ESHG

www.nature.com/ejhg

ARTICLE

Check for updates

DAG1 haploinsufficiency is associated with sporadic and familial isolated or pauci-symptomatic hyperCKemia

Monica Traverso[1,12], Serena Baratto[2,12], Michele Iacomino[3], Marco Di Duca[3], Chiara Panicucci[2], Sara Casalini[2], Marina Grandis[4], Antonio Falace[1], Annalaura Torella[5,6], Esther Picillo[5,6], Maria Elena Onore[5,6], Luisa Politano[5,6], Vincenzo Nigro[5,6], A. Micheil Innes[7,8], Rita Barresi[9], Claudio Bruno[2,10], Federico Zara[3,10]✉, Chiara Fiorillo[10,11]✉ and Marcello Scala[3,10]✉

DAG1 encodes for dystroglycan, a key component of the dystrophin-glycoprotein complex (DGC) with a pivotal role in skeletal muscle function and maintenance. Biallelic loss-of-function *DAG1* variants cause severe muscular dystrophy and muscle-eye-brain disease. A possible contribution of *DAG1* deficiency to milder muscular phenotypes has been suggested. We investigated the genetic background of twelve subjects with persistent mild-to-severe hyperCKemia to dissect the role of *DAG1* in this condition. Genetic testing was performed through exome sequencing (ES) or custom NGS panels including various genes involved in a spectrum of muscular disorders. Histopathological and Western blot analyses were performed on muscle biopsy samples obtained from three patients. We identified seven novel heterozygous truncating variants in *DAG1* segregating with isolated or pauci-symptomatic hyperCKemia in all families. The variants were rare and predicted to lead to nonsense-mediated mRNA decay or the formation of a truncated transcript. In four cases, *DAG1* variants were inherited from similarly affected parents. Histopathological analysis revealed a decreased expression of dystroglycan subunits and Western blot confirmed a significantly reduced expression of beta-dystroglycan in muscle samples. This study supports the pathogenic role of *DAG1* haploinsufficiency in isolated or pauci-symptomatic hyperCKemia, with implications for clinical management and genetic counseling.

European Journal of Human Genetics; https://doi.org/10.1038/s41431-023-01516-4

INTRODUCTION

Dystroglycan, encoded by the *DAG1* gene (MIM #128239), is post-translationally cleaved into a transmembrane β-subunit (β-DG) and a highly glycosylated extracellular α-subunit (α-DG) [1]. This glycoprotein plays a crucial role in skeletal muscle function and maintenance, serving as a key component of the dystrophin-glycoprotein complex (DGC) [1]. This structural network connects the extracellular matrix to the intracellular cytoskeleton, providing stability and integrity to the muscle membrane during contraction and relaxation [2]. α-DG interacts with various extracellular matrix proteins, such as laminin and agrin [3]. β-DG binds non-covalently α-DG, connecting it to the intracellular cytoskeleton through dystrophin [4].

Dystroglycan participates in signaling pathways that regulate muscle development and function [1]. The interaction between dystroglycan and its extracellular ligands is crucial for muscle development, regeneration, and maintenance. The binding of α-DG to laminin in the extracellular matrix provides structural support to the muscle fibers and contributes to their alignment and organization [5]. Additionally, α-DG interacts with agrin, a signaling molecule, that is essential for the clustering of acetylcholine receptors at the neuromuscular junction, ensuring proper muscle contraction [6]. Dystroglycan is also involved in signaling pathways that regulate muscle cell differentiation and survival [1]. Activation of dystroglycan signaling can influence intracellular pathways, such as the focal adhesion kinase and the mitogen-activated protein kinase pathways, which play pivotal roles in muscle development, repair, and adaptation [7, 8].

Pathogenic variants in *DAG1* cause different muscular dystrophies and congenital muscular disorders characterized by muscle weakness, atrophy and impaired muscle function, collectively known as dystroglycanopathies [9]. Dysfunctional dystroglycan compromises the stability of the DGC, leading to increased muscle membrane fragility and damage susceptibility [10]. The clinical spectrum of dystroglycanopathy associated with biallelic pathogenic variants in *DAG1* ranges from a muscle eye-brain disease-like phenotype, also reported with multicystic leukodystrophy, to mild limb-girdle muscular dystrophy or asymptomatic hyperCKemia, consisting in an increased value of serum CK beyond 1.5 times the upper limit of normal [11]. Recently, a novel 1-bp deletion heterozygous variant in *DAG1* has been reported in a single Japanese family with asymptomatic hyperCKemia [12]. Isolated

