Journal of Clinical and Experimental Genetics
Volume 2 | Issue 1

Case Report Open Access

Identification of Novel De-Novo 12q14 -12q22 Gene Mutations and MTHFR C677T Gene Polymorphism Increase Genetic Susceptibility in Hirschsprung Disease - A Rare Case Report

Saxena AK*

Human Cytogenetic and Molecular Genetics Laboratory, Department of Pathology/Lab Medicine, All India Institute of Medical Sciences, India

***Corresponding Author:** Saxena AK, Human Cytogenetic and Molecular Genetics Laboratory, Department of Pathology/Lab Medicine, All India Institute of Medical Sciences, Patna, Pin code: 801507, Bihar, India. Tel: 7781012677, Email: draksaxena1@rediffmail.com

Citation: Saxena AK (2022) Identification of Novel De-Novo 12q14 -12q22 Gene Mutations and MTHFR C677T Gene Polymorphism Increase Genetic Susceptibility in Hirschsprung Disease - A Rare Case Report. J Clin Exp Gen 2(1): 102

Abstract

Background: Hirschsprung's disease (HSCR) is a complex congenital genetic aganglionic disorder of intestine fall under paediatric age group of "Birth Defects". Etiopathology reveals that more than 80% cases are sporadic in nature with known etiological factors and remaining 20% are still unknown.

Objectives: The curiosity has been arises to evaluate the genetic analysis based on karyotyping and also assess the risk factor using methylenetetrahydrofolatereductase (MTHFR) C677T gene polymorphism.

Study Design: Clinically diagnosed male proband shows typical Mongolian type features like flat broad nasal septum with protruded tongue and broad forehead with epicanthal folding.

Results: Karyotype showing four new breakpoints mapped on 12q14, (10.08Mbp), 12q15 (5.04 Mbp), 12q22 (4.41Mbp) and 12q21(16.38 Mbp) with the loss of euchromatic and heterochromatic DNA fragments (ASI software version 7.2.7.34276). HSCR with Down's syndrome is rare case confirmed by karyotype 47, XY+ 21 and fluorescence in-situ hybridization (FISH) analysis. Amplification-refractory mutation system (ARMS) PCR technique is used for MTHFR C677T gene polymorphism and findings reveals that melting temperature (*Tm*) values significantly change with respect to controls Glyceraldehyde-3-posphate dehydrogenase (GAPDH), resulting there is change of single nucleotide cytosine substitute into thymidine followed by changes of amino acid alanine to valine.

Conclusion: The involvement of new "breakpoints" 12q14-21 (mutations) increases genetic susceptibility due to deficiency of folates, suggesting confirm the "risk factor" in heterozygous condition.

Keywords: Hirschsprung's Disease, Breakpoint 12q21, MTHFR C677T Gene Polymorphism, Trisomy-21

Introduction

Epidemiology, reveals that HSCR is a rare congenital anomaly and the prevalence occurs higher in male population (1:5000). Clinical features includes the involvement of enteric central nervous system leading to defective intestinal disorders and include complications enterocolitis, megacolon, bowel obstruction with perforation [1]. The half of the isolated cases of HSCR are sporadic in nature linked to specific genetic mutations, and 20% are familial seems to be associated with other genetic disorders like Down syndrome or Waardenburg syndrome [2]. Earlier study shows that multiple genes and specific regions of chromosomes (12q21) have been identified with association of mutations including RET proto-oncogene, a tyrosine kinase receptor in HSCR [3]. The genetic susceptibility of *SOX10*, TGF-β genes includes specific signalling pathways that are involved in different cellular programmes crucial for the normal development of the enteric nervous system including neuregulin 1 (*NRG1*) and *NRG3* in Chinese population [4]. Besides, these two genes are also associated with enteric nervous system – one gene is located on the long arm of the chromosome 15 (15q23) and other is colonic aganglionosis involve deletion of chromosome 10 (q11.21 q21.2). *De novo* reciprocal balanced translocation t(3, 17) (p12, q21) leads to the mutations of *L1CAM* gene, which encodes a neural adhesion molecule often involved as isolated X-linked disease [5-7]. Hence, there is a need for further characterization of new loci in the expansion of the predisposing genes possibly leading to HSCR, to understand the genesis of enteric phenotype [8]. Therefore, the present study has been designed with the aim to explore the expansion and characterization of new locus in interstitial deletion of 12q21 region. The study further extends to determine genetic heterogenicity using MTHFR C677T gene polymorphism to assess the "risk" and try to correlate with transforming growth factor β (TGFβ) in HSCR as independent factor during proliferation of cells.

