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Molecular versus classical cytogenetics – evaluation of 20 Prader-Willi syndrome patients

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Abstract. Prader-Willi syndrome (PWS) is a developmental disorder caused by a deficiency of paternal contribution of the chromosome region 15q11.2-q13 arising from differently sized deletions, maternal disomy, or rarely imprinting mutations. We have analyzed 20 PWS patients using combined cytogenetic high resolution technique (HRT), fluorescence in situ hybridization (FISH) and molecular studies to identify parental origin (uniparental disomy) or molecular defect (deletion) of the Prader-Willi region. Lack of a paternal copy of 15q11.2-q13 resulting from its deletion was found in 16 patients. Using high resolution GTG banding on prometaphase chromosomes, deletion in the 15q11.2-q13 region was detected in only 8 patients. Application of FISH with different sets of PWS specific unique sequence probes (D15S11, SNRPN, D15S10, GABRβ3) revealed microdeletions in 12 patients. In 12 out of 20 cases FISH confirmed HRT studies, while in 8 cases inconsistent results were obtained. No discrepancies between results of FISH and molecular studies were found, although the latter had a higher sensitivity. We conclude that FISH appears to be a rapid and reliable method of microdeletion identification and should be performed as a method of choice in cytogenetic diagnosis of Prader-Willi syndrome.

Key words: chromosomal deletions, cytogenetics, fluorescence in situ hybridization (FISH), Prader-Willi syndrome.

Introduction

Prader-Willi syndrome is a complex developmental and neurobehavioural disorder caused by the lack of paternal contribution of genes within human

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chromosome region 15q11.2-q13. It occurs at a frequency of about 1/10 000-25 000 and is characterized by neonatal hypotonia and feeding difficulties, developmental delay with mental retardation, obesity associated with uncontrolled appetite, behavioral disorder, hypogonadism, short stature and dysmorphic facial features (BUTLER 1990, HOLM et al. 1993).

About 70-75% of PWS patients have the deletion involving the 15q11.2-q13 region. In the majority of the remaining 20-25% patients the inheritance of two chromosomes 15 of maternal origin can be identified (uniparental disomy – UPD). About 1% of the cases represent abnormal methylation patterns resulting from putative imprinting center (IC) abnormalities (BUITING et al. 1995). Parental origin of deletion in PWS, as well as the occurrence of maternal UPD suggest that the PWS genes are active on the paternal chromosome only. So far, four loci expressed from the ~350 kb Prader-Willi critical region (PWCR) on the paternal homologue have been identified: *SNRPN* (small nuclear ribonucleoprotein N) (ÖZÇELIK et al. 1992), *PAR-1*, *PAR-5* and *IPW* (imprinted gene in the Prader-Willi syndrome region) (WEVRICK et al. 1994). This contiguous gene syndrome is distinct from the Angelman syndrome (AS), most probably monogenic disorder, caused by a deficiency within the maternal 15q11.2-q13 chromosomal region. The frequency and diversity of this ~4 Mb region's (containing > 100 genes) abnormalities suggests that it is structurally complex, unstable and prone to breakages and rearrangements (KNOLL et al. 1993, TOTH-FEJEL et al. 1995). Variations of these chromosome aberrations include (micro)deletions, pericentric inversions, (micro)du- or triplications, translocations and supernumerary inv dup (15) chromosomes (RIVERA et al. 1990, SCHINZEL et al. 1994, WOODAGE et al. 1994, JAUCH et al. 1995). It has been postulated (ZACKOWSKI et al. 1993, BUTLER 1994) that chromosome 15 with a large short arm heterochromatic region (classical satellite DNA - *D15Z1*) may be more prone to de novo deletions (BUTLER 1994). The approximately 10 times higher rate of meiotic recombination within this region indicates that in the Prader-Willi chromosome region gene density is much higher than the human genome average (ROBINSON, LALANDE 1995). This seems also to be confirmed by exceptional CpG clustering which indicates that genes in chromosome 15 are not preferentially distributed to T bands (telomere located subset of R bands) but along 15q (CRAIG, BICKMORE 1994). This region has also been investigated in relation to replication timing, showing allele specific asynchrony (KNOLL et al. 1994).

Recent advances in molecular cytogenetics using fluorescence in situ hybridization (FISH) with chromosome-specific DNA probes have made the cytogenetic diagnosis of microdeletion syndromes a much easier and quicker

task (RAO et al. 1995). The construction of a complete YAC contig of the Prader-Willi chromosome region (MUTIRANGURA et al. 1993) enabled the creation of YAC and cosmid contig probes, which are now used in FISH studies.

