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Bone Aneurysmal Cysts and Pathologic Fracture Associated with Supernumerary Ring Chromosome 6 in Two Unrelated Patients

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Abstract

Small supernumerary ring chromosome 6 (sSRC(6)) is a rare chromosomal abnormality characterized by a broad clinical phenotype. The spectrum of this disorder can range from phenotypically normal to severe developmental delay and congenital anomalies. We describe two unrelated patients with small SRCs derived from chromosome 6 with a novel bone phenotype. Both patients presented with a complex bone disorder characterized by severe osteopenia, pathologic fractures and cyst-like lesions within the bone. Imaging revealed decreased bone mineral density, mutiple multiloculated cysts and cortical thinning. Lesion pathology in both patients demonstrated a bland cyst wall with woven dysplastic appearing bone entrapped within it. In patient 1, array comparative genomic hybridization (CGH) detected a tandem duplication of region 6p12.3 to 6q12 per marker chromosome. Cytogenetic analysis further revealed a complex patient of mosaicism with some cell lines displaying either one or two copies of the marker indicative of both tetrasomy and hexasomy of this region. Patient 2 was mosaic for a sSRC that encompassed a 26.8 Mb gain from 6p21.2 to 6q12. We performed an in-depth clinical analysis of a phenotype not previously observed in sSRC(6) patients and discuss the potential influence of genes located within this region on the skeletal presentation observed.

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Keywords

osteochondrodysplasias; mosaic marker chromosome; pathologic fracture; bone cysts

INTRODUCTION

A small supernumerary marker chromosome (sSMC) is a rare chromosomal abnormality characterized by presence of cytogenetic content in addition to the normal 46 chromosome karyotype. While the frequencies of these variants are extremely rare, 0.044% (1/2500) in new born patients, supernumerary marker chromosome 6 has been described in fewer than 35 patients. Majority of sSMC(6) patients are found in conjunction with the presentation of other chromosome abnormalities and to date only 30 patients involving pure sSMC(6) have been described [Aalfs et al., 1996; Callen et al., 1991]. This disorder is characterized by a large spectrum of clinical severities that can range from phenotypically normal to a myriad of congenital anomalies. Intrauterine growth retardation (IUGR), mild to severe developmental delay, transient neonatal diabetes mellitus, congenital heart defects, hypotonia, hypogenitalism, hydronephrosis and vesico-ureteral junction obstruction are clinical features observed in this disorder. Dysmorphic features can include epicanthic folds, thick upper lips, broad nasal bridge and tip, hypertelorism and prominent cheeks [Huang et al., 2012; Liehr et al., 2006; Oldak et al., 2006].

Here we report on new clinical symptoms associated with small supernumerary ring marker chromosome 6 in two unrelated patients who both present with severe abnormalities in bone formation and turnover. These patients exhibit cyst-like lesions of the skull, spine and appendicular skeleton, osteopenia and pathologic fracture. Patient one harbors a complex case of mosaicism with two copies of 6p12.3-q12 per ring chromosome. Cytogenetic analysis revealed mosaicism for both one and two copies of this sSRC(6), indicating both tetrasomy and hexasomy of this region that has not been previously described in literature. Patient two presents with a mosaic sSRC(6) encompassing 6p21.2-6q12. Comparison with current literature on sSMC(6) failed to exhibit any patients noted with bone abnormalities. We propose expansion of the phenotype observed in patients with sSMC(6).

RESULTS

CLINICAL CHARACTERIZATION

Patient 1—Patient 1 is an 18-year old Caucasian male who was the fourth pregnancy to a non consanguineous couple. Maternal history of pregnancy is not known. His family history was significant for multiple fractures with relatively trivial trauma in his mother. She died in a minor car accident from multiple craniofacial fractures and family reports that she had issues with bone fragility and multiple fractures. Pregnancy, labor and delivery were unremarkable and birth weight was 4.41 kg (90–95 %). Other birth parameters are not known. Early medical history was notable for reactive airway disease, bronchiolitis and 6 weeks of vomiting around one year of age. Both of which resolved over time. Patient 1 was initially examined at our institute when he was 10 years old following complaint of back pain from a fall. Physical examination was negative for dysmorphic features, however a

bluish tint to the sclera was observed. At the present time, mild developmental delays were noted with a failure to read until third grade.