Materials and Methods

Pedigree analysis of the proband indicates that this is a fifth male child of 2-year-old having 3.2 kg weight attend the out-patient's department of Paediatric surgery, All India Institute of Medical Sciences, Patna (India). This study is approved by Institute Ethical Committee (IEC) Clinical diagnosed the case of HSCR show typical Mongolian phenotypic features (figure-1) with low set ears, wide nasal septum, protruded tongue. There is also complaint of constipation since birth and defective intestinal disorders that includes complications enterocolitis, megacolon and bowel obstruction and referred to Human Cytogenetic and Molecular Genetic Laboratory in the Department of Pathology / Lab Medicine for karyotyping and molecular analysis using specific markers.

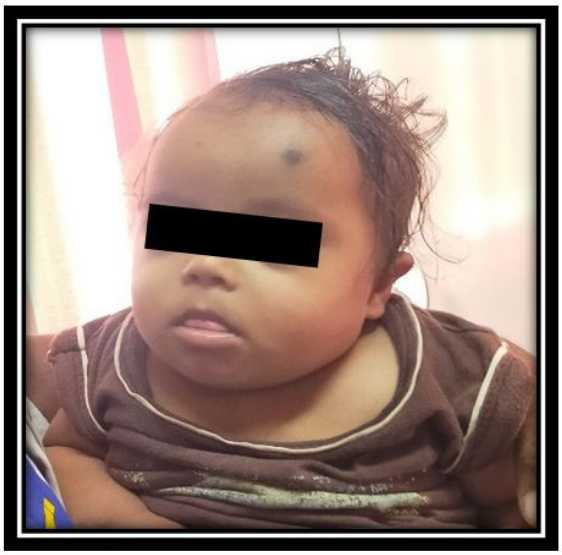

Figure 1: Clinical feature of the HSCR with Down syndrome. Karyotype showing 47, XY + 21

Blood sample (1.0 ml) was obtained in sterile vial for short- term lymphocytes cultures in RPMI 1640 media, supplemented with foetal bovine serum (FBS) (5%) and phytohemagglutinin-M for 72hrs in CO_2 incubator at 37°C after written consent from legal guardian. Karyotypes were prepared after giemsa trypsin giemsa (GTG) banding for chromosomal and FISH analysis was carried out further confirmation of diagnosis using DNA probe labelled with spectrum orange of 220 kb and their cytogenetic location 21q22.13 - q22.21 obtained from Abbott Vysis (LS121). The cells were counter-stained with DAPI and vied under fluorescence microscope Olympus Cytoscan system of applied spectral imaging (ASI) of software version 7.2.7.34276 [9].

Briefly, genomic DNA were isolated using a Promega DNA isolation kit and MTHFR C677T gene polymorphism was studied by RT-PCR using SYBR green as fluorescence dye using specifics

MTHFR-T,5'–GCACTTGAAGGAGAAGGTGTCTGCGGGCGT-3',

MTHFR-C-polyG,5'GGCGGGCGGCCGGGAAAAGCTGCGTGATGATGAAATAGG-3'

MTHFR-cf,-5'-TGTCATCCCTATTGGCAGGTTACCCCAAA-3',

MTHFR-cr, 5' -CCATGTCGGTGCATGCCTTCACAAAG-3' set of primers. Earlier, the detailed procedure of polymerase chain reaction (PCR) and calculated *Tm* values are documented earlier by Saxena et. al [10]. Similarly, the TGFβ analysis was also studied using PCR method with specific forward TTTCGCCTTAGCGCCCACTG and reverse GAAGTTGGCATGGTAGCCCTT primers with PCR program initial denaturation 95°C for 5min (Denaturation 95°C-30 sec, annealing 56°C-45 sec, elongation 72°C-1 min) 35 cycles followed by final extension 72°C 8 minutes.

