Chapter

Genetics of Sirenomelia, the Mermaid Syndrome

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Abstract

Sirenomelia (SML) is a rare, almost universally fatal congenital malformation presenting pathognomically with fused lower extremities and absent or malformed perineum. The classic Sirenomelia sequence includes a uniform spectrum of caudal malformations, spinal defects, and a single umbilical artery. SML is postulated to be due to a genetic predisposition, unmasked by biochemical or environmental triggers. Primary developmental defects in the formation of caudal mesoderm or embryonic caudal vessels with resultant local tissue hypoperfusion are proposed hypotheses for its pathogenesis. SML occurs sporadically in humans, presumably due to a spontaneous mutation, and is speculated to have an autosomal dominant inheritance pattern. In mutant mice, specific defects in Cyp26a1 and Bmp 7 genes are demonstrated to produce offsprings with SML. Bmp 7 is a signaling protein, which belongs to the transforming growth factor-β (TGF β) superfamily. Tsg 1, a Bmp and chordin-binding protein, functions as an activator-inhibitor of Bmp signaling in the embryonic caudal region (ECR). Loss of Bmp7 genes combined with a complete loss or half-dose of Tsg 1 is demonstrated to produce an invariable SML phenotype. SML is also demonstrated to occur with increased Retinoic acid (RA) signaling in the ECR. The Cyp26a1 gene is involved in coding for an enzyme, which expresses in ECR and degrades RA. A specific defect in this gene leads to excess local RA concentration and SML generation with a reported 20% penetrance in mutant mice. However, the mutational screening of Cyp26a1 and Bmp 7genes has failed to confirm their involvement in mankind and the molecular defect and genetic inheritability of SML in humans remain undefined.

Keywords: Sirenomelia, Blastogenesis, Vascular steal hypothesis, Retinoic acid, BMP 7 signaling, Cyp26a1gene

1. Introduction

Sirenomelia (SML) is a rare and almost universally fatal congenital malformation characterized by the pathognomonic feature of fused lower extremities, with absent or malformed perineum and Potter facies (**Figure 1**) [1]. SML, also called mermaid syndrome, is one of the most striking phenotypes among human anomalies. The exact etiopathogenesis of SML is undetermined, and the syndrome is postulated to be due to a genetic predisposition that is unmasked by a biochemical or environmental trigger factor. Several hypotheses have been proposed for the pathogenesis, among which the most accepted ones are vascular steal phenomenon, defective blastogenesis, and mechanical compression of the fetal caudal body. [1–8] Studies in mutant mice have provided significant and relevant information towards the understanding

Figure 1.
Sirenomelia in a newborn infant showing merged hind extremities and Potter facies.

of the genetic aspect of the anomaly. [1] SML occurs sporadically, and even though not recognized as familial, it is documented to be more common in twin monozygotic pregnancies, among whom the relative risk is stated to be increased by 100-fold. About 9–15% of all cases of SML are products of twin gestation. Environmental and teratogenic factors, such as cocaine, retinoic acid, heavy metals, cyclophosphamide, and certain antibiotics, have been linked to SML in humans and animal models [1, 9]. In addition, nicotine, alcohol, radionuclides, diethylpropion- an appetite suppressor, organic solvents of fats, and even air pollution have been associated with SML and caudal regression syndrome, which is controversially considered as its minor form [4, 9]. Other authors have reported fetal exposure to cadmium, lithium, phenytoin, sodium valproate, carbamazepine, warfarin, methylergonovine, diethylpropion, trimethoprim, and ochratoxin-a type of fungus as possible triggers for the anomaly. [7–9] Some of the maternal complications, such as diabetes mellitus, hyperthermia during the 1st trimester of pregnancy, amniotic bands, and age below 20 years or over 40 years at conception, have also been implicated in the pathogenesis of SML. [1, 9]. Recently a case report tentatively associated persistent early gestational maternal Chlamydia trachomatis (CT) infection with SML. CT is an obligate intracellular pathogen that is recognized for its cytopathogenic effects like cellular disruption, tissue dysgenesis, and genomic instability and is known to invade the placenta and cause fetal demise. [10] SML has no ethnic or geographical preferences, and a gender preponderance with a male to female ratio of 2.7:1 has been described. [1] The prevalence of SML is reported to be between 1.1 and 4.2 per 100,000 births [1, 6]. The molecular basis of the defects in SML is yet to be defined.