He had a history of bone fragility and four prior fractures. He had a femur fracture at age 2 years, a right arm fracture, a left hand fracture as well as a rib fracture and back pain (Figure 1A,C). Imaging revealed severe osteopenia, pathologic compression fractures and numerous cystic lesions throughout the spine (Figure 1C). An initial bone density scan was performed and this study demonstrated a whole body Z-score of −1.1 with a lumbar spine Z-score of −2.6. A subsequent bone scan revealed fracture of the right eighth rib, T8 vertebral body and ankles bilaterally. Laboratory studies determined his electrolytes were within normal limits and alkaline phosphatase was elevated (593 U/L) (normal: 146–333 U/L). His calcium, osteocalcin, Vitamin D and intact PTH were within normal limits. Collagen I and III studies performed on fibroblast cultures were normal.

He was placed on pamidronate therapy and the scan closest to the completion of pamidronate demonstrated a whole body Z-score of −0.2, with the lumbar spine Z-score of −0.7. The R1 regions of the right and left femur were, +4.1 and +3.0 SD respectively, indicating some degree of increased bone density. Other femoral scores ranged between −0.8 and −0.2. Following completion of the pamidronate, at age 15, he was noted to have a fracture of the proximal humerus through a cystic structure (Figure 1A).

At age 17 he had a height of 177.1 cm (50–75 %), weight 80.0 g (95 %) and head circumference of 59.4 cm (>2 SD). Overall heath and cognitive development appeared normal, however there were mild craniofacial abnormalities, including macrocephaly, midface hypoplasia, prominent supraorbital ridges and micrognathia. Following fracture to his right patella, MRI demonstrated multiple cystic lesions. Bone cysts were present in the proximal tibial metaphysis as well as the distal femoral metaphysis. A bone biopsy was performed on his tibial cyst at this time (Figure 2A–C). Mild scoliosis was centered in the mid thoracic region with mid thoracic curve convex to the left. No significant thoracolumbar kyphosis or lumbar lordosis was noted.

Last seen he presented with acute back pain with urinary hesitancy and findings suggestive of a compressive myelopathy. An MRI done at that time demonstrated an expansile lesion coming off the posterior elements of T7 and also T6, causing compression of the thoracic cord. He underwent decompression surgery and went on to recover all neurologic function. He was then placed back on pamidronate approximately 6 months after surgery. He has not had any new fractures since and remains asymptomatic. He continues to have persistent cysts within his spine and appendicular skeleton, which are being monitored.

Patient Two—Patient two is a 14 year old male born at 40 weeks gestation to a 25 year old G3P2 mother weighing 2.21 kg. There is no significant family history of bone abnormalities. He presented prenatally when the mother screened positive for elevated levels of serum alpha feto protein (AFP). Amniocentesis showed a normal amniotic fluid AFP, but identified a mosaic supernumerary ring chromosome (47,XY,+mar[12]/46,XY[3]). He was noted at birth to be dysmorphic with IUGR and was discharged at 2 days of age. At two months of age he was placed with a G-tube due to poor feeding and vomiting. The subsequent course

was marked by seizures, visual and developmental delays, particularly in speech, hydronephrosis, and reoccurring otitis media. Craniofacial dysmorphism consisted of biparietal prominence, bitemporal narrowing, asymmetry, and coronal indentations, high nasal bridge, deep-set eyes, thin upper lips, and microglossia. He presented with amblyopia and latent nystagmus upon ophthalmological examination.

Evaluation at nine years of age showed a height of 123cm (3 %), weight of 30kg (55 %) and a head circumference of 51.5 cm (25 %). He no longer used a G-tube for feeding but reported frequent vomiting once or twice a day due to oral phase dysphagia. He had persistent absence seizures and continued to have gross motor and developmental delays with decreased balance and frequent falling. Some urinary incontinence was observed that was improved with tethered cord surgery. There was also mild conductive hearing loss.

Orthopedic evaluation showed marked cystic changes and severe osteopenia through his spine, pelvis and proximal femurs. His entire spine has fishtail deformities of the vertebrae as well as intervertebral disks that have invaginated into the bone above and below (Figure 1D). CT scan of the spine shows the same, with no burst component and no spinal canal encroachment. Medical history revealed that a cystic lesion of the femur was incidentally noted at age six, and multiple compression fractures a year later. In 2011, he began having fractures with minimal trauma including his left distal tibia, proximal humerus and distal humeral shaft. There was also progressive vertebral compression evident in the lumbar spine.

