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Review Article

Research advances in Apert syndrome

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ABSTRACT

Apert syndrome is one of the several genetic syndromes associated with craniosynostosis, a condition that includes premature fusion of one or multiple cranial sutures. There has been significant clinical variation among different sutural synostoses and also within particular suture synostosis. Enormous progress has been made in identifying various mutations associated with Apert Syndrome. Although a causal gene has been defined, the precise role of this mutation in producing craniofacial dysmorphology and other related abnormalities is in the process of discovery. Most of the understanding regarding this rare disorder has been possible due to mouse models that have helped in deciphering the elements of this rare human disease. Thus, molecular and cellular understanding of the disease has taken a leap and further with the advent of technology definitive diagnosis of the syndrome is no more of an issue. In this review, we have discussed and consolidated the possible molecular studies that have contributed in understanding of this rare syndrome. This article may help clinicians and researchers to inform about the latest progress in Apert syndrome.

Contents

1. Introduction

Apert syndrome (AS) is a rare genetic disorder characterized by craniosynostosis, acrocephaly, syndactyly of the hands and feet and often combined with anomalies of other organs. Craniosynostosis involves premature fusion of one or more neurocranial sutures and associated dysmorphologies of cranio-facial complex. AS incidence is reported to be 1 in 65,000 live births and its diagnosis is sometimes clinically overlapped with that of Crouzan, Pfeiffer, Muenke and Saethre-Chotzen syndrome since they all are characterised by craniosynostosis.[1,2] Other malformations include abnormalities of the skin, skeleton, brain and other internal organs. Mental ability varies widely from normal to severe deficiency, although not lethal but many cases result in death due to abnormalities in respiratory or cardiovascular systems.[3–5] Studies report 98% cases to be a result of one of two heterozygous mutations in exon IIIa of fibroblast growth factor receptor 2 gene (*FGFR2*) encoding the amino acid substitutions Ser252Trp or Pro253Arg. FGFR2 is one of four transmembrane FGFRs that mediate signalling downstream of fibroblast growth factor ligands and plays a vital role in skeletal development and disease.[6]

The two identified mutations are known to affect the highly conserved linker region between immunoglobulin-like II and III

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domains which result in increased affinity and altered specificity of fibroblast growth factor (FGF) ligand binding.7,8 The first mutation S252W is the most common and is associated with severe craniofacial anomalies while the other mutation P253R contributes to severe syndactyly.4,9 Signalling activities involving FGFR2 regulate multiple activities such as stem cell proliferation affecting different cell lineages such as osteoblasts and chondroblasts.10–13 Further, it plays crucial role in control of cell migration, proliferation, differentiation and survival by activation of the mitogen activating protein kinase pathway and protein kinase C pathway.14–17

Recent studies have explored various means to understand the diverse cellular processes that explain mechanisms that cause disturbances in normal growth process and manifest as AS in newborns. Thus, in the present article, we have consolidated the studies conducted in this area that have contributed in understanding the pathogenesis of this syndrome at molecular level.

1.1. Mouse models

Genome manipulation using mice is a rich resource to study molecular genetic diversity and is a powerful and informative way to study and model human diseases. Chen et al. 18 created the first transgenic mouse model $Fgfr2^{+/S250W}$ having a mutation targeted for the 252 conserved serine residue. This mouse model exhibited features similar to that of AS such as a small body size, midface hypoplasia, wide-spaced eyes, brachycephaly, short presphenoid bone and malocclusion. Other significant observations were long bones with abnormalities, height of growth plate correlating with smaller size of mutants and column of proliferating chondrocytes being shorter in older mice (postnatal day 10). Their detailed study suggested dysregulated apoptosis to play a vital role in AS like phenotypes.18 Similarly the mouse model by Wang et al. 19 used the cre-mediated knock-in method and introduced the $Fgfr2^{+/S252W}$ mutation into the mouse genome and explored the other possible malformations of internal organs and midline sutural abnormalities. Since this mutation alters the proliferation and differentiation of osteoblasts at the midline calvarial sutures, the resultant mice showed ectopic cartilage formation in the neurocranium, cartilaginous abnormalities in several organs and long bones.19 Yin et al. 20 in their study involving a knock-in mouse model carrying P253R mutation exhibited smaller body size and brachycephaly. Analysis of mutant skulls and long bones revealed coronal suture fusion, shortened cranial base and growth plates of long bones. Additional studies employing the *in vitro* organ culture methods revealed that the mutation effects growth retardation of femur, cranial base and premature closure of coronal sutures and that Erk1/2 signalling pathway to partially mediate the effects of P253R mutation.20

