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SHORT COMMUNICATION

Pure de-novo 5 Mb duplication at Xp11.22–p11.23 in a male: phenotypic and molecular characterization

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Abstract Males with duplications within the short arm of the X chromosome are rare and most cases are inherited from a maternal heterozygote. Here we describe the first detailed characterization of a de-novo Xp duplication delineated to Xp11.22 → Xp11.23 in a 15-year-old male with moderate mental impairment, autistic-like behaviour, short stature, and mild dysmorphic features. Chromosome analysis (550 band resolution) was normal and comparative genomic hybridization (CGH) analysis on metaphase spreads detected duplication on Xp11. Further characterization of the duplication by array CGH, FISH experiments with specific BAC probes, and genotyping with microsatellite markers helped to determine proximal and distal breakpoints giving a size of the duplication of approximately 5 Mb. As far as we are aware this is the first described male with isolated microduplication on Xp11.22–Xp11.23. Among the genes included within the duplicated region, and particularly those which are outside copy number polymorphisms, we discuss the relationship of *FTSJ1*, *PQBP1* and *HDAC6* with the clinical symptoms of our patient.

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Introduction

Pure duplications of the short arm of chromosome X in males are rare (Zhang et al. 1997), and the aberrant phenotype associated with these duplications is usually attributed to excess gene dosage, because of functional disomy of the duplicated region. Most cases are inherited from a heterozygote mother. Surviving males with Xp duplication are almost invariably mentally retarded, have a variety of minor anomalies, and are small in stature (Nielsen et al. 1982; Cianchetti et al. 1992; Lugtenberg et al. 2005). Several cases of Xp duplication had sex reversal related to the presence of two active copies of the DAX1 gene located in Xp21 (Baumstarck et al. 1996; Swain et al. 1998). In this communication we report phenotypic and laboratory findings for a male proband with a pure de-novo Xp duplication identified by using metaphase comparative genomic hybridization (CGH), with further delineation of the duplicated region by array-CGH to Xp11.22 → Xp11.23.

Clinical report

This boy is a second child of a healthy 30-year-old woman and a non-consanguineous 37-year-old man whose family history was unremarkable. The spontaneous pregnancy was uneventful. He was born at 35 weeks of gestation. Height, weight, and head cir-

cumference were in range for the term. The first six months were reported as normal but motor and verbal milestones were secondary delayed with walking reported at 20 months and the first words after 30 months. Alteration of social contacts, with poor eye contact, was also reported early. Hyperkinesia was described at 3 years with poor peer interaction. At the same age the child's language skills were below expectations for his developmental quotient with, in addition, verbal stereotypes. Owing to the autistic-like behaviour, he received special education in a child psychiatric centre for autistic patients. Sleep was reported as normal.

At 4 years, he had a normal male karyotype and was negative for a CGG repeat expansion in the FMR1 gene responsible for the fragile-X syndrome. The EEG showed irregular spikes in the left temporal area, accentuated by intermittent light stimulation. Encephalic MRI examination was performed at the age of 9 years and showed evidence of frontoparietal cortical atrophia. On physical examination at the age of 15 years he had reached his final height, because of early onset of puberty. His height was 164.6 cm (–1.8 SD), unlike the parental target height (185 cm, +2SD). Facial morphology was normal except for a synophrys, a low posterior hairline on the neck, and over-folded ear helices (Fig. 1). He was moderate mentally retarded, but full scale IQ testing had not been performed.

Cytogenetic and molecular investigations

Chromosome analysis was performed on peripheral blood lymphocytes by means of RBG banding. A CGH analysis was performed on the metaphase spreads of the patient in accordance with standard procedures in our laboratory (Bourdon et al. 2004) with Quips XL image-analysis software (Vysis, Downer's Groves, IL, USA). Fluorescence in-situ hybridization analysis (FISH) with a whole-chromosome painting probe for the X chromosome (Vysis) was performed on the patient's metaphases. Other FISH experiments were conducted with bacterial artificial chromosomes (BAC) clones containing chromosomes Xp11.2-specific sequences from several locations, in accordance with publicly available genome resources (NCBI Map Viewer: http://www.ncbi.nlm.nih.gov; Santa Cruz Human Genome Browser: http://www.genome.ucsc.edu). BACs were obtained from the RPCI-1 and RPCI-11 libraries (BACPAC Resources Center, CHORI, Oakland, CA, USA) and selected according to their position on chromosome X: RP11-552E4 (Xp11.23), RP11-107C19 (Xp11.23), RP11-363G10 (Xp11.22), RP1-290F12 (Xp11.22), and RP11-258C19 (Xp11.22). BAC DNA was labelled with biotin by nick translation. The labelled probes were visualized with fluorescein isothiocyanate–avidin (Vector Laboratories, Burlingame, CA, USA).