2. The phenotype

The typical phenotype of SML is characterized by a partial or complete merging of the lower extremities into one single limb. The deformity, which gives the mermaid appearance to the syndrome, is invariably associated with smooth perineum, malformed or absent perineal structures, and in most instances, Potter facies (**Figure 1**). The classic Sirenomelia sequence consists of a uniform spectrum

of visceral malformations involving urogenital and gastrointestinal systems, pelvic and lumbosacral spinal defects, and a single aberrant umbilical artery. Renal agenesis or severe renal malformation are almost invariably present and lead to oligohydramnios and consequent pulmonary hypoplasia. Other commonly observed abnormalities in SML are sacral agenesis, abnormal vertebrae, hemivertebrae, meningomyelocele, and less frequently, cleft palate, cardiac defects omphalocele, and pentalogy of Cantrell [1, 11]. It is hypothesized that a defect in blastogenesis leads to impaired angiogenesis in the embryonic caudal region, and the resultant vascular compromise causes anomalous development of the area structures. The lower extremity phenotype is evidenced to occur due to merger and not by fusion of the two bilaterally positioned fetal hind limb buds as suggested by the histopathological persistence of epithelial linings in the conjoined limb fields. [1] The merged lower extremities are abnormally rotated by 180 degrees to the normal position of legs (**Figure 1**). Normally, during development, the hind limb buds rotate medially, and the original ventral surface turns to become dorsal [12] In SML, due to the early midline posterior fusion of the embryonic hind limbs, this rotation does not occur, as a result of which, the soles of the affected fetus face anteriorly, and the fibulae end up being placed medially between the tibiae. [1] Some researchers believe that the typical phenotype in SML is secondary and not a primary defect. As the developing leg fields in the fetus normally lie along the lateral body wall, it is thought that a developmental failure of caudal midline structures, such as ventrally positioned cloaca and urogenital sinus, and dorsally positioned somites and neural tube, results in the approximation and final merging of the two analgen of fetal limb buds [12, 13]. In SML, kidneys are almost always affected, and although the gonads may be spared, the urethra is undeveloped and external genitalia either absent or represented by abnormal tissue tags [14]. Other almost invariably present anomalies involve the gastrointestinal tract and are represented by a blind-ending colon, rectal atresia, and imperforate anus [15]. In the classic form, SML is fatal during the perinatal period, whereas, in the minor forms, reconstructive pelvic and limb surgery may prolong the life if the renal function is compatible with survival. [16] Notably, the neurodevelopment of the surviving babies is reported to be normal.

3. Pathogenesis

Although unsubstantiated, it is believed that SML is an autosomal-dominant genetic condition in humans, and each case is the result of a new spontaneous mutation. It is speculated that the syndrome is induced by a combination of genetic and environmental influences. The most accepted non-genetic hypotheses for the pathogenesis of SML are the vascular steal phenomenon and defective blastogenesis. These two theories are not mutually exclusive as any deficiency in blastogenesis could potentially lead to fetal vascular and visceral maldevelopment. Genetically, SML phenotype has been demonstrated in mutant mice with both gain-of-function of retinoic acid (RA) signaling and loss-of-function of bone morphogenetic protein (Bmp) signaling [11].

3.1 Non-genetic theories

3.1.1 Vascular steal hypothesis

SML is characterized by an anomalous single umbilical artery that diverts blood away from the caudal fetal limb buds to the placenta, thereby compromising the regional blood supply during the early developmental period. In the early fetal

stage, the vitelline artery complex serves as an embryonic vascular network that supplies the yolk sac. [2, 11] In SML, the umbilical vasculature develops abnormally and connects to the vitellus in an anomalous way, resulting in the formation of a large aberrant aorta-like vessel that originates from the vitelline artery high in the future abdominal cavity. The normal aortic branches, such as the celiac artery, are either separated from this abnormal abdominal aorta or are absent or hypoplastic. This large anomalous vessel functions as a single umbilical artery and diverts blood away from the lower part of the fetus to the placenta, thus severely limiting the blood and nutrients supply to the mesoderm of the caudal fetal body. In autopsies, the tissues dependent upon the lower branches of this aberrant steal aorta are found to be malformed or in different stages of incomplete or arrested development. However, case reports of SML with two umbilical arteries have been described, and an aberrant single umbilical artery, which was considered as the hallmark of SML and a major differentiating feature from caudal dysgenesis, is now not considered to be so [2, 15, 17–19].

