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Research Article

Impact of Genetics and Stem Cells Factors Increase Risk in the Cases of Anorectal Malformations during Development

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Abstract

Anorectal Malformations (ARMs) are one of the rare anomalies called "Birth Defects" associated during development of digestive system. During embryogenesis, the abnormal hindgut develops either isolated or along with other congenital abnormalities perhaps due to involvement of genetic and epigenetic factors like methylenetetrahydrofolate reductase (C677T) gene polymorphism. However, little is known about etiopathology of ARMs cases. In the present case study, we have evaluated genetic changes based on karyotyping with GTG banding to identified rare Complex Chromosomal Rearrangements (CCR) in different cases that includes structural aberrations (8.58%) including t[6,9[6p23.4;9q34.1] and monosomy of chromosome 18 (4.30%) in proband and family members (in mother 6.00% and father 4.16%). Because, ARMS is a developmental defect the curiosity has been arises to characterize the role of stem cells using cells markers like Oct4, Sox2 and Nanog form blood samples of cases (proband), family members with their respective controls. Statistical analysis showing significant differences between cases *vs.* controls in Sox2 ($p<0.0064$) and Oct4 ($p=0.0016$), while between case *vs.* mother Oct4 showing p value (0.0020). Similarly, again the highly differences were observed between case *vs.* father in Sox2 and *Nanog* the p values are 0.0014 and 0.0041, respectively. Interestingly, PCR based findings reveal complete disappearance of 577 bp band (null mutations) of Oct4 in the proband of ARMS family. Data was further characterize using Sanger's sequencing which showing "point mutation" in *Nanog* at position 83`, where nucleotide guanine change in to thymine at 84`(G →T). *MTHFR* gene polymorphism showing in heterozygous (CT) condition increase "risk" factor of the disease due to defective folate metabolism.

Keywords: Anorectal malformations; Stem cell; Complex chromosomal rearrangements; Congenital anomalies; Pluripotency marker

Introduction

Anorectal Malformations (ARMs) are complex "birth defect" that is linked with abnormal of distal hindgut differentiation that ranges from mildly stenotic anus to imperforate anus with a fistula between urinary and intestinal tracts. The incidence of ARMs is high (~1:5000 live births) and more often seen in boys than in girls with a ratio of 1.7 [1,2]. Further, ~45%-70% ARMs cases present along with other congenital anomalies involving central nervous system, cardiovascular system, genitourinary tract, skeleton (vertebrae/limbs), and remaining gastrointestinal tract [3-6].

The etiopathology of ARMs is complex and multifactorial. Till date, very limited genetic and epigenetic "risk factors" have been associated with ARMs cases [7]. A chromosome abnormality has been identified in 4.5% to11% of the patients with ARM and mainly includes trisomy 13, trisomy 18, and trisomy 21[8]. Interestingly, ARMs also present as isolated non-syndromic cases suggesting specific genes in hindgut development. The molecular genetics elucidation of a few syndromic forms (SALL1 zinc-finger protein causing Townes-Brocks syndrome, *GLI3* gene involved in Pallister-Hall syndrome and Sonic Hedgehog (Shh)) [9,10], the current knowledge of genetic factors underlying most ARM is very limited. Despite the advancements in molecular genetics, no single gene or chromosomal locus has been identified that can be linked even to minority of ARM cases. Since ARM are associated with abnormal embryonic development, understanding the genetic basis of critical embryonic and prenatal developmental process becomes crucial in the understanding the development of ARMs.

Stem cells play a crucial role in the developmental process and the major identified factors involved in pluripotency are *OCT-4*, *SOX-2* and *Nanog* 3 [11,12]. Although the precise embryologic defect that causes the spectrum of malformations described as imperforate anus has not been determined, it is suggested that the genes involved in stem cell regulation may play an important role. Thus, in order to understand the genetic basis of the disease, we studied the role for stem cell markers *OCT-4*, *SOX-2* and *Nanog* in a case of ARM. Identification of such possible genetic cause/specific genes involved in ARM is warned for diagnosis of this anomaly, so that the prognosis of the patient after proper treatment can increase.