Laboratory tests revealed alkaline phosphatase (720 U/L) and alkaline phosphatase bone isoenzyme were elevated. PTH, Phosphorus, Viatmin D, 25-Hydroxy Vitamin D, creatinine, and calcium were within normal limits. CBC, CHEM7, collagen crosslinked N-telopeptides, osteocalcin, and acute phase proteins, CRP and ESR were also within normal limits.

Fractures were observed in the left femoral neck and he was placed on pamidronate therapy, however there was no definite improvement. DEXA at age 11 showed low bone density with Z scores of −1.1 in the lumbar spine, −3.4 for total hip, and −4.6 for femoral neck. Subsequent fractures were seen in the right superior pubic ramus and left proximal humerus. Skeletal survey showed diffuse osteopenia with multiple compression fractures of the thoracic or lumbar spine and bowing of the tibia. There is also involvement of the included upper cervical spine with height loss of C3 and C4 vertebra. Multiple cystic changes were noted within the femora as well as right humerus, right radius, sternum and calvarium. Intracranially, the CSF spaces and ventricles appear unremarkable without evidence of hydrocephalus. Some of the calvarial lesions had mild internal table protrusion without significant intracranial mass effect. No other cerebral malformations were observed. He was fracture free for one year following treatment with Zolendronate. He had subsequent fractures of two metacarpals and left proximal radius and ulna.

CYTOGENETIC AND HISTOLOGICAL ANALYSIS

Patient 1—Cytogenetic analysis was performed on a tissue biopsy from a tibial cyst. G band staining (GTG) revealed a small SRC in 17/48 cells and two small SRCs in 3/48 cells, 47,XY, + r[17]/48,XY,+rx2[3]. Fluorescence *in situ* hybridization (FISH) and CGH analysis

were performed by Signature Genomics using DNA from peripheral blood lymphocytes. FISH analysis of metaphase cells identified a ring chromosome in 9 out of 30 cells, ish r(6)(RP11-325M4++,RP11-665C2−)[9/30]. Two hybridization signals with this probe were identified, indicating a two copy gain of this region per marker indicative of partial tetrasomy of this region. In 3 out of 30 cells, two copies of the marker chromosome were observed indicating mosaic partial hexasomy. Microarray analysis using a whole genome oligonucleotide array detected an 18.2 Mb gain, 6p12.312 (46,078,398–64,326,404)×3 from the pericentromeric region of chromosome 6 with break points at 6p12.3 and 6q12. Genome coordinates were based on UCSC 2006 hg 18 assembly build at the time of testing. Updated coordinates using GRCh37 (hg19) assembly are chr6:45,970,440 –64,268,445 for genomic region within the sSRC.

Histological analysis of a cyst isolated from the right proximal tibia showed simple cystic structure with no evidence of fibrous dysplasia or any other type of pathologic process present (Figure 2). Microscopic examination revealed a fibrous cyst wall which incorporated attenuated woven bone surrounding trabecular bone and hematopoietic cells. In some foci, the cyst contains red blood cells and surrounding hemosiderin and focally, a rim of giant cells along the cyst wall. These features are most compatible with a simple bone cyst. Fluid from within the cyst was also obtained and numerous red blood cells with admixed fat and scattered hematopoietic cells were noted. Immunohistochemistry evaluation with vascular markers CD31 and D2-40 were negative within the cyst walls. Cytogenetics from the lining cells demonstrated a ring chromosome.

Patient 2—Patient two’s chromosomal abnormality was identified following amniocentesis with an abnormally high serum AFP. Subsequent AF/AFP was normal however a supernumerary marker chromosome was identified *in utero*. Parental karyotypes were normal. GTG analysis from amniocytes revealed a small ring SMC in 12 out of 46 cells, 47,XY,+mar[12]/46,XY[3]. Parental karyotypes were normal. FISH analysis of metaphase cells confirmed the origin of the marker from chromosome 6 in 15 out of 20 cells. Whole chromosome paint indicated the entire marker is derivative of chromosome 6, mos 47,XY, +mar[15]/46,XY[5].ish r(6)(p11.2q13)(D6Z1+,wcp6+) with break points 6p11.2-6q13. Array CGH revealed an 26.83 Mb gain from band 6p21.2 to 6q12, mos 47,XY,+mar[15]/46,XY[5].arr[hg18] 6p21.2q12(37,324,401–64,154,444)×3. Genomic coordinates based on UCSC 2006 hg 18 assembly build at the time of testing. Updated coordinates using GRCh37 (hg19) assembly are chr6:37,216,424 –64,096,485 for genomic region within the sSRC. Bone biopsy on distal femur did not include a cyst and was read normal by the pathologist.

