A molecular, cytogenetic, and clinical evaluation of mosaic tandem duplication 17p and Charcot-Marie-Tooth type 1A neuropathy

K S Reddy, M B Larsen

Abstract

An 8 year old girl with partial duplication of the short arm of chromosome 17 had a mosaic 46,XX,der(17)?del(17)(p12)dup(17)(p11.2p12).ish dup(17)(p11.2p13.3)(D17S379x2, p53x2, D17S122x2, D17S29+) karyotype. The extent of mosaicism was 20% in lymphoblasts and 100% in fibroblasts. Fluorescence in situ hybridisation (FISH) proved invaluable in defining the abnormality precisely. The cytogenetic morphology by FISH assay ruled out a microdeletion of the Miller-Dieker syndrome (MDS) region. However, there was no MDS deletion but a duplication of this region. The duplication was extensive and included proximal p53 and D17S122, Charcot-Marie-Tooth type 1A (CMT1A), but not D17S29, the Smith-Magenis syndrome (SMS) region. This patient has the clinical features and generalised decreased peripheral nerve conduction velocity characteristic of CMT1A.

The clinical management of paediatric cases of mosaic trisomy 17p cases would entail testing for CMT1A duplication. If duplicated, a decrease in nerve conduction velocity (NCV) of the peripheral motor neurones would be necessary to ensure the manifestation of CMT1A neuropathy. The parents of probands with delayed NCV should be counselled about the risk of CMT1A in later life.

(J Med Genet 1998;35:169-172)

Keywords: mosaic; dup(17)(p11.2p13.3); CMT1A

Partial trisomy for the short arm of chromosome 17 is relatively rare.¹⁻¹² The clinical findings in reported cases were pre- and postnatal growth retardation, craniofacial abnormalities including microcephaly, hypertelorism, micrognathia, apparently low set ears, high arched palate, and a short, webbed neck, flexion abnormalities of the fingers, and foot abnormalities. Localised to 17p are four disorders that could result from alterations in gene dosage. Miller-Dieker syndrome (MDS) and Smith-Magenis syndrome (SMS) involve deletion of 17p13.3 and 17p11.2, respectively.¹³⁻¹⁴ Charcot-Marie-Tooth type 1A (CMT1A) is a demyelinating peripheral neuropathy that is commonly associated with a submicroscopic duplication of 17p11.2p12.¹⁵ Hereditary neuropathy with liability to pressure palsies (HNPP) is associated with a deletion resulting from a reciprocal recombinant product of the

CMT1A duplication.¹⁶⁻¹⁸ The specific features of CMT1A are progressive distal muscle atrophy, weakness of the feet and hand deformities which appear later, gait abnormalities, and a completely penetrant electrophysiological phenotype of symmetrical reduction in motor nerve conduction velocity (NCV <40 m/s).¹⁹ The frequent 1.5 Mb and other reported duplications encompass a gene that codes for a peripheral myelin protein of 22 kDa (PMP22)^{9-12, 20-22} and this gene is highly expressed in peripheral nerves.²³⁻²⁶ The increased gene dosage of PMP22 is strongly associated with the CMT1A phenotype.²⁰

Mosaicism for 17p trisomy is very rare. Shabtai *et al*²⁷ described a case of trisomy 17p with multiple malformations and psychomotor retardation at 11 months of age; 60% of the lymphocytes had trisomy 17p. Morrison *et al*²⁸ reported a partial trisomy 17 resulting from a ring chromosome in 13% of cells from peripheral blood culture. In this paper we describe the molecular and clinical findings in a patient with mosaic partial duplication of the short arm of chromosome 17.

Case report

An 8 year old girl with psychomotor retardation and no family history of this condition was examined. Her parents, two older sisters, and a brother are normal without any birth defects or muscular, skeletal, or neurological disorders. The mother had cytomegalovirus infection during her pregnancy. Labour was induced. At birth the baby had cytomegalovirus infection, a right calcaneovarus foot deformity, microcephaly, high arched palate, a heart murmur, and a renal abnormality. A cerebral CT scan done because of the small head size was normal. At 6 months chromosome study showed extra material on the short arm of chromosome 17. Motor development was delayed; she sat at 10 months, stood at 16 months, and walked at 22 months. At 2-3 years she began uttering single words, at present speaks only in short phrases, and is unable to read or write. She had bilateral inguinal hernias. Cardiovascular examination showed a regular heart rate and rhythm and a faint 2/6 systolic murmur. A dark naevus was present on her upper posterior medial thigh and the dorsal aspect of her wrist. The spine appeared straight although flattening of the lumbar region with some lordosis was observed. Her reflexes were depressed and planter responses were equivocal. She had an obvious drift or dysmetria. Sensory examination showed normal reaction

**Cytogenetics
Laboratory, Quest
Diagnostics
Incorporated, 33608
Ortega Highway, San
Juan Capistrano, CA
92690-6130, USA
K S Reddy**

**Shriners Hospital,
Chicago, IL, USA
M B Larsen**

Correspondence to:
Dr Reddy.