Animal model studies by Holmes et al. 21 assessed the process of coronal suture fusion in a modified Apert Fgfr2 (S252W) mouse model. This study determined the molecular and cellular processes as how the disturbances in increased osteoblast proliferation, differentiation and apoptosis observed in craniosynostosis lead to abnormal suture fusion. The *in vivo* observation of this work reports that vital events like early loss of basal sutural mesenchyme as the osteogenic front and expression of activated Fgfr2 leads to contiguous skeletogenic membrane only after an initial increase in osteoprogenitor proliferation and osteoblast maturation followed by apoptosis that helps in fusion restricted to bone fronts in contact with one another. Further, correlation of the observed outcome with intrinsic effects of activated Fgfr2 S252W mutation in primary osteoblast culture shows an increased capacity for proliferation and differentiation and the major determinant of craniosynostosis to be the failure to respond to signals and halting of recruitment of the advanced osteoprogenitor cells at sites of suture formation.21 Hajihosseini et al. 22 strongly suggested Fgf10 gene to cause AS-like pathologies in their generated mice deficient in or heterozygous for Fgf10. The genetic knockdown of Fgf10 rescued the mice from phenotypic changes like skeletal and visceral defects and restored near normal level of Fgfr2 signalling by involving a switch between ERK (p44/p42) and p38 phosphorylation.22 On the other hand study by Aldridge et al. 23 made use of two inbred strains of mouse by Wang et al. 19,49 that had both the mutations $Fgfr2^{+/S252W}$ and $Fgfr2^{+/P253R}$ and analysed the inbred strains and their littermates C57BL/6J strain, at postnatal day P0 using data from 3-D magnetic resonance microscopy (MRM) and morphometric methods. This strategy helped in studying the effects two genetic mutations on brain phenotypes and suggested that brain is primarily affected rather than showing a secondarily response to skull dysmorphogenesis. Their hypothesis suggested both the skull and the brain getting affected in craniosynostosis and shared phenogenetic development process affecting both types of tissues in AS. The analysis using mice P0 also reduced the effect of environmental factors pattern on post-natal growth thus revealing the real effect of mutations on phenotype.23

