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Genetic conundrum of Radioulnar synostosis

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Abstract

Radioulnar synostosis is a rare congenital anomaly of skeleton that is inherited in autosomal dominant pattern. Although, it has mostly been described in several syndromic conditions, but occurs in isolated form as well. Many recent studies have associated different genes with syndromic RUS however; no specific genetic cause has been linked to isolated condition thus far. Since 1793 more than 400 cases have been described but the genetics of the phenotype have been studied in syndromic form. We review recent development in molecular genetics of RUS that may provide clues to find possible inheritance pattern of the phenotype and to know which genetic pathway is responsible for the disease.

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Introduction

The skeleton provides a structural framework for the muscle attachment, protecting organs, assisting movements, and maintaining the homeostasis in the vascular system (Baldridge *et al.,* 2010). However, a perturbation in the development of joints, cartilage, or bone can lead to skeletal dysplasia. It affects about 1 in 5000 live births (Krakow and Rimoin 2010). This group of skeletal abnormalities includes genetic disorders of skeleton caused by genetic alterations in several genes. Molecular genetic characterization of skeletal dysplasia has revealed many different disease forms (Mortier 2001). A remarkable phenotypic, allelic and genetic heterogeneity exists in these disorders. In other words, a specific skeletal phenotype might arise due to mutations in many different genes and conversely different clinical phenotypes may arise mutation in the same gene (Geister and Camper 2015).

Limb Development

The limb development starts early at the time of embryogenesis. The upper limb buds can be recognized after 26 days of fertilization and the length becomes 20-22mm at 53rd day of pregnancy (França Bisneto 2012). A secretion of protein sonic hedgehog provides a stimulus for bud formation (Al-Qattan *et al.,* 2009) and in-between the 4-8 week of pregnancy, a number of congenital anomalies can potentially arise (Bisneto 2013).

The limb skeleton originates from the lateral plate mesoderm which gives rise to serosal mesoderm. A series of connection and overlying ectoderm lead to limb bud formation. Initially, mesenchymal cells of growing limb-bud start differentiating to form numerous limb tissues in a proximodistal sequence. Accordingly, the differentiation of each cell is controlled by the 3D-coordinate system containing the proximodistal, dorsoventral, and anteroposterior axes. Each of these axes is controlled by a specific set of signaling pathways shaped by a distinct population of cells. Three distinct signaling regions have been recognized: These include proximodistal axis (ectoderm which covering bud sides), AER (apical ectodermal ridge), and ZPA (zone of polarizing activity). Only a few signaling molecules have been identified and characterized that are produced by these signaling centers (Capdevila and Belmonte 2001).

In fact, the bud formation takes place under the ectoderm through the introversion of mesoderm. Later, the somatic mesoderm cells contribute to the formation of vessels, nerves and muscles while lateral plate mesoderm make tendons, cartilage and bones. Gradually, the differentiation process leads to the creation of three axes i.e. proximodistal, anteroposterior and dorsoventral. Among these, the AER regulates proximodistal area and functions through covering the mesodermal bud and endorsing its differentiation. It has been observed that removing the AER tissue, results in severe transverse deformities (França Bisneto 2012). On the other hand, the ZPA regulates anteroposterior axis and controls the growth of radioulnar in pre- and post-axial directions. A defect in ZPA leads to several predominate conditions like polydactyly. Finally, the wingless type, Mouse mammary tumor virus (MMTV), regulates dorsoventral plane of axis and helps differentiate the palm and dorsum of the hand. An alteration in the region, among other consequence, can result in the duplication of palm (Al-Qattan and others 2009).

Synostosis

The synostosis is a generic term use for fusion of bones that are normally not fused. The deformity can occur at any location, but it carries significant clinical importance when the union between bones is in the elbow. The anomaly may go unnoticed in newborns because the elbow is smaller and radiographs appear normal (França Bisneto 2012).

The radiohumeral synostosis has been associated with dysplasia of the ulna or as a part of multiple synostosis syndrome. The functional capacity of the shoulder, upper limb, and hand depends on the elbow position. The proximal radioulnar synostosis might not be recognized till adolescence. The pronation and supination are absent and upper limb movement is compensated by the shoulder and wrist. The radioulnar synostosis (RUS) is an isolated deformity

but it is also present in the syndromic form (James and Bednar 2005).