DISCUSSION

We report on two unrelated patients with sSRC(6) that results in severe osteopenia, cystic lesions involving the skull, spine and appendicular skeleton, and frequent, pathologic fractures. At least 14 patients with pure supernumerary chromosome 6 abnormalities have been described encompassing regions similar to our patients described here and there are no reports of bone abnormalities (http://ssmc-tl.com/chromosome-6.html#sym). Our patient one has a duplicated 18.25Mb gain encompassing 6p12.3-q12 and a complex case of tetrasomy and hexasomy of the duplicated region. Patient two has a ring chromosome that

encompasses 6p21.2-q12. In reported sSMC(6) with clinical manifestations, a positive course is most commonly marked by facial dysmorphism, congenital heart defects, feeding difficulties and various neurological deficiencies such as psychomotor delay, epilepsy or abnormal behavior. A patient with sSMC encompassing 6p21.2-q11.2 presented with IUGR, severe developmental delay, facial dysmorphism, scoliosis, lax joints and transient neonatal diabetes [Crolla et al., 1997]. An additional patient with a 6p21.2-q11.2 sSMC was described with IUGR, facial dysmorphism, developmental delay and epilepsy [Huang et al., 2012]. The remaining patients of characterized sSMC6 consist of small duplicated regions encompassing 6p11.1-q12. Phenotypic severity consists of psychomotor delay, hypotonia, congenital heart defects and facial dysmorphism to phenotypically normal [Huang et al., 2012; Leite et al., 2006; Liehr et al., 2006; Oldak et al., 2006; Stankiewicz et al., 2000]. Intriguingly, this bone phenotype has been described once before. Reoccurring cyst formation associated with pathologic fracture was observed in a suspected osteogenesis imperfecta (OI) patient [Stig Jacobsen 1997] which is suggestive of a similar chromosomal abnormality to our patients.

There is a large spectrum of clinical phenotypes observed in patients with marker chromosomes and sSMC's consisting of similar cytogenetic content can manifest with extreme phenotypic variability. While there are some genotype-phenotype correlations observed in sSMC(6) cohorts, we did not observe any reports of pathologic fracture or bone cyst formation in any other patients with sSMC(6). Phenotypic effects of sSMC's are largely multifactorial and can depend on the whether the marker is *de novo* or familial, the size of the marker, presence of UPD and the extent of euchromatin material within the marker. Marker mosaicism adds an additional layer of complexity. Precise tissue distribution of the sSMC and extent of expression within these tissues can all play a role in the clinical manifestations observed in our patients.

There are over 75 genes within the region of overlap in these two patients (Figure 3). Several of which have been associated with bone development and maintenance. An elevated alkaline phosphatase level in both of our patients alludes to increased bone formation and turnover. *Runt-related transcription factor 2 (RUNX2)* is a major regulator of bone formation and osteoblast differentiation and is close in proximity to the region of duplication. Increased Runx2 activity is associated with pathologic bone formation in radial cysts [Kusafuka et al., 2006] and mutations in *RUNX2* cause Cleidocranial dysplasia (OMIM 119600) and metaphyseal dysplasia with maxillary hypoplasia (OMIM 156510) [Mundlos et al., 1997]. *Translocation associated membrane protein 2 (TRAM2)* is also found in the sSRC region and is integral in the late stages of osteoblastogenesis and its activity can be regulated by Runx2. [Pregizer et al., 2007]. Altered *RUNX2* and *TRAM2* expression could play a role in the profuse osteopenia and increased bone turnover observed in these patients.

In addition to key regulators of bone formation and development, several pro-inflammatory cytokines are found within the duplicated region. Pro-inflammatory cytokines within this region include, *Tumor Necrosis Factor Receptor Superfamily, member 21 (TNFRSF21), interleukin 17F (IL-17F)* and *interleukin 17A (IL-17A)*. IL-17A can increase proliferation of mesenchymal stem cells [Huang et al., 2006] and enhance bone loss via osteoclastic bone resorption [Won et al., 2011]. Dysregulation of inflammatory cytokines could play a role in

progression of pathologic fracture through upregulation of osteoclast activity and elevated inflammation resulting in cystic lesions.

