

ARTICLE

A 2.3 Mb duplication of chromosome 8q24.3 associated with severe mental retardation and epilepsy detected by standard karyotype

Maria Clara Bonaglia^{*1}, Roberto Giorda¹, Romano Tenconi², Marco Pessina¹, Tiziano Prampero³, Renato Borgatti¹ and Orsetta Zuffardi^{3,4}

¹IRCCS Eugenio Medea, Bosisio Parini (LC), Italy; ²Servizio di Genetica Clinica ed Epidemiologica, Dipartimento di Pediatria, Università di Padova, Italy; ³Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy; ⁴IRCCS Policlinico San Matteo, Pavia, Italy

Chromosome duplications are found in about 2% of subjects with a typical chromosomal phenotype but their frequency is likely to be higher, as suggested by the first array-CGH data. According to the orientation of the duplicated segment, duplications may be in tandem or inverted. The latter are usually associated with a distal deletion. We studied a *de novo* 2.3 Mb inverted duplication of 8q24.3 without apparently associated deletion in a subject with profound psychomotor retardation, idiopathic epilepsy and growth delay. In spite of its small size, the presence of the rearrangement was suspected on standard karyotypes (approximately 400 bands) and later confirmed by Fluorescent *in situ* hybridization (FISH) analysis. We hypothesize that the GRINA gene, a glutamate binding subunit of NMDA receptor ion channel lying within the duplicated segment, may be responsible for the epilepsy. This paper confirms that small subtelomeric *de novo* duplications may be responsible for mental retardation, facial dysmorphisms and/or congenital malformations, although their presence may be overlooked by FISH analysis.

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Introduction

Chromosome duplications have an approximate frequency of 1:4000 in the general population¹ and are found in about 2% of subjects with a typical chromosomal phenotype.² Moreover, the first array-CGH screenings on these patients indicate that cryptic duplications have a much higher frequency, even considering that some of them might represent just polymorphisms.^{3,4} According to the orientation of the duplicated segment, the duplications

may be either in tandem or inverted.⁵ For both types of rearrangement segmental duplications play a primary role in causing nonallelic homologous recombination.⁶ Unlike direct duplications, inverted duplications are usually associated with the deletion of the distal region of the duplicated chromosome. Between the duplicated and the deleted region, a single copy region may be present flanked by homologous segmental duplications. The *inv dup(8p)* is the paradigmatic example of this type of rearrangement.^{7,8} We describe here an inverted duplication of 8q with apparently no associated deletion in a child with profound psychomotor retardation, idiopathic epilepsy and growth delay. The rearrangement was rather obvious in 400 band karyotypes, although molecular techniques demonstrated that the duplication involves only the distal 2.3 Mb of 8q.

*Correspondence: Dr MC Bonaglia, IRCCS Eugenio Medea, Via Don Luigi Monza, 20, 23842 Bosisio Parini (Lecco) Italy. Tel: +39 031 877 111; Fax: +39 031 877 499; E-mail: bonaglia@bp.inf.it
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Materials and methods

Case report

MA, aged 6 years, is the second child of a healthy 37-year-old woman and a nonconsanguineous 39-year-old man, whose family history was unremarkable.

The pregnancy was uneventful, except for feeble foetal movements, which began at 6 months of gestation. Ultrasound findings at 5 and 8 months were referred as normal, with only a slight reduction of the biparietal diameter. Caesarean section was required at the 39th gestational week for breech presentation. Birth weight was 2850 g (between 10th and 25th centile) and birth length was 52 cm (between 50th and 75th centile). Head circumference was not recorded.

Owing to sucking and swallowing difficulties, poor growth and psychomotor retardation at the age of 3 months, the child was admitted for diagnosis to another hospital where a cerebral echo scan, ophthalmologic evaluation and visual-evoked potentials and EEG were performed, all with normal results. Cerebral MRI scan and a standard chromosomal analysis were also reported as normal.

Screening for aminoacidaemias, organic acidurias and fatty acid disorders were normal.

Cerebral MRI, repeated at the age of 1 year and 8 months, and brain stem auditory-evoked potentials at the age of 2 years and 4 months were normal.

At the age of 3.5 months, repeated episodes of generalized seizures were observed, controlled with barbiturate and vigabatrin. No seizures have been observed after the age of 11 months. Only the barbiturate therapy was maintained after the age of 2.5 years.

At the age of 5 years, generalized spike-wave discharges of typical absence seizures were detected without apparent clinical effect.