[1]Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [2]Centre of Translational and Experimental Myology, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [3]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [4]IRCCS Ospedale Policlinico San Martino, Genoa, Italy. [5]Department of Precision Medicine, University "Luigi Vanvitelli", Naples, Italy. [6]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy. [7]Department of Medical Genetics and Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [8]Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [9]IRCCS San Camillo Hospital, Venice, Italy. [10]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy. [11]Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [12]These authors contributed equally: Monica Traverso, Serena Baratto. ✉email: federico.zara@unige.it; chiarafiorillo@gaslini.org; mscala.md@gmail.com

Received: 25 August 2023 Revised: 31 October 2023 Accepted: 27 November 2023
Published online: 04 January 2024

asymptomatic hyperCKemia remains a common diagnostic challenge being an unspecific manifestation of different muscular dystrophies, metabolic conditions, and several other neuromuscular disorders [13].

In this study, we identified seven novel truncating variants in *DAG1* in a cohort of 12 individuals from seven unrelated families presenting with isolated asymptomatic or pauci-symptomatic hyperCKemia, providing evidence that these variant lead to reduced expression of the dystroglycan complex on muscle fibers and supporting the causative role of *DAG1* haploinsufficiency in this rare condition.

METHODS

Patient enrollment and clinical assessment

This study adheres to the principles set out in the Declaration of Helsinki. The study was approved by the Research Ethics Committees of the Gaslini Children's Hospital (Comitato Etico della Regione Liguria (163/2018). Informed consent was obtained by the enrolled subjects or their parents or guardians. For clinical assessment, hyperCKemia was defined as a creatine kinase (CK) level beyond 1.5 times the upper limit of normal, as defined in the European Federation of the Neurological Societies guidelines [11].

Next generation sequencing panels

Next-generation sequencing (NGS) custom panels including 74 (#1-11) or 90 (#12) different genes (Table S1) associated with limb-girdle muscular dystrophy, glycogen or lipid metabolism, muscular myopathies, or channelopathy were performed on genomic DNA extracted from the peripheral blood of enrolled subjects. The target gene panels were performed in the Laboratory of Medical Genetic of Giannina Gaslini Institute (Genova) and Department of Medical Genetics and Pediatrics of Cumming School of Medicine (University of Calgary, Calgary, Alberta) with Ampliseq/Ion Torrent PGM technology. The variants were analyzed using IOn Reporter and CLC Bio Genomics Workbench 7.5.1 software (CLC Bio, Aarhus, Denmark).

Exome sequencing

Exome sequencing (ES) was performed for the Family GE-1266 as previously described [14]. Variants were filtered out for minor allele frequency (MAF) ≤ 0.01 in genomic databases (GnomAD, https://gnomad.broadinstitute.org). Then, in silico tools were employed to predict the impact of candidate variants on protein structure and function, including CADD score (https://cadd.gs.washington.edu), SIFT (https://sift.bii.a-star.edu.sg), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and Splice AI (https://spliceailookup.broadinstitute.org). Candidate variants were validated by Sanger sequencing (Table S2) [14]. All variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines. Further details available in Supplementary Information. *DAG1* variants are reported according to the NM_001177643.2 RefSeq transcript.

Histopathology studies

Muscle biopsies were obtained from three participants (#3, #5, and #12) from the quadriceps muscle. Samples were prepared according to standard procedures and routine staining on 7 µm sections including Hematoxilin and Eosin (H&E). Mouse monoclonal Anti-α-DG Antibody, clone VIA4-1(Merck), and mouse monoclonal anti-Beta-dystroglycan 43DAG1/8D5 (Monosan) were employed for immunofluorescence study, as previously described. Western Blot (WB) on total protein lysates in Urea10M/DTT50 mM was performed with NCL-b-DG mouse monoclonal antibody (Novocastra) and anti-Vinculin hVIN-1 mouse monoclonal antibody (Sigma), to assess sample normalization. Band intensity was quantified with ImageJ 1.52i (https://imagej.nih.gov/ij). Further information available in the Supplementary Material.

RESULTS

Clinical data

The study cohort consisted of twelve individuals from seven unrelated and nonconsanguineous families, including eight familial cases (Table 1). The age of the enrolled subjects ranged from 8 to 61 years, with a 1:1 sex ratio. In eight out of twelve subjects hyperCKemia was an incidental finding in the context of routine laboratory testing, whereas it was found in association with mild muscular manifestations in the remaining four individuals. These included muscle cramps (#2), myalgia (#4 and #5), and exercise intolerance (#6). In some cases, additional features were observed over time, including weakness affecting the lower girdle (#6), decreased deep tendon reflexes (#1 and #2), and exercise intolerance (#2). The age at the first identification of hyperCKemia ranged from 4 to 41 years. Overall, CK levels ranged from a minimum of 195 U/L to a maximum of 3054 U/L. Muscle MRI was available in four subjects, showing absence of fat or fibrotic infiltration in all cases.