Radioulnar Synostosis

RUS is a bony or fibrous continuity between radius and ulna (forearm bones) causing supination and limited pronation. The common cause of the anomaly is a congenital defect (Farzan *et al.,* 2002) and posttraumatic synostosis is a less frequent one (Colton 1973). Head injury (Garland *et al.,* 1980) and burn (E BURKE 1991) are the prominent among other causes. In adults, the most prevalent cause is posttraumatic synostosis in which iatrogenic origin is rare. Postoperative RUS covers only 2-5% of all the cases of posttraumatic forearm-synostosis (Dohn *et al.,* 2012; Gofton and King 2001). The fractures of the radius and proximal third of the ulna are the highest risk for postoperative synostosis (Bauer *et al.,* 1991). The forearm synostosis has been classified based on the anatomical location of the bony bridge (Hastings 2nd and Graham 1994).

Types of Radioulnar Synostosis

Radioulnar Synostosis is divided into acquired and congenital disease forms.

Acquired Radioulnar Synostosis

It is most likely developed following forearm fracture surgery and commonly these cases arise after breaking of bones in many pieces. Reported data state that it is because of the absence of forearm supination and pronation.

Congenital Radioulnar synostosis

Congenital RUS is a rare forearm malformation as described in museum Anatomicus by Sandiford in 1793 (Wilkie 1913). At the time of early morphogenesis longitudinal segmentation produces separation of the distal ulna and radius, although for the time the proximal ends are united and share the same pericardium. Factors responsible for the disease include environment and alternations in genes that function at the time of development of radius and ulna. The mechanism of function involves interruption in the subsequent proximal-radioulnar joint separation (Elliott *et al.,* 2010; Simmons *et al.,* 1983).

The anomaly may also be associated with thumb aplasia, syndactyly, polydactyly, and other limb malformations. Additionally, it is associated with other syndromes like Carpenter's syndrome, Arthrogryposis, William's syndrome, Apert's syndrome and Klinefelter's syndrome. Predominantly, more males are affected than females comprising 60% bilateral cases (Cleary and Omer Jr 1985).

The deformity may be unilateral or bilateral. It is usually sporadic and percentage of familial occurrence is very low (Hansen and Andersen 1970). Since the first description of congenital RUS in 1793 about three hundred cases have been reported (Finidori *et al.,* 1978; Griffet *et al.,* 1986). The Italian Registry on Congenital Malformation has collected only one case of RUS in the period from 1986 to 1992 in a total of 0.766 million births. The anomaly of the reported case was associated with right thumb duplication, club hands, absent left thumb, anomalous umbilical vessels and cervico-vertebral schisis (Group 2002). In 1997 an isolated case was reported by Camera in Genova (Rizzo *et al.,* 1997). Though, recurrence may be underestimated as at birth the anomaly is not perceivable. In the largest island in the Mediterranean Sea named Sicily, about eighteen cases of the deformity have been observed and seven incidents that belonged to two families were associated with microcephaly (Giuffrè *et al.,* 1994). One incident had isotretinoin exposure (Rizzo *et al.,* 1991) while another had Nager syndrome (Pavone *et al.,* 1988). Of these, four events had syndromic basis with Brachman De Lange syndrome.

Renata Rizzo *et al,* in 1997 reported a three-generation RUS affected family containing five affected relatives. Unrelated sporadic cases were also reported with unilateral and bilateral synostosis. According to the study, familial cases show a complex form of RUS (Rizzo and others 1997).

Types of Congenital Radioulnar Synostosis

Congenital radioulnar synostosis can be classified in various ways, but the classification devised by Omer and Cleary is mostly used. It is classified into four different radiographic types (Fig. 1).

The type I anomaly has normal visible radial head with no osseous and fibrous synostosis is reduced. A short osseous synostosis has an anteriorly dislocated radial in type II deformity. Whereas, type III osseous synostosis manifests a normal radius and type IV shows a posteriorly hypoplastic radial head (Cleary and Omer Jr 1985).

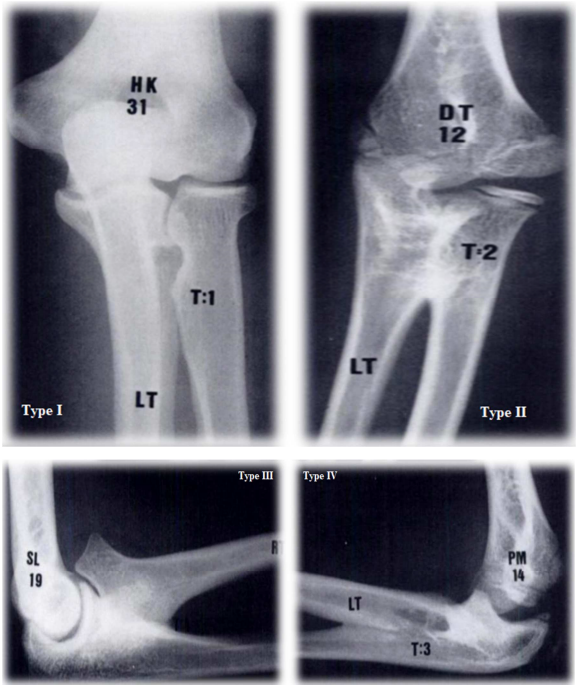

Fig. 1. Radiographic types of Congenital RUS (Cleary and Omer Jr 1985).