Fig. 1 Facial appearance of the patient at 15 years of age

To analyse the chromosome duplication at higher resolution, array CGH analysis was performed (Bonnet et al. 2006). Patient and reference genomic DNAs (Promega, Madison, WI, USA) were split into two samples for differential labelling with Cy3 and Cy5-labelled nucleotides (Amersham Biosciences, Orsay, France) in accordance with standard random priming procedures. The patient Cy3-labelled DNA was mixed with the Cy5-labelled reference DNA and the patient Cy5-labelled DNA was mixed with the Cy3-labelled reference DNA. Each mix was denatured, and applied to 1 Mb resolution human DNA microarrays, in accordance with the manufacturer's recommendations (Spectral Genomics, Houston, TX, USA). Slides were hybridized for 48 h, washed in 50% formamide and 2× SSC solutions at 45°C, and scanned on a GenePix 4000B Axon scanner. Images were analyzed with SpectralWare software provided by Spectral Genomics.

Genotyping of the patient and his mother was performed with 14 chromosome X microsatellite markers selected from the Genome Database (http://www.gdb.org): DXS8085 (Xp11.4), DXS8054 (Xp11.3), DXS8026 (Xp11.3), DXS1003 (Xp11.3), DXS 337 (Xp11.3), DXS1367 (Xp11.23), DXS1126 (Xp11.23), DXS1470 (Xp11.23), DXS573 (Xp11.23), DXS8023 (Xp11.22), DXS8024 (Xp11.22), DXS1039 (Xp11.22), DXS2505 (Xp11.22), and DXS2507 (Xp11.22). Amplification products were run on an ABI Prism 310 Genetic Analyser machine and fragment sizes were determined by use of GeneScan software (Applied Biosystems, Forster City, CA, USA).

Results

At the age of 15 years, the proband had a normal 46 XY karyotype at 550-band resolution. Both parents were karyotypically normal.

By use of CGH on the patient's metaphases, a gain was observed in DNA material corresponding to Xp11 (Fig. 2a). Because CGH analysis of the mother's DNA did not reveal any gain in Xp11, this additional material arose de novo. FISH analysis performed on lymphocyte metaphase spreads of the patient using a whole-chromosome painting probe for the X chromosome revealed hybridization along the entire length of the X chromosome and no signal on any other chromosome (data not shown), confirming the Xp11 duplication.

Resolution of the duplication was refined by using array-CGH. A gain of chromosomal material was noted in the patient (Fig. 2b); the probes identified as duplicated were RP11-107C19 (Xp11.23), RP11-58H17, RP11-637B23, and RP11-363G10 (Xp11.22) which extends from nucleotide 48–51.9 Mb.

FISH experiments confirmed the presence of the chromosome duplication identified by microarray analysis (Fig. 2c). Large insert clones mapping within and immediately adjacent to the duplicated segment were hybridized to metaphase and interphase nuclei from cultured peripheral leucocytes from the proband. The proximal breakpoint mapped between clones RP11-363G10 (duplicated) and RP1-290F12 (not duplicated) whereas the distal breakpoint was not determined by FISH (the more distal clone RP11-552E4 was duplicated).