Material and Methods

The cases were referred to Cytogenetic and Molecular Genetics laboratory from the OPD of AIIMS-Patna for genetic analysis. All of the investigations and procedures were performed in accordance with the approved protocol from Institutional research committee. Written informed consent was taken from parents of the cases and age matched controls. The study has been approved by Institution Ethics

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Cytogenetic analysis

Blood samples (0.5 ml) were collected in heparin vials for cytogenetic studies at the time of initial diagnosis. Samples were cultured in RPMI 1640 (Gibco, USA) medium supplemented with fetal calf serum and incubated at 37°C for 72 h. Colchicine (Sigma) was added 2 hr prior to harvesting the culture for chromosome preparation. Culture was centrifuged and fresh suspension of cells was made with hypotonic KCl. Cell suspension was dropped on clean slides and flame dried. Giemsa Trypsin Giemsa (GTG) banding was performed after trypsinization. At least 20 metaphases were analyzed at each examination. If less than 20 metaphases were observed, the result was considered to be uninformative. Karyotyping was performed according to the International System for Human Cytogenetic Nomenclature (ISCN) 2016 [13].

DNA isolation and PCR: Blood samples (1.0 mL) were collected in EDTA vials from clinically diagnosed ARMs patients; family members (mother and father) and age matched respective controls. Genomic DNA was isolated from whole blood using DNA isolation kit (Promega, U.S.A.), quantified using Nanodrop spectrophotometer (Thermo scientific, USA) and samples were kept at -20°C until further analysis.

MTHFR SNP analysis of MTHFR C677T alleles: MTHFR C677T polymorphism was analyzed by RT PCR (Bio Rad, USA) Syber green method. MTHFR C677T primers were designed for tetra plex RT PCR assay (http://cedar.genetics.soton.ac.uk/public_html/primer1.html) and BLAST program at http:// www.ncbi.nlm.nih.gov/blast to determine the specificity of the primers. To increase the specificity of the reaction a mismatch at the 2 positions of the 3' end both the allele-specific primers were selected and confirmed by software. The selection of the primers were based on the amplicons 'Tm' values and following primers used in present study: MTHFR-T, 5'–GCACTTGAAGGAGAAGGTGTCTGCGGGCGT-3'; MTHFR-C-poly G, 5'–GGCGGGCGGCCGGGAAAAGCTGCGTGATGATGAAATAGG-3'; MTHFR-cf, 5'-TGTCATCCCTATTGGCAGGTTACCCCAAA-3';MTHFR-cr,5'-CCATGTCGGTGCATGCCTTCACAAAG-3'. To obtain amplicons with distinct melting points, 'Tm' values were calculated using known software (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/). We have plan to select these primer ARMS PCR based on "Tm" analysis, part of plan of our interest to detect SNP of mutant of MTHFR allele(s), same PCR product further confirmed by agarose gel (2.0%) electrophoresis and bands were visualized and characterized after Ethidium Bromide staining on Gel Doc system with software (Bio-Rad USA).

A total volume of 20 µl containing 10 µl of SYBR Green PCR Master Mix (Bio red USA), 1 µl of each primer per reaction, 40 ng of genomic DNA, and distilled water was taken to performed Real-time PCR. The PCR protocol on the Light Cycler (BioRad USA) was as an initial denaturation step (95 "C for 7 min) was followed by amplification and quantification steps repeated for 30 to 40 cycles (95 "C for 10 s, 60 "C for 10 s, 72°C for 20 s), with a single fluorescence measurement at the end of the elongation step at 72°C, a melting-curve analyzed the data and reaction was terminated by cooling to 400°C. Melting curves were constructed by lowering the temperature to 65°C and later increasing the temperature by 0.2 "C/s to 98°C to measuring the change in fluorescence consistently. Tm values were assigned to develop plot generated by the RT PCR of the negative derivation of fluorescence *vs.* temperature (dF/dT) of the melting curve for amplification products measured at 530 nm.

