CHAPTER 3

RESULTS

1. Autistic patients

A total of 30 autistic children were recruited for this study. These patients included 1 female and 29 males. The mean age was 8.2 years old, ranging from 3 to 17 years old.

Three out of the 30 autistic children were diagnosed by clinicians from Yuwaprasart-withayopatham Hospital, while the remaining subjects were diagnosed by clinicians from Rajanukul Institute.

1.1 Cytogenetic study

1.1.1 High-resolution chromosome analysis

Karyotype analysis on peripheral blood of each patient examined 20 metaphases at the 550-850 band levels. High-resolution chromosome analysis revealed an interstitial deletion of chromosome 7p in one patient. The karyotype of the patient was defined as 46,XY,del(7)(p12.2p12.3) (Fig. 6).

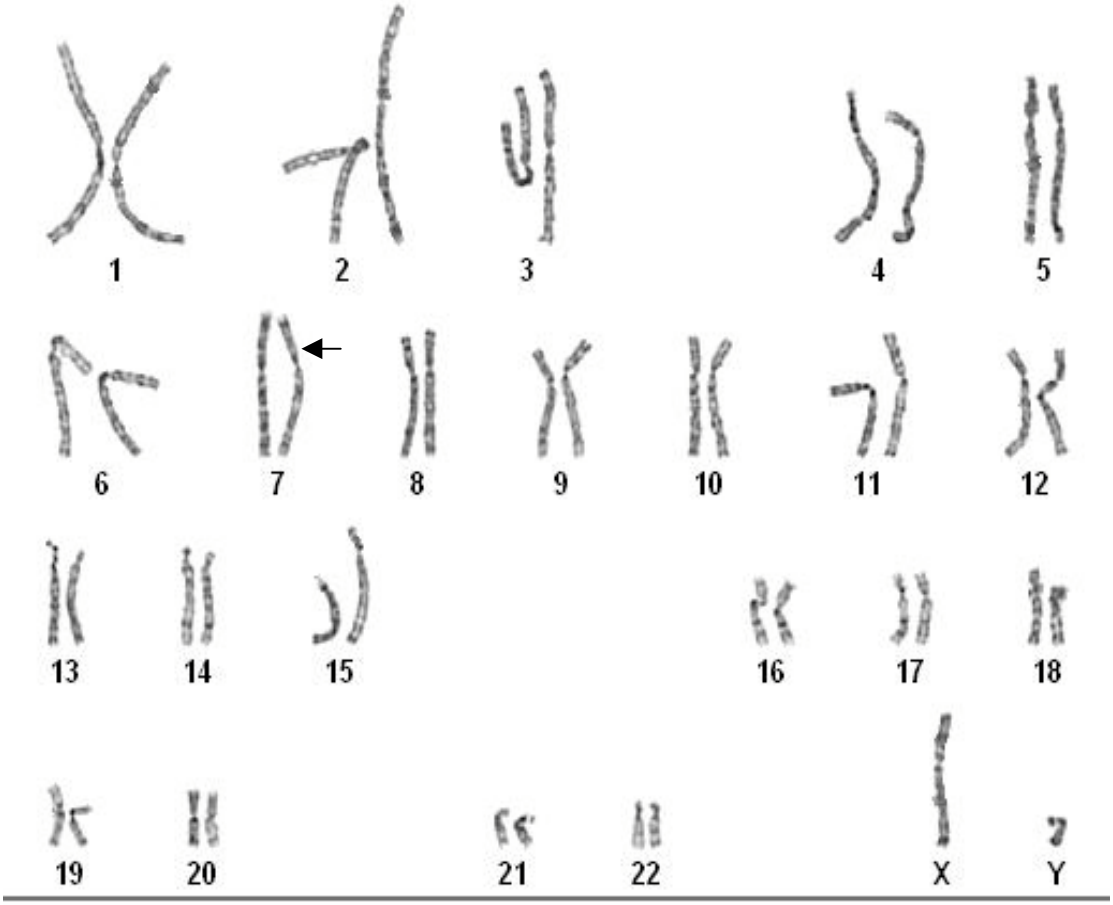


Figure 6 A G-banded karyotype of an autistic patient at the 700-850 band levels. The arrow indicates del(7p).

