

A DUPLICATION dup(4)(q28q35.2) *DE NOVO* IN A NEWBORN

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We report here a case of a newborn with hypotrophy and somatic stigmatization: microcephaly, facial dysmorphism, heart defect and immunodeficiency syndrome. The proband's karyotype was 46,XY,dup(4)(q28q35.2) *de novo* with chromosomal breaks in 4% of metaphases. We demonstrate the usefulness of a combination of physical examination, classical cytogenetics, FISH and PCR techniques in order to establish correct diagnosis because of overlap of some clinical and cytogenetic features of Nijmegen breakage syndrome (NBS) and duplication 4q in our patient. Although FISH technique detected translocation t(14q;21q) in 4 metaphases, deletion 657del5 in exon 6 of the NBS1 gene associated with NBS in Slavic population was not confirmed. We compare in this report similarity of the clinical picture of our patient, NBS cases and other patients carrying a duplication of the distal part of 4q as described in the literature.

INTRODUCTION

Duplication of the long arm of chromosome 4 has been already described in more than 60 patients^{1,2}. Most of them result from the meiotic segregation of a parental balanced translocation and associate with a partial monosomy of various segments of other chromosomes. Patients with partial trisomy 4q have variable clinical stigmatization, which is both related to the size of duplicated segment of the chromosomal arm 4q and to specific associated monosomy. A review of the literature² revealed approximately 20 cases of a pure duplication of 4q spanning the region from q12 to q35. Although simple or pure forms of duplications are less common they serve as a basis for a better understanding of relationships between chromosomal anomalies and their clinical manifestations.

In this communication we describe clinical, cytogenetic, FISH and PCR findings in a male newborn with an overlap in some clinical and cytogenetic features of Nijmegen breakage syndrome and duplication 4q. We illustrate here the usefulness of the combined views of the clinical geneticist, the cytogeneticist and the molecular geneticist in establishing the correct diagnosis. To our knowledge this is the second case of the pure *de novo* duplication 4q.

MATERIAL AND METHODS

Cytogenetic and FISH analysis were performed on cultured PHA-stimulated peripheral blood lymphocytes of the proband and his parents. Classical cytogenetics was carried out using G-banding. The whole chromosome painting probes 4, 7, 14 and 21 (Vysis) and chromosome specific telomeric probe 4q (Vysis) were used for detection of (the) suspected rearrangements by FISH.

PCR diagnosis was performed using a DNA extracted by salting-out method from peripheral blood as described previously³.

RESULTS

Clinical report

The boy was born in the 40th week of physiologic pregnancy. His birth weight was 2600 g and his length was 52 cm. The child was referred for genetic counselling at the age of 1 month because of somatic stigmatization.

The newborn was hypotrophic and manifested slight hypertonic syndrome. Physical examination at the age of 1 week revealed microcephaly with head circumference of 31 cm (– 4 SD). The facial features included antimongoloid slanted palpebral fissures, epicanthic folds, strabismus, broad nasal bridge, prominent beaked nose with anteverted nostrils, low-set malformed ears, high arched palate, micrognathia and a pigmented naevus in



Fig. 1. Photography of reported patient aged 7 months.



Fig. 2. Idiograms of the normal and abnormal chromosome 4 (left) and partial G - banded karyotype.

the occipital area. Other findings included slight bilateral contractures of the knee and bilateral coloboma of optic papilla. The patient also suffered from congenital heart defect – foramen ovale apertum and immunodeficiency syndrome.

A follow-up physical examination at the age of 7 month confirmed microcephaly (head circumference was 39 cm, i.e. – 3.6 SD) with the same dysmorphic features (Fig. 1). Moderate psychomotor delay was observed (developmental age estimated at 3 to 4 months). However, there was no growth retardation.

The propositus is the second child of young, healthy and unrelated parents (gravida 2, para 2). The first child of the parents is a healthy daughter. There was no previous history of manifestation of any inherited developmental defects or mental retardation in the family. Several malignancies have occurred in previous generations.

Classical cytogenetic analysis

Cytogenetic and FISH analyses were performed on cultured PHA-stimulated peripheral blood lymphocytes of the propositus and his parents. The lymphocyte culture showed a low mitotic index. The karyotype was 46,XY,dup(4)(q28q35.2)*de novo* with chromosomal breaks in 4 % of the metaphases. Both parental karyotypes were normal. Repeated cytogenetic investigation of the propositus at the age of 7 months revealed structural chromosomal aberrations in 2 % of the metaphases.