The purpose of this study was to asses the usefulness of the FISH technique in diagnosis of PWS and to compare it with the standard cytogenetic high resolution technique with reference to the results of molecular studies.

Material and methods

A total of 20 families with a typical or suspected PWS proband were ascertained using Holm's criteria (HOLM et al. 1993) by a clinician in our Department.

Classical cytogenetic study

Chromosome preparations were obtained from PHA stimulated lymphocyte cultures synchronized with Metotrexat or thymidine according to the Yunis method (YUNIS 1976). GTG banded chromosomes were analyzed at the resolution level of ~850 bands (19 patients) or ~550 bands (1 patient). Each case was analyzed by two cytogeneticists.

Fluorescence in situ hybridization (FISH) procedure

Digoxigenin labelled, unique sequence PWS/AS molecular probes were purchased from Oncor (Gaithersburg MD, U.S.A.). These products include four cosmid probes of 30-80 kb: D15S11, SNRPN, D15S10 and GABRβ3. The region A probe – D15S11 containing approximately 60 kb cosmid contig DNA and the region B probe – GABRβ3 consisting of ~80 kb of DNA sequence flank the PWCR region on both sides. The SNRPN probe is composed of two overlapping cosmids which span approximately 40 kb, and is mapped within PWCR, whereas D15S10 within the Angelman syndrome critical region (ANCR) (Fig. 1). Each PWS/AS chromosome region probe included a chromosome 15 control probe PML, located in 15q22 to facilitate chromosome identification. These were applied and detected according to the protocols provided by the manufacturer. In each case, two or more probes were used. Slide preparation and microscopy are described elsewhere (BOCIAN et al. 1996). A minimum of 30 metaphase cells were analysed paying special attention to signal intensity differences (SUN et al. 1996), as well as to possibilities of marker chromosome presence or mosaicism.