Received 5 June 1997
Revised version accepted for
publication 12 August 1997

Figure 1 (A) A G banded metaphase in which the normal and duplicated 17 are shown by arrows. FISH showing duplicated 17p with two signals for the probes (B) D17S379, Müller-Dieker syndrome region, (C) p53 locus, and (D) D17S122, Charcot-Marie-Tooth 1A region. (E) The D17S29, Smith-Magenis syndrome region probe gave only one signal.

to touch. The fingers on both hands were very tapered. The elbows lacked about 20° of terminal extension bilaterally. Recently her feet have turned in, but walking is independent with a varus position of both feet. She has a heel-toe reciprocal gait but she walks on the lateral border of her right foot. Her feet have talipes varus deformities, much greater on the right than on the left. She has bilateral pes cavus and cock up deformities of both big toes, the right much greater than the left. X ray showed calcaneovarus feet. The motor nerve conduction and electromyography studies were abnormal with evidence of a significant generalised neuropathy affecting both upper and lower extremities. The primary pathology appears to be demyelination rather than an axonopathy. The nerve conduction velocities were: left common peroneal 14.2 m/s (normal for age >45), left posterior tibial 11.6 m/s (normal for age >45), right common peroneal 12.8 m/s (normal for age >45), and right median 17.4 m/s (normal for age >47). Electromyography showed that the left anterior tibialis spontaneous activity had increased insertion activity, ++ fibrillation, and some fasciculation, without a positive sharp wave or myotonia. Similarly, the left vastus had increased insertion activity, + fibrillation, without fasciculation, a positive sharp wave, or myotonia.

CYTOGENETIC AND FISH STUDIES

Peripheral blood cultures were synchronised with methotrexate (amethopterin 0.05 µg/ml, Sigma M6770) for 17 hours and released with thymidine (2.5 µg/ml, Sigma T5018) for 5.5 hours. The cultures were harvested following

the addition of colcemid (0.05 µg/ml, Gibco) 20 minutes before harvest. The skin was minced and divided into two. One was cultured after mincing and the other was subjected to 10 minutes digestion in 0.05% trypsin (Gibco 25300-054) followed by 10 minutes in 1 mg/ml collagenase IIS (Sigma C-1764). The digested pellet was seeded onto coverslips. Chromosomes were GTG banded. FISH studies were initiated using D17S379 (MDS), p53, D17S122 (CMT1A), and D17S29 (SMS) digoxigenin labelled probes (ONCOR). The slides were denatured in 70% formamide/2 × SSC at 70°C for two minutes, followed by dehydration in 70%, 80%, 90%, and absolute alcohol. Ten microlitres of the probe were applied to the slide, coverslipped, and sealed. Following incubation overnight in a humid chamber at 37°C, the slides were washed in 2 × SSC at 72°C for five minutes. Detection was carried out using rhodamine labelled antidigoxigenin and counterstain 4,6-diamidino-2-phenylindole (DAPI). In G banded metaphases, a chromosome 17 appeared to have a duplication of the short arm in ~20% (6/30 cells) of the lymphoblasts and 100% of the skin fibroblasts (30/30) (fig 1A). To rule out Miller-Dieker syndrome, a FISH study was initiated on fibroblast chromosomes. The Miller-Dieker region probe (D17S379) gave two signals on the duplicated 17p (fig 1B). To assess the extent of the duplication, Charcot-Marie-Tooth syndrome (D17S122) (fig 1C) and p53, a tumour suppressor gene probe (fig 1D) were used. Two copies of each probe were detected on the duplicated 17p. The Smith-Magenis region probe (D17S29) gave only one signal

(fig 1E). Therefore, the duplication is tandem and includes the region distal to D17S29 and beyond D17S379.

Both parental peripheral blood karyotypes were normal. Therefore, the duplication in the proband is most probably a de novo event.

Discussion

Partial trisomy of 17p because of a duplication is most often tandem and occasionally inverted. The clinical findings in reported patients are intrauterine and postnatal growth retardation, developmental delay, mental retardation, craniofacial abnormalities, and limb deformities.¹⁻¹² The features may vary depending on the extent of the duplication. Although 17p is involved in four known disorders, Miller-Dieker syndrome (MDS), Smith-Magenis syndrome (SMS), Charcot-Marie-Tooth type 1A (CMT1A), and hereditary neuropathy with liability to pressure palsies (HNPP), CMT1A is the only one that is a duplication syndrome. Therefore, 17p duplication cases should be tested for CMT1A duplication and motor nerve conduction velocity.