Prognosis of Congenital Radioulnar Synostosis

In congenital RUS proximal end of ulna and radius are usually involved and commonly the forearm fixing in pronation results in difficulties in the daily activities (Cleary and Omer Jr 1985). The child has difficulty holding the glass or ball in the hand, and problem in handling shirt buttons or washing face whenever the dominant side of forearm is involved (Simmons and others 1983).

It is difficult to treat congenital RUS but there are two different surgical option for treating the anomaly. First one is called mobilization operation that serves restoration of forearm rotation by separation of radius and ulna but the outcome are disappointing because of high recurrent fusion rate (Hansen and Andersen 1970; Sachar *et al.,* 1994).

To bypass this problem Oka K *et al* blocked postoperative reappearance of the radius and ulna fusion by placing free vascularized fascial-flap between forearm bones, however, it is not much promising.

The second one is osteotomy to readjust forearm in a suitable position to perform daily activities. Mostly patients do not go for this extensive operation because they are not disabled enough. Any supination deformity or disabled pronation can be readjusted through osteotomy. In children, the osteotomy is indicated with bilateral hyperpronation but in unilateral cases patient adopts easily. Rotational osteotomy is required for severe cases to achieve exit and accurate functional forearm position (Fujimoto *et al.,* 2005). Generally, the outcome of surgical treatment of RUS is reasonable with loss of one half intraoperative rotation and high failure rates.

Pathophysiology of Congenital Radioulnar Synostosis

The anomaly contains varying degrees of ulnar fusion and proximal radial with or without the radial head involvement. It might be dislocated posteriorly or anteriorly if the radial head is involved (Mital 1976). In fact, at embryogenic stage upper limb bud arises at 25 to 28 days from unsegmented body wall. At day 34 the elbow becomes visible and at day 37 radius, ulna, and humerus become visible. Initially, before segmentation, the three-cartilaginous analogue connect to radius, ulna, and humerus. Thus, radius and ulna, for a short time, share same perichondrium. At this time any irregular event can lead to failure of segmentation possibly results in RUS. The degree of the synostosis will determine the severity and duration of the abnormal event. Congenital RUS occurs between the ulna and proximal radius in the forearm. The condition is present at birth also but not revealed clearly till early adolescence. Initially, the union of radius and ulna are more synchondrosis but with time as the skeleton becomes mature, the osseous bridge also becomes more apparent radiographically (Kelikian 1974).

Epidemiology of Congenital Radioulnar Synostosis

The congenital RUS is not a common anomaly and occurs rarely. There are approximately 350 reported cases. The anomaly is rare according to IPMC registry i.e. 1 incidence out of 766,000. The uncommonness of this deformity leads to often late clinical diagnosis. Omer and Cleary in their study reported an average age ratio of patient to be 6 years at diagnosis and occurs between 6 months to 22 years (Cleary and Omer Jr 1985). There is no predictable inheritance pattern apparent in congenital RUS without any sex bias. The 60% cases of congenital RUS are bilateral because it is associated with other abnormalities. The distance is present in syndromic form mainly and caused by an *in utero* abnormal events. Approximately, one-third cases of RUS are linked with common skeletal abnormalities like knee anomalies, syndactyly, polydactyly, hip dislocation, GI systems, neurological impairments, renal, cardiac issues, ligamentous laxity, carpal coalition, thumb hypoplasia, and Madelung deformity. While others are associated syndromes and abnormalities and determined genetically, such as Apert syndrome, acrocephalosyndactyly, Willian syndrome, Carpenter syndrome, Klinefelter syndrome, arthrogryposis, Holt Oram syndrome, fetal alcohol syndrome, microcephaly, mandibulofacial dysostosis, and multiple exostoses (Giuffrè and others 1994; Jaffer *et al.,* 1981).