A simultaneous study by Martinez-Abadias et al. 24 investigated the variation in cranial phenotypes using mice models of Wang et al. 19,49 i.e. $Fgfr2^{+/S252W}$ and $Fgfr2^{+/P253R}$. Using high resolution micro computed tomography (μCT) on skulls of newborn P0 mice they estimated the morphological variation within and among groups and also detected the local shape differences resulting from phenogenetic processes that may contribute to this syndrome development. Their study reports that not only coronal sutures as found earlier, but facial sutures and bones of the cranial vault, cranial base, face and palate along with brain to be the affected region of cranium. Further, extension of the study by the same group investigated the cleft palate in AS using the same 2 mouse models. A novel combination of morphometric, histological and immunohistochemical analysis was applied to precisely quantify the differences in the distinct palatal phenotypes. Palatal suture scoring and 3D shape analysis of 120 new-born mouse skulls revealed $Fgfr2^{+/S252W}$ mice to display severe palate dysmorphologies with contracted and separated palatal shelves and aberrant development of inter-premaxillary suture. Palatal defects were associated with abnormal cellular proliferation, differentiation and apoptosis leading to suture-specific patterns. The study also suggested the posterior region of the palate to be a potential target for therapeutic management of cleft palate.24,25 Another interesting approach apart from mice models was the tissue engineering method by Yang et al. 26 wherein they used a 3D hydrogel culture model that provided an effective *in vitro* setting to study this syndrome. Use of tissue engineering strategies helped in studying the $Fgfr2^{+/S252W}$ mutation in differentiating osteoblasts postnatally. Isolated cells from the long bones of mutant and wild type mice were used for studying different parameters. Their cell culture observation found increased proliferation and altered responses to FGF ligands with varying binding specificity only in mutant cells. Further, for efficient mimicking of *in vivo* disease development, cells were encapsulated in 3D hydrogels and the 3D *in vitro* culture was compared to that of *in vivo* tissue specimen. The results found a 2.8-fold and 3.3-fold increase in collagen type I and osteocalcin respectively as compared to wild type controls. Apart from these effects the mutant cells showed decreased bone matrix remodelling and 87% less expression of metalloprotease-13 and 71% less Noggin. This novel approach thus applied the tissue engineering methods to successfully study genetic disease and in proposing *in vitro* 3D culture system as a valuable alternative method for future studies.26

1.2. Identified Mutations and gene expression studies

Identification of chromosomal aberrations and mutations play a vital role in diagnosis of genetic disorders and specific molecular or

cytogenetic tests are carried out based on the type of genetic syndrome. Early analysis of AS found FGFRs as candidate genes and further studies report mutations in FGFR2 exon IIIa among AS patients.27 However, with increasing number of studies other mutations have also been found to be present among patients. Oldridge et al. 28 in their study involving 260 patients reported only 2 patients to have *Alu*-element insertion of ~360 bp within exon 9 and in 1 case upstream of exon 9. This *de novo* insertion was found only on paternal chromosome and was suggested to have pathological implications. Further, the study examined the splicing of exon 9 in RNA from fibroblasts and keratinocytes in a patient having *Alu* insertion and 2 patients with Pfeiffer syndrome having nucleotide substitution of exon 9 and found ectopic expression of keratinocyte growth factor receptor (KGFR) isoforms but not in fibroblast from patients with canonical Apert mutations. Thus, the first evidence of signalling through KGFR to be a possible reason for syndactyly in AS was suggested.28

While the study by Britto et al. 29 revealed TGFβ-3 to co-regulate with molecules of FGFR signalling throughout the stages of human palatal fusion suggesting their controlling influence on apoptosis and palatal cleft pathological mechanism.29 Another study tested the global gene expression profile of coronal suture periosteal cells among 7 AS patients (S252W) and matched controls revealing 263 significantly altered genes involved in positive regulation of cell proliferation and nucleotide metabolism and several downregulated genes to inhibit cell proliferation, gene expression regulation, cell adhesion and extracellular matrix organization. Further, experiments with selected set of genes through real-time PCR using patient, control and control cells treated with high FGF2 concentration and analysis of genes involved in FGF-FGFR signalling suggested for the first time the periosteum to be involved in pathophysiology of AS. The study reported osteogenic potential of periosteal cells and found them to be committed towards osteoblast lineage.30 Later, Tiozzo et al. 31 studied the cellular and molecular basis of tracheal stenosis (uniform cartilaginous sleeve instead of normally ribbed cartilaginous trachea) in mouse model $Fgfr2c^{+/\Delta}$ mice that revealed ectopic expression of Fgfr2b in mesenchymal tissues. This study reported Fgf10 to have a critical role in tracheal stenosis as its genetic knockdown rescued the mice model from this phenotype. This phenotype was found to be associated with increased proliferation of mesenchymal cells with expression of Fgf10 and upstream regulators Tbx4 and Tbx5 to be abnormally elevated.31