Our report describes novel clinical symptoms associated with sSRC(6). The overlapping region in both of our patients consists of several important genes involved in bone development, bone maintenance and inflammation. We hypothesize that a combination of dysregulation of critical genes involved in bone development via *RUNX2, TRAM2* and inflammatory pathways involving *IL-17A* contribute to pathology of disease. We propose an expansion of the phenotype associated with sSMC(6) and our clinical data supports the importance of this region 6p12.3-q12 in the normal development and maintenance of bone.

Acknowledgments

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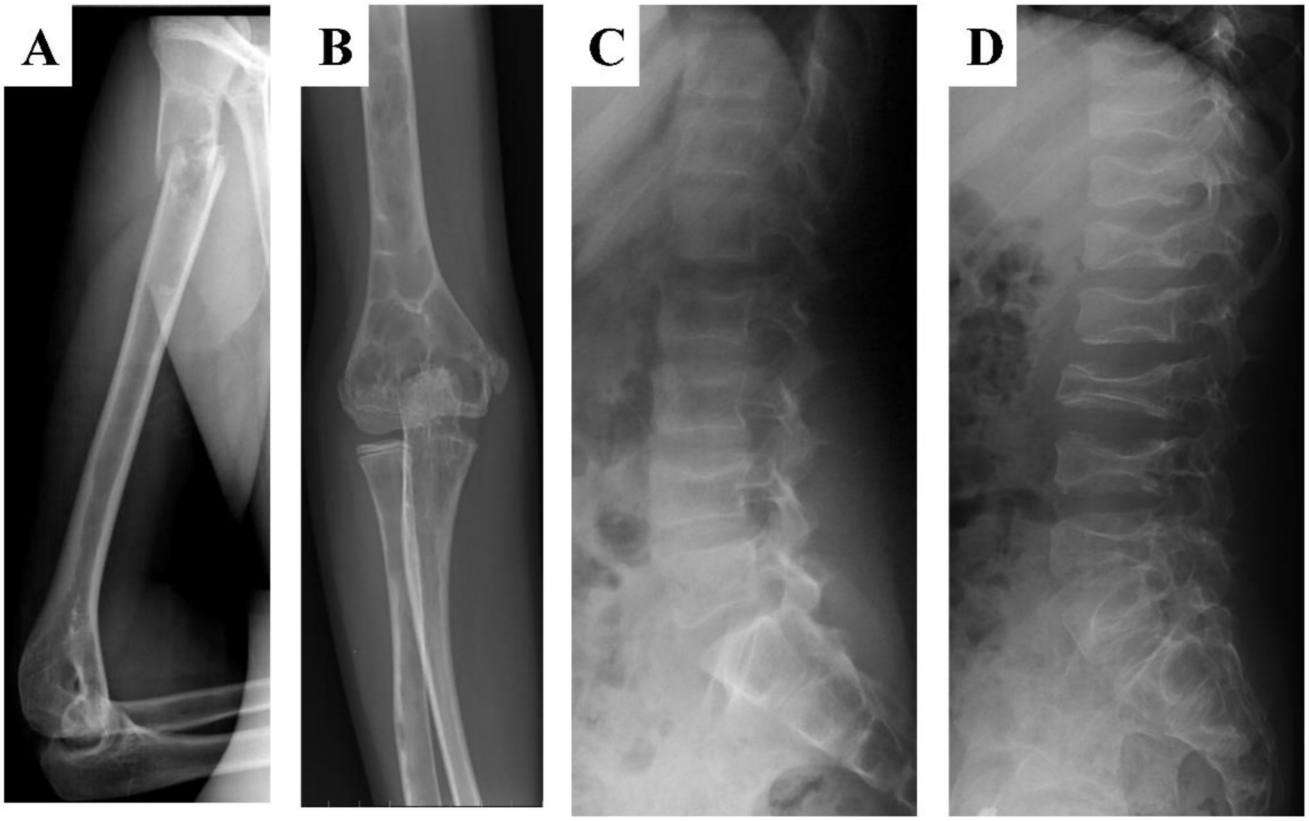

Figure 1.