Inheritance of Congenital Radioulnar Synostosis

In 1985, Omer and Cleary reported inheritance of RUS with variable penetrance with autosomal dominant in 20% of their affected cohorts (Cleary and Omer Jr 1985). It shows that one mutated copy of disease-causing gene is sufficient to cause the deformity in the affected individuals. The mutated gene may be inherited from an affected parent or mutated for the first time. As the disease is inherited to offspring in a dominant pattern therefore each child has 50% risk of getting the mutated copy. The deformity is also associated with a genetic syndrome or other chromosomal abnormalities. The pattern of inheritance depends on existence of genetic abnormality (Wiltfong *et al.,* 2016). In such cases, the phenotype also occurs sporadically for which the cause may be unknown.

Genetics of RUS

The deformity has been inherited in autosomal dominant pattern (Spritz 1978), and is associated with chromosomal anomalies (Rizzo and others 1997). The chromosomal anomalies include X, Y or polysomy X in both male and female patients with supernumerary. The reported X chromosome anomalies contain 49XXXXX, 49XXXXY, 48XXXY, 48XXYY, 48XXXX, 47XXY (Mazauric-Stüker *et al.,* 1992; Townes *et al.,* 1965) and other anomalies that include Del (10) (pter-p13), Del (11) (q23), Del (12) (q24-qter), Del (13) (q22-qter), Dup (14) (q23-qter), Partial trisomy 11q, trisomy 8 mosaicism, and trisomy 18p (De Smet and Fryns 2008; Syed and Quinton 2008).

The RUS has been associated with more than sixty five different syndrome like Arthrogryposis, Apert syndrome, Carpenter syndrome, Berant syndrome, Cenani-Lenz syndactyly (Cenani and Lenz 1967), Der Kaloustian syndrome, De Lange syndrome, Fetal alcohol syndrome (Froster and Baird 1992), Jorgenson syndrome, Mandibular syndrome, Holt-Oram syndrome, Multiple dysostoses, Michels syndrome, Noonan syndrome, Nail-patella syndrome, Poland syndrome, Williams syndrome, and Fetal vitamin-A syndrome (Rizzo and others 1997).

In isolated RUS none of the developmental gene has been linked to disease phenotype thus far (Elliott and others 2010). A novel missense mutation in *B4GALT7* in a family with Ehlers-Danlos syndrome and exhibiting other facioskeletal anomalies i.e. osteopenia and RUS was reported (Faiyaz-Ul-Haque *et al.,* 2004). In addition, a *FGFR1* 1300T mutation has been reported in a child with AB syndrome and RUS (Hurley *et al.,* 2004). 63.6% of the Nager syndrome patients with other pre-axial limb defects such as include proximal RUS were reported with *SF3B4* mutation (Czeschik *et al.,* 2013). Moreover, a 325 kb de novo deletion in chromosome 10q25.3 that includes *ATRNL1* was reported in a patient with cognitive impairment, autism, RUS, toe syndactyly, postnatal growth retardation, and ventricular septal defects (Stark *et al.,* 2010). A Turkish family was reported with *MASP1* mutation with Michels syndrome and with some other characteristics i.e.

facial dysmorphism, mixed hearing loss, coccygeal appendage, periumbilical depression, and RUS (Sirmaci *et al.,* 2010). Additionally, a patient was reported with *FGFR2* mutation in exon 7 with multiple phenotype i.e. proptosis, cloverleaf skull, RUS, broad thumb, and great toes representing Pfeiffer syndrome (Schaefer *et al.,* 1998).

In 2000, Thompson and Nguyen reported that mutation in *HOXA11* resulting in amegakaryocytic thrombocytopenia in two pedigrees with autosomal dominant RUS. In 2002, Fujino T *et al.,* reported a point mutation in inherited amegakaryocytic thrombocytopenia with RUS in the third helix of *HOXA11* homeodomain. A novel missense mutation in *SALL4* on exon 3 is also associated with RUS and thumb agenesis (Diehl *et al.,* 2015). In 2015, de novo mutation in *MECOM* was reported, associated with RUS and amegakaryocytic thrombocytopenia which encodes for an oncoprotein EVI1 (Niihori *et al.,* 2015). In a instance, a patient with RUS and brain abnormalities was reported to have 17q21.31 microdeletion (Zarate *et al.,* 2015).

Homeobox Genes

The homeobox genes are key development controlling genes and act on the top according to genetic hierarchies regulating aspects of cell differentiation and morphogenesis in animals. These genes contain highly conserved regions of 180-183 base pairs, and the genes contain homeobox sequence called homeobox genes. The homeobox genes were first discovered in *Drosophila melanogaster*. Thereupon, these genes were shown to arise in metazoa form sponges extending to vertebrates, plants, fungi and therefore these are evolutionarily conserved all over the three kingdoms of multicellular organisms (Gehring 1994).

