GeNeViSTA

Radial Ray Defects: Genetics and Syndromic Etiologies

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Limb anomalies are a commonly occurring group of malformations, deformations and disruptions due to the developmental complexity of the limbs, their extended period of morphogenesis and their position outside the body wall. Limb malformations can be a part of chromosomal aberrations or an array of single gene disorders or may occur due to environmental teratogens. Radial ray defects are a group of limb malformations characterized by unilateral or bilateral absence of the radial ray which consists of the radius and thumb (Fig.1). The prevalence of radial ray defects is low and varies between 1 in 30,000 to 1 in 100,000 with syndromic causes accounting for approximately two-third of cases.[1] The common syndromes associated with radial ray defects are Holt-Oram syndrome, Fanconi anemia, TAR syndrome and VACTERL association. In addition, chromosomal disorders such as trisomy 18 can also cause radial ray defects along with significant growth and developmental delay and other congenital anomalies. In this review syndromes associated with radial ray defects are discussed.

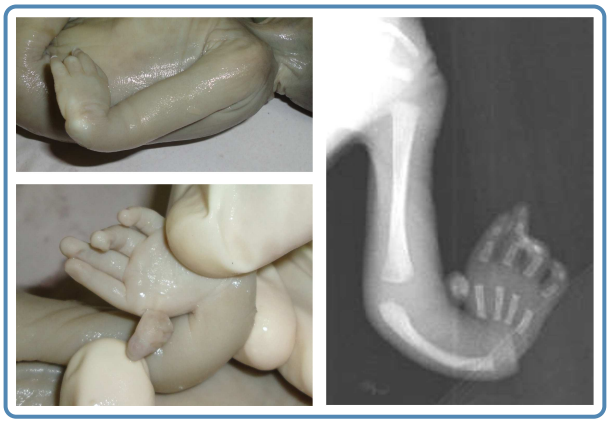

Figure 1 Characteristic hand abnormality in radial ray defects and X-ray showing absent radius and rudimentary thumb.

Molecular Embryology

Limbs develop from embryonic limb buds. Upper limb buds are first visible in the embryo on Day 26-30 as an elevation on the anterolateral aspects of the body wall. Limb development includes limb initiation and growth (proximo-distal axis) and its polarization in the antero-posterior and dorso-ventral axis. It involves several coordinated processes characterized by a constant equilibrium between cell mitotic activity and programmed cell death. Limb bud formation and growth (proximo-distal axis) are due to rapid cell proliferation in the progress zone (PZ) induced by the overlying apical ectodermal ridge (AER). The proximo-distal growth is closely linked to polarization along the antero-posterior axis (under control of the zone of polarizing activity, ZPA) and the dorso-ventral axis (limb patterning).[2]

Limb development involves coordinated functioning of various interlinked genes which work by forming a network of signals. Limb bud outgrowth is promoted by *WNT* and *FGF10*. Upper limb anatomy is specified by *TBX5* and lower limb anatomy by *TBX4* genes.[3] Mutations in T-box genes are associated with syndromes characterized by limb anomalies, the location of which is in agreement with the expression profile of the respective gene i.e. in either the arms only (TBX5-Holt Oram syndrome) or both arms and legs (TBX3-ulnar mammary syndrome). Mutations in the *SALL4* gene (SAL-like4), which also encodes a transcription factor, can cause limb anomalies. Mutations in another gene in the same pathway *SALL1* (SAL-like1) are known to cause Townes-Brocks syndrome. Proximal-distal growth is controlled by the apical ectodermal ridge (AER) whose formation requires induction by the bone morphogenic protein (BMP) and the homeobox gene *MSX2*. The important gene in establishment of antero-posterior polarity is the sonic hedgehog (*SHH*) gene.[4] Its expression

is confined to the ZPA. A number of molecules involved in the SHH pathway are known and include patched-1, smoothened, GL1-1, GLI-2, GLI-3 and TWIST.[5] WNT7A is a major determinant of dorsal development accomplished through upregulation of LMX1B and WNT7A is repressed by Engrailed 1 (En1).

Syndromes with radial ray defects

• Holt-Oram Syndrome (OMIM 142900): Holt Oram syndrome (HOS) is an autosomal dominant disorder occurring in approximately one in every 10,000 live births and is characterized by cardiac and upper limb malformations. Affected individuals exhibit limb defects that range from subtle carpal abnormalities, absent digits and triphalangeal thumbs to sloping shoulders and various grades of reduction abnormalities of the radius (Fig.2). Limb defects are usually bilateral but may be more prominent on the left side. This is frequently associated with cardiac defects like ostium secundum atrial septal defect, ventricular septal defect or asymptomatic conduction disturbances in most cases. More complex anomalies like tetralogy of Fallot and pulmonary arterial hypoplasia occur rarely.

