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Apert's syndrome: study by whole exome sequencing

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Running Title: Aperts syndrome study by exome sequencing

Abstract

In the present study we attempted a parent-child trio, whole exome sequencing (WES) approach to study Apert's syndrome. Clinical characteristics of the child were noted down and WES was carried out using Ion Torrent System that revealed the presence of previously reported P253R mutation in *FGFR2* gene. Presence of two SNPs rs1047057 and rs554851880 in *FGFR2* gene with an allelic frequency of 0.5113 and 0.001176 respectively and 161 complete damaging mutations were found. This study is the first reported case of exome sequencing approach on an Apert's syndrome patient aimed at providing better genetic counseling in a non-consanguineous relationship.

Key words: Apert syndrome, Craniosynostosis, Exome sequencing, *FGFR2* gene, Parent-child trio study

1. Introduction

In humans abnormal sutural development and skull growth leads to a severe autosomal dominant condition called as Apert syndrome (AS) (OMIM no. 101200) [1]. Genetically this syndrome is known to be caused by a very restricted number of mutations where 99% of reported cases reveal two missense mutations in neighbouring amino acids (Ser252Trp and Pro253Arg) of fibroblast growth factor receptor 2 (*FGFR2*) mapped to 10q25-26 [2, 3]. Both the mutations are known to affect the highly conserved linker region between immunoglobulin-like II and III domains which result in increased affinity and altered specificity of fibroblast growth factor (FGF) ligand binding [4, 5]. However, there exists clinical diversity of brain phenotypes among affected individuals, ascribed to different causes which involve secondary effects of pattern of suture fusion [6, 7] and potential action of mutations on varying genetic background and environment [8-10]. It is a developmental malformation characterized by craniosynostosis, a cone-shaped calvarium (acrocepahly), hypertelorism, midface hypoplasia, pseudo cleft-palate, a parrot beak-shaped nose, pharyngeal attenuation and syndactyly of hands and feet [11-13]. Suture progenitor cells with FGFR2 mutation cannot transduce signals from FGFs and thus these cells fail to form necessary fibrous material required for normal calvarial suture [13].

AS is a rare genetic disorder with an incidence of 1:160 000 and an advanced paternal age has been consistently noted with it. Thus, AS is an active research area of developmental disorders and a number of studies have tried to understand the signalling mechanisms that cause failure of proper sutural closures. Further, most of the case reports describe on the clinical aspects however, in the present study we have explored the molecular genetics understanding of this syndrome using exome sequencing to study the possible incomplete penetrances and also to provide appropriate genetic counselling.

Materials and Methods

Subject

A male child was delivered on 26-11-2014, at Civil Hospital, Bathinda, Punjab, India, through normal vaginal delivery. The child was diagnosed with congenital conditions which seemed to fall under the broad classification of craniofacial/limb abnormalities. On

examination by a paediatrician the clinical characteristics were noted down with a suggested Magnetic Resonance Imaging (MRI) report of the brain. Written informed consent was obtained from the family for molecular analysis and routine laboratory investigations like blood picture etc. were carried out using standard procedures.

Methods:

Peripheral blood (5ml) was collected in EDTA vaccutainers from the proband and its genetic parents. Informed written consent was obtained from all the individuals participating in the study. Exome sequencing and molecular analysis was done over a period of 3 months (September 2015-January 2016). 2 µg of genomic DNA of all the participating samples was used for the whole exome capture process using Ion Torrent system (Life Technologies, USA). Exome enrichment and Library preparation was done using Ion AmpliSeq™ Exome RDY Kit which targets >97% of CCDS with 5bp padding around exons. Processing of the raw data was done using the pipeline Genome Analysis Tool Kit (GATK) [14], sequence alignment using Burrows-Wheeler Aligner [15] (BWA) and the reads obtained were analysed by mapping against Human Genome Build 18. This was followed by detection of Single nucleotide Polymorphisms (SNPs) using unified Genotyper from GATK and dbSNP138 as the reference to call the variants. SNPs obtained were annotated using human Gene transfer format (gtf) file using bed tools and unannotated variants and variants not lying in 1KB gene region were discarded. The score of 20 was maintained as minimum phred-scaled confidence threshold at which variants were called and score 10 was the cut-off to eliminate low quality variants. Further, PolyPhen-2 was used to determine the deleterious effects of SNPs and Variant Effect Predictor (VEP) was used for the annotation of variants [16, 17].

