Molecular cytogenetic characterisation of partial trisomy 9q in a case with pyloric stenosis and a review

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Abstract

Partial trisomy 9q represents a rare and heterogeneous group of chromosomal aberrations characterised by various clinical features including pyloric stenosis. Here, we describe the case of a 1 year old female patient with different dysmorphic features including pyloric stenosis and prenatally detected partial trisomy 9q. This partial trisomy 9q has been analysed in detail to determine the size of the duplication and to characterise the chromosomal breakpoints. According to the data gained by different molecular cytogenetic techniques, such as fluorescence in situ hybridisation (FISH) with whole and partial chromosome painting probes, yeast artificial chromosome (YAC) probes, and comparative genomic hybridisation (CGH), the derivative chromosome 9 can be described as $\text{dup}(9)(\text{pter} \rightarrow \text{q22.1}::\text{q31.1} \rightarrow \text{q22.1}::\text{q31.1} \rightarrow \text{q22.1}::\text{q31.1} \rightarrow \text{qter})$. Four breakpoint spanning YACs have been identified (y806f02, y906g6, y945f5, and y747b3) for the proximal breakpoint. According to this new case and previously published data, the recently postulated putative critical region for pyloric stenosis can be narrowed down to the subbands 9q22.1-q31.1 and is the result of either partial trisomy of gene(s) located in this region or a gene disrupted in 9q31.

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Partial trisomy 9q was first described by Turleau *et al*¹ in 1975. It is a rare and heterogeneous group with respect to the chromosomal region involved in the aberration and the clinical phenotype. As recently pointed out,² pyloric stenosis is a common feature in 15 cases described so far with partial trisomy 9^{1–14} involving bands q22.1–31.3. Here, we describe a further case with pyloric stenosis and partial trisomy 9q, which was analysed in detail by comparative genomic hybridisation (CGH) and fluorescence in situ hybridisation (FISH) with region specific probes. Pyloric stenosis might be the result of a gene interrupted by one of the chromosomal breakpoints or of a gene which is trisomic in 9q. Including published data,^{1–17} we were able to narrow down one putative critical region for pyloric stenosis to

q22.1–q31.1. Although imprinting effects might play a role in the development of pyloric stenosis, according to published data the most likely assumption is that a gene for pyloric stenosis is located in the region of the distal breakpoint in 9q31.

Case report

The female patient is the first child of a 42 year old woman. Pregnancy was accomplished by in vitro fertilisation (ICSI) because of a long standing inability to conceive. During late pregnancy (in week 35 of gestation), growth retardation was diagnosed, while the length of the fetus had been within the normal range during the previous examination in week 22. Amniocentesis was performed and because of cardiac depression the child was born by caesarian section in the 36th gestational week. The female patient was hypotrophic at birth (weight 1400 g, length 36 cm) and showed multiple craniofacial anomalies like dolichomicrocephaly (head circumference 26.5 cm, –4 SD below normal), saddle nose, mandibular hypoplasia, facial asymmetry (left microphthalmia, coloboma of the retina and the choroid) and hand anomalies (clinodactyly V, camptodactyly, overlapping fingers). Pyloric stenosis was diagnosed, which made a pylorotomy necessary. Growth retardation was apparent during postnatal development (–4 SD) together with severe mental retardation. Further investigations showed low levels of insulin-like growth factor I (IGF-I), IGF-II, and insulin growth factor binding protein 3 (IGFBP-3), while the values for IGFBP-2 were increased (data not shown but published in Hübler *et al*¹⁸). At the age of 1 year, the patient was neither able to sit nor to say words, but could lift her head and fix on objects. A picture of the patient at the age of 8 months is shown in fig 1.

Figure 1 The female patient at the age of 8 months showing (A) the chest, arms, and head and (B) the eyes. (Photograph reproduced with permission.)

Figure 2 Localisation of FISH probes used; YACs are derived from the CEPH-MEGA-YAC library and the "midi" probes are microdissection derived probes created in the Institute of Human Genetics and Anthropology, Jena.