The initial genetic study of sequence homology with ultrabithorax and antennapedia genes led to similar genes isolation in other species form nematodes to zebra fish, xénope, mouse and human (Vieille-Grosjean *et al.,* 1997).

The homeobox which encodes a 63-amino acid protein domain called homeodomain and it binds to specific DNA sequences (Gehring *et al.,* 1994). The homeobox gene family extended with time as positive achievements and now a total of 300 loci with 65 pseudogenes and 235 functional genes in human are found (Holland *et al.,* 2007).

The “Homeo” is a Greek word means alike and Drosophila homeotic (*HOM)* genes are named because of their ability. As these genes mutate, these transform one of the insect’s body segment into a resemblance to another. The *UBX* is a homeobox gene. whenever the loss of function mutation occurs in *UBX* it leads to the change of the halter-bearing third thoracic segment, thereby resulting in the creation of four-winged Drosophila flies (McGinnis and Kuziora 1994).

The *HOX* genes of mammals are well-defined by their homology with *HOM-C* of *Drosophila*. In human and mouse at least 39 genes are organized in *HOXA, HOXB, HOXC,* and *HOXD*. These four clusters are localized at different chromosomes, e.g. *HOXA* at 7, *HOXB* at 17, *HOXC* at 12, and *HOXD* at human chromosome number 2. Each cluster contains 9-11 genes (Fig. 2).

The earliest expression of *HOX* genes in mammalian embryos can be detected at gastrulation stage and are expressed in all three germ layers with overlying domains that extend to sharp anterior limit from the caudal end of the embryo (Duboule and Morata 1994). In the above schematic representation Drosophila HOM-C, ancestral homeotic complex and HOX complexes of human are shown which represent phylogenetic relationships (Favier and Dolle 1997). Each gene is shown by a colored box. Each color showing anterior expression domain. HOX and HOM expression are schematized in fly and pre-vertebrae of the human fetus and in the CNS. Abbreviation of HOM gene are: Dfd (Deformed), lab (Labial), Scr (Sex combs reduced), pb (Proboscipedia), Abd-A (Abdominal-A), Abd-B (Abdominal-B), Antp (Antennapedia), and Ubx (Ultrabithorax).

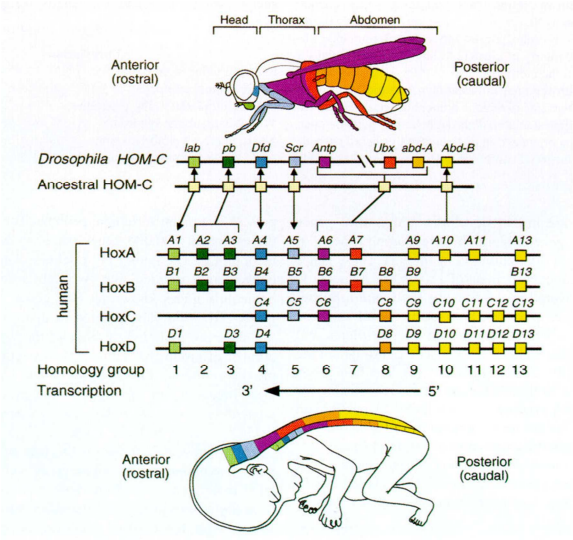

Fig. 2. Pattern of colinear expression and genetic organization of mammalian *HOX* and *Drosophila HOM* genes.

HOX genes in Limb Morphogenesis

The *HOX* gene family of mammals contains fifteen genes related to the *Abdominal-B* gene of *Drosophila*. Most of the *Abdominal-B* related genes are expressed by overlapping domains in the hind limb role in the specification of the digits pattern (Duboule and Morata 1994). The outcome of these genes is delayed ossification of the forelimb, change in shape, and in size reduction e.g. *HOXD9 (Fromental-Ramain et al., 1996)*, *HOXD11*, and hind limb *HOXA10 (Favier et al., 1996)* (Favier B *et al.,* 1996). The Favier B and Dollé P in 1997 reported that these genes control the growth and allocation of prechondrogenic condensations and eventually the ossification sequence.

