria	Number of subtelomeric rearrangements	Method
Slavotinek et al., 1999	IMR + 3DF	2/27 (7.4%)	Microsatellite marker
Knight et al., 1999	Mild IMR / moderate to severe IMR	1/182 (0.5%) 21/284 (7.4%)	FISH
Joyce et al., 2001	IMR group control group[a]	0/200 (0.0%) 2/150 (1.33%)	FISH
Anderlid et al., 2002	Mild/severe IMR+ DF and/or FH	10/111 (9%)	FISH+SKY
Clarkson et al., 2002	IMR	3/50 (6%)	FISH+SKY
Helias-Rodzewicz	Mild / moderate to	3/33 (9%)	FISH

et al., 2002	severe IMR		
Rio et al., 2002	Severe IMR	15/150 (10%)	FISH+ genotyping

Table 10 (continued)

Reference	**Selection criteria**	**Number of subtelomeric rearrangements**	**Method**
Barker et al., 2002	IMR / IMR+DF	9/250 (3.6%)	FISH
van Karnebeek et al., 2002	IMR / FH	1/184 (0.5%)	FISH
Bocian et al., 2004	mild/severe IMR +DF and/or MCA+FH	10/84 (11.9%)	FISH
Harada et al., 2004	IMR+MCA	4/69 (5.8%)	FISH+Microarray CGH
Rodriguez-Revenga et al., 2004	Mild/severe IMR	2/30 (6.7%)	FISH+DNA polymorphism
Velagaleti et al., 2005	IMR	2/18 (11%)	FISH
Sogaard et al., 2005	IMR / IMR+DF	9/132 (6.8%)	FISH
Present study	**IMR / IMR+MCA**	**1/35 (2.85%) 4/47 (8.51%) [5/82 (6.1%)]**	**FISH**

DF=dysmorphic features, FH=family history of mental retardation, IMR=idiopathic mental retardation, MCA =multiple congenital anomalies,

[a] = control group consisted of relatives of probands with numerical or structural chromosome abnormality

Specific subtelomeric deletion may reveal recognized syndromes such as Wolf-Hirschhorn syndrome (deletion of 4pter), Cri-du-chat syndrome (deletion of 5pter), Miller-Dieker syndrome (deletion of 17pter) and 18p- syndrome (Helias-Rodzewicz et al., 2002). In addition,

terminal deletion of 1p36 is commonly considered as a contiguous gene deletion syndrome characterized by distinct facial features, mental retardation, seizures, hearing impairment, growth failure and cardiac defects (reviewed in Ballif et al. 2004). Recent studies, have characterized the deletion size of 60 subjects with monosomy 1p36 using telomere-region-specific-probes and showed that the deletion size can vary widely from ~1 Mb to >10.5 Mb of 1p36 with no single common breakpoint (Ballif et al., 2003; Ballif et al., 2004). In the current study, there was one case with terminal 1p36 deletion (patient 2). The size of the deletion have been refined the breakpoint to a region 4.3 Mb from telomere.

In our study, we also found terminal deletion of 4p using subtelomeric-specific probes in one IMR patient with MCA (patient 4). This chromosomal abnormality is compatible with Wolf-Hirschhorn syndrome (WHS) which is caused by a deletion of the region 4p16.3, a deletion which may be submicroscopic. To detect such submicroscopic deletions requires molecular techniques such as microsatellite analysis, or molecular-cytogenetic techniques such as FISH. The characteristic clinical signs consist of mental retardation, seizures, congenital heart defects and craniofacial phenotype features such as microcephaly, hypertelorism, prominent glabella, broad and/or beaked nose, short philtrum, micrognathia, downturned corners of the mouth, dysplastic ears, or preauricular tags. Most patients with WHS had a de novo deletion, whereas a familial translocation was responsible for only 5-13% of the patients (Wieczorek, 2003).

All ends of human chromosomes must have a telomeric cap to be structurally stable and protect against end-to-end joining. There are two general mechanisms by which broken chromosomes such as those that have suffered terminal deletion from some cause can acquire this cap, telomere healing and telomere capture. First mechanism is ‘telomere healing’. The result of this event is terminal deletion that the telomeric repeat sequences at the site of healing must be found. The broken chromosomes can be stabilized by the addition of new telomeric repeats onto the end of a broken chromosome through the action of the enzyme telomerase; alternatively, the broken chromosomes can be stabilized through telomerase-independent mechanisms by acquisition of telomeric sequences from another chromosome end. The second mechanism for stabilizing a telomere end is ‘telomere capture’, in which the broken chromosome can be stabilized by acquiring a new telomeric sequence from another chromosome. Telomere capture events can occur between sister chromatids, homologues or nonhomologous chromosome ends

and result in a derivative chromosome (Knight and Flint, 2000a; Ballif et al., 2003; Ballif et al., 2004).