Molecular typing showed that 12 of 14 microsatellite markers were informative. Both maternal alleles were transmitted to the patient, for six informative markers; the duplication therefore arose from an interchromosomal event involving both maternal X chromosome

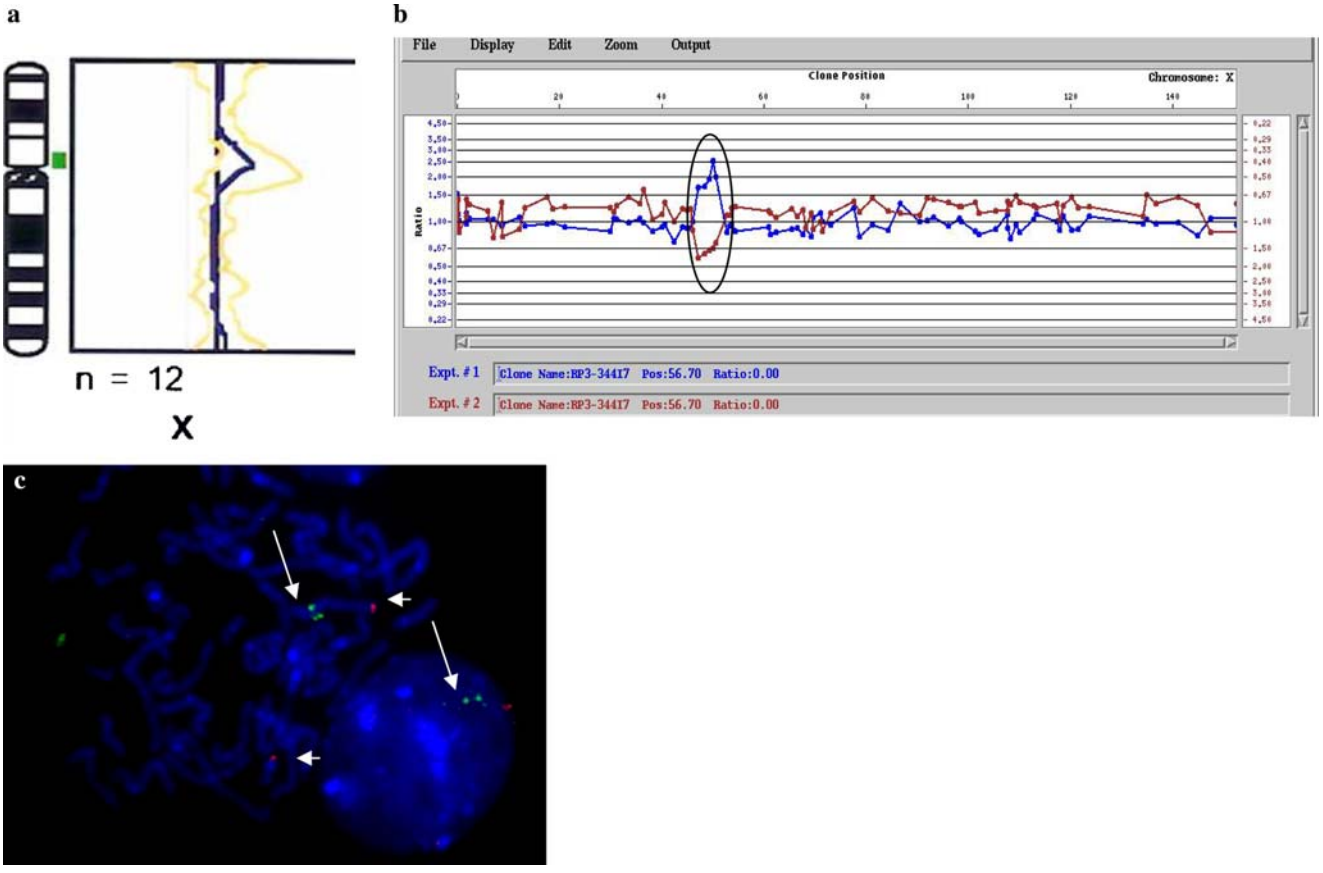

Fig. 2 **a** CGH ratio profiles of chromosome X showing an excess of material from Xp11 (data were obtained from analysis of 12 chromosomes X). **b** Chromosome X ratio plot with deviations from the expected 1:1 ratio from clone RP11–107C19 (Xp11.23) to clone RP11-363G10 (Xp11.22). The *blue* and *red lines* represent, respectively, the Cy5-patient DNA compared with the Cy3-reference (*first slide*) and the Cy3-patient DNA compared with the Cy5-reference (*second slide*) ratio profiles. Placement of the *blue line over the red* indicates gain of material (SpectralWare software, Spectral Genomics). **c** FISH analysis of the clone RP1-552E4 showing the duplicated green signal (*arrow*) on metaphase chromosome X and in the nucleus. The red signal (*arrow head*) corresponds to the telVysion Xq/Yq probe (Vysis)