Radiographic Features. (A) Lateral view of the humerus shows a pathological fracture through a cystic medullary lesion in Patient 1. (B) AP image of the elbow showing multiple cystic lesions in the humerus and radial cortex in Patient 2. (C) Lateral view of the lumbar spine showing osteopenia and compression fractures at L2, L4 in Patient 1. (D) Lateral view of the lumbar spine with osteopenia and compression fractures at multiple levels in Patient 2.

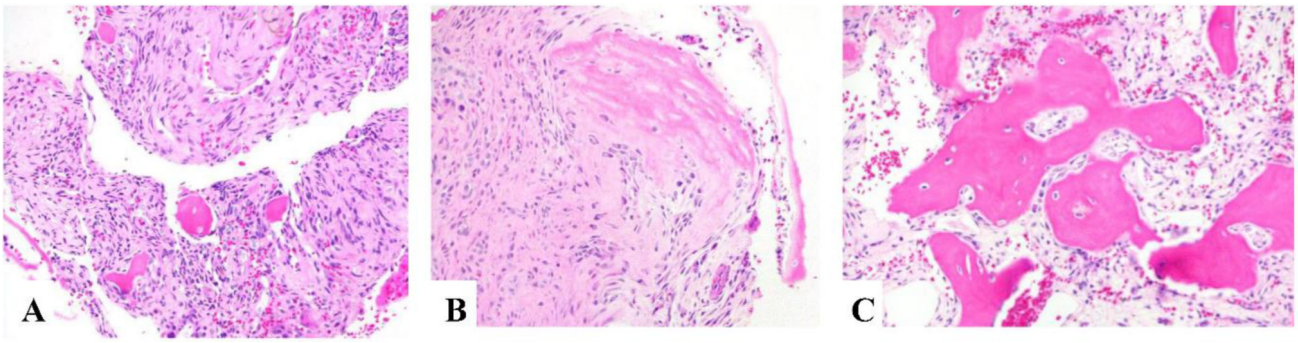

Figure 2.

Patient 1 histology of biopsies from a proximal right tibial lesion and a thoracic spine lesion. A. Hematoxylin and eosin (H &E) staining of cyst wall with fragmented bone from the tibial lesion (400×). Consistent with a simple bone cyst. B. H& E (400×) of cyst wall with attenuated woven bone with surrounding hemorrhage. C. H& E (400×) from the thoracic spine biopsy exhibiting dysplastic woven bone that may be seen with fibrous dysplasia, other areas of intervening fibrohistiocytic and vascular components, as well as normal cellular marrow.

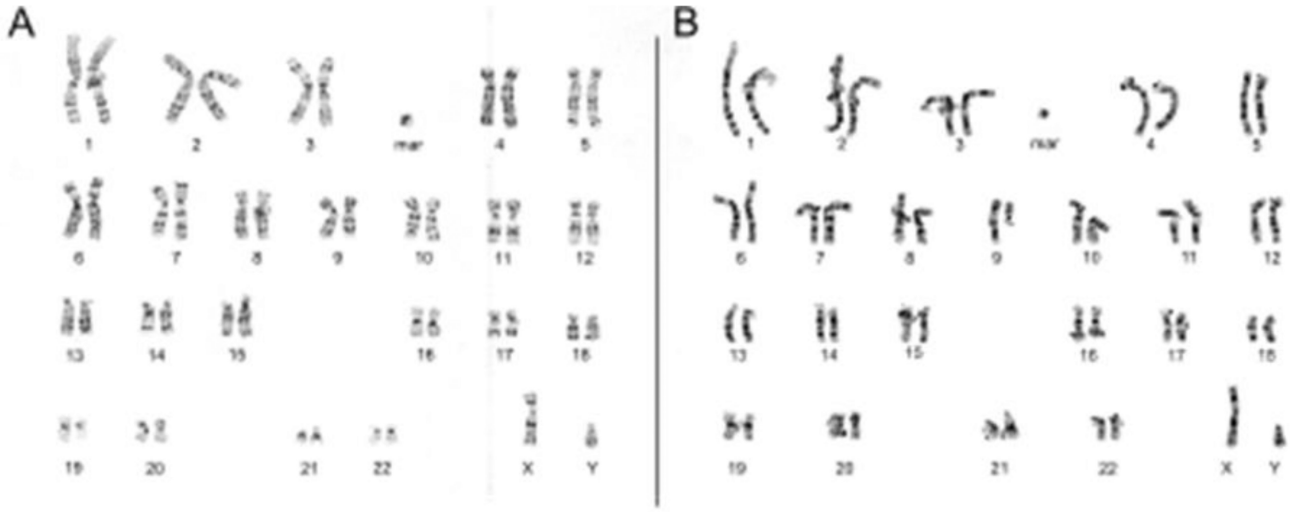

Figure 3.