3.1.2 Defective blastogenesis hypothesis

According to this theory, SML results due to an impairment in blastogenesis during the final stages of gastrulation. A primary defect in blastogenesis leads to abnormal angiogenesis and poor blood supply to the embryonic caudal region at the tailbud stage during the third human gestational week with consequent deficiencies in the area structures' growth and development (**Figure 2**) [18]. Presumably, the timing, duration, and severity of the disruption determine the clinical presentation's phenotypical variability. Developmentally, during the gastrulation phase, epiblast cells move massively through the primitive streak and transform a two-layered blastocyst into a three germ layered embryo, with the endomesoderm emerging in a rostrocaudal sequence [20]. At late gastrulation, the regressing primitive streak forms the tailbud, which is composed of a mass of loose mesenchyme covered by ectoderm [13, 21]. As the tailbud grows, the remnants of the primitive streak continue to involute ventrally, eventually forming a distal thickened ectodermal area called the ventral ectodermal ridge (VER) [21], which basically is a continuation of the posterior primitive streak.

Meanwhile, the surface mesoderm precursors continue to internalize and contribute to the tail elongation and caudal structures formation. This process is confirmed in both chick and mouse embryos by performing analysis of the concerned cells' molecular markers [22]. As demonstrated in chicks, the VER cells can undergo an epithelial-mesenchymal transition process and thus accumulate mesoderm cells in the lateral and ventral tailbud region. The VER controls cell proliferation in the underlying mesoderm and is presumably the signaling center responsible for tail elongation [22]. A disruption in this embryological process is postulated to produce the SML phenotype. The questions arise about the triggering factors' identity and source in the induction of the specific blastogenesis defect. These factors could be environmental or more recently recognized pathological condition of excess accumulation of retinoic acid and its metabolites in the embryonic caudal buds, which hypothetically may superimpose upon an unidentified genetic susceptibility.

3.1.3 Mechanical compression of embryonic caudal region

Abnormalities in the amniotic forces may adversely impact the embryo's caudal body and lead to its hypoplasia. It is also suggested that abnormal excessive distension of caudal neural tube may lead to lateral rotation of the developing mesoderm and cause fusion of lower limbs and abnormal closure of primitive gut and

