



# An Additional Case of the Recurrent 15q24.1 Microdeletion Syndrome and Review of the Literature

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We report a 9-year-old girl with 3 Mb interstitial deletion of chromosome 15q24 identified by oligonucleotide array comparative hybridization. She is of Chinese ancestry and shared some typical features of previously reported 15q24 deletion cases such as mild dysmorphism with developmental and speech delay. She also had mild hearing loss that was reported in one other case. We compared all 19 cases that are identified from array-CGH. The deletion occurred within an 8.3 Mb region from 15q23 to 15q24.3. The minimum overlapping deleted region is less than 0.5 Mb from 72.3 Mb to 72.7 Mb. The functions of the nine annotated genes within the region and how they might contribute to the microdeletion phenotype are discussed.

■ **Keywords:** 15q24, developmental delay, dysmorphism, microdeletion, qRT-PCR, speech delay

The use of chromosomal microarray in congenital pediatric disorders has led to the identification of a number of chromosomal aberrations. One of the recurrent regions is a microdeletion in the 15q24 region, with 18 cases reported thus far. With more reports of patients who have similar deletions in this region and share a set of phenotypic presentations, it has been recognized as a new syndrome recently. Common presentations include mild to moderate developmental delay, growth deficiency, dysmorphism, hand anomalies, and genital malformation in males (Cushman et al., 2005; Sharp et al., 2007; Van Esch et al., 2009). Depending on the size of the deletion and the genes involved, other features that may be present include abnormalities of the heart, central nervous system, skeletal system and ophthalmic system.

We report an additional case with identified deletion involving the critical region of this recurrent 15q24 syndrome. This patient has a number of phenotypic traits that are defining features of this syndrome. We compare this case to previously reported cases for common phenotypic presentations and overlapping deletions and suggest candidate genes for the observed traits. We further define the smallest region deleted in all reported cases.

## Materials and Methods

### Subject Description

The Chinese girl was referred to the hospital's Genetics Service at 10 months for evaluation of craniosynostosis and suspected syndromic disorder. She is the second child of a consanguineous marriage between first-degree cousins. The father and mother were 32 and 31 years old at the time of her birth. The father's two siblings were reported to be slow in learning to walk independently when young. The father had G6PD deficiency but was otherwise normal. The mother and an elder sister were apparently normal.

The patient was born at 35 weeks of gestation after an uneventful pregnancy. Her birthweight was 2220 g. Her head circumference was 32 cm and her body length was 45 cm. Delivery by cesarean was uneventful with Apgar scores of 9 and 10 at 1 and 5 min.

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She had labial adhesions that were surgically corrected shortly after birth. She presented with mild dysmorphism with midface hypoplasia, depressed nasal bridge, frontal bossing, plagiocephaly, slight downslanting palpebral fissures, and right epicanthus inversus with strabismus and right convergent squint; and mild conductive hearing loss for the right ear. She also had long thin fingers and left simian crease that was reported in one other 15q24 deletion case (El-Hattab et al., 2010). There was no hypoplasia of distal phalanges or broad thumbs.

Her muscle tone was within normal limits with no evidence of hypotonia. 3-D computed tomography at 12 months showed premature fusion of the coronal and lambdoid sutures with mild left occipital phagiocephaly and prominence of the right frontal bone. Magnetic resonance imaging of the brain at 28 months showed relatively small orbits relative to cranium size. There was no significant abnormality in the brain parenchyma. The two globes, extra-ocular soft tissue and optic nerves were all normal and symmetric. Bilateral strabismus surgery for correction of squinting was performed at 3½ years of age. She had impacted cerumen and otitis media, and had bilateral myringotomies at 5 and 8 years of age.

She was able to sit unsupported at 8 months and walked at 16 months. She started speaking at 2 years but progress was slow. At 8 years of age, she could say about 10 words. Her physical growth was normal with height and head circumference between 50th and 75th centile but her weight was at about 25th centile. She is presently attending a special school and undergoing language therapy and occupational therapy.

