ELECTRONIC LETTER

Constitutional de novo interstitial deletion of 8 Mb on chromosome 22q12.1-12.3 encompassing the neurofibromatosis type 2 (NF2) locus in a dysmorphic girl with severe malformations

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We report on the cytogenetic and molecular characterisation of a constitutional de novo interstitial deletion of chromosome 22q12.1-q12.3 in a dysmorphic girl. The deletion extends over approximately 8 Mb including the NF2 gene region. The corresponding deletion syndrome is characterised by severe developmental delay accompanied by multiple malformations at an age when clinical manifestation of NF2 is not expected.

CASE REPORT

The proband is the second daughter of a 28 year old, gravida 2, para 2 woman and her unrelated 35 year old husband. The pregnancy was uncomplicated until 30 weeks, when polyhydramnios and congenital heart disease were diagnosed by ultrasonography. Because of these abnormalities, karyotype analysis was performed on fetal blood obtained by percutaneous umbilical cord blood sampling. Spontaneous delivery occurred at 31 weeks of gestation after premature labour (birth weight 1360 g, 25th centile; length 41.5 cm, 50th centile; OFC 29.8 cm, 75th centile). Dysmorphic features (fig 1) consisting of depressed nasal root, hypertelorism, small and downward slanting palpebral fissures, short philtrum with a tented upper lip, mandibular hypoplasia, low set, and posteriorly rotated ears, overlapping fingers and toes, and a large cleft palate were noted. The child presented with visceral situs inversus with dextrocardia, interrupted aortic arch type A, a large ventricular septal defect, subaortic stenosis secondary to

conoventricular malalignment, hypoplastic ascending aorta, and bilateral superior caval veins with drainage of the right sided caval vein via a coronary sinus into the right atrium. Prostaglandin E₁ infusion was given to maintain ductal patency until the age of 4 months when surgical repair of the aortic arch abnormality and subaortic stenosis was done. Furthermore, duodenal stenosis with annular pancreas were detected requiring surgical intervention at the age of 1 week. Cranial ultrasound showed a congenital malformation of the central nervous system with dilated, abnormally shaped lateral and third ventricles, abnormally enlarged gyri (pachygyria), and a hypoplastic corpus callosum. The girl is severely neurodevelopmentally disabled. At the corrected age of 8 months, she cannot focus on a face or follow an object, has few and uncoordinated spontaneous movements, has no head control, and has to be fed by nasogastric tube. Another problem is failure to thrive. At the age of 10.5 months (corrected age 8 months), her weight is 4.3 kg (2 kg below the 3rd centile), length is 57 cm (8 cm below the 3rd centile), and head circumference is 38 cm (6.5 cm below the 3rd centile).

Abbreviations: NF2, neurofibromatosis type 2; YAC, yeast artificial chromosome; BAC, bacteria artificial chromosome

Figure 1 Our patient at 10.5 months.

Table 1 Characterisation of the deletion in the proband by FISH analysis

Probe used for FISH analysis (GenBank accession number)	Probe specific STS marker(s)	Presence (+)/absence (-) of FISH signal on the deleted chromosome 22
Oncor CATCH22 probe	D22S75	+
Oncor M-bcr probe	(BCR)	+
322B1 (z84718)	D22S449, WI-7416	+
CTA-221G9 (z99916)	D22S1019E	+
RP3-462D8 (al022332)	B14425	+
CTA-246H3 (al022324)	D22S1040, D22S925, D22S926	+
CTA-390C10 (al008721)	WI-30096, D22S419	+
CTA-407F11 (al022329)	D22S258, D22S315, D22S650	Reduced
CTA-125H2 (z98949)	D22S429, D22S1148	-
bk256D12 (ac005529)	D22S929 (NF2)	-
CTA-415G2 (z83846)	D22S411E	-
CTA-282F2 (al008630)	D22S11720	-
CTA-566G5 (al023577)	AQ512838	-
RP1-75E8 (z76736)	AQ516245	-
LL22NCO3-20A6 (z69713)	SHGC-143268	-
LL22NCO3-13E1 (z54073)	R60425, T05310	-
LL22NCO3-32F9 (z73429)	D22S1162	+
LL22NCO1-90C2 (z82182)	B32366	+
CTA-221H1 (al008717)	D22S684	+
YAC 948b02	WI-356	+
YAC 826d12	WI-88, D22S1141	+

BCR, Breakpoint cluster region; NF2, neurofibromatosis type 2 gene.