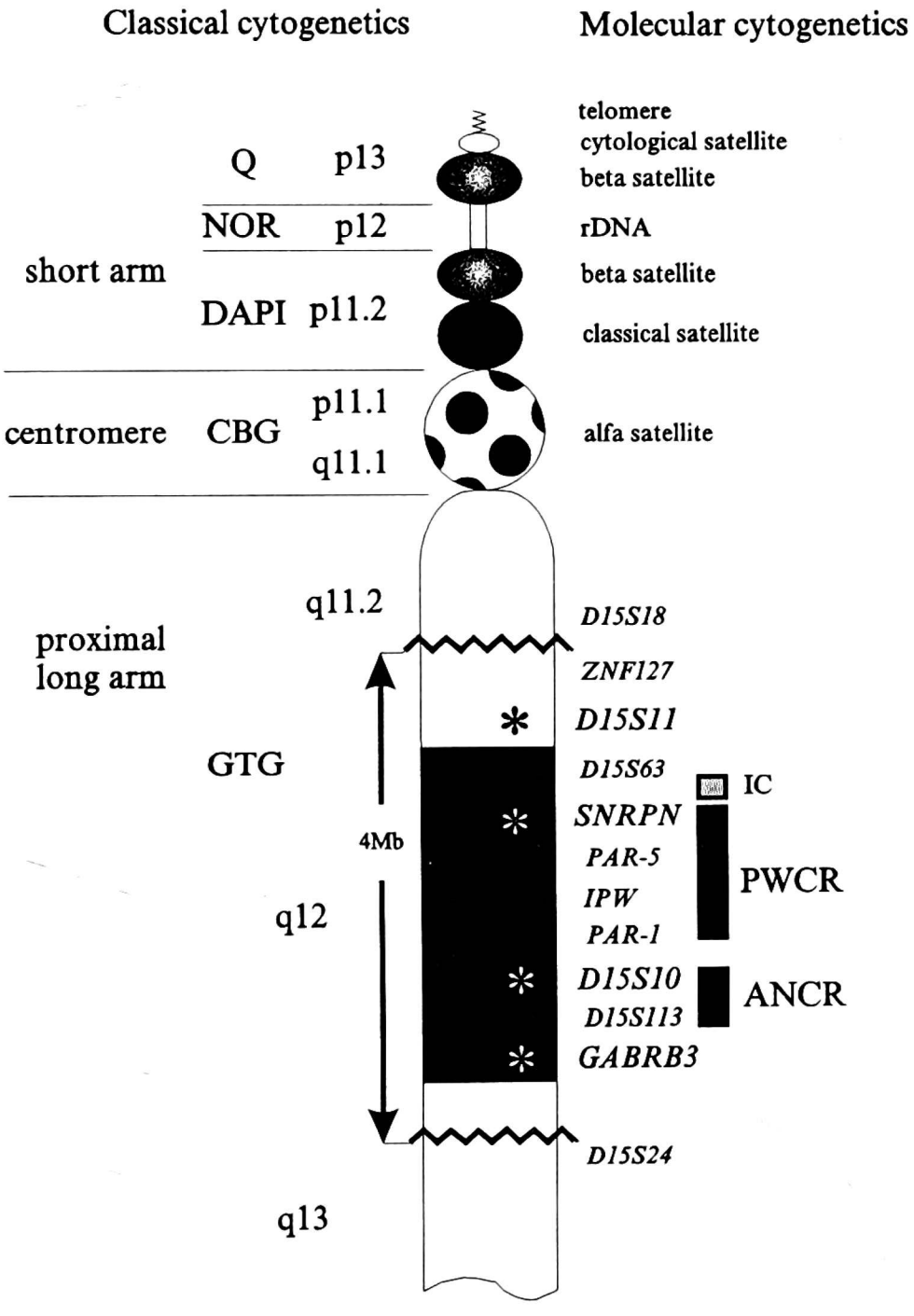

Fig. 1. Schematic representation of chromosome 15 short arm and proximal long arm with a common deleted Prader-Willi/Angelman chromosome 15q11.2q13 region containing PWCR and ANCR (map not to scale) (FRIEDRICH et al. 1996)

Molecular investigations

DNA methylation analysis

Southern blot analysis on genomic DNA from peripheral blood lymphocytes was performed according to standard procedures in 19 probands. DNA methylation patterns were analysed in the D15S63 locus using the PW71B probe after HindIII + HpaII or BglII + CfoI digestion, and in the ZNF127 locus using the pML34 probe after HpaII + Hind III digestion.

Table 1. Comparison of HRT, FISH and molecular studies in patients referred for Prader-Willi syndrome

No	Patient	Classical cytogenetics HRT karyotype	FISH probes D15S11	SNRPN	D15S10	GABRβ3	Molecular result	Concordance HRT/FISH	FISH/molecular
1	M.M.	46,XX	–	–			del	No	Yes
2	Z.A.	46,XX,del(15)(q11.2q13)	–		–	–	del	Yes	Yes
3	T.A	46,XX	–	–			del	No	Yes
4	R.E.	46,XX	–	–			del/UPD	No	Yes
5	L.M.	46,XX,del(15)(q11.2q13)	–		–	–	del/UPD	Yes	Yes
6	M.P.	46,XY	+			+	0	Yes	Yes*
7	S.P.	46,XX,del(15)(q11.1q13)	–		–	–	del/UPD	Yes	Yes
8	K.J.	46,XX	+	–		+	del	No	Yes
9	R.P.	46,XY,del(15)(q11.2q13)	–	–	–		del	Yes	Yes
10	W.A.	46,XX,del(15)(q11.2q11.2)	+	+			del	No	Yes*
11	A.A.	46,XY	+	+			UPD	Yes	Yes
12	S.P.	46,XY,der(14;15)	–	–	+	+	del	Yes	Yes
13	S.Ł.	46,XY	+	+			part. UPD	Yes	Yes
14	T.M.	46,XY	+	+			0	Yes	Yes
15	D.K.	46,XY,del(15)(q11.2q11.2)	+	+			0	No	Yes
16	S.B.	46,XY,del(15)(q11.2q13.1)	–		–	–	del/UPD	Yes	Yes
17	S.M.	46,XX		+	+		del	Yes	Yes*
18	S.P.	46,XY		+	+		impr.	Yes	Yes
19	S.K.	46,XY**		–		–	uninf.	No	Yes
20	K.K.	46,XY		–		–	del/UPD	No	Yes

– signal was present only on one chromosome 15 – deletion was found
+ signal was present on two chromosomes 15 – no deletion was found
* no discrepancy was found
** 550 GTG bands
del/UPD – molecular analysis could not distinguish between UPD and deletion
part. UPD – partial UPD
impr. – imprinting mutation
uninf. – uninformative results
0 – laboratory studies showed no abnormalities

Microsatellite polymorphism and *SNRPN* deletion analysis

Polymerase chain reaction (PCR) amplification was performed for $(CA)_n$ repeats polymorphism in 17 families at the D15S11, SNRPN, D15S10, D15S113, GABRβ3, GABRβ5 or D15S144 locus. Analysis of deletion of the *SNRPN* gene was assessed in 12 patients using Southern blot hybridization with the SmN1 probe after BglII + CfoI digestion.