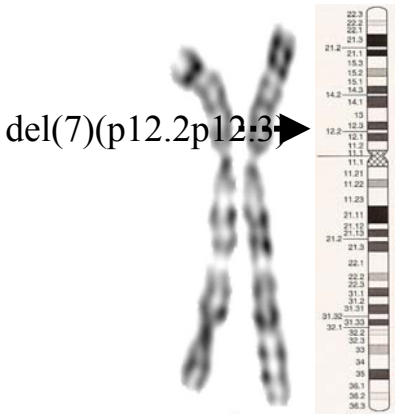


Figure 7 Partial G-banded karyotype of del(7p)(p12.2p12.3)

1.1.2 FISH study

FISH, using a gene-specific probe for a Prader-Willi/Angleman (SNRPN; RP13-487P22) with subtelomeric specific region (RP11-89K11; 15qter) was performed in all autistic patients. There was no duplication of any PWS/AS region in all cases examined.

A DiGeorge/VCF (TUPLE1; RP11-186O8) with subtelomeric-specific region (RP4-579N16; 22qter) probe was also performed in all cases. All cases were negative.

In addition, subtelomeric-specific probes for all chromosome ends were used to check for subtelomeric rearrangements in 6 of the 30 cases, but no subtelomeric abnormalities were detected.

1.2 DNA analysis of Fragile X syndrome

Fragile X syndrome screening using multiplex PCR for the *FMR1*, *FMR2* and *SRY* genes was performed on all cases. No case showed abnormal PCR.

Table 4 Summary of high-resolution chromosome analysis, FISH and FXS screening of autistic patients.

	Number of Patient		
	Total	**Normal result**	**Abnormal result**
Male	29	-	-
Female	1	-	-
High-resolution chromosome analysis	30	29	1
FISH analysis			
-Subtelomeric-specific probes*	6	6	0
-PWS/AS region probe	30	30	0
-DiGeorge/VCF region probe	30	30	0
FXS screening	30	30	0

* randomly selected cases

2. Idiopathic mental retardation (IMR) patients

82 IMR patients with and without MCA were recruited for this study. These patients included 50 males and 32 females with a mean age of 4 years old, ranging from 1-39 years old. Mental retardation was present in only 35 cases (22 males and 13 females) and mental retardation with multiple congenital anomalies in 47 cases (28 males and 19 females). Table 5 shows the data of all the patients in this study.

Table 5 Summary of clinical data and subtelomeric FISH results

Diagnosis	Number of patients			Subtelomeric FISH result	
	Total	Male	Female	Normal	Abnormal
MR	35	22	13	34	**1** (Male)
MR with MCA	47	28	19	43	**4** (2 Males, 2 Females)
Total	**82**	**50**	**32**	**77**	**5 (6.1%)**

MR=Mental retardation, MCA=Multiple congenital anomalies

Subtelomeric-specicfic FISH study

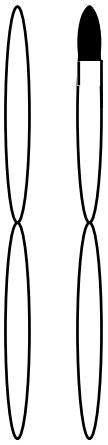
82 IMR patients with and without MCA, with normal high-resolution chromosome analysis, were further examined using subtelomeric-specific probes for all chromosome ends. Subtelomeric rearrangements were detected in five cases (Table 6). These included two terminal deletions and three derivative chromosomes (Fig. 8).

Parental chromosome and FISH analysis were performed in two cases. The results showed de novo rearrangement in both cases.

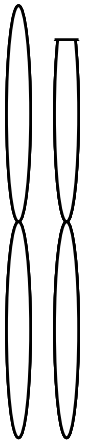
Table 6 Summary of the subtelomeric rearrangements detected in this study.

	Diagnosis	Karyotype	Parental karyotype
Patient 1	MR	46,XY.ish der(20)t(X;20)(pter+,qter-)	NA
Patient 2	MR+MCA	46,XX.ish del(1)(pter-)	Normal
Patient 3	MR+MCA	46,XY.ish der(3)t(X;3)(pter+,pter-)	Normal
Patient 4	MR+MCA	46,XY.ish del(4)(pter-)	NA
Patient 5	MR+MCA	46,XX.ish der(10)t(7;10)(pter+,qter-)	NA

MR= mental retardation, MCA= multiple congenital anomalies, NA= not available



A. derivative



B. terminal deletion

Figure 8 Diagram of structural chromosome aberrations. (A) A derivative chromosome is a structural chromosome aberration which results from two chromosomes rearrangement. (B) A terminal deletion chromosome is a structural chromosome aberration which results from the loss of a segment of a terminal chromosome.

