

FLUORESCENCE IN SITU HYBRIDIZATION AND SINGLE NUCLEOTIDE POLYMORPHISM OF A NEW CASE WITH INV DUP DEL(8p)

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Summary: *Fluorescence In Situ Hybridization and single nucleotide polymorphism of a new case with inv dup del(8p):* Inverted duplication deletion of 8p [inv dup del(8p)] is a complex chromosome rearrangement leading among others to deletion of the chromosome region distal to the duplication in 8p. A new case with an inverted duplication deletion of 8p and the results of SNP-array analysis and fluorescence in situ hybridization (FISH) are reported here. Our results are in concordance with earlier reported inv dup del(8p) cases.

Key-words: Inv dup del(8p) -- FISH -- SNP-array analysis

INTRODUCTION

Inv dup del(8p) is a well known chromosome abnormality with the p-arm of chromosome 8 consisting of a deleted distal segment, an intact intermediate segment and a duplicated proximal segment (1, 3, 7). The estimated prevalence is 1/10,000–30,000 newborns (3). The phenotype of the inv dup del(8p) patients involves anomalies of the central nervous, orthopedic and cardiac systems, mental retardation with specific involvement of speech and multiple facial dysmorphisms (2, 5, 8). Inverted duplications of 8p have been shown to consistently occur in maternally derived chromosomes, presumably at meiosis I (1, 3, 4, 7). Molecular analysis of both 8p duplications and 8p interstitial deletions showed that all cases shared similar chromosomal breakpoints located within regions containing olfactory receptor gene clusters (1, 4, 7). The high incidence of chromosome abnormalities involving this region suggests that there is an underlying propensity for rearrangement in the DNA. Here, we report on the postnatal diagnosis of a case of inverted duplication deletion of 8p.

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CLINICAL REPORT

The patient, a girl, was the first and only child of non-consanguineous healthy parents of Turkish origin. At time of birth, the father was 30 and the mother was 29 years old. Their family histories were normal. There was no exposure to toxins or known teratogens. The baby was delivered by normal spontaneous vaginal delivery at 34 weeks. Birth weight was 1830 (25-50 percentile), length was 44 cm (25-50 percentile), and head circumference was 30.5 cm (25-50 percentile). She had one artery and one vein in her umbilical cord. Transfontanel USG and cranial MRI showed agenesis of the corpus callosum. At the age of 7 months, she was referred to the genetic clinic for further diagnostic evaluation. Psychomotor development was moderately retarded (developmental level of 3 months on the Denver developmental scale). Weight was 5.7 kg (3-10 percentile), length was 64 cm (25-50 percentile), and head circumference was 39 cm (<3 percentile). Except for manifest axial hypotonia, neurological findings were normal. Her facial appearance (Fig. 1) was mildly dysmorphic with anteriorly rotated ears, strabismus, iris and optic disc coloboma, anteverted nares, thin upper lip and high arched palate (Table I). Now, at 2 years of age, psychomotor development is severely retarded with a motor developmental level of 10 months and cognitive level of 13 months (Bayley developmental scale). Routine screening of blood and urine aminoacids, cardiac and renal echographies, and computed tomography of the brain showed no abnormalities.



Figure 1: General view of the patient.

Table I: Summary of the clinical findings.

Clinical manifestation	Present Case
Sex	F
Craniofacial	
Microcephaly	+
Frontal bossing	+
Malformed ears	+
Thin upper lip	+
High arched palate	+
Abnormal maxilla or mandible	-
Ocular	
Strabismus	+
Coloboma	+
Cardiac	-
Skeletal	-
Cns	
Early hypotonia	+
Mental retardation	+
Agenesis of corpus callosum	+

GENETIC STUDIES

Chromosomes were prepared from peripheral blood lymphocyte cultures following standard procedures. Analysis of GTG-banded chromosomes was done at a resolution of at least 550 bands per haploid genome. Fluorescent *in situ* hybridization (FISH) was done on metaphase spreads as previously described (6) with a chromosome 8 specific paint, and with probes RP11-16H11, RP4-660C16 and 75P13 specific for chromosome band 8p23.2, chromosome band 8p21.3 and chromosome band 8p12, respectively.