Results

The cytogenetic findings of the proband includes the new breakpoint spanning regions extend 12q14, (10.08Mbp), 12q15 (5.04 Mbp), and 12q22(4.41Mbp). These breakpoints indicate the loss of euchromatic DNA fragments and 12q21(16.38 Mbp) region consist of heterochromatin after mapping of chromosome shows in 16% of the cells (figure-2A). Besides this, the major findings are were observed in the GTG banded karyotypes 47, XY+21 in 66% of lymphocytes and further confirm by FISH in interphase as depicted in fig.2B.

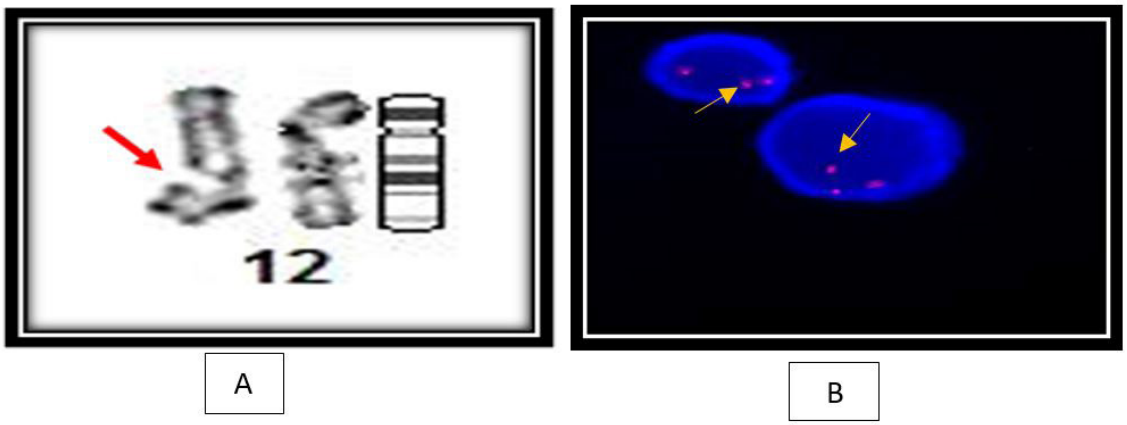

Figure-2A & B. Partial karyotype showing unconstitutional break-point at position 12q14-22 with loss of 35. 91 Mbp DNA fragment (arrow) involving both euchromatin and heterochromatin regions after mapping of chromosome (arrow) as shown in fig.2A. FISH analysis in interphase cell showing extra copy of chromosome-21 (fig.2B, arrow) in HSCR

ARMS-PCR is highly sensitive technique used to determine genetic heterogeneity after SNP of MTHFR C677T allele analysis by calculating *Tm* values between case and control (GAPDH used as genomic controls) as shown in figure-3. The findings shows that homozygous CC (wild type) genotypes are shifting to heterozygous (CT) condition of MTHFR C677T gene due to shift of *Tm* values from 86.00 (case) to 88.00 (GAPDH), when compared with controls group i.e. 85.00 to 87.50 followed by substitution of cytosine to thymidine resulting change of amino acid alanine to valine. Interestingly, there is lack of mutant genotype TT in homozygous condition. Interestingly, the finding showing the significant shift of *Tm* values from C →T allele and confirm to increase the genetic heterogenicity and increase risk factor of the disease like HSCR.

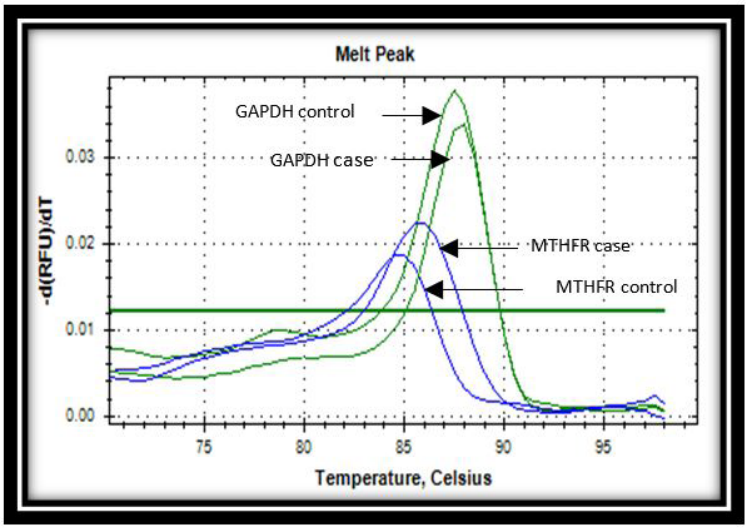

Figure 3: RT-PCR analysis of MTHFR C677T gene polymorphism between HSCR and control. The melt peak analysis showing the significant shift of *Tm* values between case and control (arrow)