Details of molecular investigations are described elsewhere (BUITING et al. 1994, SZPECHT-POTOCKA et al. 1994).

Results

The overall outcomes of HRT, FISH and molecular studies in patients referred for Prader-Willi syndrome are summarized in Table 1. Our studies confirmed previous clinical PWS diagnosis in 17 cases and questioned it (but did not rule it out) in 3 cases. Molecular results showed the highest efficiency identifying 14 deletions, 2 uniparental disomies (one of them identified as a partial UPD) and 1 imprinting mutation. Eight cases were found to be chromosomally abnormal using G-banded prometaphase chromosomes. In 5 of them, common, large deletions were identified (Fig. 2). In 2 cases, the deletion was interpreted as 15q11.2. In the eighth one, unbalanced Robertsonian translocation t(14;15)(p11.2;q12) was found. Of these cytogenetically visible aberrations, FISH confirmed the presence of 6 deletions and did not show both the small q11.2 deletions although two most proximal probes (D15S11, SNRPN) were used. This suggests false positive HRT results. In 6 cytogenetic non-deletion cases, FISH identified microdeletions of various sizes. In 6 cases only FISH enabled the identification of deletion. In one case, the microsatellite polymorphism analysis was not possible because the probands parents were unavailable and in 5 cases molecular results were insufficient to distinguish between disomy and UPD. In 3 patients, in which results of CA repeats polymorphism were uninformative in some loci, FISH helped to assess more precisely the size of the deletion. FISH results showed no discrepancy with molecular results in all the studied cases.

Discussion

Cytogenetic deletion of proximal 15q is difficult to detect due to the size of this region, its proximity to the centromere, its heteromorphic nature with a tendency to bend and variability in its homologue condensation (KNOLL et