Psychomotor retardation was clearly evident from the first months of age. The child's language skills were below expectations for her developmental quotient (DQ). At the Brunet-Lezine Psychomotor Development Scale at the age of 2 years and 4 months and of 3 years and 8 months DQ was, respectively, 48 and 56 corresponding to a profound psychomotor retardation. Growth has been delayed with weight constantly around the 3rd centile, length between 3rd and 10th centile and head circumference between -0.5 and -1.0 s.d.

On physical examination at the age of 3 years and 9 months, she presented with short proportionate stature, prominence of the metopic suture with small but proportionate skull, mild ptosis of the left eyelid, large ears with prominent antitragus, large nose, malar hypoplasia, prominent and short philtrum and high palate. Bilateral clinodactyly and camptodactyly of the five fingers was observed. A *café-au-lait* spot with a diameter of 1 cm on the chest was observed. The remaining of the physical examination was normal.

From the age of 3 years till the last evaluation at the age of 6 years and 2 months isolated plasma transaminase (AST, ALT) and gamma glutamyl transpeptidase (GGT) levels have exceeded 2–3 times the normal values. Abdominal sonography and liver biopsy were normal.

Cytogenetic and molecular analysis

Routine cytogenetic analysis was performed on proposita and parents' blood using standard G-bands with a resolution of approximately 400 bands.

Fluorescent *in situ* hybridization (FISH) with whole chromosome painting (wcp) (Multi-M kit, Cytocell Ltd, Adderbury, England) and with telomere-specific probes (Multi-T kit, Cytocell Ltd, Adderbury, England) for all chromosome was carried out on patient's metaphases.

Other FISH experiments were performed with bacterial artificial chromosome (BAC) and prokaryotic artificial chromosome (PAC) clones containing chromosome 8q24-specific sequences from several locations according to the publicly available genome resources (NCBI Map Viewer: <http://www.ncbi.nlm.nih.gov>; Santa Cruz Human Genome Browser: <http://genome.ucsc.edu>; <http://genome.imb-jena.de/>). DNA clones were labelled with biotin and/or digoxigenin using standard nick translation reactions. Double-colour FISH was performed with BAC clones RP11-520P18 (AC083982, ctg NT_037701) and RP11-1022M7 (AC083959 ctg NT_031818) labelled with biotin and digoxigenin, respectively. The probes were visualized with fluorescein isothiocyanate-avidin (Vector Laboratories, Burlingame, CA, USA) or rhodamine-labelled anti-digoxigenin (Oncor Inc., Gaithersburg, MD, USA) and the chromosomes were counterstained with DAPI (Sigma-Aldrich, Milano, Italy). Hybridizations were analysed with an Olympus BX61 epifluorescence microscope and images were captured with the Power Gene FISH System (PSI, Newcastle upon Tyne, UK).

DNA was extracted from 3 ml of peripheral blood with standard protocols. Polymorphic loci were selected based on the NCBI Human July 2003 Assembly viewed on the UCSC browser; amplifications were performed with ABI fluorochrome-labelled primers and analysed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Monza, Italy).

Results

Cytogenetic investigation revealed an alteration on one chromosome 8 consisting of a slight difference in size at the G-negative 8q24.3 band between the two homologues. This difference was evident even if the banding resolution was no higher than 400 bands (Figure 1). Wcp with libraries of all chromosomes revealed that the abnormal chromosome 8 was entirely composed by chromosome 8 material. FISH analysis with all subtelomeric regions was performed to exclude the presence of a cryptic

translocation that could have been missed by the use of painting probes. This analysis demonstrated that the 8q subtelomeric clone was present at the end of the abnormal 8q, although the corresponding signal was much bigger than the one detected on the homologue. In metaphase, this 8q signal was compact and not interrupted by unstained regions, while in interphase two clearly distinguished and separated signals were evident (Figure 2a). FISH analysis with BAC and PAC clones from 8q (Table 1) demonstrated a duplication of about 2.3 Mb from RP11-520P18 clone (AC083982, ctg NT_023684, 144.1–144.3 Mb) to AF235103 (RP1-291P5, RP1-316H24, RP4-659B21, RP5-1047A19, RP5-1109M23, ctg NT_037704, 145.98–146.2 Mb).