Because the subtelomeric-specific probe for the X-chromosome is located on a psuedoautosomal region which is located on both the X and Y chromosomes, further studies using locus-specific probes, which are located on the p-arm of both X and Y chromosomes, was used to characterize the subtelomeric abnormalities of the subtelomeric-specific probe for the X-chromosome.

Table 7 Clones used for characterizing the p-arm of the X and Y chromosomes.

Clones	Location (Mb) from telomere
Xp clones	
RP11-465A24	3.91
RP5-46C18	4.01
RP11-501G22	3.63
RP11-315B16	4.13
Yp clones	
RP11-112L19	2.73
RP11-4D2	2.96
RP11-414C23	2.83

Patient 1

A 3-year-old boy was referred for evaluation of mental retardation. G-banding chromosome analysis did not identify chromosomal rearrangement, while subtelomeric-specific FISH probes revealed derivative chromosome 20 (Fig. 9). Further study was done using locus-specific probes to examine the metaphase chromosomes. Locus-specific probes of the p-arm of the X chromosome showed an extra signal on the q-arm of chromosome 20, while there was one

signal on the p-arm of the Y chromosome when the p-arm of the Y chromosome was probed. This result indicated that the extra material on the q-arm of chromosome 20 originated from the p-arm of the X chromosome. The karyotype of the patient was defined as 46,XY.ish der(20)t(X;20)(p22.3;q13.3)(RP13-465B17+;RP11-11M20-). This patient was comparable to an individual with duplication Xp and monosomy 20q. Parental peripheral blood was not available.