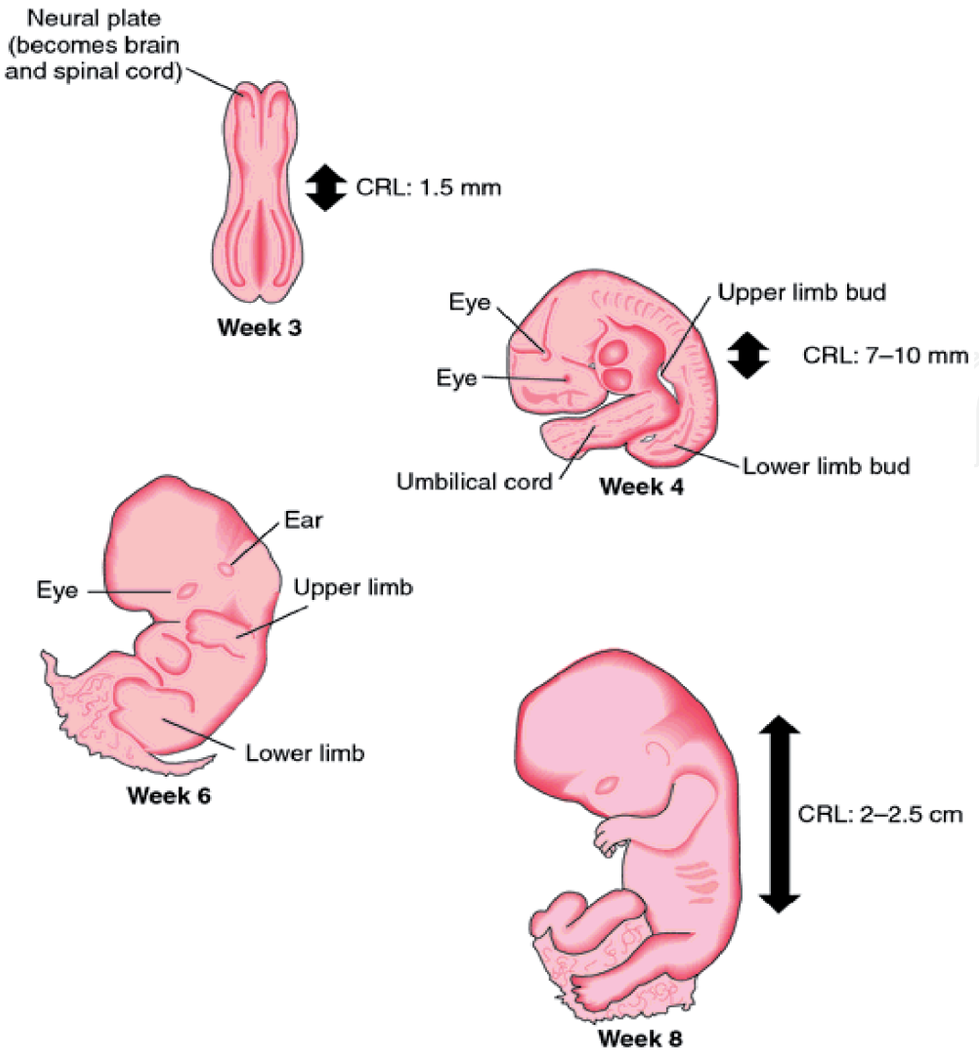

Figure 2.
Diagrammatic representation of the early development during 3–8 weeks after fertilization in humans. CRL = crown to rump length.

urethra. [1] Finally, it is speculated that the merger of the lower extremity in SML is a consequence of the allantois abnormally intervening or inhibiting the cleavage of the lower limb bud field into two lateral masses. These hypotheses, however, are not confirmed.

3.1.4 Environmental factors

RA, maternal diabetes mellitus and heavy metals have been reported to be important environmental risk factors for caudal malformations. In a mouse model, RA exposure resulted in multiple caudal structural anomalies, such as tail agenesis, caudal vertebral defects, spina bifida occulta or aperta, imperforate anus, rectovesical or rectourethral fistula, renal malformations, cryptorchidism, gastroschisis, limb malformations, and the classical SML phenotype. [23] On serial histopathological examinations, progressive hemorrhage, edema, cell death, vascular disruption, and tissue deficiencies were demonstrated as the adverse effects of fetal RA exposure. The embryos treated with RA displayed early cell death, particularly in the caudal median axis, hindgut, and neural tube, and a failure to develop the tailbud. The symmelia appeared to be due to the failure of fission or the merger of limb fields rather than a fusion of two limb buds [1, 23]. As RA levels can be modified by genetic, nutritional, and iatrogenic factors, their involvement in the

causation of SML could occur via multiple pathogenic routes. Still, regardless of RA's association in the genesis of caudal anomalies in mice models, RA has not been so far documented to induce or trigger SML in humans. Also, even though maternal diabetes mellitus is associated significantly with caudal dysgenesis, its association with SML is not confirmed. Only 0.5–3.7% of SML cases are reported to have mothers with the disorder as opposed to about 15% in caudal dysgenesis [9]. Exposure to heavy metals is another group of environmental toxins, and their anecdotal and unconfirmed association with SML in humans and experimental animals has been described. [24–26].

