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**ORIGINAL ARTICLE**

# Detection of Novel mutation in VANGL1 gene indicating genetic association of Myelomeningocele

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## ABSTRACT

**Aim:** To detect the novel mutation in VANGL1 gene indicating genetic association of Myelomeningocele.
**Methodology:** The study design was cross sectional. It comprises of sixty individuals, of them fifty were diagnosed cases of myelomeningocele and ten were healthy individuals taken as controls. The cases were collected from Jinnah Postgraduate Medical Center. The study was carried out in Dow Diagnostic and Research Laboratory (D.D.R.L.). Most of the patients were less than one year of age. The cases were evaluated for various other parameters like site and size of cyst and associated features like presence of hydrocephalus in the individuals. Since folic acid deficiency is the key component in the causation of the disease so mothers were also asked about the consumption of folic acid. Blood was drawn from patients after a written permission from the parents of the concerned patient. It was followed by the conduction of PCR to seek for any mutation in VANGL1 gene.
**Results**: We found a rare mutation in VANGL1 gene revealing substitution of valine to serine at position 239 i.e. V239S. Hydrocephalus being the associated anomaly was present in 32% of the patients. Most of the affected individuals were males. 98% mothers of the sufferers did not take folic acid during pregnancy. In most of the patients, lump was present on the lumbar region.
**Conclusion:** Myelomeningocele is a congenital birth defect with lifelong complications. Its prevalence can be decreased by taking certain measures. Periconceptional intake of folic acid has been established to lessen the risk of the disease. We identified a rare mutation in VANGL1 gene that may result in the causation of myelomeningocele.
**Keywords:** Neural tube defects, mutation, myelomeningocele.

## INTRODUCTION

Neural tube is the embryonic precursor of brain and spinal cord. Failure of its closure will lead to neural tube defects (NTDs). The second most common anomaly after congenital heart defects are the lesions in the neural tube[1]. Recent studies show that the birth prevalence for NTDs ranges between 0.7 and 2.2 per 1000 newborns globally[2]. Anencephaly and myelomeningocele (MMC) are the most common anomalies of neural tube[3]. The defects which influence the brain are fatal, whereas MMC, although compatible with life but mostly lead to critical disability. Closure of neural tube occurs through a process known as neurulation, in which the neural plate arch upward and finally unites to form a hollow tube that will form the future brain and spinal cord. The dynamic force for the closure of neural tube is contributed and sustained by the cells which undergo convergence and extension[4]. The process of neurulation is divided among mammals into primary and secondary neurulation[5]. During primary neurulation, the union occurs at spine and end up in final closure at the posterior neuropore. Closure begins at hindbrain/ cervical boundary (Closure 1) and extends bidirectionally into the hindbrain and along the spinal section. Independent closure commencement areas happened at midbrain-forebrain boundary (Closure 2) and at the rostral end of forebrain (Closure 3)[6]. If Closure 1 fails to occur it would result in craniorachisis, whereas if Closure 1 is accomplished but the cranial neural tube is defective it would lead to anencephaly[1]. Closure 3 failure is not common but if exist would yield split face with anencephaly. In the spinal region, failure of final closure at posterior neuropore yields open spina bifida, in which upper limit can be of varying axial level. By contrast, defective secondary neurulation leads to closed form of Spina Bifida[7].

The most common NTD linked with survival is Myelomeningocele (MMC)[8]. It is a lethal form of spina bifida that occurs due to failure of neural tube closure at the caudal end leading to protrusion of meninges and spinal cord[9]. Majority of babies born with MMC remain alive but have lifelong disabilities. Deficiency of maternal folic acid levels has been proved to be a significant factor in the causation of NTD[10]. Both the genetic and environmental factors are involved in the etiology of the disease. PCP genes are related to noncanonical Wnt pathway that was initially recognized through studies of fruit fly eye and wing pattern genetic screens and was subsequently found to play significant roles during closure of neural tube in mice[11]. PCP signaling controls process of convergent extension and cell polarity[12] which is essential for gastrulation, neurulation, motile cilia orientation, left- right asymmetry initiation and other procedures in the growth of kidney, liver and other organ. There are various genes linked to PCP which if mutated would cause NTDs[13]. These genes comprise of VANGL1, VANGL2, CELSR1, FZD and DVL.VANGL1 and VANGL2 are mammalian homologues of Drosophila gene Van Gogh (Vang). In humans, VANGL1 gene is situated on chromosome 1p11- p13.1. It encodes

