Detailed discussion of prenatal findings in patients carrying variants of unclear significance

In **individual F14:II.1**, two compound heterozygous missense variants of uncertain significance in *SLC6A9* were detected. Bi-allelic variants in *SLC6A9* have been associated with glycine encephalopathy with normal glycine levels (OMIM #617301), characterized by arthrogryposis multiplex congenita, severe hypotonia, respiratory failure, and global developmental delay. To our knowledge only ten affected individuals from five independent families have been reported to date.[1] The patient described in this study presented with reduced fetal movements, multiple joint contractures, and retrognathia in ultrasound scan. Autopsy following termination of pregnancy in 27 WGA showed no further organ malformations and a congenital myasthenic syndrome was suspected. Both variants identified in the patient have not been reported in literature today and change weakly conserved amino acids. In addition, there is only small or moderate physiochemical difference between alanine and glycine or serine and cysteine, respectively. The change *SLC6A9* c.167C>G, (p.Ser56Cys) is listed once in an in-house database and three times in the gnomAD browser (10/2021) in a heterozygous state. The variant *SLC6A9* c.245C>G, p.Ala82Gly is observed once in both databases. Taken together, phenotypic overlap and allelic distribution are in line with a disease causal role of the identified missense variants. Nevertheless, additional functional studies or the identification of these changes in further patients with similar phenotypes are needed to establish their pathogenicity.

In **individual F15:II.1**, we identified a missense variant of uncertain significance in *BICD2*. Variants in *BICD2* have been associated with spinal muscular atrophy with predominant involvement of the lower extremities (OMIM #615290 and #618291). Affected individuals present with decreased fetal movements, congenital contractures, benign or slowly progressive muscle weakness and atrophy, delayed motor development, and dysmorphic facial features. Severity of the disease is reportedly highly variable.[2] The patient reported here is an 8-year-old girl who presented with reduced fetal movements in the perinatal period. She was born with bilateral hip dislocation, contractures of knees and ankles, and exhibited foot deformities. She developed atonic seizures, cognitive impairment, muscular hypotonia, atrophy of lower leg muscles, and positive Babinsky sign. A syndromal disease with arthrogryposis multiplex congenita was suspected. The identified missense variant in *BICD2* is listed once in ClinVar as likely pathogenic (ID 648279), however, without detailed clinical information and to our knowledge no additional evidence of pathogenicity in the literature. The variant changes an evolutionary highly conserved glutamine residue to arginine, with just small physiochemical difference between the two amino acids. Because of its position at the 3' end of exon 5 an alternative consequence could be altered splicing efficiency. *BICD2* c.2105A>G, (p.Gln702Arg) is absent from an in-house database and the gnomAD browser(10/2021). Taken together, a relevance of the identified variant is conceivable, but not secured yet. A first step towards proof of possible clinical relevance would be carrier testing of the parents to verify a *de novo* status. However, at the time of the investigation no parental samples were available.

SUPPLEMENTARY REFERENCES

1. Mademont-Soler I, Casellas-Vidal D, Trujillo A, Espuna-Capote N, Maroto A, Garcia-Gonzalez MDM, Ruiz MD, Diego-Alvarez D, Queralt X, Perapoch J, Obon M. GLYT1 encephalopathy: Further delineation of disease phenotype and discussion of pathophysiological mechanisms. Am J Med Genet A 2021;**185**(2):476-85 doi: 10.1002/ajmg.a.61996[published Online First: Epub Date]|.
2. Storbeck M, Horsberg Eriksen B, Unger A, Holker I, Aukrust I, Martinez-Carrera LA, Linke WA, Ferbert A, Heller R, Vorgerd M, Houge G, Wirth B. Phenotypic extremes of BICD2-opathies: from lethal, congenital muscular atrophy with arthrogryposis to asymptomatic with subclinical features. Eur J Hum Genet 2017;**25**(9):1040-48 doi: 10.1038/ejhg.2017.98[published Online First: Epub Date]|.