

Discussion and Conclusion: This clinical case highlights the difficulty that clinicians experience in GC when the index case is a pregnant and the fetus has similar features, but the clinical diagnosis is not yet confirmed by molecular testing. One should always keep in mind that the optimal time for determination of genetic risk and GC regarding prenatal testing is before pregnancy, even when the prognosis is likely good as in DA, as well as address associated psychological aspects.

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WHOLE-EXOME SEQUENCING ANALYSIS OF ADULT PATIENTS WITH RARE GENETIC DISEASES: WHAT HAVE WE LEARNED?

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Introduction: Next-generation sequencing (NGS) is accelerating clinical genetics research and diagnostics, given its capacity to generate genomic data in a faster and cheaper way. NGS may avoid the usual stepwise gene-by-gene analysis by performing targeted resequencing of several loci simultaneously (gene panels). Wider NGS applications, such as whole-exome sequencing (WES), may even enable the identification of new genes associated with human diseases. Nevertheless, WES applicability is challenging considering the large number of variants obtained which require specific analytical strategies and bioinformatic resources. The authors describe the use of WES in three adult patients, exemplifying its diagnostic potential but also difficulties encountered during analysis.

Materials and Methods: WES was performed using the Ion Proton system in five individuals: Case #1- a female patient with a childhood-onset progressive muscular dystrophy (35 years of clinical follow-up) and her parents; Case #2- an infertile male with situs-inversus and total sperm immotility; Case #3- a male patient presenting limb-girdle muscular dystrophy with onset during early adulthood. Bioinformatic analysis was performed using several algorithms for variant annotation, filtering and to identify autozygosity through runs of homozygosity.

Results and discussion: In case #1, analysis assumed an autosomal recessive (AR) disease model and focused on genes implicated in hereditary myopathies. This analysis suggested the choline kinase beta (*CHKB*) gene as a possible candidate, where the detailed scrutiny of sequence alignments revealed the causal variant (c.1031+3G>C). Although the mutation was successfully detected its zygosity was incorrectly called suggesting a possible pitfall in WES.

A similar approach was used for case #2, resorting to candidate genes known to be associated with sperm immotility due to flagellar abnormalities. Variants in additional loci were also filtered by Gene Ontology. As a result, two novel variants were identified: a homozygous missense variant (p.Arg35Pro) in the *CCDC103* gene and a novel frameshift variant in the *INSL6* gene (c.262_263delCC).

The experience gathered in the study of these first two patients was important to delineate the analysis strategy for case #3, which shall be presented in this work. We propose a new bioinformatic pipeline for the analysis of AR diseases using WES, combining variant filtering and autozygosity mapping.

Concluding remarks: Considering the present state of the art, WES should be seen as a screening method. There are technical and analytical limitations to be properly addressed in WES before incorporating it in routine diagnostics. Our experience, in line with recent scientific reports, suggests that WES is presently one of the most efficient and cost-effective approaches to study highly heterogeneous rare diseases.

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PHENOTYPIC SPECTRUM OF *DCX* PATHOGENIC MUTATIONS IN FEMALES: FROM CHILDHOOD TO ADULTHOOD CLINICAL ONSET

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Introduction: *DCX*-related disorders (MIM#300067) are caused by pathogenic mutations in the *DCX* gene (MIM*300121; Xq23) that result in abnormal neuronal migration patterns, including isolated lissencephaly sequence (ILS) and subcortical band heterotopia (SBH; also called double cortex). Often occurring in males, ILS causes intellectual disability (ID) and childhood-onset epilepsy. More common in females, SBH is associated with a broad spectrum of clinical features, from ID and epilepsy to normal intelligence without epilepsy. We report two families that illustrate the phenotypic spectrum of *DCX* pathogenic mutations in females, from childhood to adulthood onset.

Patients and methods: Family 1: A 3 years old boy, born to non-consanguineous parents, presented with global developmental delay, seizures and microcephaly. Brain MRI diagnosed fronto-parietal classic lissencephaly. Sequence analysis of *DCX* detected a novel likely pathogenic variant, c.806G>T, p.(Gly269Val), in hemizyosity. Segregation analysis confirmed that his mother, who has mild ID and a frontal simplified gyration pattern shown by brain MRI, carries this variant in heterozygosity. Family 2: A 15 years-old girl, born to non-consanguineous parents, had epilepsy since 4 years old and global developmental delay. Brain MRI showed SBH and the previously reported pathogenic variant c.1150C>T, p.(Arg384*) was identified in *DCX* gene, in heterozygosity. Her mother, who carried the same mutation, had epilepsy with onset at 19 years old, an unremarkable brain MRI and normal intelligence.

Discussion: Pathogenic *DCX* mutations are identified in approximately 40% of males with classic lissencephaly (more severe anteriorly than posteriorly), as well as in 85% of patients with SBH. Given the well-known genotype-phenotype correlation in *DCX*-related disorders, the decision of testing this gene was made based on the clinical and cerebral imaging features of the probands.

A novel likely pathogenic variant was identified in *DCX*, increasing the genotypic spectrum of mutations in this gene. *DCX* mutations were also detected in the probands' mothers, who had previously non-investigated mild ID (family 1) and adult-onset epilepsy (family 2).

DCX-related disorders may not be clinically recognizable in females due to its clinical heterogeneity. Consequently,