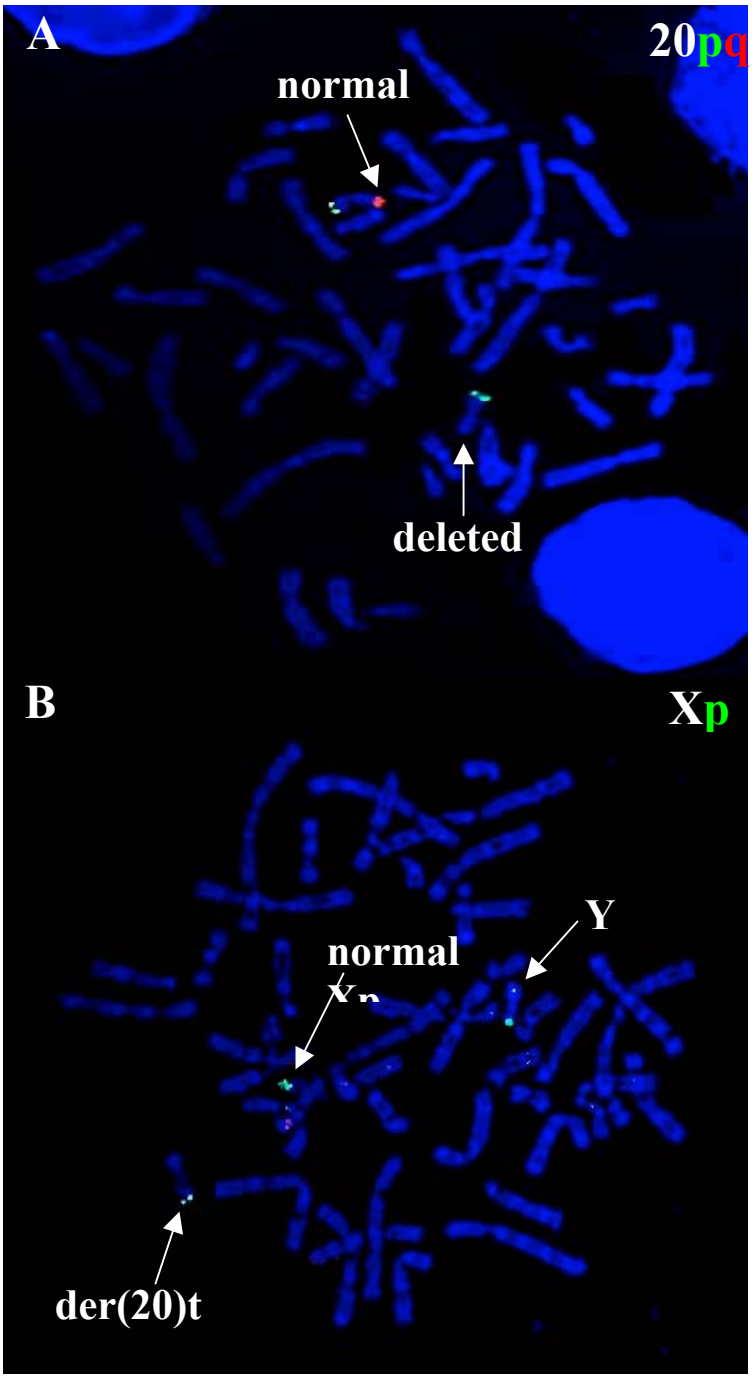


Figure 9 Subtelomeric FISH studies of the metaphase chromosomes from Patient 1 (A) The results from a subtelomeric-specific FISH probe for 20pq; RP11-530N10 (20pter; green), RP11-11M20 (20qter; red). The signal of 20p showed on both chromosome 20, while the signal of 20q is absent from the other homologue of chromosome 20q. (B) The results from a subtelomeric-specific probe for XYpq; RP13-465B17 (XYpter; green), RP11-954J6 (Xqter; red). There is an extra XYpter (green) signal on the long arm of chromosome 20.

Patient 2

Patient 2 was an 8-year-old girl with mental retardation and multiple congenital anomalies. High-resolution chromosomal analysis was normal. The karyotype of the patient was defined as 46,XX.ish del(1)(p36.32)(RP11-465B22-)dn using FISH (Fig. 10). Parental karyotypes were normal using G-banding and FISH analysis.

The deletion size was approximately 4.3 Mb from 1pter, involving 5 BAC/PAC clones located on chromosome band 1p36.32-36.33. Clones used for breakpoint mapping are listed in Table 8.

pq

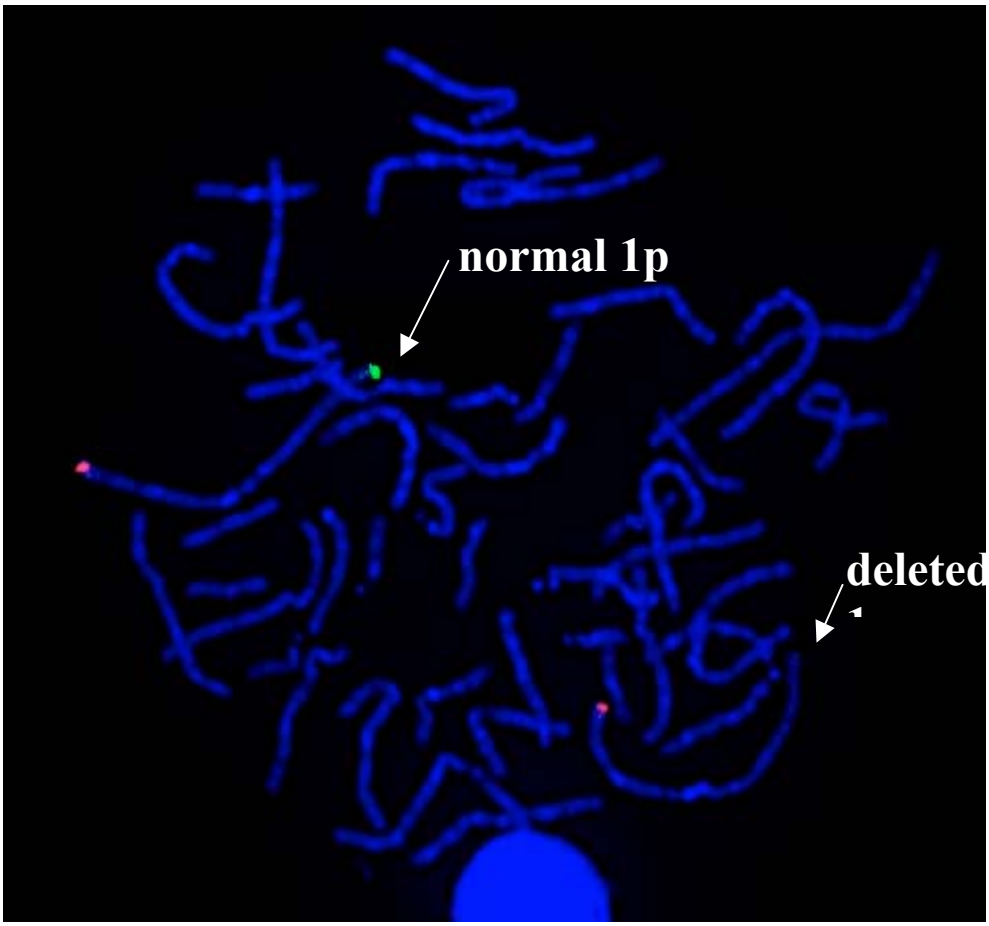


Figure 10 Subtelomeric FISH studies of the metaphase chromosomes from Patient 2, showing the subtelomeric-specific probe for 1pq; RP11-465B22 (1pter; green), RP11-438F14 (1qter; red). A green signal on one 1p is missing, indicating a deletion.