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524 amino acid and its size is 56kb. VANGL2 is situated on chromosome 1q22-q23 and encodes 521 amino acid; its size is 28 kg[14]. Expression of VANGL1 gene is limited to midline floor plate cells and to notochord[15]. Mutation in VANGL1 gene is associated with NTDs in humans[16].

In this study we have hypothesized that mutation in VANGL1 gene would lead to Myelomeningocele and these mutations are present in our population.

## MATERIAL AND METHODS

This is a cross sectional study which consist of fifty diagnosed cases of myelomeningocele and ten healthy individuals taken as controls. The patients were recruited at Jinnah Postgraduate Medical Center (J.P.M.C.) and the study was conducted at Dow University of Health Sciences (D.U.H.S.) Most of the mothers did not take folic acid supplementation periconceptionally. Few patients have positive family history. The experimental protocol was approved by the I.R.B. of D.U.H.S. and a consent form was signed by the patient's attendants.

**Inclusion Criteria:**

1) The age of the patient ranges from 0 -10 years.
2) The patients were selected regardless of sex, socioeconomic and ethnic background.

**Exclusion Criteria**

1) Other cases of neural tube defects.
2) Patients older than the given range of the study.

A trained phlebotomist was hired who drew 2c.c of blood and then placed in EDTA tubes. DNA was obtained from whole blood by QiAmp DNA mini kit. Extraction was done according to specifications provided by the fabricator. It was followed by the PCR amplification of VANGL1 gene. PCR was performed in a tube which contains a reaction mixture of 20µl. The mixture was prepared by following constituents 500 µM of four deoxynucleotides, 10 pmol of each forward and reverse primers for VANGL1 gene, 2 U of Taq polymerase (Promega), 10 x PCR buffer and 1.5 mM MgCl2. The thermal cycler was designed to initially incubate the product for 10min for 95°C followed by 35 cycles comprising of 94°C for 30s. 64°C for 1min and 72°C for 1min with final extension for 10min at 72°C for 1min with final extension for 10min at 72°C. The amplified products were run on 2% Agarose and visualized under transilluminator. The length of the product was calculated according to migration pattern of a 50bp DNA ladder. The image of the products was taken using DOC gel documentation system. The amplified products were of 404bp and were then sent for commercial sequencing. Mutation was analyzed by aligning the sequence with the reference sequence.

## RESULTS

The selected patients were known cases of myelomeningocele. The diagnostic factor was the presence of lump on the vertebral column which is mostly on the lumbosacral region. Most of the mothers (98%) did not take folic acid periconceptionally which may be the causative factor of MMC. Patients were assessed for any associated anomaly like hydrocephalus and it was found that 32% of patients were associated with it. Molecular analysis was further carried out to seek for any mutation in VANGL1 gene. Sixty blood samples were taken, of them, fifty were known cases of MMC and ten were healthy individuals which were labeled as controls. Extraction of DNA and PCR amplification of VANGL1 gene was carried out and the products were sent for commercial sequencing. One of the sample, among fifty cases indicated mutation in the targeted region of exon 4 of VANGL1 gene and amplification was seen at 404 base pairs. The mutation V239S was evident on the position 239 of exon 4 of VANGL1 gene and it was verified by DNA sequencing. Our result revealed the substitution of valine with serine at position 239 in one of the sample. The mutation has not been observed previously and is present in our population as shown in figure 2.

A mutation (V239S) was shown in sample # 16 in which a nine month old boy had myelomeningocele cyst at lumbosacral region (Figure 1). His family didn't show any sign of neural tube defect. His mother did not take folic acid during pregnancy. Due to low socioeconomic status, no antenatal ultrasound was performed to rule out the anomaly in the fetus.