In 1996 Muragaki Y *et al* described that the familial limb abnormalities are caused by a mutation in *HOXA13* and *HOXD13*. The human synpolydactyly results from in-frame insertion of polyalanine stretches in N-terminal region. Interestingly in both heterozygous and homozygous states the human limb phenotype is more severe than disruption of *HOXD13* gene in mice phenotype (Fig. 3) (Dollé *et al.,* 1993).

Dorsal view phalanges, carpals, and metacarpals in adult HOX mutants and wild-type (WT). The limb defects result from a disruption in HOX. Each digit is numbered in Roman, digit V most posterior and digit I the most anterior. HOXD13 mutant A and B, homozygotes (d-13$^{-/-}$, arrow) having a supernumerary postaxial digit and heterozygotes (d-13$^{+/-}$) having a supernumerary carpal bone.

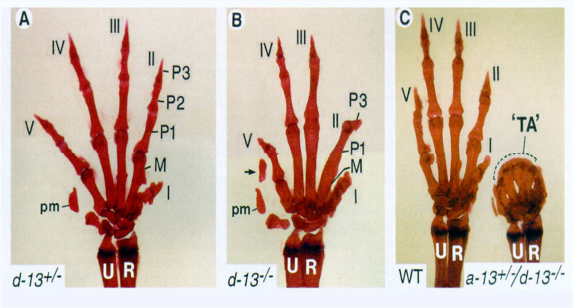

Fig. 3. Forelimb dorsal view autopod.

HOXA11

Normally, *HOX* genes regulate differentiation of mullerian duct and are expressed in epithelial ovarian cancer subtypes but not in the normal epithelial ovarian surface. In tumorigenic mouse epithelial ovarian surface cells, ectopic expression of *HOXA9* gives rise to papillary tumors, whereas, *HOXA11* induces morphogenesis of mucinous like and endometrioid like tumors (Cheng *et al.,* 2005).

The *HOXA11* expression has been reported in both human and mouse uterosacral ligaments. The uterosacral ligaments from 18 women showing lower expression of collagen and *HOXA11* approximately 75-fold and 17-fold lower while the MMP2 increase in patient tissue nearly 2-fold. The *In vitro* experiments on murine embryonic fibroblast disclosed that *HOXA11* decreases MMP-2 expression and increases collagen-III expression. These findings were consistent with extracellular metabolism pathway involving MMP2, HOXA11, and COL3A1. The *HOXA11* is essential for development of uterosacral ligaments and change in the signaling pathways might result in weakened connective tissue in women with pelvic organ prolapse (Connell *et al.,* 2008).

Chromosomal Location of HOXA11

The *HOXA11* containw two exons and a total of 4,067 bp from 27,181,157 to 27,185,223 chromosomal coordinates having the cytogenic location 7p15.2, at short arm (p) on chromosome number 7 at position 15.2 (Fig. 4) (Acampora *et al.,* 1989).

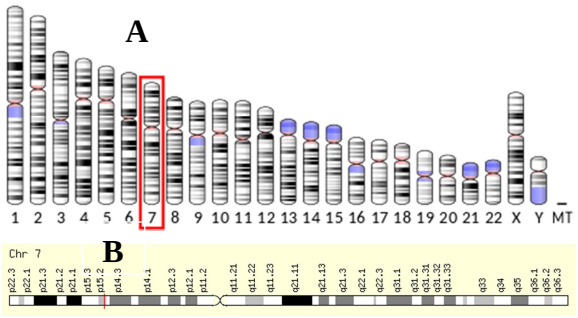

Fig. 4. Chromosomal Location of *HOXA11*.

The above fig. is the schematic representation of the chromosomes and the locus of gene. A) The general view of all the chromosomes. B) Represent all the locus on chromosome 7. The red mark shows the specific locus of HOXA11.

Mapping of HOXA11

A 90kb stretch of DNA containing 8 homeoboxes was identified on chromosome 7 from 5′ to 3′ in the following order: *HOXA13. HOXA11. HOXA10, HOXA9, HOXA7, HOXA6, HOXA5,* and *HOXA4* (Acampora and others 1989).

Animal Model of HOXA11

The complex of 38 *HOX* genes in the mouse encodes transcription factors that give regional information to lateral embryonic axis. In earlier vertebrate evolution, a shared ancestral complex with invertebrates rises to four linkage groups *HOXA, HOXB, HOXC,* and *HOXD*. The *HOX* divided further into 13 paralogous groups based on sequence similarities. A mouse-based study reported with distinct mutations in the paralogous *HOXA11* and *HOXD11*. By breeding both the strain together results in double mutants which then show dramatic phenotypes in mice. The radius and ulna were eliminated of the forearm, homeotic transformations were seen in the axial skeleton, and serious kidney defects were also seen, not present in either single mutant mice before (Davis *et al.,* 1995).