Patients with mosaic trisomy 17p are extremely rarely reported. Shabtai *et al*²⁷ described a 20 month old child with pure trisomy 17p11.2p13.3 in 60% of lymphoblasts. The clinical findings were psychomotor retardation, hypotonia, frontal bossing, low set and malformed ears, ptosis, micrognathia, and antimongoloid slant of the eyes. Morrison *et al*²⁸ reported a mosaic partial trisomy 17 resulting from a ring chromosome identified by FISH to have a breakpoint probably distal to the Smith-Magenis region and proximal to the Miller-Dieker region. The ring was found in 13% of the lymphoblasts (6/45). This patient was a 3 year old girl who had feeding difficulties and bilateral dislocation of the hips. Delayed speech and motor skills were observed. Her eyes were deep set and she had macroglossia and macrostomia. Limb defects included a smaller right hand than the left, long, narrow feet, generalised joint laxity, and scoliosis. The present case has psychomotor and speech delay, microcephaly, high arched palate, a systolic murmur, renal abnormality, and bilateral hernias, which are features recorded in other cases with trisomy 17p. The recent development of foot abnormalities and the symmetrical decrease in nerve conduction velocity is consistent with CMT1A. Although, this supports a gene dosage mechanism for CMT1A, the mosaicism (20% lymphoblasts, 100% fibroblasts) raises questions about the proportion of neurones with the duplication that are necessary for phenotypic manifestation. The clinical picture suggests that the neurones probably have a high percentage or almost all abnormal dup(17) cells as observed in the fibroblasts. However, if a peripheral nerve biopsy had been available, the mosaic distribution of the duplication 17p, the neuronal histopathology of onion bulb formation, and the decreased axonal compared to fibre diameter could have been assessed. Although the percentage of abnormal cells in the blood

was low, this appears to have no obvious impact on the CMT1A neuropathy.

The only other report of a child of comparable age with mosaicism involved full trisomy 17.²⁹ The proband was an 8 year 8 month old boy who had full trisomy 17 present in his fibroblasts but not lymphoblasts. This patient had mental and growth retardation, microcephaly, and minor abnormalities. However, he did not have delayed nerve conduction velocities, which is consistently found in CMT1A cases. The authors proposed that the trisomy 17 mosaicism in the peripheral neurones may have been insufficient to cause abnormal nerve conduction. Therefore, the ratio of abnormal cells in the fibroblasts may not reflect the ratio present in the peripheral neurones. The only way to confirm if a mosaic patient will develop CMT1A neuropathy is by nerve conduction velocity studies. Trisomy 17 has been found rarely in spontaneous abortions and never in a livebirth. Mosaic trisomy 17 has been detected in three amniotic fluid samples sent for prenatal diagnosis.³⁰⁻³² Two cases resulted in normal liveborns with a normal karyotype in peripheral blood.³⁰⁻³¹ The third case was terminated and the fetus appeared normal and trisomy 17 was not detected in the second amniotic fluid sample taken at the time of the abortion.³² There are only two reports of mosaic trisomy 17 in livebirths,²⁹⁻³³ one of which had no details.³³ Shaffer *et al*²⁹ did not find trisomy 17 in the lymphoblasts but detected trisomy 17 in 18% of the fibroblasts at first evaluation and in 80% of the fibroblasts five years later. Based on two mosaic cases²⁹ (present study), partial or complete trisomy 17 appears to be better tolerated by fibroblasts than lymphoblasts. The precedent for preferential expression of mosaicism in fibroblasts has been observed in cases of trisomy 8, 9, and tetrasomy 12p. The clinical implications are that if there are strong clinical grounds for suspecting trisomy 17, a negative blood chromosome study probably should be pursued with cytogenetic analysis of skin fibroblasts. Similarly, for confirmation of prenatal mosaic full trisomy 17 or trisomy 17p, a normal blood chromosome result should be ascertained in fibroblasts. Paediatric cases with partial duplication of 17p should be tested for duplication of the CMT1A region. The parents of probands with the duplication need to be counselled about the child having CMT1A later in life (mean 12 years (SD 7.3)). In mosaic cases with a CMT1A duplication, a nerve conduction velocity study will be necessary to ascertain the manifestation of CMT1A.

We thank Dr K Kohli for all the help she gave us in coordinating efforts to obtain a skin biopsy.

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