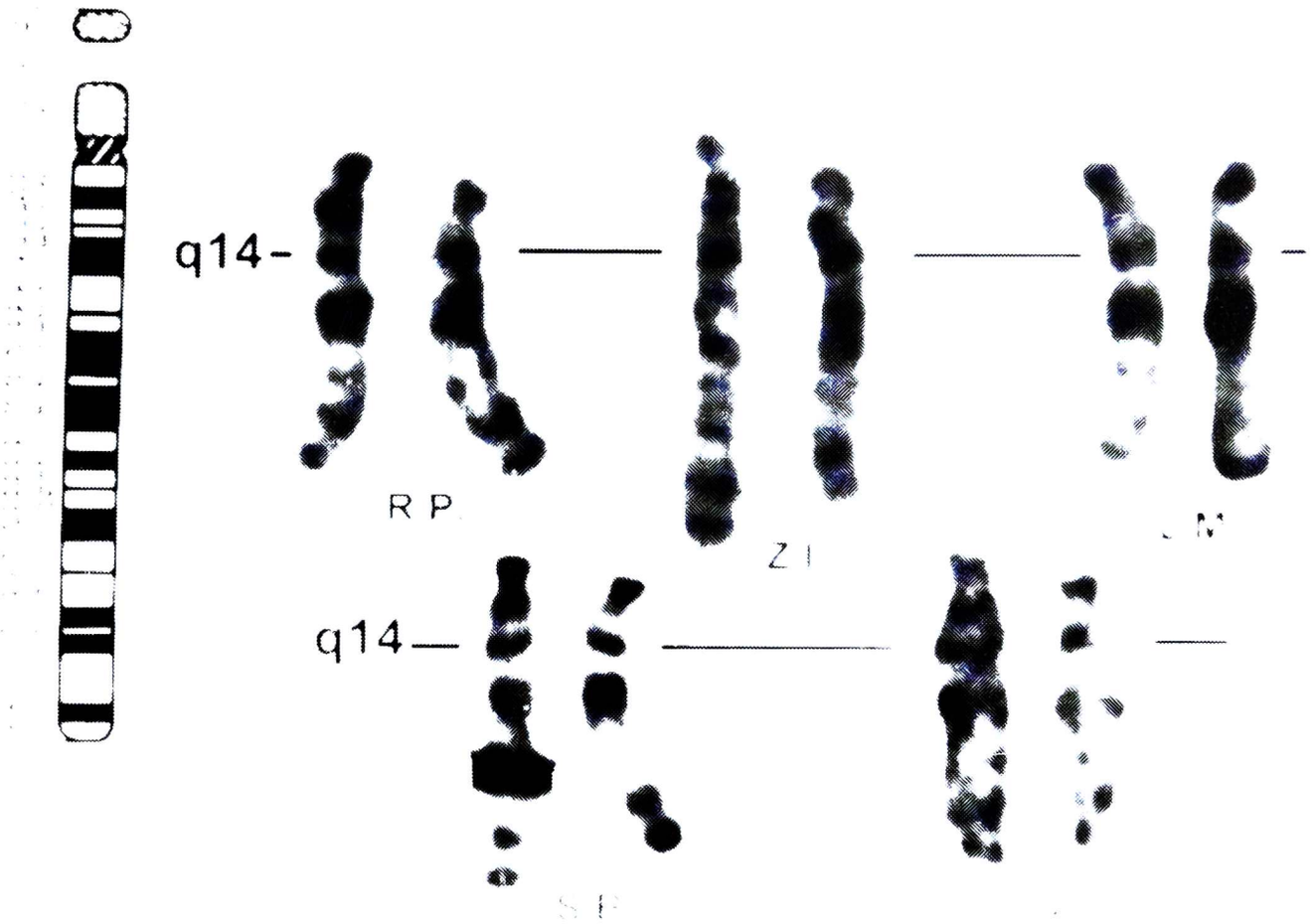

Fig. 2. Ideogram and G banded chromosomes from five cytogenetic detectable large deletions del(15)(q11.2q13). Abnormal chromosomes in each pair are placed on the right