Figure 1(a) A nine month old boy having MMC cyst at lumbosacral region

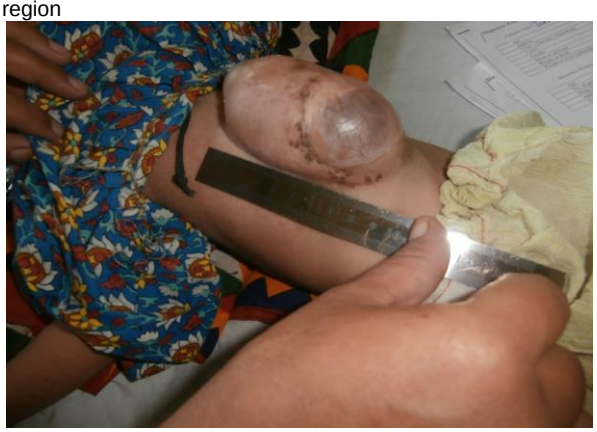
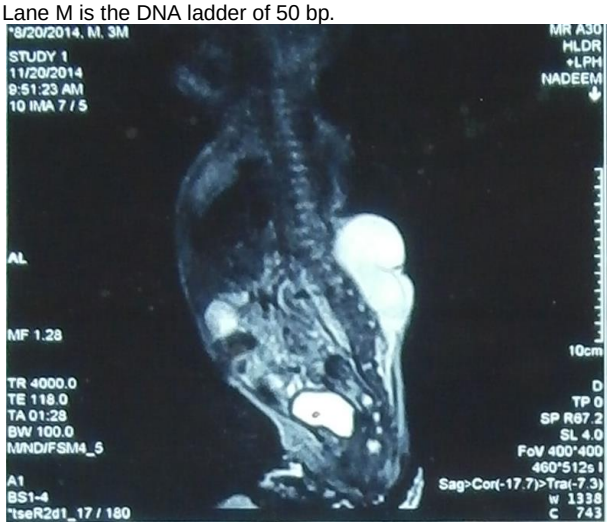


Figure 2: The analysis of VANGL 1 exon 4 DNA sample, Lane 1. 2 3, 5,6,7, and 8 were negative with the VANGL 1 exon 4 mutation whereas Lane 4was identified as positive for VANGL exon 4 mutation Lane N is negative control, Lane P is positive control and Lane M is the DNA ladder of 50 bp.



(b) MRI of the same boy having MMC cyst

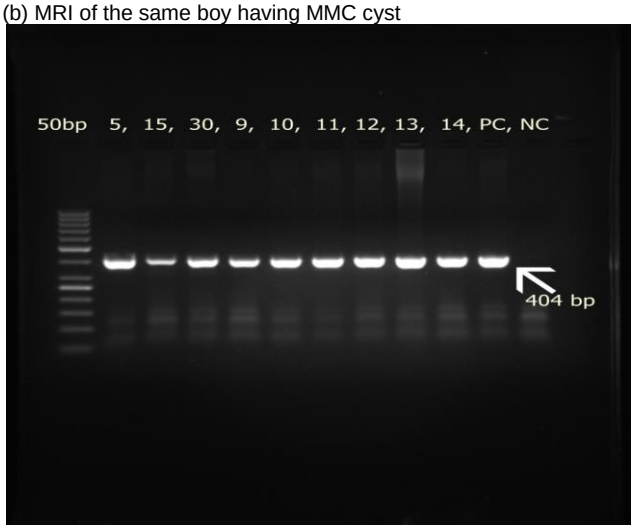



Figure 3: Chromatogram of patient with MMC showing substitution of valine with serine at position 239 in sample number 16 compared with control