Two of terminal deletion in our study may have been stabilized through telomere healing by acquisition of a telomeric repeat sequence from another chromosome. Patient 2 was a de novo terminal 1p deletion, but the parental karyotype of patient 4 (4p-) was not available. Molecular studies may help to confirm that the telomeric repeat sequences were added to the broken chromosome and may help to elucidate the underlying mechanism involved in generating the deletions.

One derivative chromosome in our study was de novo. The broken chromosome was stabilized by a wide range of telomeric rearrangements from other chromosomes and resulted in a derivative chromosome, although the mechanism by which the terminal deletions were generated and/or stabilized are not well understood. Moreover, the mechanism of double strand break (DSB) repair may have resulted in a telomere capture event, in which the DBS used a homology sequence from another chromosome as a template. This process is known as break-induced replication (BIR), and with this mechanism, the repair of a DNA double-strand break can occur through either homologous or non-homologous mechanisms (Johnson and Jasin, 2001; Billif et al., 2004).

Studies on autistic patients have reported a considerable variety of chromosomal aberrations. To date, both numerical and structural chromosomal abnormalities have been reported in autism. The positive rate of chromosomal abnormalities in the present study was 3.33% corresponding to other studies. We found one case with an interstitial deletion at the 7p12.2p12.3 region, which has not been previously reported. Also, the candidate gene on chromosome 7p is *HOXA1* which functions in hindbrain development (reviewed in Ingram et al., 2000). The most common chromosomal abnormalities on chromosome 7 in autistic children are at the 7q21-q22 and 7q31-36 regions. Molecular studies such as linkage or association studies may identify a new candidate gene for autism (Muhle et al., 2004; Xu et al., 2004). In addition, the 15q11-q13 region is a common region for abnormalities to be found in autistic patients, however, we did not find any aberrations in this region by using gene-specific (PWS/AS) probes for FISH.

Both terminal deletion of 22q13 and deletion of 22q11 have been reported in patients with autistic behaviors in several studies. The clinical manifestations of deletion 22q11 include learning disability, attention-deficit-hyperactivity, low IQ and autistic spectrum disorders

(Niklasson et al., 2005; Nair-Miranda et al., 2004; Campbell et al., 2006). The clinical features of a 22q13 deletion are associated with hypotonia, development delay, absent or severely delayed speech and language and autistic behaviors (Manning et al., 2004). However, in our study using the DiGeorge/VCF region and 22qtel as a control probe, no abnormality was detected in this region, although this may have been due to the small number of cases in our study.

Since the subtelomere region is gene rich and abnormalities in several genes have been found to result in syndromes, subtelomeric analysis has been studied in many reports. The first report of a subtelomeric abnormality in an autistic patient was reported by Wolff et al. (2002). On the other hand, Battaglia and Bonaglia (2006) found no subtelemeric rearrangements in 71 autistic children. This present study randomly selected 6 cases for subtelomeric FISH, however, none showed subtelomeric rearrangements. Not surprisingly, subtelomeric rearrangements have rarely been found in autistic patients (Table 11). For this reason, it might not be necessary to test for subtelomeric rearrangement in autistic patients except in those cases of a mentally retarded autistic child with dysmorphic features.

Table 11 Cytogenetic study and FXS screening results in autistic patients in the present study compared with previous studies.

Reference	No. of patients	Result of chromosome abnormalities	Result of FISH (Abnormal case)			Result of FXS screening
			PWS/AS	DiGeorge	Subtelomeric	
van Karnebeek et al., 2002	25	2/25 (8%) 45,X/46,XY, +mar(idic15)	0/25	0/25	0/25	0/25
Wolff et al., 2002	10	-	-	-	1/10 (10%) del(2)(q37)	-
Medne et al., 2003	108	7/108 (6.5%)	-	-	1/108 (0.9%) der (18)t(13;18) (q34;q23)	-
Reddy, 2005	433	14/421 (3.32%)	0/9	0/5	0/7	7/316 (2.2%) Full mutation=3/7 mos full mutation=3/7 mos premutation carrier=1/7
Battaglia and Bonaglia, 2006	71	-	-	-	0/71	-