Bochukova et al. 32 reported novel mutations in 2 patients who resulted negative for the 2 usual mutations. Among them one carried a 1.93-kb deletion, removing exon IIIc and substantial portions of the flanking introns which happened to be the first large FGFR2 deletion in craniosynostosis. The other patient was found to have 5' truncated Alu insertion into exon IIIc (AluYk 13 subfamily) this was the third Alu insertion to be reported in AS patients.32 Interestingly, a recent case study reported a child in whom the standard genetic testing ruled out FGFR2 missense mutations and instead found a heterozygous 1372 bp deletion between FGFR2 exons IIIb and IIIc. This change was suggested to be a result of recombination between 13 bp of identical DNA sequence present in both exons and found to be absent in unaffected parents. 33 Simultaneously, another recent study discovered a soluble truncated FGFR2 molecule i.e. IIIa-TM encoded by PTC (premature termination codon) containing transcript to be upregulated in AS mice model. IIIa-TM was suggested to have arisen as a result of aberrant splicing of FGFR2 exon 7 (IIIa) into exon 10 (transmembrane domain TM). IIIa-TM molecule is glycosylated and modulates the binding of FGF1 to FGFR2 molecules in BIAcore-biinding assays and can negatively regulate FGF signalling *in vitro* and *in vivo*. Further, the study also suggests AS phenotypes to be a result of IIIa-TM contributing to loss-of-FGFR2 function and FGFR2 signals to be a regulator of NMD (nonsense-mediated decay) pathway.34 Research on a transgenic mice bearing AS-type mutant i.e. Fgfr2IIIc (P253R) mouse revealed upregulation of p21, Ihh and Mmp-13 accompanied by modest increase in expression of Sox9 and Runx2, indicating acceleration of chondrocytic maturation and hypertrophy in the cranial base and suggesting acrocephalic feature of AS to be a result of primary disturbance in growth of the cranial base with precocious endochondrial ossification. They also report acquired affinity and specificity of this mutant receptor to FGF2 and FGF10 leading to activation of FGFR2 signalling in cranial base alone.35

On the other hand, a latest study carried out by Yokota et al. (2014) clarified the etiological mechanism of craniosynostosis and effect of soluble FGFR2 harbouring the S252W mutation in calvarial sutures of AS mice *in vitro*. The study result found increased Fgf10 and FgfrIIIb expression to induce the onset of craniosynostosis and suggested that suitable delivery of purified $sFGFR2IIIc^{S252W}$ to be an effective method in treating the disorder. Administration of the purified molecule inhibited Fgf2-dependent proliferation, phosphorylation of intracellular signaling molecules and mineralization of FGFR2S252W-overexpressing MC3T3-E1 osteoblasts. Delivery of the molecule using the nanogel complexed with the molecule maintained the coronal suture patency while delivery using the nanogel without the molecule led to observation of synostosis condition.36 Another experiment by Morita et al. involving $sFGFR2IIIc^{S252W}$ in Apert mouse model revealed the soluble form of S252W to partially prevent craniosynostosis.37