Dual-colour FISH (Figure 2b) revealed that the duplication was inverted. According to the whole of the data, the final cytogenetic interpretation was 46,XX,add(8)(-q24.3).ish.inv dup (8)(q24.3) (wcp8+; WI-654+; SHGC-5263+; WI-12784+; RH40626+; RH74682+; 8QTEL25++) with the duplication encompassing the last 2.3 Mb of 8q. Cytogenetic and FISH analysis with Tel8q probe (Cytocell) on proposita's parents gave normal results. A PCR-based assay to establish the origin of the duplication was performed with markers 8QTEL11, 8QTEL25 and

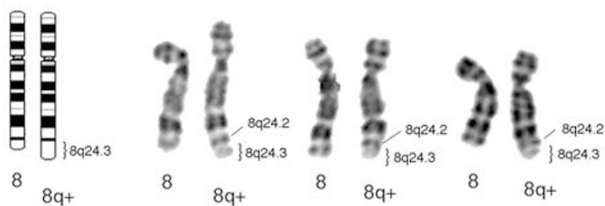


Figure 1 Ideogram of chromosomes 8 (right); cut-out of normal and abnormal chromosomes 8 (left) at resolution of 400 bands.

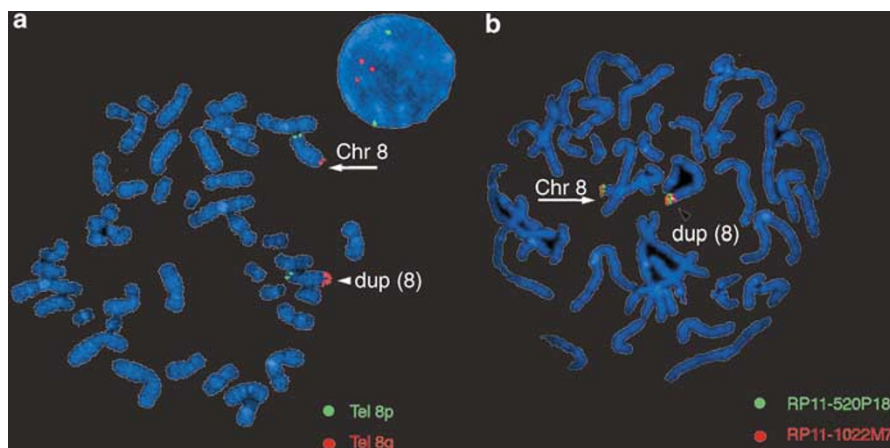


Figure 2 Proband's FISH analysis: (a) Specific subtelomeric probes for chromosome 8p (green) and 8q (red). Abnormal chromosome 8 (arrowhead) shows on 8qtel (red) a signal bigger than the one detected on the homologue (arrow). In metaphase, this 8q signal was compact and not interrupted by unstained regions, while in interphase, two clearly distinguished and separated signals are evident. (b) BAC clones RP11-520P18 (green) and RP11-1022M7 (red) demonstrating the inverted duplication (arrowhead).

RH74682. RH74682 typing demonstrated that the duplication was of maternal origin, while 8QTEL25 showed that it involved only one of the two maternal alleles (Figure 3).

A schematic map of the 8q24.3 region showing the relative location of all probes is presented in Figure 4.