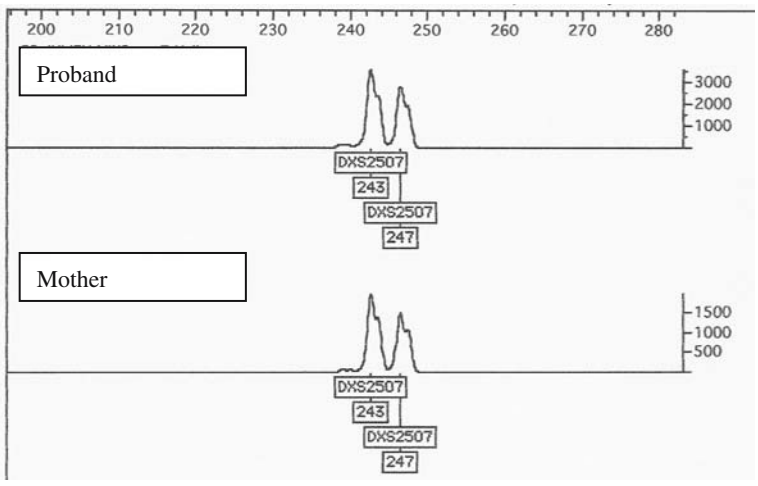

Fig. 3 Genotyping with DXS2507 (current position 52.5 Mb) within the duplicated region. Both maternal alleles were transmitted to patient confirming that the duplication arose from an interchromosomal event between both maternal X chromosome homologues

homologues (Fig. 3). The duplication extended from DXS1126 (Xp11.23 current position 48.2 Mb) to DXS2507 (Xp11.22 current position 52.5 Mb). Microsatellite markers from DXS8085 (Xp11.4 current position 42.08 Mb) to DXS1367 (Xp11.23 current position 47.5 Mb) were not duplicated. This enabled us to determine the distal breakpoint of the duplication between DXS1367 (Xp11.23 current position 47.5 Mb) and RP11-552E4 (current position 47.8 Mb) and the proximal breakpoint between DXS2507 (Xp11.22 current position 52.5 Mb) and RP1-290F12 (current position 52.9 Mb) giving a size of duplication of 4.7 and 5.4 Mb (Fig. 4).

Discussion

Duplications and translocations on the short arm of chromosome X have previously been described in males and in females (Schinzel 2001). Expression of the