Furthermore, 250K NspI SNP genotyping array analysis (Affymetrix UK Ltd, High Wycombe, UK) was performed according to the manufacturer's specifications (http://www.affymetrix.com/Auth/support/downloads/package_inserts/genomewide_snp5_insert.pdf). Briefly, 250 ng of DNA was digested with NspI; adaptors were subsequently ligated, followed by PCR amplification. Next, the PCR product was fragmented, labeled and hybridized to the 250K NspI SNP array. Genotype calls were obtained and analysed using the Affymetrix Genotype Console 2.1. For copy number estimation, data were processed

using the Affymetric Genotype Console 2.1. The SNP mapping data from the patient and her parents were analyzed sex-matched against a 25 female or 25 male reference set obtained on the same platform using unaffected individuals.

RESULTS AND DISCUSSION

GTG-banded chromosome analysis showed an aberrant p-arm in one of the chromosomes 8, suggestive for an inversion duplication (Fig. 2). To refine the nature of the chromosome aberration, FISH analyses were done on metaphase spreads. Chromosome painting showed fluorescence over the complete length of the normal and aberrant chromosome 8. FISH with probe RP11-16H11 showed a deletion of chromosome region 8p23.2->8pter (Fig. 3) and FISH with the probes RP4-660C16 and 75p13 showed 2 fluorescent signals on the aberrant chromosome 8p and 1 fluorescent signal on the normal chromosome 8 (Figs 4 and 5). Combining the results of GTG-banding, chromosome painting and FISH with the band-specific probes, we conclude that the patient has an aberrant 46,XX,inv dup del (8)(p11.1p23.2) karyotype. The father and the mother had a normal karyotype.

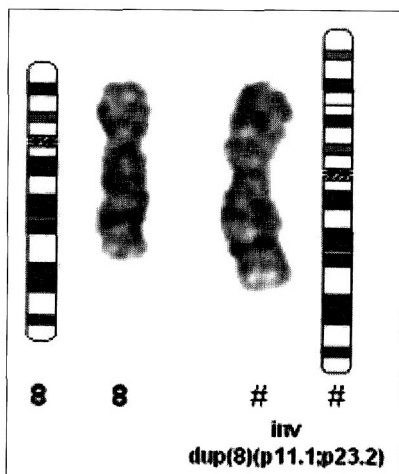


Figure 2: GTG-banded karyotype with normal chromosome 8 on the left and aberrant chromosome 8 on the right.

Subsequently, SNP data analysis of the proband showed an 6.99 Mb distal deletion, stretching out from the telomere to 8(p)(23.1), a normal 3.76 Mb chromosome region containing chromosomal material of band 8p23.1, followed by a 31.51 Mb duplication starting at 8(p)(23.1) and ending in the centromere (Fig. 6). This rearrangement sequence is in concordance with previous findings from the literature (1, 3, 7).

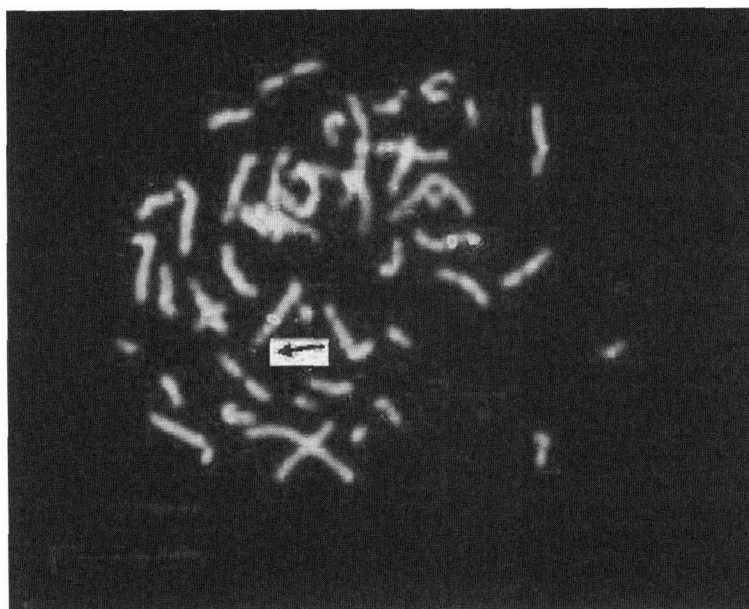


Figure 3: FISH with probe RP11-16H11 specific for chromosome band 8p23.2 showing the deletion.



