Background: Craniosynostosis (CS) conditions are included with the premature fusion of one or more multiple cranial sutures. As the second leading and most common craniofacial anomaly and orofacial clefts globally. Syndromic and nonsyndromic CS (NSCS) occur as a part of a genetic syndrome unlike Apert, Crouzon, Pfeiffer, Muenke, and Saethre–Chotzen syndromes. Approximately, 90% of the cases of CS arises from NSCS group and it is now a great challenge for the researcher and neurosurgeon for Indian-origin children, a great burden worldwide.

Material and Methods: Study design: Prospective study of analysis sequence pattern on CS and NSCS from January 2007 to 2018 was carried out. Inclusion criteria: Diagnosed cases in syndromic and NSCS patients between 3 months and 14 years of age either preoperative or postoperative were included in the study of both groups (syndromic and NSCS). Exclusion criteria: Patients with primary microcephaly (secondary CS), postural plagiocephaly, incomplete data, no visual perception, and who were lost to follow-up, and who had no interest to participate the study were excluded from the study.

Bioinformatic analysis: We have performed systematic bioinformatic analysis for all responsible genes by combining with using through the GeneDecks, Gene Runner, DAVID, and STRING databases. Genes testing: FGF family genes, MSX genes, such as Irf6, TP63, Dlx2, Dlx5, Pax3, Pax9, Bmp4, Tgf-beta2, and Tgf-beta3 were found to be involved in Cleft lip and cleft palate (CL/P), and Fgfr2, Fgfr1, Fgfr3, and TWIST, MSX, MSX1, 2 were found to be involved in both the groups of CS (SCS + NSCS). Results: FGFR, MSX, Irf6, TP63, Dlx2, Dlx5, Pax3, Pax9, Bmp4, Tgf-beta2, and Tgf-beta3 demonstrated and find out that in CL/P, and Fgfr2, Fgfr1, Fgfr3, and Twist1 had accurate sequence data with more than accuracy of 95% reported with proper order with additional anomalies CS through newly developed tools.

Conclusion: Newly developed techniques of GeneDecks, Gene Runner, DAVID, and STRING databases gave better picture to analyze the larger population, patients (SCS + NSCS) with complex genetic, maternal, parental age, environmental, and stochastic factors contributing to NSCS networking, signaling, and pathways involvement. This bioinformatic tools analyzed better...
INTRODUCTION

Craniosynostosis (CS) conditions are increasing globally.[1] CS consists of two groups: syndromic craniosynostosis (SCS) and nonsyndromic craniosynostosis (NSCS).[2] Now NSCS group of patients was developed globally. In Indian scenario, its incidence approximately is 1:1000 live births.[3] CS etiopathogenesis little complex process to understand.[4-6] The basis of multidisciplinary and lifelong care for patients with CS in different conditions were easier to understand by imaging modalities.[7] The premature fusion of metopic sutures results in the clinical phenotype of trigonocephaly were frequently occurred in India.[8] There is an association of this characteristic within the monosomy 9p syndrome was newly observed by researcher. The receptor-type protein tyrosine phosphatase gene (RPTPs) clearly. It is located in the 9p24.1p23 region and it encodes with the major component of the excitatory and inhibitory synaptic organization. We presently are considered as a good candidate gene. Because its nature is likely responsible for this form of CS. PTPRD is well-known gene to recruit multiple postsynaptic partners such as IL1RAPL1 gene. These alterations lead to nonsyndromic intellectual disability (ID)/ intelligence quotient (IQ).[9,10]

MATERIALS AND METHODS

Study design: Prospective analysis of clinical records of patients with registered in CS clinic from January 2007 to January 2018 was performed.

Inclusion criteria: Diagnosed cases in SCS and NSCS patients, who were between 3 months and 14 years of age either preoperatively or postoperatively were included in the study of both groups (SCS and NSCS).

Exclusion criteria: Patients with primary microcephaly (secondary CS), postural plagiocephaly, incomplete data, no visual perception, and who were lost to follow-up and those who had no interest to participate in the study were excluded from the study.

Genetic study: Blood sample (5 mL) was taken from both the parents along with the child in ethylenediaminetetraacetic acid (vial). For control, 500 healthy children of comparable age group, who belonged to the same geographical region, were included in this study. Genomic DNA was extracted from peripheral blood lymphocytes by phenol–chloroform extraction method. Primers were diagnosed with FGFR1, FGFR2, FGFR3, FGFR4, TWIST, and MSX mutations in this study, and for FGFR1 and FGFR2 gene, primers were custom-synthesized (Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India).