Table 8 Clones used for breakpoint mapping on chromosome 1p.

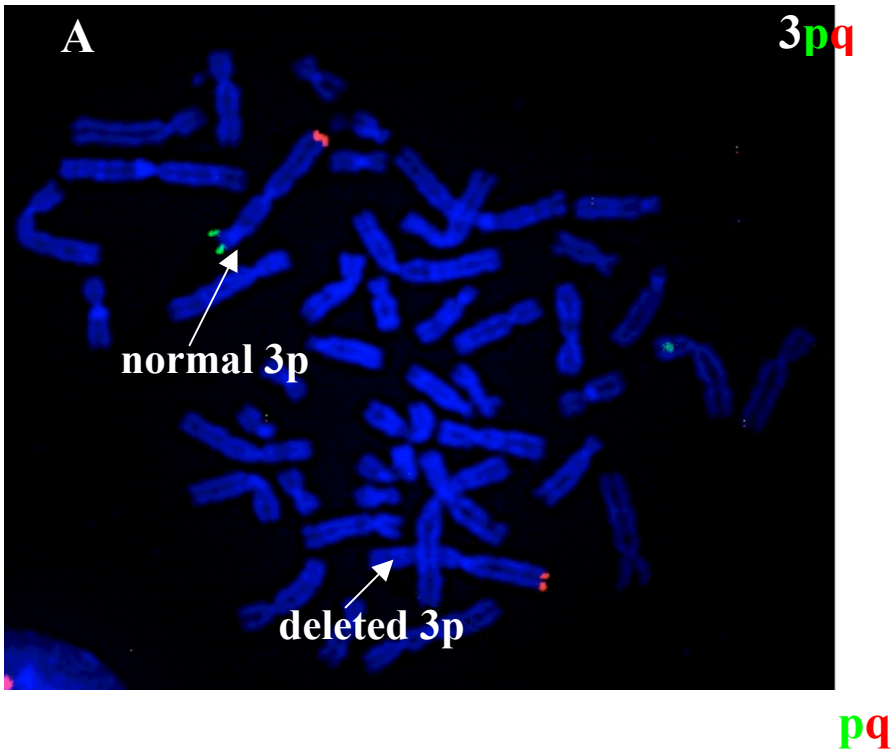
Clone	Location (Mb) from 1p telomere	FISH signal
RP11-465B22 (1pter)	1.05	+/-[a]
RP5-1092A11	3.57	+/-
RP11-374C13	4.09	+/-
RP11-51D17	4.2	+/-
RP11-168B8	4.22	+/-
RP11-111O5	4.37	+/+[b]

[a]FISH signal is present on one chromosome 1p,

[b]FISH signal is present on both of chromosome 1p

Patient 3

Patient 3 was a 6-year-old boy with mental retardation and multiple congenital anomalies. Standard cytogenetic analysis showed a normal male karyotype. However, FISH analysis showed monosomy for the short arm of chromosome 3 and duplication for the short arm of the X or Y chromosome. A further study using locus-specific probes examined the metaphase chromosomes. Locus-specific probes of the p-arm of the X chromosome showed an extra signal on the p-arm of chromosome 3, and there was one signal on the p-arm of Y chromosome when the p-arm of the Y chromosome was probed, indicating that the extra material on the short arm of chromosome 3 originated from the short arm of the X chromosome. The karyotype of the patient was defined as 46,XY.ish der(3)t(X;3)(p22.3;p26.3)(RP13-465B17+;RP11-306H5-)dn (Fig.11). Standard cytogenetic and FISH analysis of the parental peripheral blood were normal.