Etiopathology of HSCR is highly complex in nature due to involvement of various metabolism including neurone signalling pathway. The present case study TGFβ showing lack of significant difference (L1 to L3) after use of three different "concentrations" of the same amplified products (3.5 ul, 4.5 ul and 5.5 ul) to confirm the findings, when compared with control (L4) as shown in figure-3.

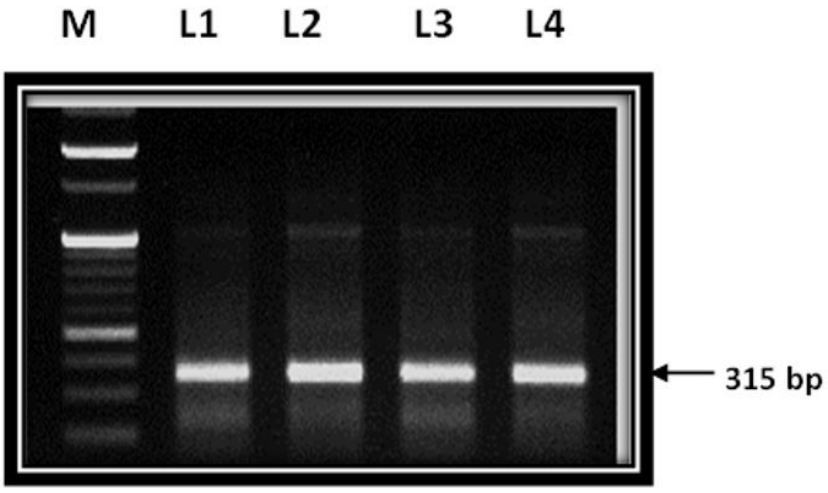

Figure 4: PCR based analysis of TGFβ (315bp) with different concentration of amplified PCR product in L1 to L3) in HSCR with control (L4), M=Ladder of 100bp

Discussion

HSCR appears to be multifactorial, a ganglionic complex genetic disease of intestinal obstructive malformations. The genetic diversity of 12q21 deletion syndrome with loss of 1.6 Mb DNA has been known in the patient with development delay along with hydrocephalus, horseshoe kidney, heart defects and other ectodermal anomalies [11]. After prediction of data tools of this region identified four major involvement of genes - *SYT1, PP1R12A, PAWR* and *OTOGL* among these four two genes *SYT1* and *PPP1R12A* play an important role in the etiopathology of HSCR. Present study of HSCR with Down's is unique case reporting first time in India, that there is loss of three euchromatin regions mapped on different chromatid breakpoints identified namely 12q14, 12q15 and 12q 22 are significantly linked to the developmental anomalies during early embryogenesis. The pedigree analysis of the family shows poor "socioeconomic condition" with four sisters having normal phenotypes with last family member is male having typical phenotypes (as shown in figure-1), after karyotyping and FISH analysis confirm the HSCR cases of Down syndrome. During collection of the family history parent failed to cooperate either due to conserved nature or illiteracy may be one the factor, although, it is difficult to find the mode of transmission based on Mendel's law. However, the author suggested that the penetrance of oligogenic mode of "sex-dependent" inheritance occurs due to the segregation of alleles in the proband. The poor socio-economic status might be increasing the genetic susceptibility followed by increase of "risk factor" of the disease which further confirm the defective folate metabolism based on MTHFR C677T gene polymorphism.

HSCR is a complex disorder since Birth Defects and TGF β might have also regulate to proliferation and differentiate of cells as observed earlier in tumour of paediatric age group [11]. Present case study showing lack of significant differences, suggesting either *SYT1* gene encoded membrane protein of postsynaptic vesicles either failed to regulated independently or synergism with *PPP1R12A* gene which encodes myosin phosphatase enzyme in muscles during development [3].