Results

Clinical features:

The child was born to non-consanguineous parents with a normal blood picture and had a five-year old normal sibling. Clinical investigations revealed a prominent forehead, oxycephalic head, midfacial hypoplasia, high-arched palate, a prominent stubby nose, telecanthus, supranasal groove, open metopic suture and gross sagittal suture dehiscence. Further, prominent Darwin's tubercle, a congenital ear condition which often presents as a thickening on the helix at the junction of the upper and middle thirds, syndactyly fingers with thumbs bearing hypoplastic phalanges and nails and gross feet syndactyly were observed. The MRI of the brain revealed turricephaly with midfacial hypoplasia and there was thinning

of corpus callosum in posterior body and splenium region with rest of the brain parenchyma revealing normal signal intensity.

Sequencing and Interpretation Analysis:

Reads were mapped against reference human genome 18, 94.9% of proband reads, 97.5% of paternal reads and 96.9% of maternal reads aligned to human reference genome and further statistics of sequencing reads are provided in table 1. Whole-exome sequencing of the proband revealed the presence of P253R mutation in *FGFR2* which was inherited by the heterozygous father (rs2278202 NM_000141.4:c.2301+15C>T p.Leu339Pro) and heterozygous mother (rs1047100 NM_022970.3:c.1343A>G p.Val232Val). List of complete mutations in *FGFR2*, *FGFR3* and *FGFR4* are listed in table 1. We report the presence of two mutations in the proband for *FGFR2* gene at Chr10 i.e. rs1047057 and rs554851880 having an allelic frequency of 0.5113 and 0.001176 respectively from Exome Aggregation Consortium browser (ExAC, Cambridge). Deleterious effects of SNPs in all 3 samples were analysed by PolyPhen2 and VEP (Table 2). Classification of total 5177 nsSNPs based on PSIC score and complete damaging number of mutations in all the samples is presented in figure 1 and 2.

AS is a severe form of craniosynostosis and there is drastic variability in phenotypic outcome among affected subjects. Identification of a child with AS is mainly via clinical detection and further confirmation requires molecular diagnostic testing. Patients with AS have affected cranial bones and clinically they overlap with Crouzan syndrome and Pfeiffer syndrome, hence rare genetic disorders like AS where genetic heterogeneity can also vary from case to case investigations with whole-exome sequencing (WES) provide valuable information [18, 19]. In the present study the patient was borne to non-consanguineous parents and had a normal elder sibling. Due to typical features and presence of the syndactyly of hands and feet with oxycephalic head, mid facial hypoplasia, high arched palate the patient was suspected to be having AS. The exome analysis revealed the presence of the previously reported P253R mutation in *FGFR2* gene. However, in addition to this the child was found to have an intronic SNP rs54851880 in the *FGFR2* gene. Further, the parents and the child were found to be heterozygous for two more SNPSs i.e. rs1047100 and rs1047057 and the proband had many other alterations in *FGFR3* and *FGFR4* genes as well. The results of exome sequencing of the family did not reveal anything interesting however, to the best of our knowledge this is the first exome report of an AS patient from India reporting P253R mutation along with an intronic mutation in *FGFR2* gene. The proband had a total of 161

complete damaging mutations out which 154 were seen in maternal and 117 in paternal sample.

Clinical characteristics of the patients were typical and consistent with description of AS. However, due to overlapping features with other closely related genetic syndromes molecular testing was necessary to confirm the diagnosis. A recent article by Polla et al., (2015) that studied 36 cases of suspected clinical diagnosis of skeletal disorders using exome sequencing and a designed 1.4 Mb panel that could test 4,800 exons in 309 genes, reports in 1 case the diagnosis to change from osteodysplasia syndrome to AS (20). Thus, it can be said that molecular technologies have proved efficient in precisely screening and defining variations in a plethora of genetic diseases and diagnostic testing in symptomatic individual has gained sophistication due to faster and accurate techniques like WES and whole-genome sequencing (WGS) (21). Although WGS is not yet clinically available but WES i.e targeted exon capture prior to sequencing helps in analyzing the coding regions of genome and helps in accurate prediction of mutations and more so where coverage is sufficient it has high concordance with Sanger sequencing as reported by Hamilton et al., 2016 [19] . Interpretation of WES is limited but medical geneticists and researchers are open to it as it is helpful in unexplained genetic disorders. Further, information from WES can act as a better aid in understanding the molecular genetics of rare syndromes and in providing appropriate genetic counseling to the patients.