1.3. Cell signalling studies:

Signalling pathways are known to be involved in proper development of cell type, cross talk between cells and organogenesis and thus any error in them implicates their role in aberrant functions of many kinds. In AS, FGFs/FGFRs signalling is known to regulate balance of cell proliferation, differentiation and apoptosis necessary for the normal formation of cranial bones whereas the gain of function mutation in FGFR2 has been suggested to disrupt balance leading to this syndrome.38 Early cell signalling studies found AS to be a result of increased affinity of mutant receptors for specific FGF ligands that activate signalling under conditions of ligand limitability. The difference between normal and Apert osteoblasts was correlated to altered balance between TGF-β1 and FGF.39,40 Yu et al. 41 were the first to report the abnormal changes governing ligand specificity of FGFR2 and suggested the severe phenotypes to be a result of ectopic ligand-dependent activation of FGFR2. Their observation showed that S252W mutation causes mesenchymal splice form (FGFR2b) of FGFR2 to be activated by FGF2, FGF6 and FGF9.41 Hajihosseini et al. 42 in their study on a mouse model found heterozygotic abrogation of FgfR2-exon 9 (IIIc) to cause a splicing switch that leads to a gain-of-function mutation and causes neonatal growth retardation, coronal synostosis, ocular proptosis, pre-cocious sterna fusion and abnormalities in secondary branching in several organs that undergo branching morphogenesis. These phenotypic features had strong parallels to AS patients and thus they suggested the use of this mice model for investigating the molecular mechanisms governing normal bone growth and craniosynostosis.42 Simultaneously study by Lemonnier et al. (2001b) reported N-cadherin to activate osteoblast differentiation marker genes in mutant osteoblasts and PKC-α signalling to be involved in increased N-cadherin and osteoblast gene expression induced by S252W mutation. They also studied the effect of S252W mutation on apoptosis and underlying mechanism of human mutant osteoblasts. *In vivo* and *in vitro* analysis revealed premature apoptosis of mature osteoblasts/osteocytes in apert suture and increased apoptosis respectively. Mutant osteoblasts showed increased activity for caspase-8 and

effector caspases (-3,-6,-7) constitutively which is related to protein kinase C activation and Apert osteoblasts also showed increased expression of IL-1α, IL-1β, Fas, Bax and decreased Bcl-2 levels. Thus, it was found that Apert S252W mutation promotes apoptosis through activation of protein kinase C, overexpression of IL-1 and Fas, activation of caspase-8, and increased Bax/Bcl-2 levels leading to increased effector caspases and DNA fragmentation in human osteoblasts. Thus, a complex FGFR2 signalling pathway involved in premature apoptosis of human calvaria osteoblasts was found to influence AS development.43

Simultaneously Lomri et al. 44 by use of cDNA microarray technology found S252W mutation to induce constitutive over-expression of PKC-α, IL-1α and small GTPase RhoA in AS. These effectors were suggested to play a role in osteoblast alterations due to this mutation.44 Another interesting report studied the crystal structures of the two point mutations in FGFR2 mutants in complex with FGF2 and suggested this as a possible model to explain phenotypic variability among AS patients. They reported interactions between FGFR2 and FGF2 that augmented FGFR2-FGF2 affinity. Findings based on these structures and sequence alignment of FGF family revealed P253R mutation to increase the affinity of FGFR2 towards any FGF. However, S252W mutation selectively enhanced the affinity of FGFR2 to restricted subset of FGFs. Thus, alterations in FGFR2 ligand affinity and specificity could be a causative factor in improper autocrine or paracrine activation of FGFR2. Another study by the same group on the basis of structural and biophysical analysis suggested mutations in FGFR2b to enhance FGFR2b ligand binding affinity and specificity and account for the dermatological manifestations in AS.7,45 Tanimoto et al. (2004) investigated the role of S252W mutation on osteoblastic differentiation and found this mutation to enhance osteoblast phenotype in humans and soluble form of FGFR2IIIc with the same mutation to control osteoblast differentiation and regulate the phenotype of osteoblastic cells through a dominant negative effect revealing a novel model for regulation of abnormal osteoblastic differentiation in AS.46