### Laboratory Methods

The study was approved by the Institutional Review Board of the hospital. Written informed consent for genetic study was obtained from the parents. Chromosome analyses were performed on metaphase spreads prepared from phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes using standard methods.

DNA was extracted from peripheral blood using using the Gentra Puregene Blood Kit (Gentra Systems Inc., Minneapolis, USA). DNA was checked for quantity and purity using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, USA). NimbleGen Human CGH Array consisting of 385K 45- to 85-mer probes with median probe spacing of 6kb (Roche NimbleGen, Madison, WI, USA) was used to test for genome imbalance. Reference DNA was from a Chinese female. Genomic DNA was labeled using CyDye (Cy3 or Cy5 9mers) fluorescent nucleotides (Sigma-Aldrich, St. Louis, MO, USA). Test DNA was labeled with Cy3-dUTP and reference DNA with Cy5-dUTP. The efficiency of the labeling was measured using a Nanodrop Spectrophotometer. The labeled reference and test DNA samples were hybridized to a 384K Human Genome CGH array at 42°C in a MAUI Hybridization System (BioMicro Systems,

Salt Lake City, UT, USA) for 18 hours. All experimental procedures were performed according to manufacturer's instructions.

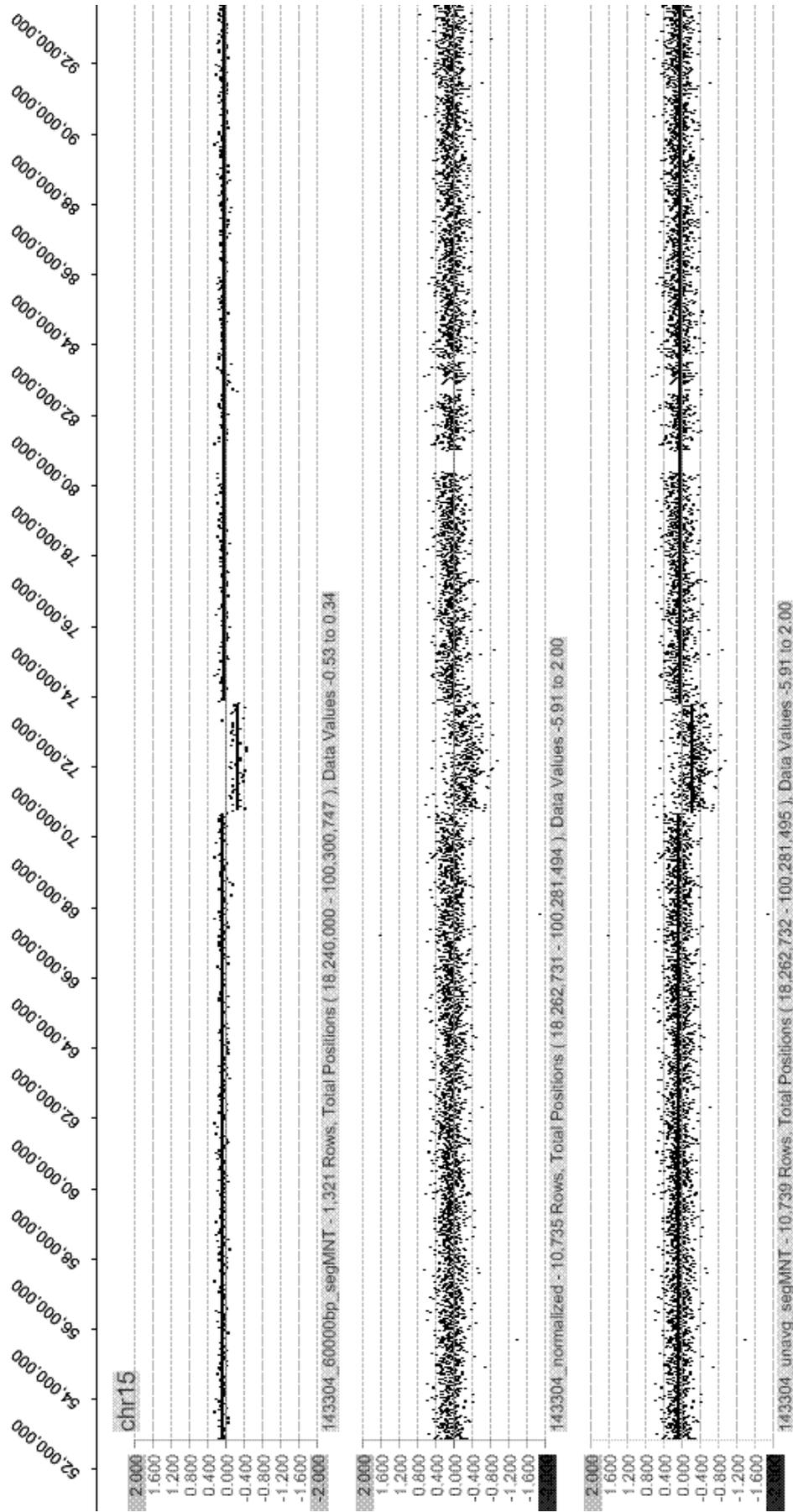
Arrays were scanned using a GenePix 4000B scanner and analyzed using the GenePix Pro 6.0 software (Axon Instruments, Sunnyvale, CA, USA). Data was extracted from scanned images and processed with SegMNT from NimbleScan (version 2.4.27) Data normalization was performed using the NimbleScan software package. The normalized intensities were subsequently analyzed with CGH-segMNT that uses the circular binary segmentation algorithm to determine the significant breakpoints in log2 intensity ratios along each chromosome. A minimum segment length of 2 probes and average window sizes of 1 kb and 10 kb were used. Results were visualized using SignalMap (version 1.9.0.03) after normalization of signal intensities.