Figure 4: FISH with probe RP4-660C16 specific for chromosome band 8p21.3

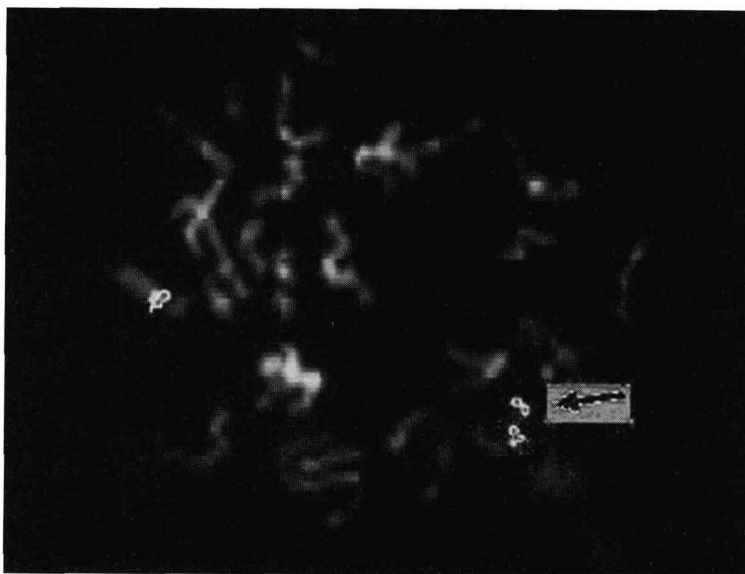


Figure 5: FISH with probe 75P13 specific for chromosome band 8p12 showing the duplication.

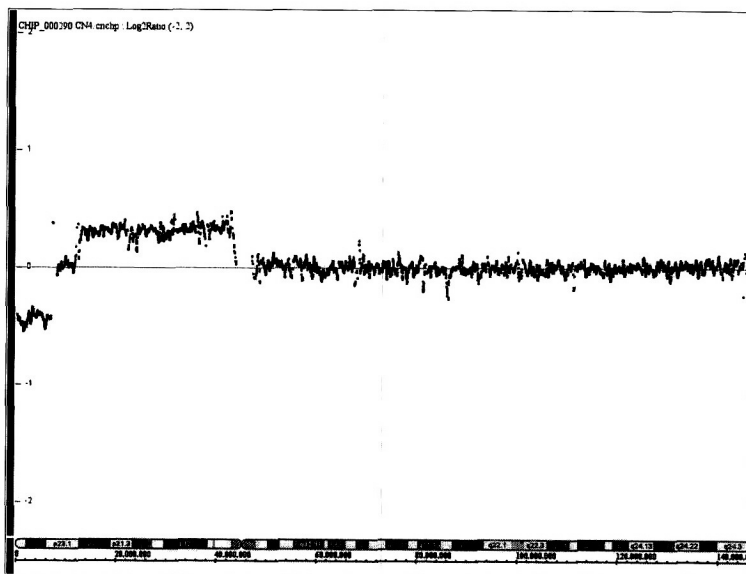


Figure 6: The Log2 AB SNP ratios in chromosome 8 from the proband. As can be seen, there is a distal deletion, followed by a normal intersection and a proximal duplication.

The deletion, which is marked by a single copy number, starts in the telomere and ends at basepair 6986630. In total, it encompasses 1518 SNP's that all showed the presence of one single copy. From these, 1271 SNP's had complete trio set (proband and parents all called) and were studied more in detail. This reveals that 81.56% (14.08% of the total SNP's) of the mismatched SNP's between proband and parents could only be explained by paternal origin, while none could be attributed to maternal origin. The remaining 19.44% did not match either parent, indicating miscalled SNP's. These results combined with a 1.91 ratio father-proband/mother-proband match showed that the aberration is of maternal origin. Between the deleted region and the 3.76 Mb normal region, a 1.12 Mb gap is present, in which the used Affymetrix 250 NspI chip does not contain any SNP. This region contains numerous repetitive DNA sequences and olfactory gene sequences (pseudo genes). The 3.76 Mb normal region, positioned between the deleted and the duplicated segments and defined by a copy number of 2, contains 480 SNPs present on the used Affymetrix 250K NspI chip. Out of these, 462 SNPs were a complete trio (proband and parents), and only in two SNP's the proband mismatched the parental SNP calls. No uniparental disomy (UPD) was found in this region. Proximal to the normal region, another gap is positioned. In this 73.7K region, devoid of any SNP on the array, olfactory genes and other repetitive DNA sequences have been reported (2). The proximal, duplicated region of the chromosome 8p arm, presenting as copy number 3 by the analysis software, is about 31.51 Mb in length and encompasses 3454 SNPs. Starting in 8(p)(23.1) and ending in the centromere region, it has its boundaries marked with SNP-less regions, being the above described gap and the centromere. In 20 SNP trios, the proband calls did not match the expected possibilities of SNP types passed through the offspring. From these, 17 indicated maternal origin of the material (85%), while 3 could not be attributed to either parent and should be dismissed as call designation errors. Moreover the 2.52 ratio mother-proband/father-proband match shows that the additional duplicated material is most likely of maternal origin.