PCR analysis for *OCT-4*, *SOX-2* and *Nanog*: The characterization of stem cell markers namely Oct4, *Nanog* and Sox2 was performed using specific upstream/downstream amplicons (primers) Oct4 F: 5'-GACCATCTGCCGCTTTGAG-3' 577 60°C/1 min; *OCT-4* R: 5'-CCCCCTGTCCCCCATTCCTA-3 [14]; *Nanog* F: 5'-CTGTGATTTGTGGGCCTG AA-3', *Nanog* R: 5'-TGTTTGCCTTTGGGACTGGT-3' [15], 56°C/30s and Sox2 F: 5'-GGCAGCTACAGCATGATGC-3', *SOX-2* R: 5'-TCGGACTTGACCACCGAAC-3' 236 60°C/30s [16]. The primers were dissolved in a total volume of 25 µL containing 50 ng to 100 ng DNA, 20 pmol of each primer, 200 µM of each dNTP with Taq buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 3.0 mM MgCl2, and 3 U Taq polymerase (New England Biolabs). Cycling conditions were 1 min for *OCT-4* (577 bp), 4 min for *Nanog*, and 2 min for *SOX-2* at 94°C for initial denaturation, 60°C/1 min, 56°C/30 s and 60°C/30 s of annealing for *OCT-4*, *Nanog*, and *SOX-2*, respectively, followed by a final extension of 35 cycles at 72°C for 5 min for all 3 stem cell markers used. PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and bands were visualized on the Gel Doc system (SR Biosystem).

DNA sequencing by Sanger's method to analyzed de-novo mutations. Sanger's sequencing was performed to evaluate different type of gene mutations in *Nanog* gene i.e. either in the form of substitution, deletion and insertion and compare the same with controls. These sequencing data was based on mutation and further searched by ensemble genome database (http://www.ensembl.org/index.html), while gene coded protein by Biological database (https://www.ncbi.nlm.nih.gov/protein). These gene mutations of *Nanog* were further confirmed by using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared from the catalogue of somatic cancer database (http://www.cancer.sanger.ac.uk/cosmic) databases as described previously [17].

Statistical analysis: Quantitative data were presented in mean (±), standard deviation and Chi square (χ^2) test was used to calculate significance ($p<0.05$) difference between cases and controls.

Results

Karyotyping analysis

Cytogenetic investigations were carried out on >50 well spread metaphase cells with Phytohemagglutinin- (PHA) stimulated peripheral blood cultures using standard procedures, and high resolution GTG banding was performed. Analysis was undertaken on metaphase chromosomes from the proband (case-I) at >550-band level according to International System for human Cytogenetic Nomenclature (ISCN) 2016 nomenclature [13], showed the unbalanced karyotype: t (6,9)[p23;q34], del (18p), monosomy 18, fragile-X, inv14 as presented in Table 1 and the representative karyotypes are shown in Figure 1. Further, the analysis of the mother's karyotype showed del 18, trisomy 18 and trisomy 16. Karyotype analysis of the father of the proband also demonstrated chromosomal aberrations demonstrating D/D and D/G association (Table 1). The second case presented with 46, XX, trisomy 18, monosomy 21, monosomy 15, trisomy 10, Del-18q23, G/G association 21, monosomy 20, and chromatic break 16p13.1.

MTHFR gene polymorphism in ARMS cases

MTHFR gene regulate folate metabolism and thus plays a crucial

Table 1: Frequency (%) of Chromosomal Aberrations in Proband, Mother and Father in ARMS case.