Fluorescence in situ hybridization

FISH analysis was performed on the propositus on the basis of classical cytogenetics using the whole chromosome painting probes 4, 7, 14 and 21 for detection of the suspected rearrangements of these chromosomes (Vysis). We explored that additive chromosomal material originated from chromosome 4 by FISH technique. Using the chromosome-specific telomeric probe 4q (Vysis), we detected only one signal on both the normal and abnormal chromosome 4. Translocation t(14q;21q) was found in 4 of 5 examined metaphases by FISH using the painting probes 14 and 21. The final karyotype of the patient was 46,XY,dup(4)(q28q35.2)*de novo*, ish dup(4)(q28qter)(wcp4+) (Fig. 2 and 3).

DNA analysis

PCR diagnosis of the suspected deletion 657del5 in NBS1 gene located on the 8q21 chromosome was performed with DNA extracted by salting-out method from peripheral blood (Fig. 4). However, this Slavic mutation of NBS1 gene was not detected in our patient.

DISCUSSION

Rapid introduction of molecular biology techniques into routine medical practice significantly improves both specificity and sensitivity of clinical diagnostics. This case report is a typical example of the rare clinical syndrome, duplication 4q, being diagnosed on the basis of molecular cytogenetics.

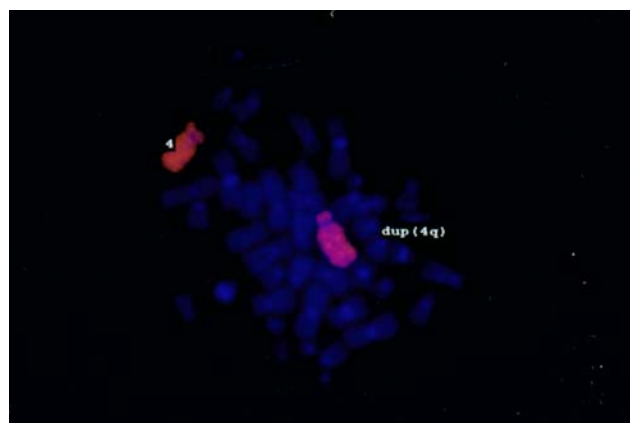


Fig. 3. FISH with whole chromosome painting probe 4 (Spectrum Orange, Vysis). Duplicated chromosome 4 is marked by arrows.

In our patient the large duplication of the distal part of 4q was detected by both the classical and molecular cytogenetic methods. Clinical and cytogenetic data are not currently sufficient to delineate a well-defined *dup(4q)* syndrome. Reviews of the literature^{4–7} and the recent report² confirm the phenotypic variability accompanying duplicated long arm of chromosome 4. The wide phenotypic variability frequently reported in cases of duplication 4q could be related to the different size of the duplicated region, the location of the breakpoints and associated monosomies of the other chromosomal parts. Well-recognized features of this duplication are growth retardation, psychomotoric retardation of variable grade, seizures, large low-set ears and high nasal bridge. Most patients have microcephaly, short narrow palpebral fissures, hypotelorism and/or broad nasal bridge, short philtrum, micro-/retrognathia, short neck, abnormalities of the extremities, feet and toes and anomalies of the hip joints. Cardiac defects are reported in patients with a large duplicated segment. Generally, severe stigmatisation has been observed in patients with a larger degree of partial trisomy and in the patients with duplications spanning the distal part of 4q. The segment 4q32→qter is genetically relatively deficient. However it contains the clinically important genes determining psychiatric illness, seizures and neurodegenerative disorders⁸. The reports of Goodman et al.⁹ and Maltby and Bennett¹⁰ show that the chromosomal segment 4q31.1→q32.3 can be duplicated with a minor or even no clinical effect.