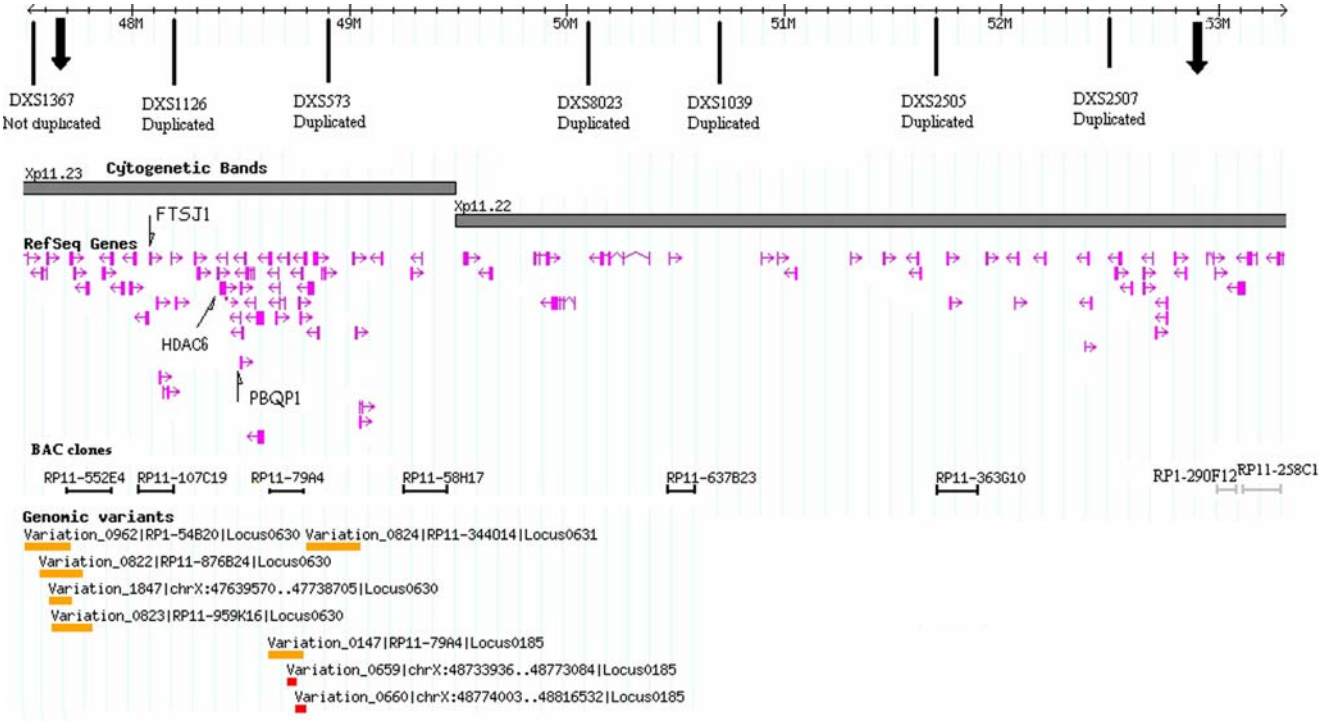

Fig. 4 Map of the Xp11.2 region showing the relative position of microsatellite markers, Ref Seq genes, BAC clones, and genomic variants. BAC clones in the duplicated region are shown in a *darker shade of grey*. *Thick black arrows* indicate the extent of the duplication and thin arrows the three candidate genes, *FTSJ1*, *PBQP1*, and *HDAC6*. The drawing is based on the UCSC map, May 2004 release (http://www.genome.cse.ucsc.edu/), and the Database of Genomic Variants (http://www.projects.tcag.ca/variation/)

X duplication phenotype in males is well supported by a gene dosage effect. In contrast, most females with X duplications have been reported to be phenotypically normal, because skewed X inactivation leaves the dup(X) inactive. Nevertheless, the X-inactivation pattern and clinical findings are variable among females with chromosome X duplication. Here we describe the first detailed characterization of a de-novo Xp duplication delineated to Xp11.22 → Xp11.23 in a 15-year-old male with moderate mental impairment, autistic-like behaviour, short stature, and mild dysmorphic features. As far as we are aware, only one of the reported Xp duplications in males overlaps with the duplication described here and seven cases of female patients (see Table 1). The commune phenotype of such duplications is mental retardation, developmental delay, and short stature. In all these cases, however, the duplication involved a larger part of Xp; in addition, a detailed molecular cytogenetic definition of the duplicated segment has rarely been provided. A reliable phenotype–genotype correlation is, therefore, difficult to draw.

The duplicated region in this case contains as many as 100 identified genes and it is unclear which of these are relevant to the disorder. The mental retardation and autistic-like behaviour are probably caused by a dosage effect of one or more genes located within the duplication on Xp11.2. We cannot, however, exclude the possibility that the disruption of gene(s) at the breakpoint(s) of the duplication and positional effects of the genes in the altered segment contributed to the phenotype of the proband.

Several independent groups have recently published genome-wide polymorphic regions detected by array CGH or representational oligonucleotide microarray analysis (Iafrate et al. 2004; Sebat et al. 2004; Sharp et al. 2005). According to the Database of Genomic Variants, a curated catalogue of large-scale variation in the human genome (http://www.projects.tcag.ca/variation/), several copy number polymorphisms have been described on Xp11.23 (Fig. 4). These data can aid identification of potential genes which are more likely to be causative of the proband's phenotype. Among these we identified three genes: *FTSJ1*, *PQBP1*, and *HDAC6*. FISH studies and genotyping with microsatellite markers confirmed that *FTSJ1* and *PQBP1* genes are duplicated and not structurally disrupted at the breakpoints of the duplication. Interestingly, *FTSJ1* and *PQBP1* located outside polymorphic regions have been involved in non-specific X-linked mental retardation (MRX) and could contribute, through their overexpression, to mental retardation and autism. FTSJ1 protein is a homologue of *Escherichia coli* RNA