In 2008, Connell KA *et al* reported that *HOXA11* as an essential for uterosacral ligament development. They further concluded that women with pelvic organ prolapse have feeble connective tissue because of changes in signaling pathway genes involve *MMP2, HOXA11,* and *collagen type III*. The late limb bud contains three proximodistal segments of all tetrapod and these segments express distinct homeobox genes. The lower limb (zeupod) expresses *HOXA11,* upper limb (stylopod) and hand/foot (autopod) *HOXA13*, even though these markers are not sufficient for limb segment identification (Roselló-Díez *et al.,* 2011). The retinoic acid, Wbt3a, and Fgf8 work together to uphold markers of primary limb mesenchyme in culture (Cooper *et al.,* 2011).

SALL4

The *SALL4* is a member of a group of genes *spalt-like* named *SALL* family. Each gene in this group gives instructions for protein production and these proteins are then involved in organs and tissue formations during embryonic development. The SALL proteins help in controlling the activity of certain genes by the mean of binding to the specific region of DNA as these are transcription factors (Tatetsu *et al.,* 2016). In 1997 *SALL* was cloned in *Drosophila melanogaster* to observe its role. It was observed in imaginal disc development in the larval stages and for terminal trunk structure formation in embryogenesis. The *SALL* encodes for a protein which contains glutamine and alanine-rich region and three distinct DNA binding zinc finger domains (Kühnlein *et al.,* 1997).

There are four SALL proteins: SALL1, SALL2, SALL3, and SALL4 with structural homology. These play numerous role in cancer, kidney function, and embryonic development (de Celis and Barrio 2009). The SALL4 protein exact function remains unclear. Based on function similarities with other organism SALL proteins e.g. mice and zebrafish, these play an important role in limbs and nerves development that control movement of the eye and septa formation. It also called a key embryonic factor. The *SALL4* encode for three protein isoforms by splicing differences named A, B, and C (Kohlhase *et al.,* 2005).

The *SALL4* also likely plays an important role in the development of abducens motoneuron based on known mutation which results in Duane-radial rat syndrome (Al-Baradie *et al.,* 2002). The gene is an oncofetal protein expressed in human fetal liver but in the adult liver it is silenced, however, it is re-expressed in a smaller group of patients having

hepatocellular carcinoma with unfavorable prognosis. A study reported in 2013, specimens of 179 patients were obtained with hepatocellular carcinoma for SALL4 expression from Singapore. The analysis showed the enrichment of progenitor-like gene signatures with overexpression of metastatic and proliferative genes in SALL4 positive hepatocellular carcinomas. The loss of function studies of SALL4 also confirmed the importance of SALL4 in tumorigenicity and cell survival (Yong and Zhong 2013).

Chromosomal Location of SALL4

The *SALL4* contain 4 exons and a total of 130.915kbp from 51,782,717 to 51,802,523 coordinates and the cytogenic location of 20q13.2 which is long arm (q) on chromosome number 20 at position 13.2 (Fig. 5) (Kohlhase and others 2005).

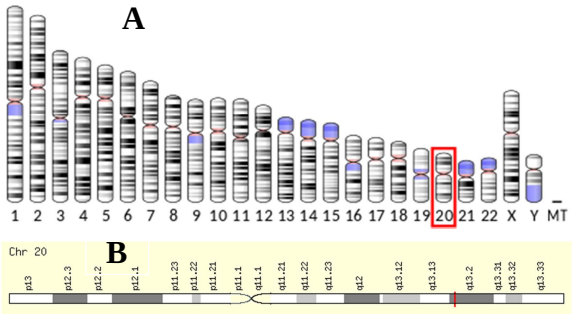

Fig. 5. Chromosomal Location of *SALL4*.

The above fig. is the schematic representation of the chromosomes and the locus of gene. A) The general view of all the chromosomes. B) Represent all the locus on chromosome 7. The red mark shows the specific locus of SALL4.