Material and methods

Metaphase chromosomes from cultured amniotic fluid cells and PHA stimulated peripheral blood lymphocytes (postnatal) of the patient were analysed by standard GTG and CGB banding. The chromosomes from peripheral blood lymphocytes were studied using the following FISH probes: whole and partial chromosome painting probes for chromosome 9 (wcp 9, pcp9p, pcp9q, midi 18, midi 23, midi 36¹⁸ and unpublished probes) and different YAC probes (y747b3, y750c6, y906g6, y945f5, y806f02, y933c05, y957h08, y931d9, y908f11, y933g6, y857g10, and y870b4). Fig 2 shows details of the localisation of the probes used. Labelling and hybridisation were carried out according to Senger *et al*¹⁹ and Liehr *et al*.²⁰

Comparative genomic hybridisation (CGH) was performed according to Chudoba *et al*²¹ using 15 single cell nuclei from the methanol/acetic acid fixed cell suspension as template.

Results

Conventional cytogenetic investigation (GTG banding) showed a derivative chromosome 9q+ (fig 3) in all metaphases analysed (20/20). This finding, which was detected during the analysis of amniotic fluid cells in the 35th week of gestation, was confirmed five months after birth in peripheral blood lymphocytes. The identified additional material in chromosome 9 was suspected to originate from 9q itself.

Molecular cytogenetic examination with whole and partial chromosome painting probes for chromosome 9, 9p, and 9q confirmed that the additional chromosomal material consists exclusively of 9q material. Subsequently performed CGH analyses (fig 3) showed the following result: rev ish enh (9q22-q31). These findings could be confirmed and refined using YAC and microdissection probes derived from the critical region (for details see figs 2 and 3).

Twelve different YAC probes were used to characterise the aberrant chromosome 9; y750c6, y933c05, and y957h08 (9q31.2-32) gave one specific signal, y870b4, y857g10, y933g6, y908f11, and y931d9 (9q22-31) gave two signals, and y806f02, y906g6, y945f5, and y747b3 (9q22.1) gave three specific signals. According to these data, loci detected by YAC probes, which map more distally in 9q31.2-32, are not involved in the duplication event and loci between 9q22 and 9q31 are duplicated. According to the distribution along the aberrant chromosome 9 of the YAC probes y806f02, y906g6, y945f5, and y747b3 and their signal intensity (fig 2E), a partial triplication is unlikely. The most proximal signal was always very weak compared to the other two more distal signals. Thus, a so called "probe splitting" is the most likely explanation for the observed FISH pattern and these four YACs must span the proximal breakpoint in 9q22.1.

This suggestion is confirmed by the fact that probes located more proximally in 9p12-9q21.3 (microdissection probe midi 23) or 9q13-9q21.1 (midi 36) do not show an aberrant hybridisation pattern on the enlarged chromosome 9.

According to these data the derivative chromosome 9 can be described as dup(9) (pter→q22.1::q31.1→q22.1::q31.1→q22.1::q31.1→qter).

Cytogenetic examination of peripheral blood lymphocytes of the healthy parents showed no abnormalities.

Discussion

To the best of our knowledge, this is the first report on a case with partial trisomy 9q which has been diagnosed prenatally. The child was born at 36 weeks of gestation and was re-examined cytogenetically five months after birth. Thus, partial trisomy 9 could be confirmed in peripheral blood lymphocytes, as well as in amniotic fluid cells, and mosaicism could be excluded for two different tissue types.

Moreover, this is the first report of such an inverted duplication event in the long arm of chromosome 9. Even though there have been many reports on pericentric inversions of chromosome 9 and speculations about what they result from,^{22,23} the only valid hypothesis for their origin is that the presence of homologous sequences in proximal 9p and 9q may predispose to rearrangements.²³ Thus, any speculations on the origin of the paracentric inversion in 9q reported here as a single case report are not warranted. However, the involvement of as yet unknown repetitive elements or transposases in the recombination event cannot be excluded.²⁴

Maraschio *et al*² were the first to point out that there is an obvious correlation between pyloric stenosis and duplication of distinct parts of 9q. However, they only included four published cases with partial trisomy 9q without pyloric stenosis,^{3,5,8,9} even though there are some more.¹¹⁻¹⁷ Assuming that 9q22-31.1 is a critical region for pyloric stenosis, there are five published cases corroborating this^{3,9,15-17} and at least six cases contradicting this point of