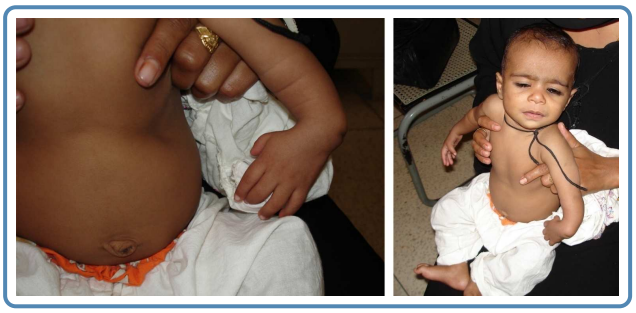

Figure 2 Holt Oram Syndrome.

Holt-Oram syndrome is caused by mutations in the *TBX5* gene and mutations are spread throughout the gene as nonsense, insertion, deletion or mis-sense mutations and rearrangements. When applying stringent clinical criteria, a detection rate of 74% can be achieved in patients with HOS.[6] Nevertheless, not all carriers of the *TBX5* mutations have the HOS phenotype, indicating phenotypic heterogeneity at this locus.

• Thrombocytopenia with absent radius (TAR) syndrome (OMIM 274000): The thrombocytopenia-absent radius (TAR) syndrome is a congenital malformation syndrome characterized by bilateral absence of the radii and thrombocytopenia. Diagnostic criteria by Hall include bilateral absence of the radii in the presence of both thumbs and a thrombocytopenia. The presence of thumbs distinguishes TAR syndrome from other disorders featuring radial aplasia, which are usually associated with absent thumbs. Bilateral absence of the radii may be accompanied by ulnar or humeral anomalies and the most severe cases exhibit phocomelia. Lower limb involvement is variable (40-47%) and includes dislocation of the patella and/or of the hips, absent tibio-fibular joint, and lower limb phocomelia. Thrombocytopenia, which may be transient, is seen in all cases and will be symptomatic in over 90% of cases within the first four months of life. Other systemic problems reported are cow milk intolerance (60%), renal abnormalities (23%), cardiac abnormalities (15%), genital abnormalities (3%) and cleft palate. Other associations reported in case series are facial capillary hemangiomas, deafness, epilepsy and neural tube defects. Differential diagnoses include other conditions with radial ray defects; however, TAR can be differentiated by the presence of the thumbs in spite of absent radii and other associated malformation.

TAR syndrome is autosomal recessive in inheritance. An inherited or denovo deletion of 1q21.2 is present in a majority of cases. However, in view of the apparent autosomal recessive inheritance an additional causative allele should be there for the development of the disease. A compound inheritance mechanism of a rare null allele and one of two low-frequency SNPs in the regulatory regions of *RBM8A*, encoding the Y14 subunit of exon-junction complex (EJC) have been found to cause TAR. This is the first disease described to be associated with the deficiency of the exon-junction complex (EJC).[7]

• Fanconi anemia: Fanconi anemia (FA) is characterized by physical abnormalities, bone marrow failure and an increased risk of malignancy. Physical abnormalities are present in 65-70% of cases which include short stature, abnormal skin pigmentation, malformations of the skeletal system and microcephaly. Upper limb malformations include anomalies of the thumb (35%) (absent,