Polymerase chain reaction: The polymerase chain reaction (PCR) for each sample was performed in 0.2 mL, thin-walled tubes by using 20 ng of DNA. A total of 2–5 pmol of each primer, 200 mm dinucleotide triphosphates, 10X PCR buffer, 1.5mm MgCl₂, and 0.5 units of DyNaZyme II DNA polymerase (Thermo Scientific; Thermo Fisher Scientific is an American multinational biotechnology product development company) were used. The PCR reaction was carried out in a T-100 DNA Engine (Bio-Rad, Hercules, CA, USA). The thermal cycles were under the following conditions: 95°C for 3 min, 35 cycles at 95°C for 30 s, annealing temperature as in for 30 s and 72°C for 1 min/kb, and a final extension at 75°C for 7 min. The size of the amplicons was verified by gel electrophoresis by running the PCR products on 2% agarose gel with the 100bp marker (ladder). After the successful amplification, PCR products were digested as per the manufacturer's instructions with the respective restriction endonucleases mentioned and they were analyzed through an ethidium bromide-stained 2.0% agarose gel with 50 bp ladder. Finally, PCR-purified products were sequenced with the Sanger’s dideoxy method and with recently available new tools and techniques.

BIOMATERIALS ANALYSIS TOOLS

The GeneDecks, Gene Runner, DAVID, and STRING databases were used for the sequence analysis on the sequences obtained after performed by PCR, PCR-Restriction Fragment Length Polymorphism (RFLP), and reverse transcription PCR (RT-PCR) confirmed by the potential candidate. FGFR1, FGFR2, FGFR2iiia, FGFRiiib, FGFRiiic, FGFR3, FGFR4, MSX, MSX1, MSX2, TWIST1, TWIST2 genes by combine we used to sequence testing for mutation analysis.

STATISTICAL ANALYSIS

Statistical analysis was performed by using the software SPSS for Windows, version 14.0 (SPSS, Chicago, IL, USA). Chi-square (χ²) test was applied for the assessment of association in two-dimensional contingency tables. Odds ratios and 95% confidence
intervals (95% CI) were calculated to measure the relationship between a potential risk factor of cases/control status. Descriptive statistics including percentage, mean ± standard deviation were calculated. Student’s t-test and Mann–Whitney U test were used for comparisons of continuous variables. Multiple logistic regression models were used to test for interaction between various genes with environmental risk factors.

**RESULTS**

FGFR, MSX, IRF6, TP63, DLX2, DLX5, Pax3, Pax9, BMP4, TGF-beta2, TGF-beta3, FGFR2, FGFR1, FGFR3, and TWIST1 with the accurate sequence data (ASD) with more than 95%. FGFR family and its isomers involved with CS within (SCS and NSCS) group. Other genes enriched into involved in different signaling and pathways (80%). At the same time different craniofacial deformities and different biological process involvement 90%. FGFR1, FGFR2, FGFR2iiia, FGFRiib, FGFRiic, FGFR3, FGFR4, MSX, MSX1, MSX2, TWIST1, TWIST2 genes and gene network including with big data set also associated with gene environment interactions 90%. Craniofacial deformities including with different biological process make us correct analyze the function of FGFR family, MSX, and other associated genes in a gene network and give appropriate sequences are enriched with big data are quite useful messages. Gene Runner provides us 98% accurate analysis, GeneDecks provides 97%, DAVID gives 96%, and STRING databases provide more than 95%. All new tools help more than excellent results to optimize the results to know the etiopathogenesis and disease progression with signaling and pathways data of CS and NSCS and associated diseases also clarified through these sequences gives better annotation.

We also describe details with boy and girl with severe ID, trigonocephaly, and dysmorphic facial features such as a midface hypoplasia, a flat nose, a depressed nasal bridge, hypertelorism, a long philtrum, and a drooping mouth, and fibroblast growth factor receptor 3 (FGFR) syndromes newly novel mutation detected both SCS + NSCS group.[13] Microarray chromosomal analysis revealed the presence of a homozygous deletion involving the PTPRD gene, located on chromosome 9p22.3. The RT-PCR amplifications all along the genes failed to amplify the patient’s cDNA in fibroblasts because of the presence of two null PTPRD alleles.[12]

Synaptic PTPRD interacts with IL1RAPL1 which defects have been also associated with ID and autism spectrum disorder. So, absence of the PTPRD transcript leads to a decreased expression pattern of the IL1RAPL1. These interesting results suggested that direct involvement of PTPRD is seen in ID. This is consistent with the PTPRD−/− mice phenotypes.