Figure 3 Images were captured on a Zeiss Axioplan microscope (Zeiss Jena, Germany) with the IKAROS and ISIS digital FISH imaging system (MetaSystems, Altlussheim, Germany) using a XC77 CCD camera with on chip integration (Sony). (A) GTG banded chromosomes of the patient with the karyotype 46,XX,ins(9;?) (q22;?). The derivative chromosome 9 is marked by an arrow in the karyotype on the left. In the frame on the right the normal and aberrant chromosomes 9 of the patient from another metaphase are shown at greater magnification. The chromosomes have been "straightened" by image analysis to get a better comparison of the GTG banding pattern with the ideograms of the normal and aberrant chromosome 9. (B) CGH profile of the chromosome 9 of the patient. Result: rev ish enh (9q22-q31). (C-G) FISH with microdissection and YAC probes: the probes midi 18 and midi 36 showed that the derivative chromosome 9 is monocentric (C-D) and because of the hybridisation pattern of midi 36 9q21.1 is not part of the duplication (D); y806f02 is one of the breakpoint spanning YACs and the resulting hybridisation pattern can only be explained by an inverted duplication in 9q (E); y933g6 showed that band 9q22.3 is duplicated (F), while the result of FISH with y957h08, located in 9q31.2-9q31.3, showed that this band is not part of the duplication (G). Thus the karyotype of the presented case is 46,XX, dup(9) (pter→q22.1::q31.1→q22.1::q31.1→q22.1::q31.1→qter).

Figure 4 Summary of published reports concerning the size of the duplicated regions of derivative chromosomes 9 and the correlation with the presence of pyloric stenosis. The numbers below the vertical lines indicating the size correspond to the reference numbers. pc = present case.

view^{5 8 11-14} (fig 4). However, some of the studies contradicting the assumption of Maraschio *et al*² were done in the early days of chromosome banding and exact breakpoints were not verified by FISH studies.^{5 12 13} On the other hand, the case of Stalker *et al*¹⁴ from 1993 is well characterised by cytogenetic and molecular cytogenetic techniques. According to Maraschio *et al*,² the duplication in this case spans the postulated critical region for pyloric stenosis in q12-q33, but no pyloric stenosis was reported.¹⁴ Moreover, in cases with complete trisomy 9 there have been no reports of pyloric stenosis.¹²

Imprinting could be another possible explanation for the fact that there are cases with duplication in 9q22.1-31.1 with and without pyloric stenosis. However, no cases with complete trisomy and pyloric stenosis have been described, which could be because all such cases which are viable are mosaic. The origin of the duplicated region of chromosome 9 has been clarified in only a few cases up to now. Sutherland *et al*¹² reported on a case without pyloric stenosis and maternal origin of the duplicated chromosome 9 material, while in two cases with pyloric stenosis^{1 7} the duplicated chromosome 9 material was shown to be of maternal origin as well. Thus, imprinting effects in chromosome 9 seem not to be involved in the formation of pyloric stenosis.

Although the mode of inheritance in pyloric stenosis is unclear, it seems to be multifactorial^{25 26} and the fact that it was present in eight of 13 cases with a duplication of 9q22.1-31.1 cannot be ignored. The cases with partial trisomy 9q22.1-31.1 summarised in fig 4 show no significant segregation with the sex of the affected children: 3/7 without and 5/8 with pyloric stenosis were female. However, a simple gene dosage effect seems, according to published reports, unlikely to be the (only) reason for pyloric stenosis in such cases. There might be a gene disrupted in the distal part of the duplicated region. Four YACs spanning the proximal breakpoint in 9q22.1 were identified

(fig 2; y806f02, y906g6, y945f5, and y747b3) in the case presented here, but unfortunately none for the distal one. A re-examination of the distal breakpoint in different cases with pyloric stenosis should be performed to confirm or exclude the possibility of a common breakpoint inactivating a putative "pyloric stenosis gene" in 9q31.