Molecular findings

ES and custom NGS panels led to the identification of seven novel variants in the *DAG1* gene segregating with the phenotypes in all the reported subjects, including three frameshift and four stop-gain changes. The variants were inherited from a similarly affected parent in four cases (#1, #3, #8, and #9), while they were presumed to have occurred de novo in patient #5 (Fig. 1A). Comprehensive segregation information was not available for patients #6, #7, and #10. All the variants are very rare, being absent in the gnomAD database, and predicted to lead to nonsense-mediated mRNA decay or the formation of a truncated transcript (Table S2). The *DAG1* variants are scattered across the protein structure (Fig. 1B). None of these changes has been previously associated with the muscular dystrophy-dystroglycanopathy complex. The p.(Arg640*) variant is reported as variant of unknown significance (VUS) in ClinVar (ID 2418757) in a subject with unknown phenotype. All the identified *DAG1* variants are classified as likely pathogenic (class IV) or pathogenic (class V) according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines. The identified variants have been deposited in the Leiden Open Variation Database database with the following accession numbers: #0000931504, #0000931505, #0000931506, #0000931507, #0000931508, #0000931509, and #0000931510.

Histological analysis and Western blot

Histological analysis of the muscle samples from #3 and #5 revealed mild nonspecific abnormalities, such as fiber size variation and presence of internal nuclei at Hematoxylin and Eosin staining (Fig. 2A). Similar abnormalities were observed in #12 (not shown). No relevant changes were observed with other histological and histochemical techniques performed. Immunofluorescence analysis revealed a reduced signal intensity of both α-DG and β-DG in subjects #3 and #5 (Fig. 2A). To confirm these results, we performed a WB analysis on muscle lysates obtained from biopsy. WB probed with anti-β-DG showed a decreased protein expression, with 43% of normalized expression in both patients compared to the control (Fig. 2B, C), although a more severe decrease appeared to be present in subject #5 by immunofluorescence. The observed discrepancy is possibly due to technical issues, such as variability in the fluorescent signal between separate test runs.

DISCUSSION

Functional and structural deficiencies of dystroglycan lead to muscular dystrophies and impair muscle integrity and function [1, 4]. Abnormalities in the glycosylation of α-DG may lead to a wide spectrum of dystroglycanopathies [1]. Primary dystroglycanopathies arise from genetic variants directly affecting glycosylation enzymes, resulting in disrupted α-DG glycosylation [15]. Secondary dystroglycanopathies are far more common and includes conditions characterized by a secondary deficit in α-DG glycosylation, such as hypoglycosylation due to metabolic defects or other disorders caused by variants in glycosylation-related genes

Table 1. Summary of clinical and laboratory features of heterozygous *DAG1* patients.

Pt #	Gender	Age	Variant (NM_001177643.2)	Status	ACMG class	Age at first identification	Symptoms at onset	CK min (U/L)	Ck max (U/L)	Weakness	Other symptoms	Muscle MRI	Muscle biopsy
1	M	10 y	c.832delG, p.(Glu278Argfs*105)	Maternal	IV	4 y	Incidental hyperCKemia	403	1832	No	↓ DTRs	NA	NA
2	F	30 y	c.832delG, p.(Glu278Argfs*105)	NA	IV	10 y	Muscle cramps	395	2240	No	↓ DTRs, exercise intolerance	No fat or fibrotic infiltration	Mild fiber size variability; ↓ αDC
3	F	8 y	c.1918C>T, p.(Arg640*)	Maternal	IV	4 y	Incidental hyperCKemia	671	1445	No	No	NA	NA
4	F	29 y	c.1918C>T, (p.Arg640*)	NA	IV	14 y	Myalgia	N	450	No	No	NA	NA
5	M	28 y	c.1925_1926delGT, p.(Cys642*)	*Presumed* de novo[a]	V	13 y	Myalgia	468	1383	No	No	Diffuse ↑↑ of muscle bulk without fat or fibrotic infiltration	Mild fiber size variability; central nuclei; 1 degenerative fiber; ↓↓ αDC
6	F	39 y	c.71_72delTG, p.(Val24Glyfs*10)	NA	V	19 y	Exercise intolerance	310	598	Yes, lower girdle (MRC 4)	No	No fat or fibrotic infiltration	NA
7	M	16 y	c.2167delC, p.(Pro723Hisfs*50)	NA	V	9 y	Incidental hyperCKemia	385	1300	No	No	No fat or fibrotic infiltration	NA
8	M	42 y	c.164C>G, p.(Ser55*)	Maternal	IV	19 y	Incidental hyperCKemia	202	911	No	No	NA	NA
9	F	61 y	c.164C>G, p.(Ser55*)	NA	IV	41 y	Incidental hyperCKemia	195	304	No	No	NA	NA
10	F	73 y	c.164C>G, p.(Ser55*)	NA	IV	51 y	Incidental hyperCKemia	220	270	No	No	NA	NA
11	M	31 y	c.164C>G, p.(Ser55*)	NA	IV	8 y	Incidental hyperCKemia	502	988	No	No	NA	NA
12	M	19 y	c.330G>A, p.(Trp110*)	NA[b]	V	9 y	Incidental hyperCKemia	511	2800	No	No	NA	Mild fiber size variaility; ↓ αDC