S.No.	Family members	Total normal cells	Total abnormal cells & (%) frequency	Types & their number (%) frequency of cells							P- value
				Asso.	Del.	Fra.	Inv.	Tran.	Mono.	Triso.	
1	Proband (Case-I)	93	12 (12.90)	5(5.37)	1 (1.07)	1(1.07)	1(1.07)	1(1.07)	1(1.07)	-	0.0038*
2	Mother (Case-I)	50	3(6.00)		1(2.00)	-	-	-	2(4.0)	-	0.004*
3	Father (Case-I)	48	2(4.16)	2(4.16)	-	-	-	-	-	-	0.005*

*χ^2 test was used to calculate p-value between normal & abnormal cells to find significant differences. Asso.: Association; Del: Deletion; Fra: Fragile site; Inv: Inversion; Tran: Translocation; Mono: Monosomy; Triso: Trisomy

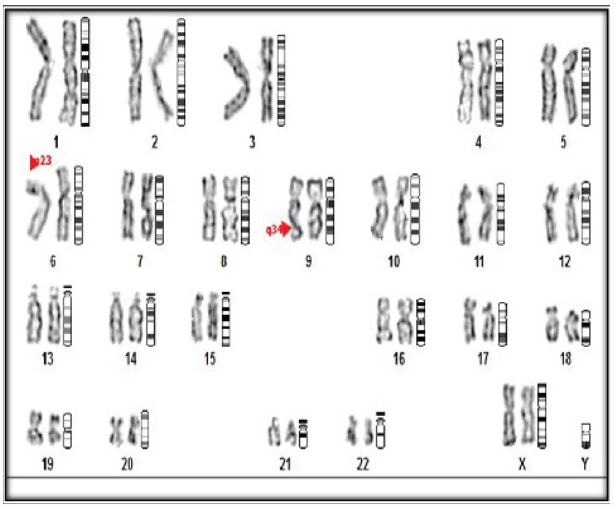

Figure 1: Representative image of GTG banding in the case of ARMs showing translocation between chromosome t[6,9][6p23.4;9q34.1]. The image was captured at 100x using upright Olympus microscope with Karyotyping analysis software. Ideograms are presented and analysis based on ISCN (2016).

role during embryogenesis as folate levels regulates DNA synthesis and DNA methylation. In order to study *MTHFR* gene polymorphism of C677T allele, SNP analysis was performed to determine "risk factor" of MTHFR C677T genotyping-wild-type (CC) allele and mutant (TT) allele in homozygous condition and heterozygous condition (CT). In the cases of ARMS, the Tm value showing shift from 82.00°C to 83.00°C, suggesting increase heterozygocity (CT) in the cases due to allele C change in to T (C→T) as mentioned in Figure 2A, suggesting increase "risk" of the disease, while rest of the three cases (75%) showing only wild type of genotype CC (Figure 2A and 2B). The findings of RT-PCR were further confirmed the appearance of additional band of 105 kb belong to mutant "T" allele (Case-3) on agarose gel electrophoresis as documented in Figure 2C.

Genetic testing for *OCT-4*, *SOX-2* and *Nanog*

Further to identify genetic association of stem cell pluripotency markers *OCT-4*, *SOX-2* and *Nanog* with the case of ARMs, the genomic DNA was isolated from peripheral blood leucocytes of the proband, proband's mother and father as well as respective case controls. PCR analysis was performed on genomic DNA for *OCT-4*, *SOX-2* and *Nanog* using specific primers as described in material and methods. The PCR products were run on agarose gel and images were captured as documented in Figure 3. The results of genetic analysis revealed null mutation in *OCT-4* in the pro band whereas the parents as well as the case controls indicated the presence of the band for 577 bp of *OCT-4* (Figure 3). In case of *SOX-2*, PCR analysis did not reveal null mutation or presence of any extra isoform; however, difference in the level of expression (may be due to variation in the copy number) was identified. Interestingly in the case of *Nanog*, 151bp band was observed in all the samples analyzed; however, an isoform of ~80 bp was identified in the case of the proband, father of the proband as well as case control for the father (Table 2).

Further, we were interested in *Nanog* due to appearance of a novel isoform in the proband, thus DNA Sanger's sequencing analysis was performed on the *Nanog* PCR product. The results identified two novel mutations in the proband as compared to the case control. A change in G→T at position 84 and A→T at position 73 in *Nanog* in the proband as compared to case control (Figure 4). Thus, overall, our findings identify a novel role for stem cell pluripotency markers *OCT-4* and *Nanog* in the case of ARM.