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- 1 Turleau C, de Grouchy J, Chavin-Colin F, Roubin M, Brisaud PE, Represse G, Safar A, Borniche P. Partial trisomy 9q: a new syndrome. *Hum Genet* 1975;29:233-41.
- 2 Maraschio P, Maserati E, Seghezzi L, Tupler R, Verri MP, Tiepolo L. Involvement of 9q22.1-31.3 region in pyloric stenosis. *Clin Genet* 1998;54:159-60.
- 3 Kleczkowska A, Fryns JP, Lemay P, Van Den Berghe H. The characteristic phenotype of distal 9q3 trisomy is due to duplication of band 9q32. *Genet Counsel* 1993;4:217-21.
- 4 Lindgren V, Rosinsky B, Chin J, Berry-Kravis E. Two patients with overlapping *de novo* duplications of the long arm of chromosome 9, including one case with Di George sequence. *Am J Med Genet* 1994;49:67-73.
- 5 Chamla Y, Bilbeissi C, Micheau M, Battin J. Partial 9q trisomy associated with a 9;21 translocation. *Hum Genet* 1977;38:245-8.
- 6 Bianchi GP, Cantone P, Dalprà L, Simoni G. Trisomia parziale 9q: riscontro della sindrome in due sorelle. *Minerva Pediatr* 1986;38:109-14.
- 7 Yamamoto Y, Oguro N, Nara T, Horita H, Niitsu N, Imai-zumi S. Duplication of part of 9q due to maternal 12;9 inverted insertion associated with pyloric stenosis. *Am J Med Genet* 1988;31:379-84.
- 8 Naritomi K, Izumikawa Y, Goya Y, Gushiken M, Shiroma N, Hirayama K. Trisomy 9q3 syndrome: a case report and review of the literature. *Clin Genet* 1989;35:293-8.
- 9 Hou JW, Wang TR. Molecular cytogenetic studies of duplication 9q32-q34.3 inserted into 9q13. *Clin Genet* 1995;48:148-50.
- 10 Nakahori Y, Nakagome Y. A malformed girl with duplication of chromosome 9q. *J Med Genet* 1984;21:387-8.
- 11 Smart RD, Viljoen DL, Fraser B. Partial trisomy 9 - further delineation of the phenotype. *Am J Med Genet* 1988;31:947-51.
- 12 Sutherland GR, Carter RF, Morris LL. Partial and complete trisomy 9: delineation of a trisomy 9 syndrome. *Hum Genet* 1976;32:133-40.
- 13 Faed M, Robertson J, Brown S, Smail PJ, Muckhart RD. Pure partial trisomy for the long arm of chromosome 9. *J Med Genet* 1976;13:239-42.
- 14 Stalker HJ, Aymé S, Delneste D, Scarpelli H, Vekemans M, Der Kaloustian VM. Duplication of 9q12-q33: a case report. *Am J Med Genet* 1993;45:456-9.
- 15 Luke S, Verma RS, PeBenito R, Macera MJ. Inversion-duplication of bands q13→q12 of human chromosome 9. *Am J Med Genet* 1991;40:57-60.
- 16 Spinner NB, Lucas JN, Poggensee M, Jaquette M, Schneider A. Duplication 9q34→qter identified by chromosome painting. *Am J Med Genet* 1993;45:609-13.
- 17 Mattina T, Pierluigi M, Mazzone D, Scardilli S, Perfumo C, Mollica F. Double partial trisomy 9q34→qter and 21pter→q22.11: FISH and clinical findings. *J Med Genet* 1997;34:945-8.
- 18 Hübler A, Seidel J, Kauf E, Schramm D, Beensen V, Heller A, Liehr T, Zintl F. Insulin-like growth factor serum concentrations reflect insufficient growth in a hypoplastic infant with partial trisomy 9q in 12th week of life. *J Pediatr Endocrinol Metab* (in press).
- 19 Senger G, Chudoba I, Plesch A. Multicolor-FISH - the identification of chromosome aberrations by 24 colors. *BIOforum* 1998;9:499-503.
- 20 Liehr T, Thoma T, Kammiller K, Gehring C, Ekici A, Bathke KD, Grehl H, Rautenstrauss B. Direct preparation of uncultured EDTA-treated or heparinized blood for interphase FISH analysis. *Appl Cytogenet* 1995;21:185-8.
- 21 Chudoba I, Henn TK, Senger G, Claussen U, Haas OA. Comparative genomic hybridization using DOP-PCR amplified DNA from a small number of nuclei. *J Pediatr* 1997;7:519-21.
- 22 Samonte RV, Conte RA, Ramesh KH, Verma RS. Molecular cytogenetic characterization of breakpoints involving pericentric inversions of human chromosome 9. *Hum Genet* 1996;98:576-80.
- 23 Park JP, Wojiski SA, Spellman RA, Rhodes CH, Mohandas TK. Human chromosome 9 pericentric homologies: implications for chromosome 9 heteromorphisms. *Cytogenet Cell Genet* 1998;82:192-4.
- 24 Reiter LT, Liehr T, Rautenstrauss B, Robertson HM, Lupski JR. Localization of Mariner DNA transposons in the human genome by PRINS. *Genome Res* (in press).
- 25 Mitchell LE, Risch N. The genetic of infantile hypertrophic pyloric stenosis: a reanalysis. *Am J Dis Child* 1993;147:1203-11.
- 26 Fried K, Aviv S, Nisenbaum C. Probable autosomal dominant infantile pyloric stenosis in a large kindred. *Clin Genet* 1981;20:328-30.