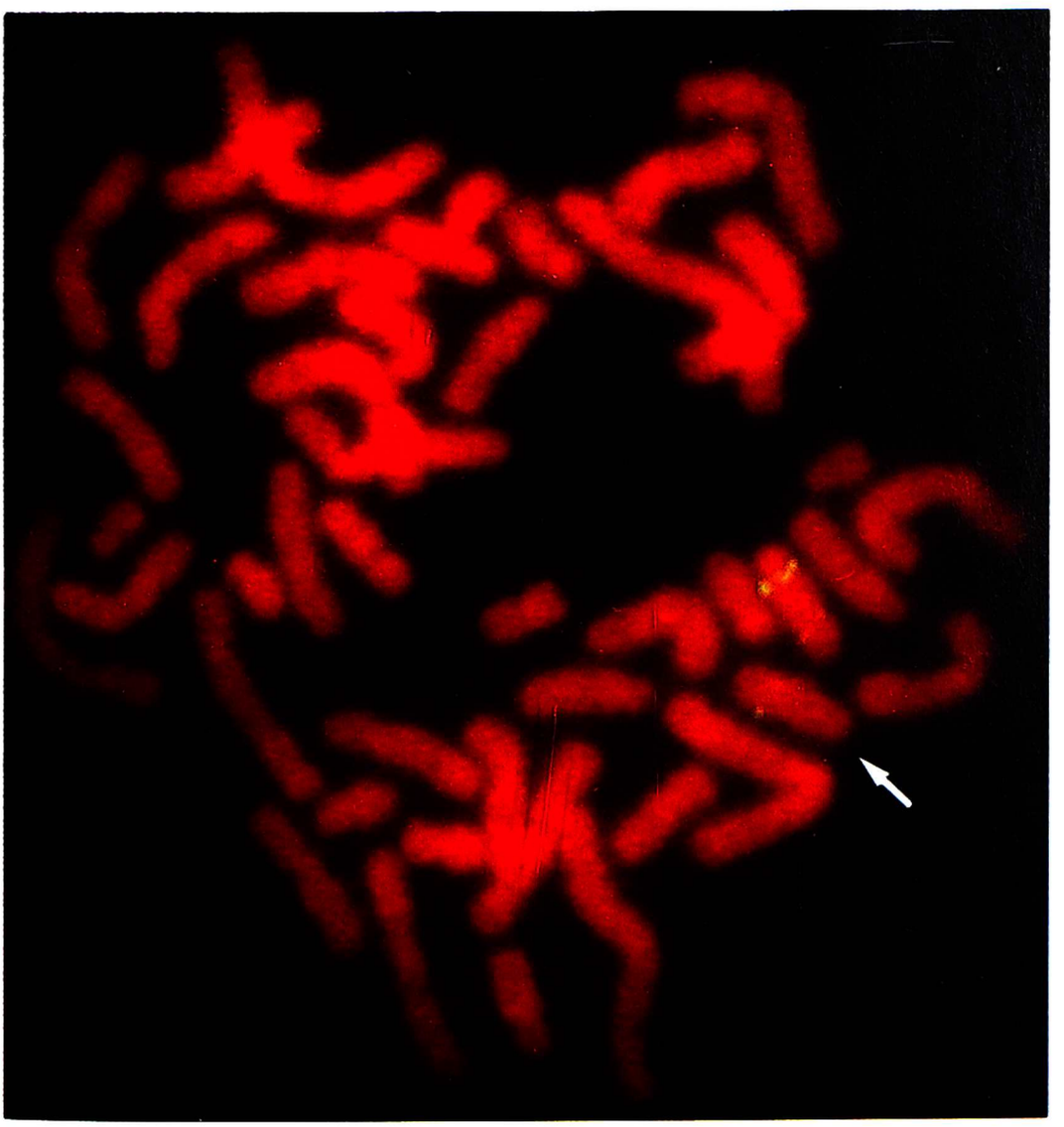

Fig. 3. Case 2 with chromosome 15 deletion identified after hybridization with D15S11 probe with PML control signal

al. 1993). Although the most common deleted fragment in PWS and AS is the same, AS deletions appear larger than PWS deletions when analyzed on prometaphase chromosomes (MAGENIS et al. 1990). This seems to be confirmed by a better concordance of FISH – HRT results in AS. This fact probably reflects differential homologue condensation – male chromosomes are more condensed (BUTLER 1995).

In 12 out of 20 cases FISH confirmed HRT studies, while in 8, inconsistent results were obtained. In 6 deletion cases in which HRT studies did not reveal any aberration, variously sized deletions were found using FISH and molecular analysis. It is difficult to say whether these cases are identifiable on prometaphase chromosomes because their physical size is unknown. Perhaps application of the RBG rather than the GTG banding technique would be more efficient in these cases (IMAIZUMI et al. 1990, ERDEL et al. 1996).

In two small deletion 15q11.2 cases false positive results of HRT studies are suggested. This may be explained by alpha satellite (*D15Z*) polymorphism or variation of some other sequences in the 15q11.2q13 region. Their variable appearance in high resolution G-banded chromosomes may lead to incorrect interpretation (DELACH et al. 1994). However, one can speculate that the deletions exist indeed, but do not encompass the investigated, more distal loci. Further studies are needed to clear these discrepancies.

Our data in this study are similar to others reported previously. Similar proportions of false positive and false negative HRT results as in the present study were found in DELACH et al. 1994, BETTIO et al. 1995, BUTLER 1995, TOTH-FEJEL et al. 1995, ERDEL et al. 1996. These results demonstrate that all patients suspected of PWS should be studied using FISH irrespective of high resolution chromosome results. FISH analysis is less time-consuming and requires less cytogenetic expertise than does high-resolution cytogenetics. In cases where the probands parents are unavailable or molecular results are uninformative, its application unequivocally distinguishes between deletion and UPD. In many cases it helps to better define the genetic size of the deletion.

In cases where a deletion has been found and parental chromosomes revealed an unusual pattern, cryptic translocations with other acrocentric chromosomes should be ruled out since overrepresentation of chromosome 15 Robertsonian translocations in the PWS population was found (KNOLL et al. 1993, BUTLER 1995, BURKE et al. 1996). That fact is very important for genetic counselling because in case of a translocation in the family, the risk of PWS in the offspring is raised from populational to 25% (BURKE et al. 1996). Therefore, the co-hybridization procedure (possibly, dual color) with a classical satellite (*D15Z1*) or an α-satellite (*D15Z*) probe in conjunction with one of the PWS probes with

PML control is suggested (BURKE et al. 1996, ERDEL et al. 1996, ASHG/ACMG REPORT 1996). At the same time, chromosomal pericentric inversion 15 inv(p11q13) could be excluded and heterochromatin polymorphism assessed when necessary.

It is not only our opinion that FISH should complement rather than replace standard chromosome analysis which may reveal other than simple deletion aberrations. The same seems to hold true for other microdeletion syndromes although in some of them (e.g. Miller-Dieker syndrome) complete replacement by FISH was suggested (KUWANO et al. 1992).

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