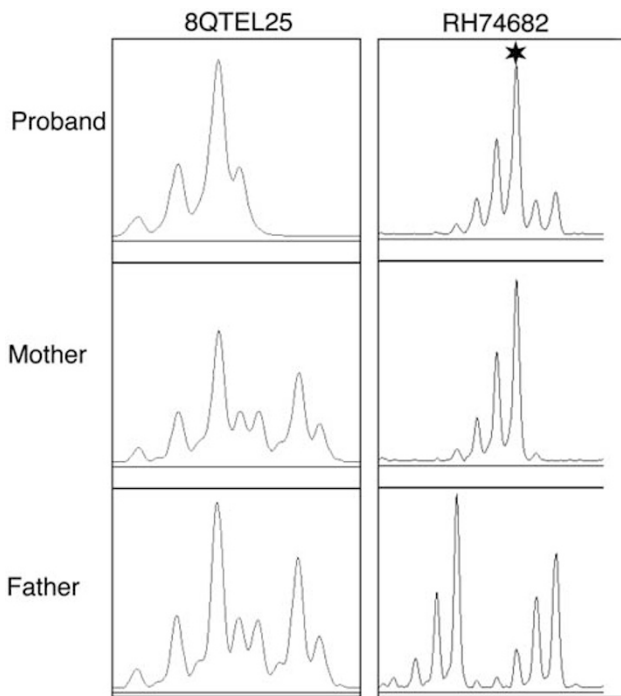
Discussion

The rearrangement

Inverted duplications are probably more frequent than suspected until 5–10 years ago. In fact, some of them, originally interpreted as direct duplications on pure cytogenetic analysis, turned out to be complex rearrangements after proper molecular analysis showing not only a duplicated but also a deleted region. The studies on the inv dup(8p), a recurrent rearrangement associated with a rather characteristic syndrome,⁹ revealed that it derives by nonallelic homologous recombination between low-copy repeats.^{7,8,10} Other examples of inv dup are those concerning 1q,^{11,12} 2q,¹³ 3p,^{14,15} 4p,¹⁶ 5p,^{17,18} 9p,¹⁹ 10p and 10q,²⁰ 18p,²¹ 18q,²² 21q²³ and the X;X or the Y;Y rearrangements leading to duplications of parts of the short or the long arm with concomitant deletion,²⁴ suggesting that the mechanism responsible for the inv dup(8p) can be generalized to all inverted duplications. In our inv dup(8q) case, we were not able to find any associated deletion distal to the last duplicated clone RP5-1109M23 nor any segmental duplication responsible for the occurrence of the inv dup(8q). In this regard, the inv dup(8q) seems to be atypical. However, in the duplicated 8q24.3 region two gaps are still present (UCSC, release July 2003). *In silico* analysis²⁵ revealed that most of the genome sequence gaps are associated with segmental duplications and that telomeric regions are significantly enriched in segmental duplication content. We believe that the results we

Table 1 FISH analysis results

Clone	Accession number (UCSC)	Distance (Mb)	Contig	STS	FISH
RP11-104C10	AC091077	143.2	NT_028251	—	+
RP11-304E6	AC073385	144.0	NT_008127	WI-654	+
RP11-520P18	AC083982	144.1–143.3	NT_0023684	SHGC-5263	++
CTA-393G12	AF205589	145.5–145.6	NT_037704	WI-12784	++
RP11-1022M7	AC083957	145.9–146.1	NT_037704	RH40626	++
RP5-1111B24	AC087720	146.2	NT_037704	RH74682	++
RP1-291P5	AF235103	145.6–146.2	NT_037704	8QTEL25	++
RP3-316H24	AF235103	145.6–146.2	NT_037704	8QTEL25	++
RP4-659B21	AF235103	145.6–146.2	NT_037704	8QTEL25	++
RP5-1109M23	AF235103	145.6–146.2	NT_037704	8QTEL25	++

**Figure 3** Molecular typing with polymorphic STS located within the duplicated portion of chromosome 8; in RH74682, the duplicated allele is indicated by a ★ symbol.

obtained are not compatible with the current sequence release of the distal 8q and it seems possible that a refinement of the sequence will support a mechanism similar to the classic inv dup rearrangements. Indeed, the finding that interphase FISH analysis with the subtelomeric probe (TEL8q Cytocell) (Figure 2a) showed two clearly separated signals suggests that a single copy region might be present distal to the duplicated one. Although we could not demonstrate the presence of the distal deletion associated to the duplication, we cannot exclude it and it seems likely that the original product of the abnormal maternal meiosis originating the rearrangement present in the proposita were a dicentric chromosome 8pter→

8q24.3::8p24.3→8pter that, at meiosis II or during early embryogenesis,²⁶ underwent a breakage leading to the present inv dup(8)(q24.3) characterized by a very distal 8q deletion and a 2.3 Mb duplication.

Genotype/phenotype correlation

The phenotype of our patient is characterized by severe psychomotor and mental retardation, idiopathic epilepsy (seizure without apparent clinical effect) and elevated blood levels (2–3 times higher than normal values) of plasma transaminase (AST, ALT) and GGT.

Among patients with epilepsy and intellectual disability, about 6% have chromosomal abnormalities and this figure climbs to 50% in patients with seizures and multiple congenital anomalies.^{27,28} To date no less than 400 different chromosomal imbalances have been associated with seizures or EEG abnormalities consisting in either deletions or duplications.²⁹

The 8q24 region was previously implicated in different forms of epilepsy (MIM 601068; MIM 603210; MIM 606970; MIM 607876; MIM 600131; MIM 602232; MIM 121201; MIM 138251). The KCNQ3 locus, whose mutations are associated to BFNC (benign familial neonatal convulsions, MIM 121200), lies 10 Mb upstream from the 8q segment duplicated in our patient. Thus, any involvement of this gene in the aetiology of our patient's epilepsy is excluded. However, the GRINA gene (MIM 138251) (Figure 4), a glutamate binding subunit of NMDA receptor ion channel proposed as a candidate for the idiopathic generalized epilepsy mapping at 8q24 (MIM 600669), is located in the duplicated 8q region. We hypothesize that duplication of the GRINA gene is responsible for the epilepsy of our patient. The finding that ion channel genes are dosage sensitive, clearly demonstrated at least for haploinsufficiency,³⁰ reinforces our hypothesis.