The present results are concordant with findings in previous reports. Indeed, SNP data analysis confirmed the previously described maternal origin of the rearranged chromosome (1, 3, 4, 7) and the breakpoints were found to occur, as previously described (1, 3, 4) within the gaps corresponding to the position of the olfactory receptor gene clusters.

In summary, we studied for the first time in the literature a Turkish family with an inverted duplication deletion of 8p [inv dup del(8p)] using fluorescence in situ hybridization (FISH) and SNP array analysis.

REFERENCES

1. *BUYASSE K., ANTONACCI F., CALLEWAERT B., LOEYS B., FRÄNKEL U., SIU V., MORTIER G., SPELEMAN F., MENTEN B.*: Unusual 8p inverted duplication deletion with telomere capture from 8q. *Eur. J. Med. Genet.*, 2009, 52, 31-36.
2. *DE DIE-SMULDERS C.E., ENGELEN J.J., SCHRANDER-STUMPEL C.T., GOVAERTS L.C., DE VRIES B., VLES J.S., WAGEMANS A., SCHIJNS-FLEUREN S., GILLESSEN-KAESBACH G., FRYNS J.P.*: Inversion duplication of the short arm of chromosome 8: clinical data on seven patients and review of the literature. *Am. J. Med. Genet.*, 1995, 20, 369-374.
3. *FLORIDIA G., PANTANIDA M., MINELLI A., DELLAECCHIA C., BONAGLIA C., ROSSI E., GIMELLI G., CROCI G., FRANCHI F., GILGENKRANTZ S., GRAMMATICOP P., DALPRAL, WOOD S., DANESINO C., ZUFFARDI O.*: The same molecular mechanism at the maternal meiosis I produces mono- and dicentric 8p duplications. *Am. J. Hum. Genet.*, 1996, 58, 785-796.
4. *GIGLIO S., BROMAN K.W., MATSUMOTO N., CALVARI V., GIMELLI G., NEUMANN T., OHASHI H., VOULLAIRE L., LARIZZA D., GIORDA R., WEBER J.L., LEDBETTER D.H., ZUFFARDI O.*: Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. *Am. J. Hum. Genet.*, 2001, 68, 874-883.
5. *GUO W.J., CALLIF-DALEY F., ZAPATA M.C., MILLER M.E.*: Clinical and cytogenetic findings in seven cases of inverted duplication of 8p with evidence of a telomeric deletion using fluorescence in situ hybridization. *Am. J. Med. Genet.*, 1995, 58, 230-236.
6. *LICHTER P., CREMER T., TANG C.J., WATKINS P.C., MANUELIDIS L., WARD D.C.*: Rapid detection of human chromosome 21 aberrations by in situ hybridization. *Proc. Natl. Acad. Sci. U S A.*, 1988, 85, 9664-9668.
7. *SHIMOKAWA O., KUROSAWA K., IDA T., HARADA N., KONDOH T., MIYAKE N., YOSHIURA K., KISHINO T., OHTA T., NIKAWA N., MATSUMOTO N.*: Molecular characterization of Inv dup del(8p): analysis of five cases. *Am. J. Med. Genet.*, 2004, 128, 133-137.
8. *YENAMANDRA A., PERRONE R., MCLAUGHLIN J., MEHTA L.*: Inverted duplication/deletion of chromosome 8p: mild clinical phenotype. *Am. J. Med. Genet.*, 1999, 82, 91-93.

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