Our proband has pure *de novo* duplication *dup(4)(q28qter)* confirmed by FISH. The duplicated region is relatively large and overlaps other previously reported cases. Celle et al.² described monozygotic twins with partially discordant phenotypes and the duplication 4q28.3→qter, originated from the unbalanced maternal translocation *t(4;22)(q28.3;p13)*. Thus our patient is the second case with the same partial trisomy and the first description of pure duplication without associated monosomy^{1,2}. Comparison of the clinical manifestations

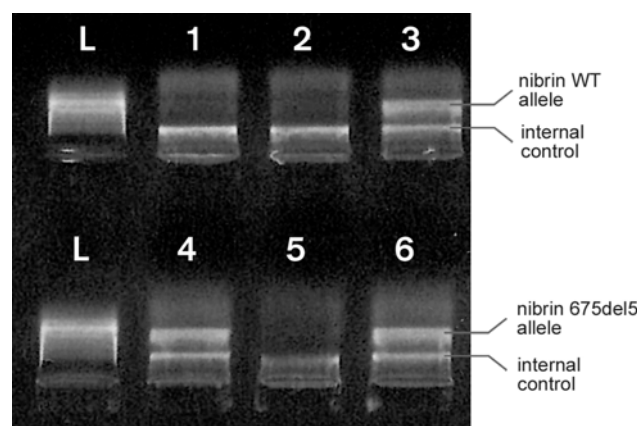


Fig. 4. Molecular diagnostics of 675del5 mutation of the nibrin gene using the sequence specific polymerase chain reaction with sequence specific primers. Shorter amplicon represents nibrin gene amplification product (wild type (WT) or 675del5 mutation) and longer internal control amplicon. L- DNA ladder (TopBio), 1- positive control for nibrin WT allele; 2- negative control for nibrin WT allele; 3- patient sample; 4- positive control for 675del5 mutation; 5- negative control for 675del5 mutation allele; 6- patient sample. 675del5 mutation in the patient was not confirmed.

in our patient and cases with pure duplication of 4q reported previously^{7,11–15} is summarized in Table 1. Our patient demonstrates majority of clinical features according to the criteria established by Halal et al.⁷ and Jeziorowska et al.¹⁵. On the other hand, he has neither renal hypoplasia nor thumb anomalies, which are consistently associated with the duplication of the interstitial segment 4q21→q22, as suggested by Zollino et al.¹⁶.

Clinical features of 4q duplication in our newborn share a lot of similarities with Nijmegen breakage syndrome. Nijmegen breakage syndrome belongs to the group of inherited chromosomal instability syndromes. The disorder is an autosomal recessive in nature, characterized by growth and mental retardation, craniofacial dysmorphism, immunodeficiency, chromosomal instability, predisposition to lymphoid malignancies and radiosensitivity¹⁷. Varon et al.¹⁸ identified the NBS1 gene on chromosome 8q21. The gene encodes protein nibrin, which participates in DNA repair. Interestingly, the authors detected the deletion 657del5 in exon 6 of the NBS1 gene in 46 out of 51 patients in the NBS register. This mutation, leading to the formation of truncated protein, was found to be of Slavic origin. However, in our patient mutation 657del5 was not detected, although it does not exclude other alterations in nibrin, since the whole gene was not sequenced.

The clinical diagnosis of Nijmegen breakage syndrome was suggested in the newborn because of his obvious microcephaly and immunodeficiency. Microcephaly is one of the major criteria of NBS. The head circumference at birth below the 3rd percentile was observed in 75 % of

NBS patients. On the other hand, this cranial anomaly has been described in most of the patients with duplication of the distal segment of 4q, although the immunodeficiency syndrome has never been reported in those cases. Repeated cytogenetic investigation revealed low mitotic activity of cultured peripheral lymphocytes, which is consistent with clinical immunodeficiency, and only 4 %/2 % of chromosomal breaks/rearrangements, respectively. We also detected the rearrangement between the long arm of chromosomes 14 and 21 by FISH method. Chromosomal instability, which is basic characteristic of the Nijmegen breakage syndrome, occurred in our patient in a few metaphases only and it was probably coincidental. The growth of the child showed that the clinical features are more specific to duplication of 4q than to the NBS than was suspected prior to laboratory examination.

CONCLUSION

A newborn suspected from NBS was assessed using classical and molecular cytogenetics and karyotype 46,XY,dup(4)(q28q35.2)*de novo* was revealed. The diagnosis of supposed NBS was excluded by molecular PCR based diagnostics, although translocation t(14q;21q) typical for this syndrome was found in 4 of 5 assessed metaphases using FISH.

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