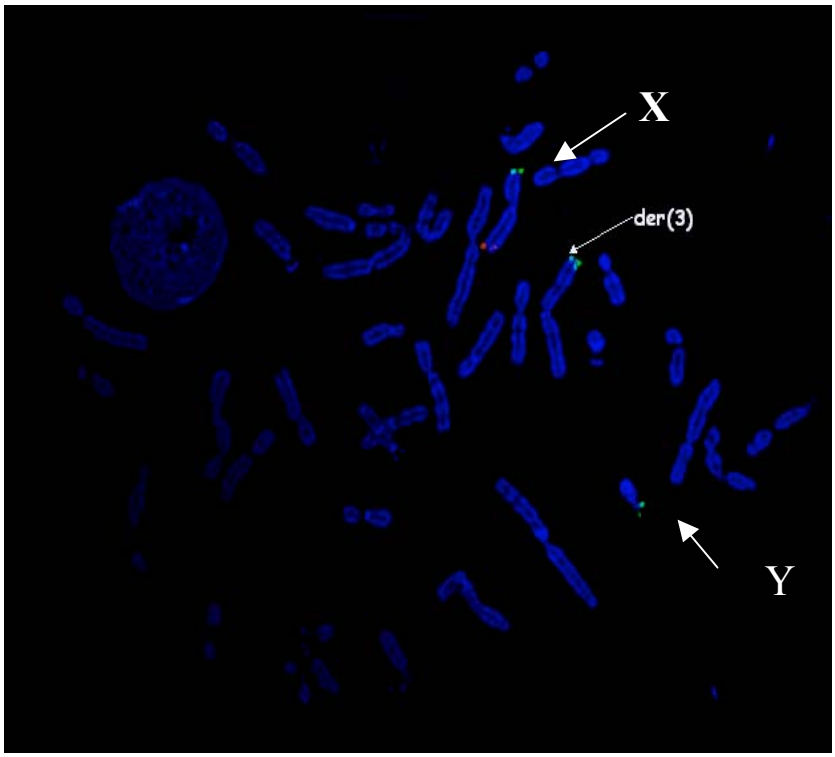


Figure 11 Subtelomeric FISH studies of the metaphase chromosomes from Patient 3. (A) A subtelomeric-specific probe for 3pq; RP11-306H5 (3pter; green), RP11-114F20 (3qter; red) found a green signal on one 3p missing, while two red signals were found on 3q. (B) A subtelomeric-specific FISH probe for Xpq; RP13-465B17 (XYpter; green), RP11-954J6 (Xqter; red), found an extra Xpter (green) signal on the short arm of chromosome 3.

Patient 4

A 5-year-old boy was referred for evaluation of mental retardation and multiple congenital anomalies. Subtelomeric-specific FISH analysis was performed and showed that he had a terminal 4p deletion (Fig.12). The karyotype of the patient was defined as 46,XY.ish del(4)(p16.3)(GS-36-P21-). Parental peripheral blood was not available.