Abnormality in copy number was confirmed by relative quantitative real-time-PCR with SYBR Green dye and CYP1A1 (Cytochrome P450, subfamily 1, polypeptide 1 gene) as the target gene for quantifying gene copy number. Primers were designed using Primer Express (Version 3.0), and the experiment was carried out in triplicate. The patient's and mother's DNA samples were amplified in the same experiment with HBB (hemoglobin beta gene) as the internal reference. Amplification was done using Applied Biosystems StepOnePlus real time PCR system (Applied Biosystems, Foster City, CA, USA). Results were analyzed using Applied Biosystems StepOne software (version 2.1).

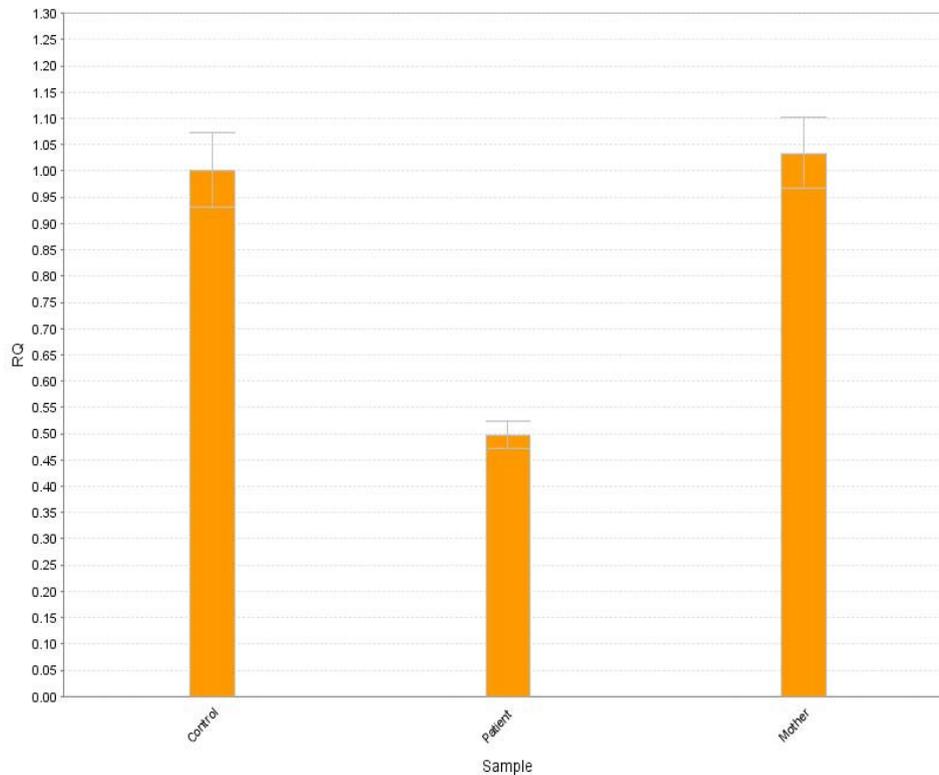
### Results

Chromosomal analysis carried out using standard G-banding of cultured lymphocytes reported normal female karyotype of 46,XX (data not shown). Array CGH analysis using a high resolution 385K platform from NimbleGen showed copy number loss for chromosome 15q24. The minimum size of the deletion was estimated to be about 3.1Mb from 70,687,579 to 73,856,393 (Figure 1) based on NCBI build 36/hg18. The last probe with normal copy number was at position 70,681,477 (probe ID CHR15P070681477) while the first probe with copy number loss was at position 70,687,578 (Probe ID: CHR15P070687578). The last probe with copy number loss was at position 73,856,392 (probe ID: CHR15P073856392) while the first probe with normal copy number was at position 73,868,960 (Probe ID: CHR15P070681477). The minimum size of loss is 3,168,814 bp and the maximum size of deletion is 3,187,483 bp.

Investigation using quantitative real-time PCR confirmed the deletion in the patient while the mother had normal copy number (Figure 2). The DNA of the father was not available for analysis. Both parents were reported to be normal.



**FIGURE 1**  
SignalMap screen capture showing the region with copy number loss.

**FIGURE 2**

Confirmation of gene dosage loss in the patient relative to her mother and a control sample. The copy number for the mother is normal and comparable to the control DNA.