Cytogenetic investigations

QFQ and G banded metaphases from lymphocytes of the proband showed a small interstitial deletion of the long arm of chromosome 22 (fig 2). Parental chromosomes were normal, indicating that the deletion originated *de novo*. The presence of an insertion of chromosome 22 in the patient, with the segment missing in the deleted chromosome 22 being inserted into another chromosome, was ruled out by FISH with a whole chromosome 22 painting probe, since only the normal and the deleted chromosomes 22 were homogeneously painted. To characterise the extent of the deletion, we applied FISH analysis on metaphase spreads of the patient using several YAC, BAC, and cosmid probes (table 1). We identified a BAC clone (CTA-407F11), which showed a strongly reduced FISH signal on the deleted chromosome 22 compared to the regular signal on the normal chromosome 22 (fig 3). Therefore, the proximal deletion breakpoint is localised within the region covered by this BAC clone. The distal breakpoint was assigned to the region between the cosmid clones LL22NCO3-13E1 and LL22NCO3-32F9 (table 1). Thus, the patient is hemizygous for the genomic segment between markers D22S419 and D22S1162. The deleted region spans about 8 Mb according to the draft sequence of the human genome (<http://genome.ucsc.edu/>) and the Ensembl database of the Sanger Centre (<http://www.sanger.ac.uk/>). As estimated by the entries of these databases, the proband is hemizygous for at least 51 genes including the NF2 gene. We compared the deletion of the proband with the deletions extending more than 2 Mb of four NF2 patients described by Bruder *et al*^{1,2} (fig 4). These authors ascertained the extent of the deleted regions by microarray CGH using mapped BAC and cosmid clones. According to the integration of these results into the complete sequence contig of chromosome 22, patient JP has a deletion spanning about 6.6 Mb.^{1,2} The distal deletion breakpoint is located between the marker D22S29 and the myoglobin gene. Based on the FISH results, the proximal deletion breakpoint in our patient is about 4 Mb proximal to that of patient JP, and the distal breakpoints of both patients are separated by 0.8-1.8 Mb. Patients IK65 and P5 have smaller deletions spanning 2-3 Mb, and all their symptoms reported belong to the clinical spectrum of NF2. As indicated in fig 4, the deletion observed in patient P41 has the most centromeric border. Unfortunately, the proximal breakpoint of this deletion has been determined less precisely between the BACs RP5-1175D24 and RP11-

Figure 2 Normal (left) and deleted (right) chromosomes 22 from the proband after G banding.

Figure 3 FISH results with BAC clone CTA-407F11. A regular signal is present on the normal chromosome 22 (arrowhead), whereas only a weak signal is present on the deleted chromosome 22 (arrow) indicating that this BAC is spanning the deletion breakpoint.

54J16, which does not allow its direct comparison with the deletion of the proband.

DISCUSSION

The deletion in 22q12 (del(22)(q12.1q12.3)) of the patient described here spans approximately 8 Mb and extends between markers D22S419 and D22S1162. To our knowledge,

Figure 4 Schematic presentation of the extent of the deletions characterised in this report and described by Bruder *et al.*^{1,2} Markers of the BAC or cosmid clones used for FISH analysis are indicated; BAC/cosmid clones used in the present study are underlined. The relative map position of the BACs integrated into the sequence of chromosome 22 is given in Mb. Black bars indicate the extent of the respective deletions. MB, myoglobin locus; NF2, neurofibromatosis type 2 gene

this is the second observation of an interstitial deletion in 22q12 detected by microscopic analysis, and it was ascertained by examination of a malformation syndrome. Initially, Bruder *et al.*¹ reported on the severely mentally retarded patient JP with a severe form of NF2, who also displayed a large deletion in chromosome 22 detected by cytogenetic analysis. Reinvestigation of this case² showed that the deletion encompasses 6.6 Mb and partly overlaps with the deletion presented here. The centromeric breakpoint of the deletion of the proband is about 4 Mb proximal to that of patient JP, whereas the distal breakpoint of our patient is 0.8-1.8 Mb more proximal.^{1,2} This finding suggests that the severe phenotype of the patient described here is mainly caused by the loss of a gene or several genes within the proximal 4 Mb of the deletion. Currently, genes located within this region that might be associated with the phenotypic features of the patients have not been identified. Patient JP is affected with a severe form of NF2, diagnosed by the presence of bilateral vestibular schwannomas before the age of 25-30 years.³ Additionally, this patient is mentally retarded and had a grand mal epileptic fit at the age of 9 years. Recently, Bruder *et al.*² reported on further patients ascertained by the phenotype of NF2, three of whom had very large deletions in 22q12. Two patients (IK65, P5) had deletions encompassing 2-3 Mb including the NF2 gene. These patients were also affected by a severe form of NF2, but mental retardation was not observed. In the third patient (P41),² who apart from a moderate form of NF2 showed dysmorphic features

and slight mental retardation, the proximal deletion breakpoint was not precisely determined, thus excluding a direct comparison with the deletion of the patient described here. Among the patients with smaller deletions, Bruder *et al.*² reported only on two further subjects with additional symptoms besides their NF2 phenotype. Patient P12 showed mild mental retardation associated with a 530 kb deletion, and patient P135 was reported to have had seizures. His deletion encompasses cosmid LL22NC03-69B4 spanning parts of the NF2 gene. In contrast to the patients described by Bruder *et al.*^{1,2} who were all ascertained by their NF2 phenotype, the patient presented here was recorded because of her "syndromic" phenotype typical of a chromosome aberration, which manifested prenatally and developed into a complex malformation syndrome with severe developmental delay. As expected, our patient showed no clinical signs of NF2 at the age of 10.5 months. Since the report of Bruder *et al.*² was mainly focused on NF2, it is not clear if one can expect complete ascertainment of further clinical signs in addition to NF2 for the whole large series of patients reported. Owing to different modes of ascertainment at different ages, and most probably the different views of the specialists involved, the phenotypes of the NF2 patients with very large deletions cannot be compared with that of our patient. Among the patients compared here, our proband has the most severe malformation syndrome, and presumably her deletion is the largest. However, in the light of her malformations, it cannot be

excluded that she represents an extreme in an otherwise highly variable clinical spectrum of a deletion syndrome with mostly quite mild phenotypes. In our case, the detection of the deletion at an age when clinical manifestation of NF2 cannot be expected draws attention to the corresponding deletion syndrome as a separate entity, which for our patient is more relevant than her additional risk of becoming affected by NF2. We recommend that NF2 patients with atypical, complex phenotypes should be carefully clinically examined and screened for the presence of large deletions, in order to establish the clinical variability, and to understand the karyotype-phenotype correlation associated with 22q12 deletion syndrome.

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