pq

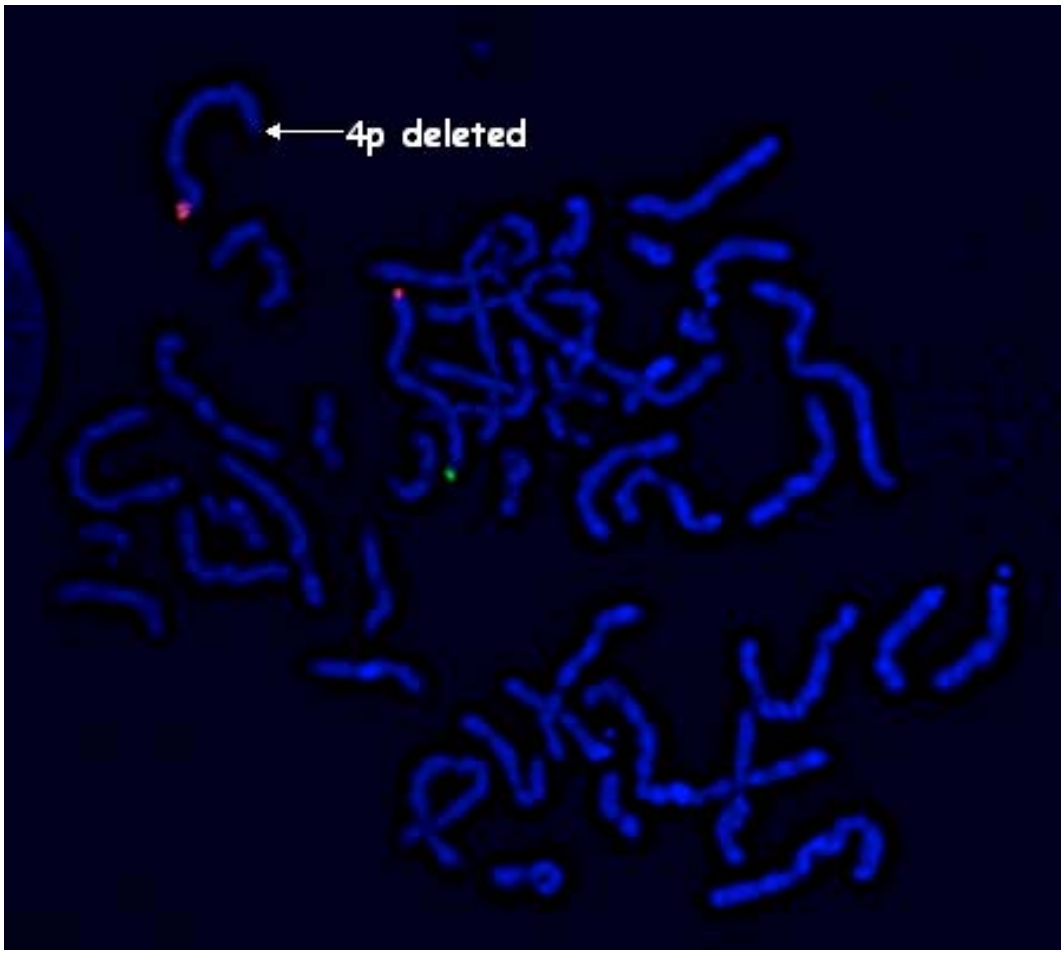


Figure 12 Subtelomeric FISH studies of the metaphase chromosomes from Patient 4. The results of a subtelomeric-specific probe for 4pq; GS-36-P21 (4pter; green), RPCI-11-463J17 (4qter; red) showed that the green signal on one 4p was missing, indicating a deletion.

Patient 5

Patient 5 was a 15-year-old girl with mental retardation and multiple congenital anomalies. Routine cytogenetic analysis showed a normal female karyotype. FISH analysis showed that this patient was monosomic for the long arm of chromosome 10 and trisomic for the short arm of chromosome 7 (Fig.13). The karyotype of the patient was defined as 46,XX.ish der (10)t(7;10)(p22.3;q26.3)(GS-164-D18+;RP11-108K14-). This patient was comparable to an

individual with trisomy 7p and monosomy 10q. Cytogenetic and FISH analysis of the parental peripheral blood were not available.

In addition, the physical examinations of five subtelomeric rearrangement patients are summarized in Table 9.