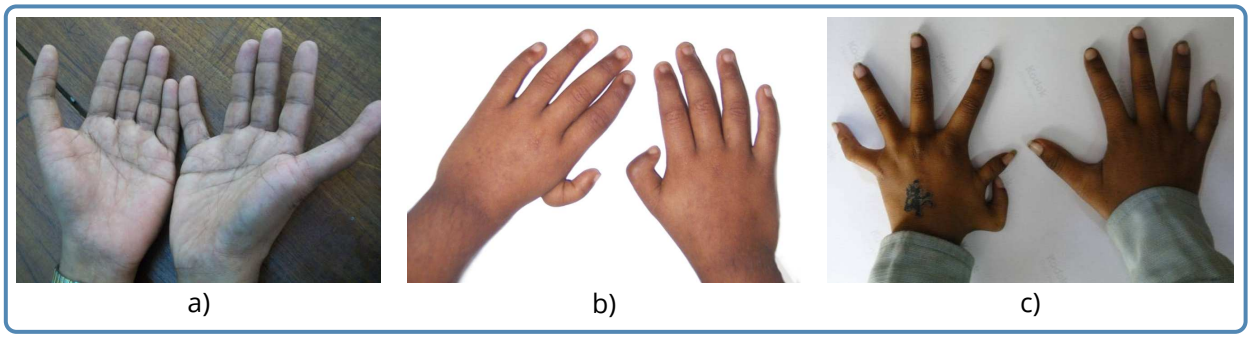

Figure 3 Thumb abnormalities in Fanconi anemia a) triphalangeal thumb b) rudimentary thumb c) duplication of thumb.

hypoplastic, bifid, duplicated, rudimentary, triphalangeal, long), radii (7%) (absent or hypoplastic with abnormal thumbs), hands (5%) (flat thenar eminence, absent first metacarpal, clinodactyly, polydactyly) and ulnae (1%) (dysplastic, short) (Fig.3). Lower limb anomalies are seen in 5% of cases which include toe syndactyly, club feet and abnormal toes. Developmental delay can occur in 10% of cases. The diagnosis of FA rests on cytogenetic testing for increased chromosomal breakages or rearrangements and formation of radial figures in the presence of diepoxybutane (DEB) or Mitomycin C. Molecular genetic testing is complicated by the genetic heterogeneity with at least 15 genes known to be responsible for the FA complementation groups. Most of these genetic abnormalities are inherited in an autosomal recessive pattern except mutations in the *FANCB* gene, which show X-linked inheritance.[8]

- SALL 4 related disorders: SALL-4 related disorders include the Duane-radial ray syndrome (DRRS), Okihiro syndrome and acro-renal-ocular syndrome (AROS), phenotypes previously thought to be distinct entities.[9] The Duane-radial ray syndrome (DRRS) and Okihiro syndrome are characterized by radial ray abnormalities which include hypoplasia/aplasia of radii, hypoplasia/aplasia of thumb, thenar hypoplasia, triphalangeal thumb, duplication of thumb (preaxial polydactyly) and Duane anomaly (characterized by uni- or bilateral limitation of abduction of the eye associated with retraction of the globe and narrowing of the palpebral fissure on adduction). Acro-renal-ocular syndrome (AROS) is clinically established in individuals with radial ray malformations, renal abnormalities (renal hypoplasia, mild malrotation, ectopia, horseshoe kidney, vesicoureteric reflux, bladder diverticula) and ocular abnormalities (ocular coloboma, Duane anomaly). Rarely, *SALL4* mutations may cause clinically typical Holt-Oram syndrome. Direct sequencing of the complete *SALL4* coding regions (exons1-4) detects mutation in more than 80% of individuals with DRRS and AROS. Exonic or whole gene deletions by quantitative real time PCR will detect a further 10-15% cases. Most mutations are private or have been observed in no more than three independent families. Inheritance is autosomal dominant with 95% penetrance. The proportion of cases caused by denovo mutations is approximately 40-50%.

Figure 4 Townes-Brocks syndrome in a father and his son. Hypoplastic radius and absent thumb are seen.

• Townes-Brocks Syndrome (OMIM 107480): Townes-Brocks syndrome is an autosomal dominant disorder. Radial ray abnormalities are reported in 50% of published cases. These consist of preaxial polydactyly (bifid thumb), triphalangeal thumb, hypoplastic thumb, broad thumb, and distal ulnar deviation of the thumb (Fig.4). Anorectal abnormality is characteristic of this condition. Other abnormalities include auricular, renal and cardiac abnormalities.[10] An important differential diagnosis is the VACTERL association, where all these abnormalities can occur. However, the presence of vertebral defects or tracheo-oeophageal malformation or both would strongly favor the diagnosis of VACTERL association. Mutation in the *SALL1* gene at 16q12.1 is responsible for this condition.