## Discussion

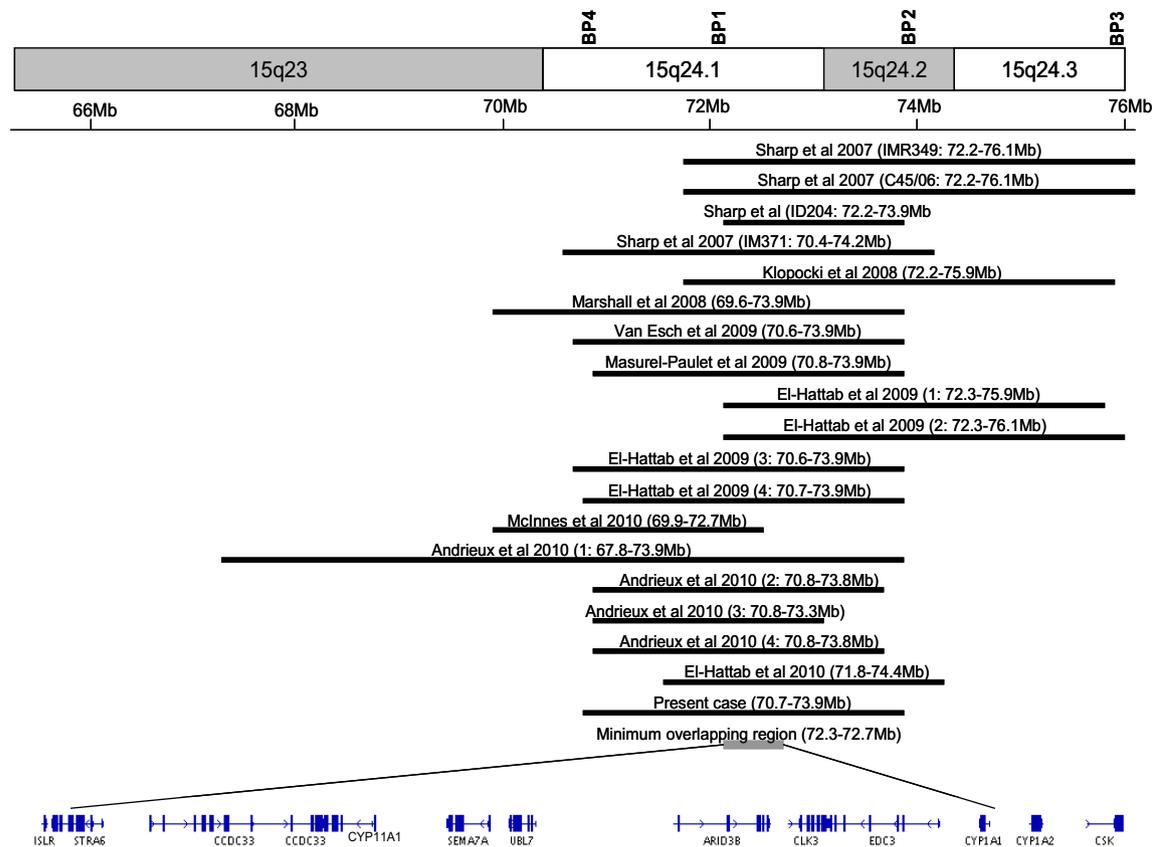
Microdeletion of 15q24 was first reported by Cushman et al. in three cases detected by FISH (Cushman et al., 2005). To date there are 18 reported cases involving 15q24.1 deletion which are detected by array-based methods (Andrieux et al., 2009; El-Hattab et al., 2009; El-Hattab et al., 2010; Klopocki et al., 2008; Marshall et al., 2008; Masurel-Paulet et al., 2009; McInnes et al., 2010; Sharp et al., 2007; Van Esch et al., 2009). The sizes of the deleted regions range from 1.7 Mb to 6.1 Mb. Fifteen of the cases had the proximal breakpoints within 15q24.1, while the starting points of the deletion of the remaining three cases were more centromeric. For the latter group, two cases from different ASD cohorts (Marshall et al., 2008; McInnes et al., 2010) had deletions starting from 69,601,300 and 69,897,977; while another case (deceased at 25 months) had the start of the breakpoint at 67,807,119 (Andrieux et al., 2009). The last patient also had the biggest deletion of 6.1 Mb. The distal breakpoints of the deletions were within 15q24 in all 18 cases, with one case within 15q24.1, 12 cases within 15q24.2 and the remaining five within 15q24.3.

In cases where parental samples were available for testing, all were reported to be *de novo*. Parental origin of the chromosome with the deletion was determined in six cases: three were paternal and three were maternal. There

was no report of any case inherited from a balanced translocation in a parent.