3.2 Genetic factors

The genetic defects specific to SML have not been so far identified in humans. Except for a few recently published cases, all human fetuses with SML are reported to have normal karyotypes. Gabriele et al. described a fetus with SML having a triploid mosaic (69, XXX/46, XX), [27] while another case was noted to have a de novo balanced reciprocal translocation 46X, t(X; 16) (p11.23; p12.3), in who the chromosomal breakpoints on the pairs of chromosomes did not disrupt the coding genes associated with early blastogenesis [28]. A big breakthrough in the genetic pathogenesis of SML was brought about by the experimental findings in mutant mice. While no genetic inheritability was established in humans, a genetic component was first detected in the SML phenotypical offsprings of mice carrying mutations at or near the T locus in the brachyury gene (short-tail strain) and in the axin1 gene (fused strain), the two genes that are involved in the structural development of tail and caudal body [1, 11]. Another spontaneous mutation called sirenomelia (*srn*) was identified to cause hind limb fusion in homozygous mice [1, 29]. In humans, mutations in the homeobox-containing gene HLXB9 gene is the only recorded genetic aberration associated with congenital caudal anomalies seen in the autosomal dominant sacral agenesis of Currarino syndrome, which presents with pelvic malformations, anal atresia, meningomyelocele, and urogenital defects but not with SML [30].

In experimental mutant mice mothers, defects in the Cyp26a1 and bone morphogenic protein7 (Bmp 7) genes are identified to induce a phenotypical SML product of conception. [1, 11] The Cyp26a1 gene is involved in coding for the enzyme that breaks down RA. RA effectuates vasculature development in the fetal caudal region, but its excessive accumulation disrupts the process. Bmp7 is an important protein related to angiogenesis and stimulates the production of endothelial cells, vessels, and tissue in the fetal caudal region in order to promote normal growth of lower extremities. Disruption of the Cyp26a1 gene is demonstrated to result in incomplete development of the embryonic caudal region and present with hind limb fusion in mice, which is also seen in mice with knockouts or mutations in both Tsg1 and Bmp7 [11].

3.2.1 Decreased Bmp signaling in the embryonic caudal region

An important link between Bmp7 and twisted gastrulation (Tsg) was reported by Zakin et al., who noted that the loss of Bmp7 combined with a complete loss or half-dose of Tsg produces an invariable SML phenotype in mutant mice models. [31]. Bmp 7 is a member of the secreted multifunctional signaling proteins, which belong to the transforming growth factor-β (TGF β) superfamily. Tsg can function as an activator of the inhibitor of Bmp signaling in the caudal embryonic region. [32] Tsg is a Bmp and chordin-binding protein that has multiple effects on BMP metabolism in the extracellular space. Bmp7 is one of many Bmps that bind to Tsg.

In mice, molecular marker studies indicate that the SML phenotype is associated with a defect in ventroposterior mesoderm formation. In Xenopus, a species of frogs, co-injection of Tsg and Bmp7 morpholino oligonucleotides showed a strong synergistic effect in substantially inhibiting the formation of ventral mesoderm, suggesting that the dorsoventral patterning of the mouse posterior mesoderm is regulated by Bmp signaling. [1, 11] It is experimentally evidenced that the single Bmp7 mutant does not generate a mermaid phenotype and the mermaid phenotype in the Bmp7; Tsg double mutant results from a reduction in Bmp signaling in the ventral caudal mesoderm. In zebrafish, deficient Bmp signaling after the mid-gastrula stage causes deficiencies in ventral mesoderm formation, with defects in kidney and excretory system morphogenesis, e.g., aberrant cloaca [33]. Thus, among the mutant mice models that have been characterized so far, the Cyp26a1 and Bmp7; Tsg engineered mice mutants are considered to be the best models to investigate the pathogenesis of SML in humans as they exhibit a phenotype that is closest to the human presentation.

Bmp signaling has important roles in early embryogenesis. Bmp ligands and their extracellular antagonists and modulators control gastrulation. During late gastrulation, in mice, several Bmp ligands and their extracellular antagonists and modulators are expressed in dynamic and partially overlapping domains [34, 35] such as Bmp2 and Bmp7 in the VER, and Bmp4, Bmp7, and the Bmp antagonist Noggin in the underlying mesoderm. These have experimentally demonstrated roles in the embryonic tailbud's growth by promoting the formation of caudal mesoderm in the VER in mice [36]. Furthermore, the termination of cell movements through the VER coincides with Bmp signaling attenuation in chicks. [36] Besides, the double Bmp7; Tsg mutant SML phenotype strongly supports Bmp signaling involvement in caudal development [31]. Physiologically, Bmp signaling is demonstrated to promote endothelial cell activation, migration, and proliferation, thereby contributing to angiogenesis, vasculogenesis, and normal vasculature remodeling of the primitive capillary plexus [1, 36, 37]. Thus, Bmp signaling is critical for the normal formation of the mesoderm and differentiation of the hematopoietic and endothelial precursor cells.