MECOM

The protein encoded by *MECOM* is an oncoprotein and transcriptional regulator, involved in apoptosis, development, hematopoiesis, cell proliferation and differentiation. The EVI1 complex locus protein also called positive regulatory domain zinc finger protein 3 or ecotropic virus integration site 1 protein homolog, is a protein that is encoded by *MECOM* in human. The EVI1 was first identified as a retrovirus integration site in AKXD murine myeloid tumors and has since been reported to have developmental role in embryogenesis in a plethora of other organisms. The protein can interact with SMAD3, KAT2B, MAPK9, CTBP1, CTBP1, and CREBBP. The EVI1 is a nuclear transcription factor involved in different signaling pathways for both co-activation and co-expression of cell cycle genes and regulating their expression. It is also involved in apoptosis through regulation of the TGF-beta and JNK signaling. The *MECOM* can influence *AML-1* resulting in the onset of leukemia and its overexpression. The variants of several transcript encoding different isoforms have been found for this gene (Métais and Dunbar 2008).

The most studied isoform of *MECOM* is 145kDa that encodes 1051 amino acids (Buonamici *et al.,* 2003). The MECOM protein has two domains characterized by 7 zinc motifs followed by a transcription repression domain that is proline-rich, three more zinc finger motifs and an acidic C-terminus (Wieser 2007).

Chromosomal Location of MECOM

The *MECOM* contains 15 exons and a total of 580.288 kbp from 169,083,499 to 169,663,786bp with cytogenic location 3q26.2 that is long arm (q) on chromosome number 3 at position 26.2 (Fig. 6).

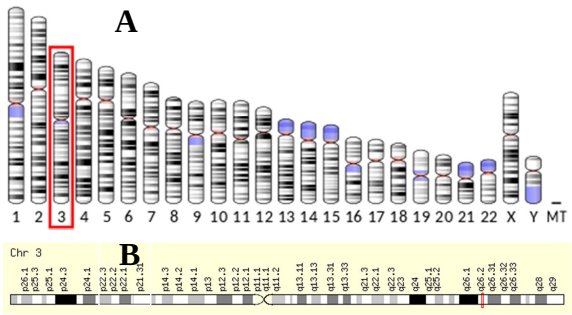

Fig. 6. Chromosomal Location of *MECOM*.

The above fig. is the schematic representation of the chromosomes and the locus of gene. A) The general view of all the chromosomes. B) Represent all the locus on chromosome 7. The red mark shows the specific locus of MECOM.

Transcript Variants of MECOM

There are various transcript variants that encode chimeric proteins and isoforms. Most common ones are:

1. Fusion transcripts with upstream genes like ETV6/MDS1/EVI1, AML1/MDS1/EVI1, and MDS1/EVI1 (Wieser 2007).

2. In mouse and human cell -Rp9 variant is relatively common, it lacks 9 amino acids in the repression domain (Wieser 2007).
3. Human cells have only one variant EVI-1a is specific and the remaining EVI-1b, EVI-1c, EVI-1d, and EVI-3L are 5 prime untranslated regions (Wieser 2007).
4. A variant Δ105 is unique to mice that result in a protein truncated at the acidic C-terminus by 105 amino acids (Buonamici and others 2003; Wieser 2007).
5. A variant Δ324 found in mouse and human cells at low levels, an alternative splice variant that encodes 88kDa protein lacking zinc fingers 6 and 7 (Wieser 2007).

Homology of EVI1

There has been 94% homology between mice and human amino acid sequence and 91% in nucleotide sequence because EVI1 is a proto-oncogene conserved across mice, rats, and humans (Buonamici and others 2003). The EVI1 is a transcription factor and by mean of conserved sequences (GACAAGATA) it binds to DNA with the potential to interact with both coactivators and corepressors (Yatsula *et al.,* 2005).

Microdeletion in RUS

In 2015 a case was reported with a 17q21.31 microdeletion that involves *EFTUD2*. The phenotypes of patient were RUS and brain abnormalities. The deletion was discovered by three different research group in 2006. The 17q21.31 microdeletion is basically a genetic disorder called Koolen De Vries syndrome and is caused by deletion of a segment that contains 6 genes of chromosome 7 (Sharkey *et al.,* 2009).

In some cases, the syndrome occurs due to deletion in *KANSL1*. The deletion is about 500-600kb in size and the common features of the syndrome are: intellectual disability, hypotonia, developmental delay, distinctive facial features, positional deformity of the feet, narrow hand, small hand, slender lower limb, congenital malformation of urogenital tract, heart and the central nervous system. The pattern of inheritance has been reported to be autosomal dominant (Koolen and de Vries 2013).

Conclusion

The above literature reveals that RUS mainly reported in syndromic form for which genetic pathways have been studied. The reported pathways are complex because it was reported with number of different phenotypes. Through that we cannot determine openly that which specific gene are responsible for RUS and which one are not. The condition has not been reported previously in isolated form. Therefore, further studies are required to unfold the genetic mystery of RUS.

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