• VACTERL association: VACTERL association comprises **V**ertebral defects, **A**nal atresia, **C**ardiac defects, **T**racheo-**E**sophageal fistula, **R**enal malformations, and **L**imb malformations. There are some single gene disorders and syndromes which resemble the VACTERL association which include Fiengold syndrome, 22q11 deletion syndrome, Townes-Brocks syndrome and Fanconi anemia. When dysmorphic features, growth abnormalities and/or learning disability are present, a syndromic diagnosis or chromosomal abnormality has to be considered.[11]

Testing strategy for individuals with typical radial ray abnormalities

- Perform cardiac evaluation, ophthalmologic evaluation and renal ultrasound examination in addition to routine physical examination.
- If no features typical of SALL-4 related disorders are found, molecular genetic testing of the *TBX5* gene is suggested as the first molecular test.
- If features typical of SALL-4 related disorders are present, molecular genetic testing of the *SALL-4* gene is suggested as the first step.
- If clinical overlap exists with Townes-Brocks syndrome, molecular genetic testing of the *SALL1* gene should be the first test if the radial

Syndrome	Craniofacial features	Limb anomalies	Other anomalies
Nager acrofacial dysostosis (OMIM 154400)	Malar hypoplasia Micrognathia Preauricular tag Cleft palate	Hypoplasia or aplasia of thumb with or without radius Proximal radioulnar synostosis with limitation of elbow	Conductive deafness Intelligence normal
Rothmund-Thomson syndrome (OMIM 268400) (Figure 5)	Frontal bossing Small saddle nose Prognathism	Small hands and feet Hypoplastic to absent thumbs Forearm reduction defects	Mental retardation Cataract Sparse hair Erythema on skin Poikiloderma Small dystrophic nails
Baller Gerold syndrome (OMIM 218600)	Craniosynostosis Micrognathia Microstomia Epicanthic fold Hypertelorism	Absent/hypoplsatic radii Curved ulna Absent/hypoplastic thumbs Fused carpal bones	Mental retardation Congenital heart disease Renal anomaly Imperforate anus
RAPADILINO syndrome (OMIM 266280)	Long face Narrow palpebral fissures Long slender nose Cleft palate	Absent thumbs Joint dislocation Stiff interphalangeal joints	Small stature Hearing defect Infantile diarrhea Pigmentation

Table 1 Other syndromes with radial ray anomalies.

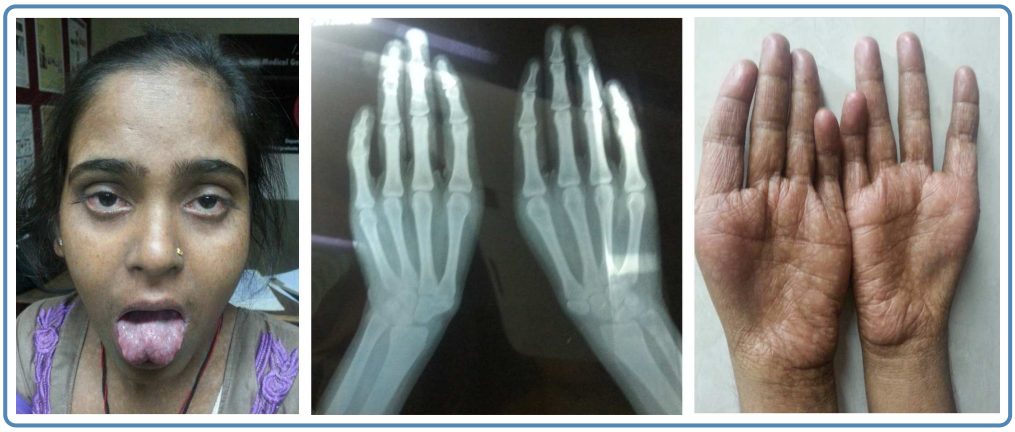

Figure 5 Bilateral absent thumb in a case of Rothmund-Thomson syndrome.

ray malformations do not include malformations of the radius itself. If malformation of the radius is present, molecular genetic testing of the *SALL4* gene is suggested as the first molecular test.

Prenatal Diagnosis

In pregnancies at risk, detailed high-resolution prenatal ultrasound examination may detect upper-limb malformations and/or congenital heart malformations. A normal ultrasound examination does not eliminate the possibility of radial ray defects in the fetus. Prenatal testing for the defect may be most useful in families with a known mutation to confirm ultrasound findings. If the disease-causing mutation has been identified in the family, prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis (usually performed at ~15-18 weeks' gestation) or chorionic villus sampling (usually performed at ~10-12 weeks' gestation). Because of the significant variable expressivity observed in most conditions especially with Holt-Oram syndrome both within and among families with the same mutation, the severity of upper-limb defects and congenital heart malformations cannot be accurately predicted by molecular genetic testing alone.

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