During embryogenesis, the process of somitogenesis is orchestrated by a wide range of genes for the precise vertebral column development. The disruption of this process results in either syndromic or non-syndromic multiple vertebral segmentation defects (MVSD). Non-syndromic MVSD is genetically heterogeneous and to date pathogenic variants in $DLL3$, $MESP2$, $LFNG$, $HES7$, $TBX6$ and $RIPPLY2$ genes are known to cause this condition. These genes are part of the Notch signaling pathway. Despite recent advances, etiology of several non-syndromic MVSD remains unknown and it is anticipated that several genes are yet to be identified for monogenic forms of MVSD.

Moreover, very few pathogenic variants have been characterized in patients with non-syndromic MVSD. Hence, we aimed to evaluate all the genes known to cause MVSD to establish the mutation spectrum as well as investigate novel candidate genes for monogenic forms.

In this study, we investigated the genetic basis of non-syndromic MVSD in a cohort of 43 patients from 38 families. Sanger sequencing of genes known to cause MVSD and exome sequencing were used for genetic evaluation. Eight patients from seven families with pathogenic variants helped us to define $DLL3$ related vertebral segmentation defects. We also identified a pathogenic variant in $MESP2$ in a patient with spondylocostal dysostosis II. Despite thorough evaluation by systematic Sanger sequencing of six genes in 21 patients (49%) from unrelated families and exome sequencing of six patients among them, we did not identify a potential monogenic cause for MVSD.

Additionally, we investigated ten patients from seven families with spondylocarpotarsal synostosis syndrome and seven novel deleterious variants in $FLNB$. Thus, our report expands and also validates key clinical (fused thoracic vertebrae and carpal and tarsal
coalition) and molecular (truncating variants in FLNB) characteristics of spondylocarpotarsal synostosis syndrome. We also observed a biallelic missense variant, c.1892C>T in FLNB as the likely cause of multiple vertebral segmentation defects in one family. Pathogenic variant was identified in PTCH1 in a patient with MVSD, which changed the diagnosis to Gorlin syndrome. Together, a molecular basis was identified for 22 individuals with MVSD in this cohort.

Our work contributes to identification and validation of several rare forms of non-syndromic MVSD. We add significant clinical and molecular data to the current literature and demonstrate clinical and molecular heterogeneity of non-syndromic vertebral segmentation defects. However, etiology of a significant proportion of MVSD still remains unknown.