3.2.2 Increased RA signaling in embryonic caudal region

RA signaling in the genesis of SML is well established in experimental animals. RA, the active metabolite of vitamin A, is degraded by the enzyme Cyp26a1, which is specifically expressed in the embryonic caudal region and the developing vascular network. [38, 39]. A deficiency of Cyp26a1 is demonstrated to result in excessive RA activity in the embryonic caudal region and induce multiple caudal defects, including that of SML with a reported 20% penetrance. This enzyme's lack or deficiency also results in diminished bone morphogenetic protein signaling in the caudal region of the embryo. Cdx2 is a transcription factor that encodes and activates the Cyp26a1 promoter, and Por encodes an enzyme that is required for the function of the Cyp26 family of enzymes. [1, 40, 41]. Even the disruption of these related factors Cdx2, and Por, are noted to result in SML. The relationship is further corroborated by the fact that a Cyp26a1 phenotype can be rescued by decreasing the production of RA by reducing the level of the enzyme haploinsufficiency of Raldh2, which is necessary for its production. [41].

Embryologically, the developing cells in caudal structures are especially sensitive to RA signaling during gastrulation. Its levels are tightly controlled by the expression of its metabolizing enzymes [1] A multitude of experiments that modified the level of RA signaling by genetic and nutritional means have demonstrated that the embryo is particularly sensitive to deviations from normal levels of RA during

gastrulation [1, 42–44]. The expression of Cyp26a1 takes place at the early gastrula stage in the primitive streak and the nascent mesoderm; and at the late gastrula stage in the neuropore, hindgut endoderm, and tailbud mesoderm [37, 39, 40]. RA signaling is generated by Raldh2 in the somites in association with specific growth factors. RA plays a key role in the proliferation and differentiation of precursor cells, and Cyp26a1 expression at the caudal area is pivotal in maintaining the physiologically appropriate RA levels. [45, 46] RA excess is shown to negatively regulate endothelial cell proliferation and impede vascular remodeling by inducing premature coalescence and differentiation of precursor cells. In animal models, abnormal development of umbilical and vitelline arteries in embryos similar to those in human SML was noted when pregnant rats were given RA. [47] However, despite strong evidence of Cyp26a1 being instrumental in the generation of SML phenotype, a mutational screening of the Cyp26a1 gene has not confirmed its involvement in caudal dysplasia in humans [48].

Even though both Bmp and RA signaling pathways are critical participants in the development of caudal structural during the early embryonic stage, it is unclear if their effects are synergistic or antagonistic and whether they modulate each other's roles. RA had been shown to decrease Bmp signal duration by reducing the level of phosphorylated Smad1, an intracellular component of the Bmp signaling pathway in the developing neural tube [1, 49], whereas Bmp signaling has been demonstrated to adversely regulates RA signaling during chondrogenesis [50]. However, such effects have not been studied or confirmed to be operative in the generation of SML phenotype. The detection of possible crosstalk between the two signaling cascades might provide helpful information regarding the pathogenesis of SML phenotype in humans.

4. Conclusion

Sirenomelia is a major, almost universally fatal human anomaly with obscure etiopathogenesis. An indeterminate genetic predisposition in combination with exposure to potentially adverse environmental trigger factors is thought to be instrumental in producing the phenotype. Several causal hypotheses have been forwarded, and studies in mutant mice have provided important insight into its generation at embryonic cellular and biochemical levels. Genetic aberrations in the Bmp and RA signaling pathways have been demonstrated to induce SML-like phenotype in the mutant mice. Defining the precise genetics, the roles of combined Bmp and RA signaling pathways, and the unidentified molecular defect in SML are subjects of future research on the subject.

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