Discussion

Genetic linkage has been identified in the cases of ARMs; however involvement of specific genes/pathways still needs to be investigated. In the present case report we have studied the genetic basis of ARMs by performing investigations at cytogenetic as well as specific genes associated with stem cell functions. Herein, we report novel findings: 1) presence of complex chromosomal rearrangements in 2 different cases of ARM and 2) identification of mutation in Oct 4 gene and *Nanog* in ARM case. Together, we hypothesize that the ARMs in present case result from one or several mechanisms including CCRs and disruption of stem cell signaling due to mutation in pluripotency marker genes- *OCT-4* and *Nanog*.

CCRs are constitutional structural rearrangements involving three or more chromosomes or having more than two breakpoints

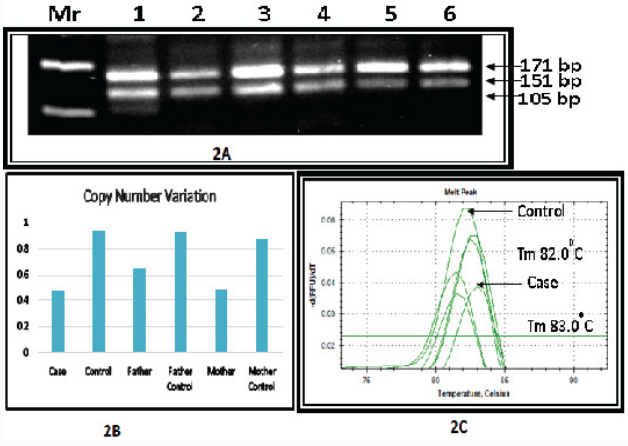

Figure 2: A, B, C: MTHFR C677T gene polymorphism showing appearance of CC&CT genotypes after ARMS PCR based analysis and additional band of 105bp confirming heterozygocity (Figure 2A), while densitometry analysis showing DNA copy number variation (Figure 2B) and melting peak (Tm) values shown in Figure 2C.

Table 2: Statistical analysis of Stem cell marker genes (*SOX-2*, *OCT-4* and *Nanog*) was carried out between patients of anorectal malformations, mother, father and their respective controls.

Case *vs.* Control				
Gene	Case No.	O.R.	C.I. at 95%	p-value
SOX-2	1	0.618	(0.4375-0.8736)	0.0064**
	2	1.233	(0.6824-2.2021)	0.4872
OCT-4	1	0.412	(0.0007-0.1810)	0.0016**
	2	1	(0.3226-2.7547)	1.01
NANOG	1	0.325	(0.1275-0.8773)	0.0260*
	2	0.432	(0.1895-1.4526)	0.2145
Case *vs.* Mother				
Gene	Case No.	O.R.	C.I. at 95%	p-value
SOX-2	1	0.523	(0.8792-1.7058)	0.2307
	2	0.752	(0.7416-2.3614)	0.3431
OCT-4	1	0.127	(0.007-0.1964)	0.0020**
	2	0.924	(0.4106-3.1283)	0.8091
NANOG	1	0.624	(0.1611-1.0488)	0.0628
	2	0.912	(0.1358-1.7403)	0.2676
Case *vs.* Father				
Gene	Case No.	O.R.	C.I. at 95%	p-value
SOX-2	1	0.431	(0.2455-1.5290)	0.0014**
	2	0.842	(0.7865-2.5265)	0.2489
OCT-4	1	0.475	(0.0033-1.0883)	0.0571**
	2	0.725	(0.3495-2.5501)	0.9703
NANOG	1	0.513	(0.4364-2.9280)	0.8007
	2	0.627	(1.5120-4.2128)	0.0041**

**Highly significant differences ($p<0.001$); *Significant difference ($p<0.05$) were observed using chi square test (two tailed).