Sharp et al. identified three regions with low-copy repeats (LCR) that might mediate non-homologous recombination, resulting in microdeletions and microduplications with recurrent breakpoints (BP1-3) located within the LCRs, causing microdeletion or microduplications in the reported cases (Sharp et al., 2007). Recently, a new breakpoint region (BP4) centromeric to BP1 was identified (Masurel-Paulet et al., 2009). The proximal breakpoint of the deletion in our case appears to coincide with BP4, while the distal breakpoint is within BP3. The deletion includes the previously identified critical region of 1.7 Mb (Sharp et al., 2007) with additional 1.3 Mb at the nearer the centromeric end. The minimum deleted region is 70.687–73.856 Mb) is most similar to case 4 described by El-Hattab et al. (70.708–73.865 Mb) in terms of size and position. However, our case had mild dysmorphism while this case had normal appearance except for cup-shaped protruding ears. Recurrent otitis media was a common feature in both.

Comparison of all reported cases thus far showed that the minimum region deleted in all patients is approximately 0.5 Mb, with nine identified genes in UCSC (hg18) (Figure 3). As all the patients with 15q24 microdeletion have developmental delay, brain expressed

**FIGURE 3**

Map of the deletion interval in the present case and 18 previously reported cases (in order of publication dates) with the approximate genome positions for the 15q23-24.3 region. The relative positions of the nine genes within the minimum region deleted in all cases are from a screen capture from the Integrative Genomics Viewer (IGV Version 1.4.1).

genes would be good candidates. One such gene is SEMA7A (Semaphorin 7A). The glycosylphosphatidylinositol-anchored semaphorin promotes axon outgrowth in the central nervous system through beta1-integrin receptors and contributes to the formation of the lateral olfactory tract (Pasterkamp et al., 2007; Pasterkamp et al., 2003).