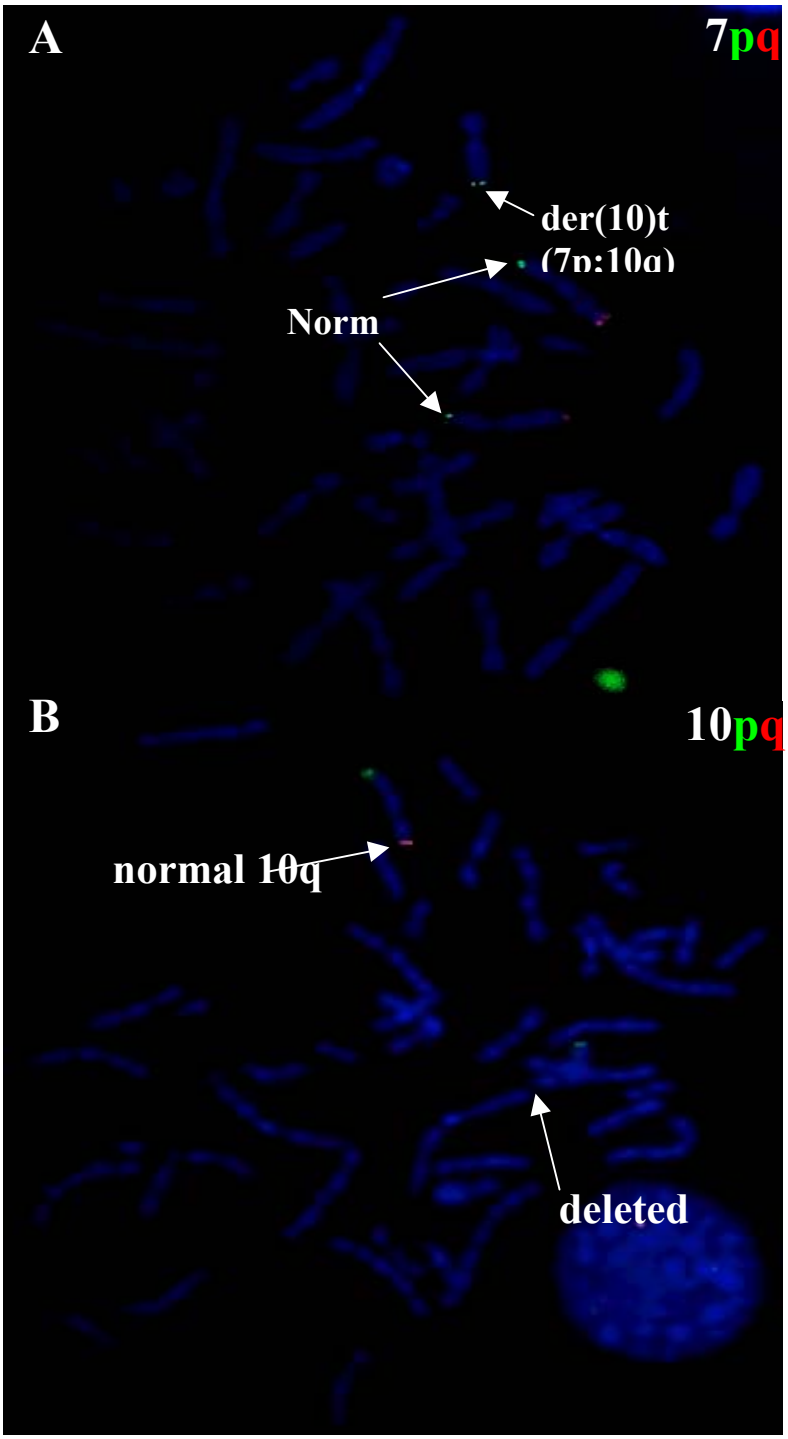


Figure 13 Subtelomeric FISH studies of the metaphase chromosomes from Patient 5. (A) The subtelomeric-specific probe for 7pq; GS-164-D18 (7pter; green), GS-3K-23 (7qter; red)) showed an extra 7pter (green) signal on the long arm of chromosome 10. (B) The subtelomeric-specific FISH probe for 10pq; RP11-468H9 (10pter; green), RP11-108K14 (10qter; red) showed that a red signal on one 10q was missing, while there were two green signals on 10p.

Table 9 Summary of physical examinations of subtelomeric rearrangement patients

Cases	Physical examinations	Other examination
Patient 1; 3-year-old boy 46,XY.ish der(20)t(X;20)(pter+,qter-)*	Mild hypertelorism, Simian crease bilaterally, Otherwise within normal limits	1. IQ = 42
Patient 2; 8-year-old girl 46,XX.ish del(1)(pter-)*	Growth retardation (height and weight below 3rd percentile) Triangular face with flat mid-face, prominent forehead, simple ears Cleft palate, congenital heart disease (VSD, PDA, coarctation of aorta)	1. IQ < 24 2. Normal brain MRI 3. Normal vision and hearing tests
Patient 3; 6-year-old boy 46,XY.ish der(3)t(X;3)(pter+,pter-)*	Dolichocephaly, prominent forehead, fair hair, upslanted eyebrows, mild hypertelorism with downslant, palpebral fissure, downturned mouth, low posterior hair line, normal chest, heart, abdomen, shawl scrotum	1. IQ < 30 2. Normal EEG, hearing test, skull x-ray
Patient 4; 5-year-old boy 46,XY.ish del(4)(pter-)*	Normal head size (head circumference = 50.5 cm at 3.8 years old), dolichocephaly, tall and prominent forehead, prominent ears with thick helix bilaterally, wide nasal bridge, normal chest, heart, abdomen, extremities and genitalia, one sacral dimple	1. IQ=28-30 2. EEG = abnormal, compatible with seizure disorder 3. Normal hearing test, normal thyroid function test

Patient 5; 15-year-old girl 46,XX.ish der(10)t(7;10)(pter+,qter-)*	Growth retardation (height and weight below 3rd percentile) Microcephaly, low posterior hair line, webbed neck, left esotropia, low set ears, downturned mouth cubitus valgus, Simian crease on right hand	1. IQ = 39 - 41

* according to ISCN, 2005