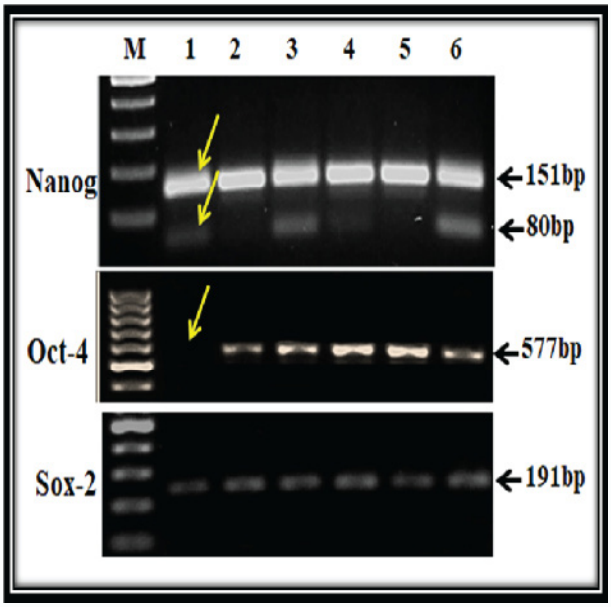

Figure 3: PCR was performed for Nanog, Oct-4 and Sox-2 gene. The PCR products were run on agarose gel and stained with ethidium bromide; images were captured by Gel documentation system. M=marker, lane-1 case, lane-2 mother, lane-3 father, lane-4 and 5 female control and lane 6 is male control.

[18,19]. While chromosomal aberrations are common in ARMs cases, Complex Chromosomal Rearrangements (CCRs) have been reported rarely in such birth defects. Here we present cytogenetic findings in two different cases referred to our cytogenetic laboratory by Department of Pediatric and Plastic surgery. The karyotype of peripheral blood showed karyotypes with 46, XX, t (6,9) [p23;q34], Del (18p), monosomy 18, Fragile X and Inv 14 in a mosaic pattern. The second case presented with 46, XX, trisomy 18, monosomy 21, monosomy 15, trisomy 10, Del-18q23, G/G association 21, monosomy 20, and chromatic break 16p13.1. To best of our knowledge these

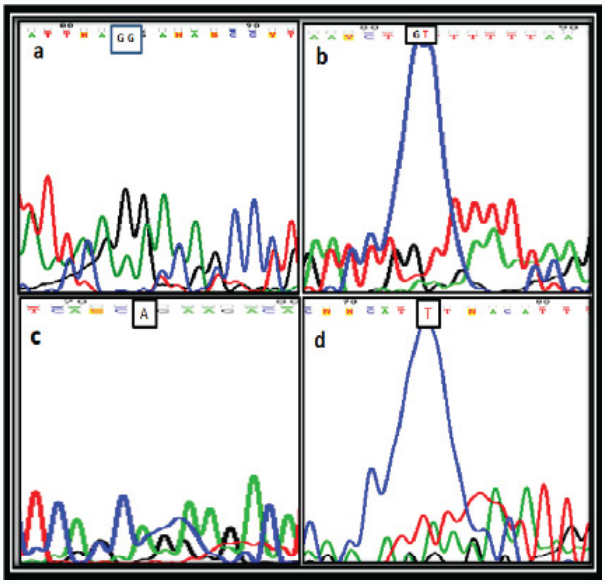

Figure 4: DNA Sanger's sequencing analysis was performed to identify mutation in Nanog gene. Chromatogram showing data a-c, control; b-d ARMs case showing point mutation at two different positions 73' A→T 74' and 83' G→T 84' between cases and control.

findings are novel and such pattern of CCRs in the case of ARMs has not been reported earlier. It is important to report such a unique chromosomal occurrence as they might give additional information on chromosomal regions and genes involved in hindgut development or putative anorectal development genes. Furthermore, based on karyotyping of probands (case-I) parents, role of de-novo mutations resulting in ARMS was suggested. As we discussed, involvement of CCRs in ARM is rare and has been reported only in few cases. In this regard, Bartels et al. [20] identified de- novo 13.2-Mb deletion of chromosome 18q22.3-qter and a 2.2-Mb de-novo duplication of chromosomal region 18pter-p11.32 located at the telomeric end of chromosome 18q in a case of ARM. Previous studies have identified involvement of chromosome abnormalities in a variety of cases with ARM, the common chromosomal abnormalities include trisomy 13, 18, and 21. Furthermore, micro-deletion 22q11.2, 5p-deletion syndrome, an unbalanced translocation with a monosomy for the proximal part of chromosome 14 and a deletion 5p15 has also been reported [8]. Taken together, although the chromosomal aberrations in cases of ARMs are common, the presence of CCRs is quite uncommon. What is the significance of such CCRs needs further investigation in the cases of ARMs.