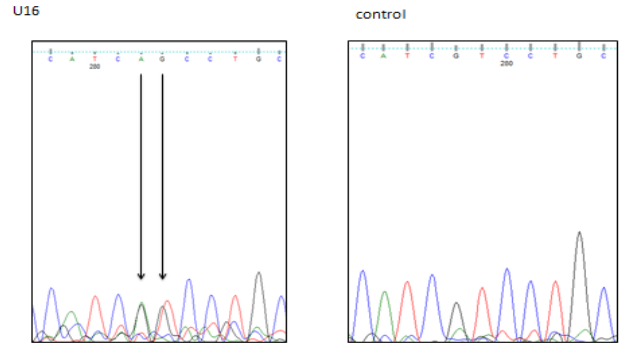
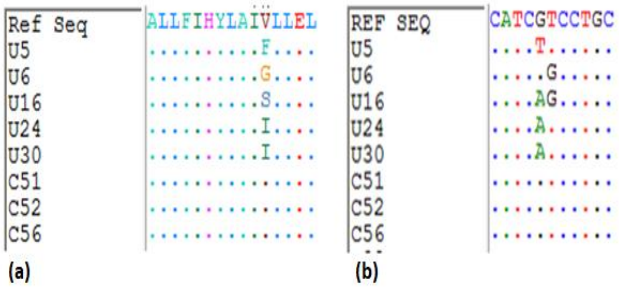



Figure 4: Sequence Alignment showing substitution of Valine to Serine confirmed by DNA sequencing




## DISCUSSION

Neural tube defects are a group of birth defects which are related to morbidity, mortality and lifelong disability. It can be prevented by periconceptional intake of folic acid[17]. Myelomeningocele being the most frequent type of NTD, have an incidence rate of 0.20–0.40/1000 births/year in Canada and the USA[18]. Patients suffering from spina bifida have ten times death risk compared with the rest of the population[19]. Infants having open NTDs have 88-96% chance of 1 year survival rate. In addition to it, such individuals have long lasting complications due to the open defects[20].The pathologies associated with the disease require constant medical and rehabilitative care.

Recognition of causative element is established by the fact that most of these defects occur by the union of environmental and genetic factors. To date about 300 genetic mutants linked with NTDs have been established in mice[21]. Such genes exist in various pathways which include planar cell polarity pathway, MAPK pathway, folate metabolic pathway and hedgehog signal transduction pathway[22].

In our study we found that among the fifty cases, only one patient showed the positive family history. Dupépé etal stated that there is relatively strong association of family history in neural tube defects[23]. It is quite possible that due to small sample size our results differ from them. Nowadays it is easy to diagnose MMC due to progress in the field of sonography. In our case, only six mothers were aware of having such babies prenatally. Requeijo et al in 2016 agreed the reality of ultrasound in visualizing the neural tube defects[24]. Unsinn et al in 2016 acknowledged the approach of ultrasound in revealing myelomeningocele[25]. The prevalence of myelomeningocele has been detoriated worldwide due to prenatal diagnosis through ultrasound. The parents are guided regarding the pros and cons of the disease and make them ready for therapeutic abortion. Unluckily, due to inadequate awareness there is increase in the incidence of myelomeningocele.

We have found mutation in VANGL1 gene V239S in which valine is substituted to serine at position 239**.** Several gene mutations have been associated with myelomeningocele. Hilman etal identified novel candidate risk gene in patients of myelomeningocele within glucose homeostasis / oxidative stress and folate/ one carbon metabolism networks[26]. Lei etal studied the CELSR1 coding region sequence among patients of spina bifida and found a novel mutation in CELSR1 gene in patients of Spina Bifida[27]. Bosoi established a rare mutation in PRICKLE1 in human neural tube defects[28]. Kibar etal identified five novel missense variants in VANGL1, pSer83Leu, p.Phe153Ser, p. Arg181Gln, p.Leu202Phe and p.Ala404Ser occuring in sporadic and familial cases of spinal dysraphisms[29]. Connealy etal established the genetic variation in GLUT3 gene in patients of myelomeningocele[30]. Merello etal found identified three heterozygous missense variants in VANGL1, p.Ala187Val, p.Asp389His, and p.Arg517His in individuals suffering from neural tube defects[31].

## CONCLUSION

Myelomeningocele is a complex birth defect. With this research we eventually establish the fact that further exploration and diagnosis of the disorder should be accomplished. In other circumstances, despite of the fact that history manifested myelomeningocele but mutation was not recognized. So keeping this point in consideration, other gene mutation should be done and more research of same area based should be carried out.

**Conflict of interest:** None

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