The human GPT gene (MIM 138200) was mapped to 8q24.3,^{31,32} within 200 kb of the 8q telomere, thus within the duplicated region (Figure 4). This finding suggests that the increase of at least the GPT values is due to gene dosage effect. An increased GPT activity was similarly reported in a patient with 8q24.2-qter duplication.³²

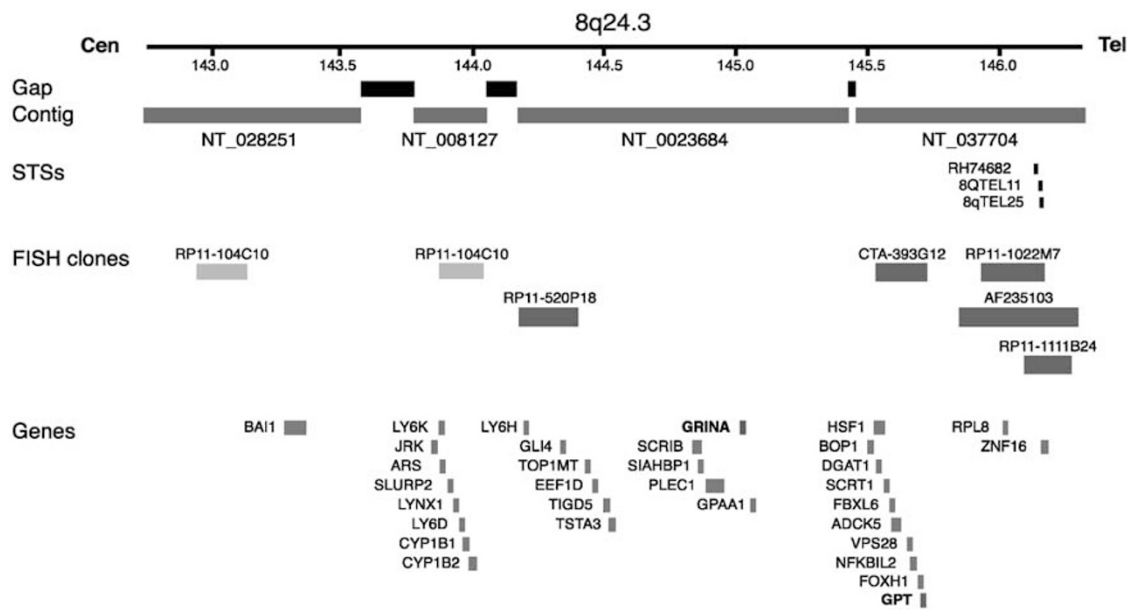


Figure 4 Map of the 8q24.3 region showing the relative position of contigs, sequence gaps, STS and FISH probes, and a selection of known genes; the drawing is based on the UCSC map, November 2003 release; FISH clones in the duplicated region, as well as the GRINA and GPT genes, are shown in a darker shade of grey.

Final considerations

Subtelomeric FISH studies demonstrating the 2.3 Mb duplication have been performed to investigate the suspicion situation of 8q noticed on 400 G-band karyotypes and not because requested by the clinicians. In the last few years, a general consensus has been reached on the fact that the more closely chromosomes are studied, the lower is the chance of finding a rearrangement with the molecular test;³³ in everyday practice, rearrangements of 5–10 Mb or even larger are easily missed.^{33,34} Thus, it is rather astonishing that our 8q duplication was suspected on 400 G-band karyotypes. We might hypothesize that the chromatin status of the duplicated distal 2.3 Mb 8q is less compact than the rest of the metaphasic chromosome, as if the duplication had induced an epigenetic decondensation of the chromatin at least in lymphocytes. Whereas no example of chromatin decondensation induced by a chromosome rearrangement is known, the opposite phenomenon, that is, the silencing of the chromatin accompanied by chromatin condensation, is well known especially in unbalanced X/autosome translocations in which up to 45 Mb of the translocated autosome may be silenced and condensed.³⁵ We shall see in the future if similar observations will confirm that chromatin decondensation induced by a chromosome rearrangement may render visible otherwise undetectable rearrangements.

Among the more than 2500 cases tested for subtelomeric rearrangements, only three *de novo* duplications have been detected³⁶ out of 99 rearrangements. Obviously, duplications are more difficult to detect by FISH, since the

operator should be on the look out for differences in the size of the signal and not simply discriminate between the presence or absence of a signal. Therefore, their frequency may have underestimated. This paper confirms that small subtelomeric *de novo* duplications may be responsible for mental retardation and congenital anomalies.

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