Hence, deletions of PTPRD suggested that a cause of trigonocephaly. Patients with monosomy 9p and genome-wide association study (GWAS) suggested variations in PTPRD are associated with hearing loss (HL) and deletion identified in the reported patient supports previous hypotheses. Additionally, its function in ID and HL with IQ. However, metopic synostosis still needs to be discussed as more investigation of patients with the 9p monosomy syndrome is required other clinical conditions of both groups in SCS + NSCS.[12]

**DISCUSSION**

Phenotypic investigation of CS patient’s SNPs in the IL-23R gene (rs11209026, rs7517847, rs11805303, rs1004819, rs17375018) patients with lower frequency are the rs17375018 GA and AA genotypes. The rs11209026 G allele frequency is higher in male patients with CS. The rs11805303 G and rs1004819 G alleles were more frequent in patients with papulopustular lesions from different ethnic backgrounds including NSCS and CS.[13] The FGFR, TWIST, and MSX genes in a gene network gene polymorphisms were associated with nonsyndromic CL/P by GWAS. It was protein–protein interaction with FGFR, MSX1, corroborate us an alternative method to perform bioinformatic analysis (BA) for genes found by GWAS and make us predict the disrupted protein function due to the mutation in a gene DNA sequences made easier to predict. These findings may guide us to perform further functional studies in the future to know the better etiopathogenesis of these (SCS + NSCS) different associated clinically observed conditions.[14]

**Fibroblast Growth Factor Receptor 3 (FGFR3)-Associated Syndrome**

Muenke syndrome (MS) is an FGFR3-associated syndrome. This was first described in the late 1990s. MS is an autosomal dominant disorder characterized by coronal suture CS. MS in the association with an identical gene mutation, an unique point mutation c.749C > G in exon 7 of the FGFR3 gene involved with MS.[11] Sagittal CS: Sagittal CS is the most common form of CS found in India. Children were the affected group, approximately one in every 5,000 newborn. In our knowledge, the first GWAS for nonsyndromic sagittal CS using non-Hispanic case-parent trios of Indian ancestry also studied with BMP genes. BMP2 and BBS9 and BMP genes played a role in skeletal development and specify the functional activities to
further understand the etiology of NSCS.\[10\] We also got nearly the same picture in the Indian population in both the groups (CS and NSCS), no extraordinary changes in our results as reported earlier in the European study. We feel need sample size more.

**CRANIOFACIAL ARCHITECTURE AND NETWORKING**

The craniofacial architecture networking of the domestic dog morphed and radiated to human whims. So far, to define the genetic underpinnings of breed skull shapes is really a question mark. Here, we can elucidate the molecular mechanisms of morphological diversification in CS. CS and NSCS framework is needed for understanding the human cephalic disorder. GWAS analysis by using whole-genome sequencing uncovers a missense mutation in BMP3 and BMP4, and validation studies in the zebrafish show that BMP3 function in cranial development is the informative one.\[17\]

**DEVELOPMENTAL PATHWAYS OF HORNS AND STRONG BONE**

Developmental pathways involved in horn development are complex and still poorly understood. The description of a new dominant inherited syndrome in the bovine Charolais breed that we have named type 2 scurs. Clinical examination revealed that despite a strong phenotypic variability. It also affected individuals show both horn abnormalities practically similar to classical scurs phenotype and skull intero frontal suture synostosis. Genome-wide linkage analysis used by the Illumina BovineSNP50 BeadChip genotyping data from 750 at half-sib and full-sib progeny.

This locus was mapped and observed that 1.7 Mb interval on bovine chromosome 4, TWIST1 gene encoding that transcription factor as considered as a strong candidate gene in both animal and human CS and NSCS. Its haplinsufficiency is quite valid and responsible for the human Saethre–Chotzen syndrome (SCS). The skull coronal and biocoronal suture synostosis sequencing of the TWIST1 gene identified a c.148_157dup (p.A56RfsX87) frameshift mutation predicted to completely inactivate gene.