In summary, this study with a clinically suspected AS patient confirms the symptomatic individual to have Apert's by authentication of variants through exome sequencing.

Conflict of interest: The authors declare that there exists no conflict of interest.

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Figure 1 Position-Specific Independent Count (PSIC) Classification of Proband nsSNPs

Figure 2 Distribution of complete damaging nsSNPs (PSIC = 1) across Paternal, Maternal and Proband

Table 1 Summary of exome sequencing statistics and mutations in FGFR2, FGFR3 and FGFR4 found in study samples

	Sequencing reads					Called coverage
	Total	**Mapped Reads**	**Unmapped Reads**	**Duplication**	**Duplication rate**	**Mean coverage**
Child	40,293,941	38,239,265	2,054,676	11,052,628	27.43%	19.17
Father	48,666,712	47,479,117	1,187,595	14,176,613	29.13%	26.39
Mother	47,342,451	46,381,186	961,265	13,511,535	28.54%	25.22

Chr	Location	SNP ID	AA Change	Allele	Present In*	Genotype	SNP Type	Gene
Chr10	123243197	rs2278202	----	G>A	F-M-C	Homozygous Alternate	Intron	FGFR2
Chr10	123279674	rs77543610	p.Pro253Arg	G>C	C	Heterozygous	Non-syn SNP	FGFR2
Chr10	123298158	rs1047100	p.Val232Val	T>C	F-M-C	Heterozygous	Syn SNP	FGFR2
Chr10	123337814	rs17542768	----	A>G	F	Homozygous Alternate	Intron	FGFR2
Chr10	123276801	rs554851880	----	A>G	C	Heterozygous	Intron	FGFR2
Ch10	123239112	rs1047057	----	G>A	F-M-C	Heterozygous	UTR	FGFR2
Chr4	1803307	rs2305183	----	T>C	F-M-C	Homozygous Alternate	Intron	FGFR3
Chr4	1803704	rs2234909	p.Asn294Asn	T>C	F-M-C	Homozygous Alternate	Syn SNP	FGFR3
Chr4	1805296	rs3135883	----	G>A	F-M-C	Homozygous Alternate	Intron	FGFR3
Chr4	1807894	rs7688609	p.Thr653Thr	G>A	F	Homozygous Alternate	Syn SNP	FGFR3
Chr5	176517797	rs376618	p.Pro136Leu	C>T	F	Heterozygous	Non-syn SNP	FGFR4
Chr5	176520243	rs351855	p.Gly282Arg	G>A	F	Heterozygous	Non-syn SNP	FGFR4
Chr5	176522560	rs201831200	p.Ala485Thr	G>A	C	Heterozygous	Non-syn SNP	FGFR4

*F- Father , M-Mother, C-Child

Table 2 Variant Predictor Analysis for damaging SNPs in Paternal, Maternal and Proband samples.

Damaging Mutation Type	Proband	Maternal	Paternal
Missense variants	27.00%	19.00%	33.00%
Non Coding Transcript Variants	19.00%	29.00%	13.00%
Intron Variants	15.00%	26.00%	14.00%
Downstream Gene Variants	12.00%	8.00%	6.00%
Upstream Gene Variants	8.00%	6.00%	6.00%
Non Coding Transcript Exon Variants	7.50%	3.00%	7.00%
NMD Transcript Variant	5.00%	3.00%	9.00%
3 Prime UTR Variants	3.00%	2.00%	7.00%
Regulatory Region Variants	1.00%	1.00%	2.00%
Unknown Significance	2.50%	3.00%	3.00%