GTG banding analysis for (A) patient 1 and (B) patient 2 exhibiting small supernumerary ring chromosomes (sSRC). FISH analysis further confirmed presence of a sSRC of pure chromosome 6 origin with an 18.2 Mb and 26.8 Mb gain in patient 1 and patient 2 respectively.

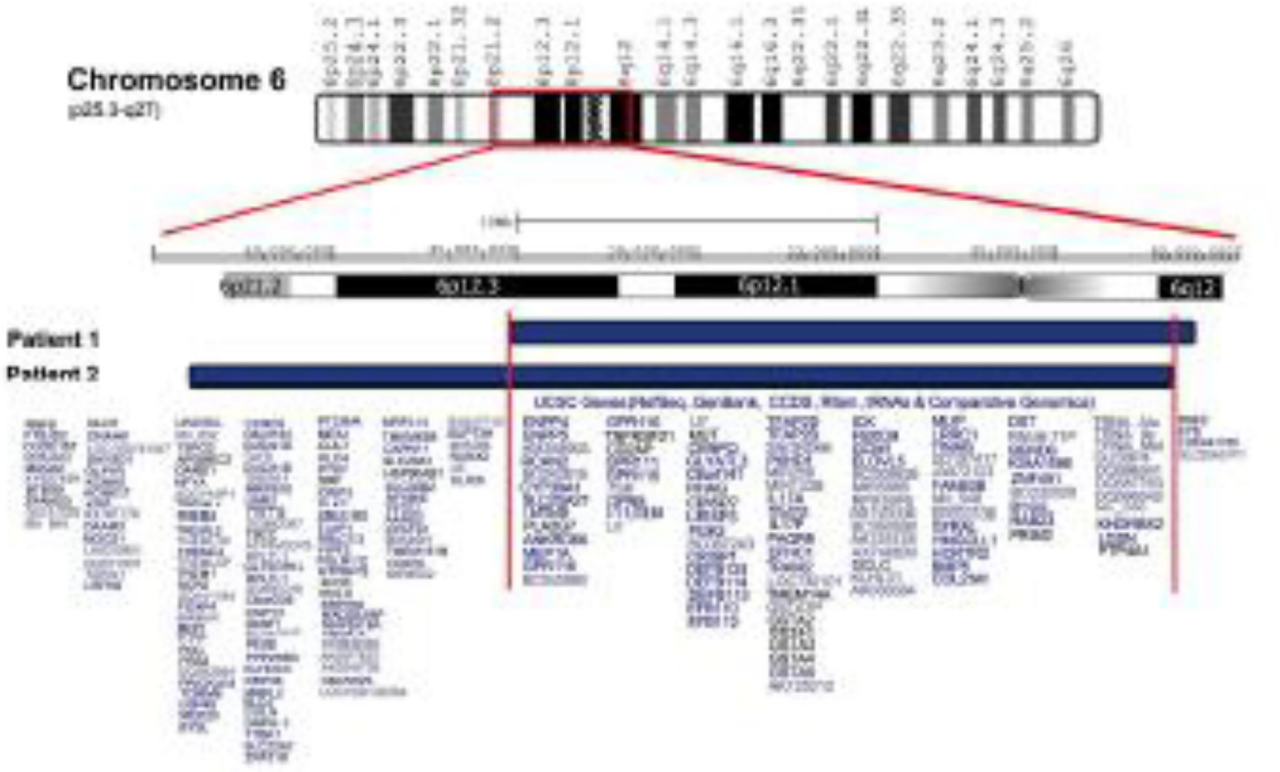

Figure 4.

Comparison of genes found within the sSRC(6) in patient 1 and patient 2 (adapted from UCSC). Overlapping regions of interest are denoted between 37,216,424 and 64,096,485 GRCh37 (hg19). Original coordinates provided at the time of testing were updated to GRCh37 (hg19) build.