Stem cells play an important role during embryogenesis in the developmental process. Stemness related factors such as *OCT-4*, *SOX-2* and *Nanog* not only maintain pluripotency but also determines cell fate and differentiation into different organs. In the present case report, we have identified a possible link between stem cell pluripotency markers *OCT-4* and *Nanog* with ARM case. Genetic analysis of the proband's and the family members identifies an involvement of *OCT-4* null mutation in the proband (case-I) but not in the parents of the proband or the case controls, suggesting de-novo occurrence of mutations in the proband DNA. Furthermore, DNA sequencing analysis of *Nanog* identified a novel mutation in *Nanog* gene in the case-I as compared to the respective control. No significant changes were observed in *SOX-2*. These results were interesting since

OCT-4 and *Nanog* are considered as a master regulator of stem cell pluripotency and regulate cell fate determination. Previously, a role for *OCT-4* and *Nanog* has been identified in cell fate determination of ectodermal origin [21]. Since hindgut development is of ectodermal origin these findings become further relevant as may provide a link between hind gut development and genes involved. Of note, a previous study by Sakaki-Yumoto et al. [22] identified role of Sall-4, which is among the key regulators in the transcriptional network in embryonic stem cells in anorectum formation. Interestingly, it was identified that Sall4 occupies the promoter regions of genes occupied by Oct-3/4, *SOX-2*, and *Nanog* and works in association with these markers to maintain stem cells [23]. Sall-4 controls its own expression and the expression of *OCT-4* and work antagonistically to balance the expressions of Sall-4 gene family members. Together, these findings indicate a possible transcription regulation feedback loop of Sall-4 and *OCT-4*, *Nanog* may be involved in hindgut development. Taken together, an alteration in any of the genes governing the development of the anorectum may result in the ARMs phenotype. It will be further interesting to identify if these different genes identified are regulating through a common pathway or act individually to develop such congenital anomaly. Since this is a case report, studies are warned involving large sample size to identify a possible involvement of stem cell regulatory gene in the cases of ARMs.

Conclusion

Our study identifies an involvement of CCRs along with mutations in stem cell pluripotency markers *OCT-4* and *Nanog* in the case of ARMs. Present study also points towards the role of these genes in the normal development of anorectum; however, a role of epigenetic factors and interaction with other genes has not been ruled out. Overall, possible genetic and epigenetic factors further need to be investigated for better management of such congenital anomalies. Number of Overall, present case study confirmed an involvement of genetic factors i.e. Complex Chromosome Rearrangements (CCRs) in the present case. To our knowledge this is the first report that suggests a possible link between *OCT-4* and *Nanog* in the case of ARMs. Together, we hypothesize that the present case of ARMs developed one or several mechanisms including CCRs interaction, disruption of stem cells signaling fail to maintain the pluripotency based on stem cell markers- *OCT-4*, *Nanog* and Sox2. The increase heterozygocity of CT alleles make complexity during differentiation of endodermal cells (stem cells) during organogenesis due to defective folate metabolism.

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Author Contribution

Author (AKS), is involved from the designing and implementation of this study, and VK are involved in clinical diagnosis, LS is responsible for data analysis and finalizing the results during writing the manuscript. UK and SK also help for editing the manuscript.

Conflict of Interest

Authors declared no conflict of interest.

