Appendix 1 - Selected examples of combined variants in the VARS2 gene, clinical manifestations and possible changes in complementary diagnostic tests.

Reference	Variants	Age of onset and prognosis	Global appearance	Impact on Growth/Weight/Feeding	Neurological Signs	Cardiovascular and respiratory signs	Laboratory and other complementary findings
Diodato D et al. (2014), Pereira S et al. (2018), Bruni F et al. (2018), Begliuomini C et al. (2019) [3,6,8,11]	c.1100C>T (p.Thr367Ile) - homozygosity	Often survive to school age, but around five years old, they may experience significant neurological decline	Microcephaly, dysmorphisms (microphthalmia, hypertelorism, epicanthic folds, depressed nose, bulky hands and feet)	Feeding difficulties and poor weight progression	Global psychomotor developmental delay, axial hypotonia and limb hypertonia, limb spasticity and hyperreflexia, ataxia, encephalopathy, and refractory epilepsy	It is not associated with hypertrophic cardiomyopathy or other cardiac alterations	High lactate values and electroencephalogram (EEG) may show increased theta-delta activity and spike-like temporal and occipital elements
Alsemari A et al. (2017) [14]	c.3650G>A - homozygosity	Manifestations since four months of life	Microcephaly, protrusion of the tongue, prognathia and wide-spaced teeth	Short stature, hyperphagia	Severe developmental delay, ataxia, speech impairment, epilepsy and tremor	-	No reported hyperlactacidemia. Severe deficiency of growth hormone, hypogonadism and severe osteomalacia
Bruni F et al. (2018) [8]	c.1135G>A (p.Ala379Thr), c.1877C>A (p.Ala626Asp) – compound heterozygosity	Manifestations since the first few months of life	-	-	Hypotonia, proximal Weakness, global developmental delay, ptosis, ophthalmoparesis, ataxia, generalized epilepsy, dyspraxia	Mild concentric ventricular hypertrophy	No reported hyperlactacidemia. Brain MRI with symmetrical bilateral basal ganglia calcification. Symmetrical increased T2 signal in the peri-trigonal white matter
Ma K et al. (2018) [2]	c.643C>T (p.His215Tyr), c.1354A>G (p.Met452Val) – compound heterozygosity	Severe phenotype since birth with death in the first week of life	Poor activity	Poor sucking	Hyporeflexia, hypertonia	Severe pulmonary hypertension and hypertrophic cardiomyopathy, cyanosis	Severe lactic acidosis with metabolic acidosis. Mild echo enhancement on the bilateral paraventricular parenchyma, left-ependymal cyst and right-choroid plexus cyst

Bruni F et al. (2018) [8]	c.1258G>T (p.Ala420Thr) - homozygosity	Manifestations since birth. Death from the third month of life	-	Poor sucking	Hypotonia, developmental delay, Epilepsy	Hypertrophic cardiomyopathy, respiratory failure	Severe metabolic acidosis
Begliuomini C et al. (2019) [11]	c.1100C>T (p.Thr367Ile), c.601C>T (p.Arg201Trp) – compound heterozygosity	Severe phenotype early in the neonatal period	Microcephaly	Failure to thrive	Hypotonia, psychomotor developmental delay and seizures	Hypertrophic cardiomyopathy with dilatation and reduced ejection fraction	High lactate levels. Brain MRI with symmetrical putamen lesions
Chin HL et al. (2019) [4]	c.1940C>T (p.Thr647Met), c.2318G>A (p.Arg773Gln) – compound heterozygosity	Manifestations since the first few months of life	-	Failure to thrive	Developmental delay	Severe primary pulmonary hypertension	Lactic acidosis
Kušíková K et al. (2021) [1]	c.1168G>A (p.Ala390Thr), c.2758T>C (p.Tyr920His) – compound heterozygosity	Severe phenotype leading to early death at two months of age	-	Could have oral intake (suction)	Neurological status was normal, with no signs of lethargy. Or hypotonia	Hypertrophic cardiomyopathy and pulmonary hypertension	Hyperlactacidemia and metabolic acidosis
Ferreira SL et al. (2023) [12]	1100C>T (p.Thr367Ile), c.1258G>A (p.Ala420Thr) - compound heterozygosity	Manifestations since birth. Survival after six months of life with progressive clinical improvement	-	Adequate growth	Hypotonia, achieved head control	Respiratory distress, need for intubation. Cardiac tamponade and hypertrophic cardiomyopathy. Progressive regression of cardiomyopathy	Increased creatine kinase and hyperlactacidemia
Constante AD et al. (2024) [10]	c.1100C > T (p.Thr367Ile), c.1258G>T (p.Ala420Thr) - compound	Manifestations since birth. Survival with no apparent deterioration of neurological, respiratory, or	-	-	Hypotonia	Respiratory distress, severe hypertrophic cardiomyopathy, cardiac tamponade and cardiomegaly	Metabolic acidosis and fluctuating hyperlactatemia

	heterozygosity	cardiovascular status to date					
Marquez J et al. (2025) [13]	c.1168G>A (p.Ala390Thr) – homozygosity	Severe phenotype detected in utero. Late prematurity. Death in first week of life	-	Small for gestational age	Encephalopathy.	Fetal bradycardia, systolic heart failure; Respiratory failure, required immediate intubation. Severe pulmonary hypertension. Biventricular hypertrophic cardiomyopathy	Lactic acidosis. Small left ventricle and left periventricular echogenicity on cranial ultrasound
Marquez J et al. (2025) [13]	c.842G>A (p.Cys281Tyr) - homozygosity	Manifestations since birth. Survival ranges from one month of life to more than five years	Bilateral ptosis	Gastrostomy for nutritional support	Generalized tonic-clonic seizures, severe proximal appendicular hypotonia, global developmental delay	Respiratory distress, pulmonary hypertension. Biventricular hypertrophic cardiomyopathy	Lactic acidosis, elevated pyruvate. Brain MRI with bilateral periventricular white matter hyperintensities, mineralization of the globi pallidi
Marquez J et al. (2025) [13]	c.673A>G (p.Lys225Glu), c.2193del (p.Leu732Cysfs*29) - compound heterozygosity	Late prematurity. Manifestations since two months of life	-	Gastrostomy for nutritional support	Hypotonia, global developmental delay	Respiratory distress, pulmonary hypertension. Right sided ventricular cardiac dilation and hypertrophy	Transient lactic acidosis. Brain MRI with mildly enlarged ventricles and subarachnoid spaces, mild anterior bilateral periventricular white matter hyperintensi-ties and borderline thin corpus callosum
Our report	c.1079C>T (p.Ala360Val), c.1258G>A (p.Ala420Thr) – compound	Severe phenotype detected in utero. Early neonatal period evolution with progressive	Dysmorphic features (unspecified), hepatomegaly	Severe fetal growth restriction. Severe feeding difficulties requiring nasogastri	Axial hypotonia, mild hypertonia of the extremities, muscle weakness, psychomoto	Severe hypertrophic cardiomyopathy, respiratory distress, need for non-invasive ventilation	Persistent metabolic acidosis and high lactate levels

	heterozygosity	worsening. Death from the second month of life		c tube, short stature	r delay, convulsions		