There is another region mapped on chromosome 12q21 which lost the DNA fragment of 16.38Mb heterochromatic in nature of unknown function. Since HSCR disease fall under the category of "Birth Defects" and is quite possible that this region (facultative heterochromatin) might have associated during early event of embryogenesis during DNA methylation. Author, also hypothesize that such Birth Defect (HSCR) occurs at the time of early organogenesis, where foetus might have exposed with strong teratogens antenatally leading to onset of disease. Therefore, the present case study concluded that HSCR fall under the category of complex disease and combination of followings - first, the involvement of complex chromosome rearrangements (CCRs) including trisomy-21 occurs due to non - disjunction event at mitosis-I leading to unequal crossing over of cell-division confirm the case of HSCR with Down's syndrome. The deletion of 12q21 regions belongs to the loss of DNA fragments of euchromatin and heterochromatin is one of the most important constitutional genetic factors. Secondly, to increase "risk factor" of the MTHFR C677T gene polymorphism in heterozygous condition due defective folate metabolism, third we also asses the expression of TGFβ in the same proband failed to correlate the role of TGβF in folate metabolism or signaling during chromatin modelling associated breakpoints 12q21 gene expression and unable to predict significant correlation due to unknown factor in present case study.

Conclusion

Complex chromosome rearrangements (CCRs) with trisomy -21 and genetic heterogenicity of MTHFR C677T in male make the study more interesting due to penetrance of gene increase "risk factor" in the same family of HSCR.

Acknowledgement

Author is thankfully acknowledged to the Director AIIMS Patna and patient and their family member who participate in this study.

References

1. Parisi MA (2002) Hirschsprung Disease Overvie- Retired Chapter, For Historical Reference Only. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. Gene Reviews. University of Washington, Seattle 1993-2022.

2. Amiel J, Sproat-Emison E, Garcia-Barcelo M, et al. (2008) Hirschsprung disease, associated syndromes and genetics: a review Journal of Medical Genetics 45: 1-14.

3. Niclass T, Guyader GL, Benrteau C, et al. (2020) 12q21 deletion syndrome: Narrowing the critical region down to 1.6 Mb including SYT1 and PPP1R12A. Am J Med Genet 1-6.

4. Dehua Yang, Jun Yang, Shuai Li, et al. (2017) Effects of RET, NRG1 and NRG3 Polymorphisms in a Chinese Population with Hirschsprung Disease. Sci Rep 7: 43222.

5. Martucciello G, Bicocchi MP, Dodero P, et al. (1992) Total colonic aganglionosis associated with interstitial deletion of the long arm of chromosome 10. Paediatric Surgery International 7(4): 308-310.

6. Angrist M, Kauffman E, Slaugenhaupt SA, et al. (1993) A gene for Hirschsprung disease (megacolon) in the pericentromeric region of human chromosome 10. Nat. Genet 4(4): 351-359.

7. Lyonnet S, Bolino A, Pelet A, et al. (1993) A gene for Hirschsprung disease maps to the proximal long arm of chromosome-10. Nat. Genet 4(4): 346-50.

8. Griseri P, Vos Y, Giorda R, et al. (2009) Complex pathogenesis of Hirschsprung's disease in a patient with hydrocephalus, vesico-ureteral reflux and a balanced translocation (3, 17) (p12, q11) Eur J Hum Genet: 17(4): 483-90.

9. Ajit KS, Aniket K (2019) Microdeletion of the AZFc locus with high frequency of mosaicism 46, XY/47XYY in cases of non-obstructive azoospermia in an eastern India population Genetics and Molecular Research 18(2): 1-14.

10. Saxena AK, Gupta RK, Kumar M (2016) ARMS-PCR based SNP analysis of MTHFR C677T allele using Syber green in pancreatic tumor. British Journal of Medicine and Medical Research 11(12): 1-6.

11. Saxena K, Singh VA, Kumar A, et al. (2019) Stop codons of TGFβ RI gene modulate the functional activity od 3D structure and their genetic susceptibility in the case of Wilms' Tumor. Journal of Cancer Science & Therapy 11(8): 251-255.