Table 1 Summary of selected findings associated with Xp11.2 duplications in the current case and for previous patients with overlapping duplications

Karyotype	Clinical features	X-inactivation status	Ref.
46,dup(X)(p11.22 → p11.23)Y	Mental retardation, developmental delay, short stature, and mild dysmorphic features	–	This case
46,dup(X)(p11.2 → p21.2)Y	Mental retardation, short stature, developmental delay, obesity, macrocephaly, hypotonia, congenital heart disease, and abnormal EEG	–	Nielsen et al. (1982)
46,X,dup(X)(p11.2 → p21.2) (Mother of case 2)	Not reported.	Selective X-inactivation	Nielsen et al. (1982)
46,X,dic inv dup(X)(p22.3 → cen)	Short stature, mental retardation, developmental delay, mild dysmorphic features, and seizures	Skewed but not completely selective X inactivation (11–89%)	Tuck-Muller et al. (1993)
46,X,dic inv dup(X)(p22.3 → cen) (Mother of case 4)	Short stature, mental retardation	Selective X-inactivation	Tuck-Muller et al. (1993)
46,X,dup(X)(p11.21 → p21.3)	Poor weight gain, developmental retardation, and several minor anomalies including pigmentary dysplasia	Random X-inactivation	Matsuo et al. (1999)
46,X,dup(X)(p11 → p21.2)	Macrosomia, polyhydrammios, macrocephaly, dysmorphic features, respiratory distress, enlarged kidneys, congenital heart disease, cerebral anomalies, and abnormal EEG, severe hypotonia The patient died aged 2 months	Random X-inactivation	Portnoï et al. (2000)
46,X,rec(X)dup(Xp)inv(X)(p11.22q21.2) Trisomy for Xp11.21 → pter Monosomy for Xq21.2 → qter	Gonadal dysgenesis	Inactivation of the rearranged X chromosome	Adamson et al. (2002)
46,XX,der(13)t(X;13)(p11.2;p11.2)	Dandy–Walker malformation, macrosomia, facial dysmorphism, congenital heart defects, and structural brain malformations	The derivative chromosome was not subject to X inactivation	Kolomietz et al. (2005)

methyltransferase FtsJ/RrmJ and may play a role in the regulation of translation (Freude et al. 2004). Mutations in this gene have been associated with MRX in four different families (Ramser et al. 2004). PQBP1 is a nuclear polyglutamine-binding protein that contains a WW domain and mediates cellular functions by binding to polyglutamine tracts via its polar amino acid-rich domain (Waragai et al. 1999). Enokido et al. (2002) examined the effects of PQBP1 on primary-cultured cerebellar neurons and concluded that overexpression of PQBP1 inhibits basal transcription in cerebellar neurons and increases their vulnerability to low-potassium conditions. In five of twenty-nine families with XLMR, Kalscheuer et al. (2003) identified mutations in the PQBP1 gene that caused frameshifts in the fourth coding exon, which contains a stretch of six AG dinucleotides in the DR/ER repeat.

The histone deacetylase-6 gene (*HDAC6*) is also located within the duplicated region and outside polymorphic regions. There is good evidence that alterations in signalling pathways involving the Rho family of small GTPases, key regulators of the actin and microtubule cytoskeletons, contribute to both syndromic and nonsyndromic mental retardation disorders (Newey et al. 2005). HDAC-6 is a microtubule-associated protein capable of deacetylating alpha-tubulin in vivo and in vitro (Zhang et al. 2003). Reversible acetylation on the epsilon-amino group of alpha-tubulin Lys40 marks stabilized microtubule structures and may contribute to regulating microtubule dynamics. Because overexpression of *HDAC6* in mammalian cells leads to tubulin hypoacetylation (Zhang et al. 2003) we speculate that deregulation of *HDAC6* could contribute to our patient's phenotype.

In summary, we report a male proband with a de-novo duplication of Xp11.2. Array CGH refined the breakpoints of the duplication at the molecular level, confirming the utility of this technology for characterization of aneusomies. Further analysis for duplications in this region in patients with MRX will give more insight in the possible involvement of Xp11.2 genes in non-syndromic mental retardation.

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