Parents with heterozygous mutation (HM) showed that homozygous mutant progenies are completely absent. Among these conditions and are consistent with the embryonic lethality (EL). Earlier, basic scientist reported that in Drosophila melanogaster and mouse suffering from TWIST1 complete insufficiency. Description of type 2 scurs symptoms allowed an opportunity to propose at different molecular mechanisms and to explain the features of this SCS + NSCS syndrome and ontogenesis provide an evidence that how to improve this study CS and NSCS.\[10\]

**GENETIC VARIANTS IDENTIFICATION (GVI) THROUGH GENOME-WIDE ASSOCIATION STUDY (GWAS)**

Now we also implemented a breed mapping approach using by moderately dense SNP arrays. The lower number of animals and breeds were carefully selected for the phenotypes. As of interest to identify genetic variants (IGVs) are responsible for breed-defining characteristics. With the help of GWAS, one of the most striking morphological traits in dogs with brachycephalic head type was observed. Although candidate gene approaches based on comparable phenotypes with mice and humans were utilized for this trait, the causative gene has remained elusive; therefore, using this method, breeds identified strong genome-wide associations (GWAS) developed for brachycephalic head type on Cfa 1. In genotyping assay additionally, dog’s model in the region confirmed the association of the genetic structure and function. Dog breeds have primarily been exploited for GWAS, and segregating the trait results demonstrated that nonsegregating traits coming under the strong selection and equally tractable to genetic analysis using smaller number of sample sizes is a new approach for both group of SCS + NSCS sequence identification.\[19\]

**FIBROBLAST GROWTH FACTOR RECEPTOR FAMILY**

HMs of three members of the fibroblast growth factor receptor family of signal transduction molecules, namely—FGFR1, FGFR2, and FGFR3—contributes significantly to disorders of bone patterning and growth of SCS + NSCS. FGFR3 mutations, predominantly, cause short-limbed bone dysplasia. CS and NSCS had three major regions (i.e., extracellular, transmembrane, and intracellular) of the protein (mainly famous for achondroplasia). The exons (IIIa and IIIc), encoding the IgIII domain and in the extracellular region, in SCS as well as NSCS. It was included with the Apert, Crouzon, or Pfeiffer syndromes (PS). Interpretation of these apparent clustering, mutations in FGFR2 has been hampered. Hence, the absence of complete FGFR2-mutation screening undertaken such a screen in 500 patients with CS. Because of FGFR2 mutations localized to the IIIa and IIIc exons, we identified mutations in seven additional exons, which included six distinct mutations of the tyrosine kinase region. Hence, the IgIIIa/IIIc region represents a genuine mutation hot spot for SCS + NSCS in our knowledge.\[20\]
So far considering these novelty and understanding the better concept of FGFR may be a great challenge and potential biomarkers and capable to reproduce the new inventory like gene therapy and molecular targeted therapy (MTT) to resolve this complex disorders SCS + NSCS.[21,22] We recently developed a new protocol any one can store the cranial samples or other human samples as per laboratory use of molecular study. It could be helpful for laboratory processing and treatment as well.[23,24] In the meanwhile, one should not ignore the medical education and recent advancement of new tools and techniques related to sample processing and handling of SCS + NSCS samples.[25,26]

Currently, new and innovative genomic discoveries data and tools such as GeneDecks, Gene Runner, DAVID, and STRING database by using these are very easy to elucidate the genetic basis for nonsyndromic cases and implicate the newly identified genes in different signaling pathways made easy. We previously found in our SCS and NSCS epidemiologic and phenotypic studies clearly demonstrate that NSCS is a complex and heterogeneous condition supporting to new innovation through new tools a strong genetic component accompanied by environmental factors, lethality, stochastic, and other additional information by this bioinformatics tools that contribute to the best pathogeneses network of this birth defect (SCS + NSCS).

**CONCLUSION**

The newly developed tools and techniques of GeneDecks, Gene Runner, DAVID, and STRING databases give a better picture to analyze the larger population. Any single, clinic or hospital-based studies is required with phenotypically homogeneous subsets of patients (SCS + NSCS) to further understand the complex genetic, maternal, parental age, environmental, and stochastic factors contributing to NSCS. Although learning about these variabilities is a key in formulating on the basis of multidisciplinary and lifelong care for patients with these conditions are quite important task. These BA tools add informative annotation to predict the sequence pattern and help the molecular study to treat the patients with CS and NSCS. It helps in the diagnosis, prognosis, and personalized medicine, and helps treatment with follow-up the study. In fact, this newly provide us new information of how to target the future opaque of MTT.

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**Conflicts of interest**

There are no conflicts of interest.

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