However, the study by McDowell et al. 47 showed that specific combinations of FGFs and glycosaminoglycans activate both alternative splice forms of mutant but not wild type FGF receptors. The study revealed that specific glycosaminoglycans (2-O-and N-sulfated heaparan sulfate) prepared via a combined chemical and enzymatic synthesis, led to antagonization of overly-activated FGFR2b (S252W) to basal levels of nanomolar concentrations thus demonstrating that glycosaminoglycans can be useful in treating FGFR signalling related diseases.47 A novel cell signalling study approach by Ahmed et al. (2008) found the altered FRS2 (FGFR substrate 2) recruitment by the mutant receptors to lead to an abnormal signalling mechanism. A new level of complexity having profound intracellular phenotypes due to extracellular receptor modification revealed a new challenge in system biology interpretation.48 The study on inbred mouse model of $Fgfr2^{+/P253R}$ by Wang et al. suggested activated p38 in addition to ERK1/2 signalling pathways to mediate mutant neurocranial phenotype and skeletal abnormalities of P253R mutation. They also suggested localized and regional variations in the phenotypes that characterise AS.49 However, the pathogeneic role of ERK1/2 signalling in abnormalities associated with FGFR2 activation was demonstrated by Shukla et al. 50. In their finding they could rescue mouse model of craniosynostosis by use of *Fgfr2* shRNA targeted against dominant mutant form $Fgfr2^{S252W}$ and administration of UO126 (specifically inhibits MEK-ERK pathway) during pregnancy and early postnatal stages.50 Miraoui et al. 51 used microarray analysis to determine the signalling pathways activated by FGFR2 mutations and found EGFR and PDGFRα expression to be higher in human apert calvaria osteoblasts as compared with wild type cells. Their research revealed that activated FGFR2 increased mRNA expression of EGFR and PDGFRα via activation of PKCa-dependent AP-1 transcriptional activity. This finding also demonstrated that inhibition of EGFR and PDGFR can reduce the pathological signs like upregulation of phenotypic osteoblast genes and in vitro matrix mineralization in apert osteoblast cells. Thus, this study revealed novel molecular crosstalks between FGFR2, EGFR and PDGFRα that functionally contribute to osteoblastic dysfunction. 51

Similarly, Martınez-Abadı'as et al. 25 explored how the mutations of Fgfr2 affect the pattern and level of integration of facial skeleton and neurocranium in inbred mouse models $Fgfr2^{+/S252W}$ and $Fgfr2^{+/P253R}$ and their non-mutant littermates at P0. Data of μCT images of skull and 3D geometric morphometric methods were used to assess the skull morphological integration (MI) that reflects the developmental interactions among traits by intensity of statistical associations. They found general pattern of MI in Apert mouse and their non-mutant littermates but found increase in magnitude of integration between and within the facial skeleton and neurocranium especially in $Fgfr2^{+/S252W}$ mice. Their findings report that Fgfr2 mutations do not disrupt skull MI and that FGF/FGFR signalling plays a significant process in modulating the intensity and patterns of skull MI and in coordination of proper skull development. Undoubtedly cell-communication and cell interactions influenced by FGF/FGFR signalling guides head morphogenesis and contributes to growth and development of functional head.52

Holmes and Basilico successfully induced the gain of expression of $Fgfr2^{S252W}$ and β-galactosidase by use of Cre/lox recombination in neural crest and mesoderm of skull and found mutation of mesoderm alone to be sufficient for craniosynostosis and thus eliminated the role of dura mater and skull base changes in craniosynostosis.53 Signalling studies by Suzuki et al. 54 suggest that altered FGFR2IIIc signalling in osteoblasts is mostly responsible for the phenotypes seen in AS and that osteoblast cell lines are highly useful for investigating the pathogenesis of this syndrome. The study involved characterisation of mutation effects (FGFR2IIIc-S252W; FGFR2IIIc-Ap and sFGFR2IIIc-Ap) in primary calvarial osteoblasts from transgenic mice. Their observations reported that osteoblasts expressing FGFR2IIIc-Ap proliferate and differentiate via highly activated MEK, ERK, and p38 pathways, while these pathways are suppressed in osteoblasts expressing sFGFR2IIIc-Ap. 54