Facial characteristics commonly found in patients with 15q24 deletion syndrome, such as high frontal hairline, long smooth philtrum, full lower lip, were not observed in our patient. However, she had frontal bossing and midface hypoplasia. She also had depressed nasal bridge, which was found in a few other cases. Various ear abnormalities were observed in almost all cases, with the present case having mild hearing loss for the right ear. Common musculoskeletal abnormalities such as joint laxity and scoliosis reported in many patients were not found in the present case. Eye abnormalities reported include strabismus, epicanthal folds, downslanted palpebral fissures, broad medial eyebrows and hypertelorism, with the first two observed in the present case. One gene that is highly expressed in sclera, retina, retinal pigment epithelium and trabecular meshwork is

STRA6 (stimulated by retinoic acid gene 6). Mutations in this gene have been found in patients with eye abnormalities, diaphragmatic hernia and mental retardation (Pasutto et al., 2007).

Male genital abnormalities are frequently observed in 15q24 microdeletion patients. One candidate gene is CYP11A1, which is highly expressed in the adrenal gland. The encoded enzyme catalyzes the side-chain cleavage reaction of cholesterol to pregnenolone, and may have an important role in genital development. It was reported that homozygous null male mice were feminized with female external genitalia and underdeveloped gonads (Hu et al., 2002). Interestingly, the CYP11A1 gene also appeared to be involved in the development of the female genitalia and reproductive system (Hu et al. 2004). The present case had labial adhesions, which to our knowledge is the first observed genital abnormality in a female patient. Other genes within the deleted region with high expression in reproductive tissues include CCDC33 (Coiled-coil domain containing 33) which has high expression in testes (Kaczmarek et al., 2009), LMAN1L (Lectin mannose-

binding 1-like) which encodes an integral membrane protein highly expressed in normal and neoplastic prostate but is also expressed in other tissues such as brain (Yerushalmi et al. 2001); and EDC3 (enhancer of mRNA decapping 3), which is highly expressed in testes in males and ovaries in females. It has been implicated in spermiogenesis and oogenesis (Rudolph et al., 2007).

Recurrent infection has been reported in some 15q24 deletion patients (El-Hattab et al., 2009; McInnes et al., 2010; Sharp et al., 2007). One immune gene within the 0.5 Mb region deleted in all reported cases is ARID3B (AT rich interactive domain 3B [BRIGHT-like]), which is expressed in leukocytes, placenta, and testis (Numata et al., 1999). ARID3B is also involved in the survival of neural crest during embryogenesis and the pathogenesis of malignant neuroblastoma (Kobayashi et al., 2006). Another immune gene slightly outside this region but is deleted in all but one patient is CSK (C-Src kinase) (Brauninger et al., 1992), which is expressed in lung tissue and macrophages.

There is a Cytochrome P450 gene within the minimal critical region. CYP1A1 is involved in the oxidization of a variety of structurally unrelated compounds including steroids, fatty acids and xenobiotics. It is an important enzyme that catalyzes activation of these procarcinogenic polycyclic aromatic hydrocarbons. Abnormal metabolism of these compounds may lead to tumor formation (Shimada & Fujii-Kuriyama, 2004).

The two remaining genes within the 0.5 Mb region are UBL7 (ubiquitin-like 7 (bone marrow stromal cell-derived)) and CLK3 (CDC-like kinase 3). Both have broad expression profiles with expression in most tissues.

In summary, this patient with confirmed interstitial deletion in 15q24 shares similar clinical presentations with previously reported cases and represents another case of this recently recognized syndrome. To our knowledge, she is the first case of Chinese ancestry. One other observation is that most of the previously reported cases are males; our case represents only the third female reported. The genes within the deleted regions would need to be further investigated in functional studies and animal models to confirm the effect of their haploinsufficiency in this syndrome.

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