References

1. Levitt MA, Peña A. Outcomes from the correction of anorectal malformations. Curr Opin Pediatr. 2005;17(3):394-401.
2. Levitt MA, Peña A. Anorectal malformations. Orphanet J Rare Dis. 2007;2:33.
3. Berrocal T, Lamas M, Gutiérrez J, Torres I, Prieto C, del Hoyo ML. Congenital anomalies of the small intestine, colon, and rectum. Radio Graphics. 1999;19(5):1219-36.
4. Ratan SK, Rattan KN, Pandey RM, Mittal A, Magu S, Sodhi PK. Associated congenital anomalies in patients with anorectal malformations-- a need for developing a uniform practical approach. J Pediatr Surg. 2004;39(11):1706-11.
5. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in patients with anorectal anomalies. Eur J Med Genet. 2007;50(4):281-90.
6. Oh C, Youn JK, Han JW, Yang HB, Kim HY, Jung SE. Analysis of Associated Anomalies in Anorectal Malformation: Major and Minor Anomalies. J Korean Med Sci. 2020;35(14):e98.
7. Holschneider AM, Hutson JM. Genetics, Pathogenesis and Epidemiology of Anorectal Malformations and Caudal Regression Syndrome. Anorectal malformations in children; 1st edition. New York: Springer. 2006:31-42.
8. Marcelis C, de Blaauw I, Brunner H. Chromosomal anomalies in the etiology of anorectal malformations: a review. Am J Med Genet A. 2011;155A(11):2692-704.
9. Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W. Mutations in the SALL1 putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet.1998;18(1):81-3.
10. Mo R, Kim JH, Zhang J, Chiang C, Hui CC, Kim PC. Anorectal malformations caused by defects in sonic hedgehog signaling. Am J Pathol. 2001;159(2):765-74.
11. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. 2005;122(6):947-56.
12. Medvedev SP, Shevchenko AI, Zakian SM. Molecular basis of Mammalian embryonic stem cell pluripotency and self-renewal. Acta Naturae. 2010;2(3):30-46.
13. Shaffer, Lisa G, Schmid M, McGowan-Jordan. ISCN: An International System for Human Cytogenomic Nomenclature Basel: Karger, 2016.
14. Henderson JK, Draper JS, Baillie HS, Fishel S, Thomson JA, Moore H, et al. Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. Stem Cells. 2002;20(4):329-37.
15. Nettersheim D, Biermann K, Gillis AJ, Steger K, Looijenga LHJ, Schorle H. NANOG promoter methylation and expression correlation during normal and malignant human germ cell development. Epigenetics. 2011;6(1):114-22.
16. Bhatia B, Singhal S, Tadman DN, Khaw PT, Limb GA. SOX2 is required for adult human muller stem cell survival and maintenance of progenicity in vitro. Invest Ophthalmol Vis Sci. 2011;52(1):136-45.
17. Saxena AK, Singh V, Aprajita, Kumar A, Tiwari M, Singh P, et al. Stop Codon of TGF βRI Gene Modulate the Functional Activity of 3D Structure and their Genetic Susceptibility in the Case of Wilms' Tumor. J Cancer Sci Ther. 2019;11(8):251-5.
18. Gardner RJM, Sutherland GR. Chromosome Abnormalities and Genetic Counselling, Oxford University Press, Oxford, UK, 2004.
19. Patsalis PC. Complex chromosomal rearrangements. Genet Couns. 2007;18(1):57-69.
20. Bartels E, Draaken M, Kazmierczak B, Spranger S, Schramm C, Baudisch F, et al. De novo partial trisomy 18p and partial monosomy 18q in a patient with anorectal malformation. Cytogenet Genome Res. 2011;134(3):243-8.
21. Wang Z, Oron E, Nelson B, Razis S, Ivanova N. Distinct lineage specification roles for NANOG, OCT4, and SOX2 in human embryonic stem cells. Cell Stem Cell. 2012;10(4):440-54.
22. Sakaki-Yumoto M, Kobayashi C, Sato A, Fujimura S, Matsumoto Y, Takasato M, et al. The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. Development. 2006;133(15):3005-13.
23. Lim CY, Tam WL, Zhang J, Ang HS, Jia H, Lipovich L, et al. Sall4 regulates distinct transcription circuitries in different blastocyst-derived stem cell lineages. Cell Stem Cell. 2008;3(5):543-54.