Yeh et al. 55 in their study found S252W mutation to elicit not only overstimulation of FGFR2 downstream pathway but other novel pathological signalling too. Their experiment involved profiling of the global gene expression of wild type and S252W mutant periosteal fibroblasts stimulated with FGF2 to activate FGFR2 and found majority of the differentially expressed genes to be divergent between each group of cell population and regulated by different transcription factors. Further, they also compared AS and Crouzan syndrome cell population but could not find any correlation and thus found the mutation in FGFR2 to cause an unique cell response to FGF2 stimulation associated with central nervous system (CNS) development and maintenance. Additionally, the validation of Strc (stereocilin) gene in newborn Apert mouse brain suggested a role for endothelial cells in the establishment of CNS abnormalities and STRC to be in the same circuitry as FGF/FGFR2.55 Effect of S252W mutation causing endochondrial ossification leading to retardation of long bone and p38 and Erk1/2 signalling pathways further partially influencing the mutation was reported by Chen et al..56 While the same mutation effect study by Heuze et al. revealed suture fusion in mouse models to be very specific resulting from a complex combination of the influence of abnormalities in biogenesis or signalling within sutures with specific variation in individual timing specific to each suture.57 Recent work by Zhang et al. reported Wnt/β-catenin

pathway to be inhibited in mutant osteoblasts and BMSCs and Wnt3a treatment to ameliorate the defects.58

2. Discussion

AS is a form of acrocephalosyndactyly and a rare congenital disease that has an exquisitely specific molecular mechanism. This syndrome is characterised by *in utero* craniosynostosis, severe syndactyly and abnormalities in skin, brain and viscera with an estimated highest prevalence rate among the Asian population.59 Decades of study reports have focussed on identifying the underlying genetic mutations and defect signalling mechanisms that contribute to its development. Given its relatively low prevalence, studies of humans may never provide data sufficient to elucidate the genotype-phenotype continuum. Nevertheless, the repercussions of the mutations and aberrant signalling pathways can be studied with the help of model organisms like mice. Thus, study of mouse models becomes critical in understanding the variation in phenotypes and the correspondence between AS transgenic mouse models and AS patients at the molecular, histological and morphological levels.60

Although the mutational spectrum (point mutation in exon IIIa, 3 *Alu* insertions and 1 deletion) in AS is limited there is drastic variability in phenotypic outcome among affected subjects. Additionally, analysis of tissues also revealed variation reflecting the severity of effects and genetic heterogeniety. Further, other known genetic syndromes like Pfeiffer, Crouzon, Jackson-Weiss, Muenke and Saethre-Chotzen syndromes have overlapping features with AS that complicates its clinical identification. Thus, molecular identification plays an important criterion in such heterogenous syndromes that result due to genes working in similar signalling cascade. Hence, molecular diagnostic approaches have been highly useful as indicated by few case-report studies over the globe suggesting case reports to focus both on clinical and molecular methods while detecting and confirming the syndrome type. A case report specifically from India identified 2 AS patients to have S252W mutation while a study involving 2 affected Indonesian subjects found both the patients to carry the 2 frequent mutations and that among Mexican patients 4 had S252W and 2 had P253R mutations but none had the mutations together.61–63 Although Apert cases are sporadic studies suggest most of the cases to occur as a result of increasing paternal age and *de novo* mutations arising in father.64,65

Similarly, experiments using mutated mouse models involving outbred and inbred backgrounds have acted as valuable resources to decipher the effect of mutations on signalling pathways and interactions with effector molecules leading to abnormal craniofacial development and other growth abnormalities.66 Further, use of innovative methods like knock-out and conditional targeting technologies, differential gene expression, tissue culture studies, 3D detection methods and other sophisticated imaging techniques further enhanced the knowledge on the pathophysiology of AS. However, most of these approaches cannot be replicated in humans or their cell lines but molecular methods like sequencing, microarray, exome sequencing and deep next generation sequencing in suspected cases can reveal interesting genetic changes responsible for individual phenotypic variability.

3. Conclusion

In conclusion, AS is still an unsolved area of investigation in congenital diseases, many of the molecular features exhibited in this syndrome are still unexplainable and requires intensive research. Although a number of studies report on various features of the syndrome the genetic heterogeneity varies from cases to cases and thus suspected cases require to be thoroughly investigated by both clinical and molecular methods. Many of the clinical features observed are still unexplainable and thus a definitive treatment plan that can improve the quality of patient's life still doesn't exist.

Conflicts of interest

None.

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