αDC alpha dystroglycan, *DTRs* deep tendon reflexes,*MRC* Medical Research Council, *N* normal, *y* years.
[a]Mother not available for segregation.
[b]Presumed de novo (father not available for segregation).

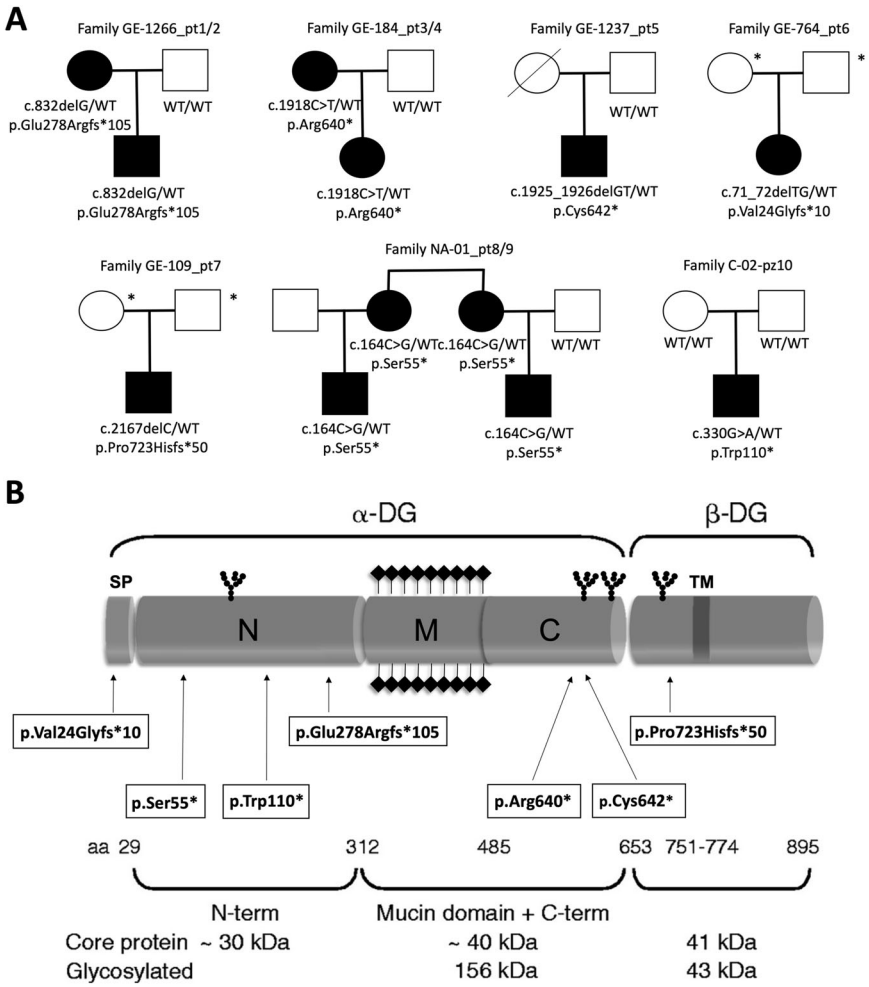

Fig. 1 Summary of genetic findings in heterozygous *DAG1* patients. A Pedigrees of *DAG1* families. Loss-of-function *DAG1* variants segregate with the hyperCKemia phenotype in all patients. In three families (Family GE-1266_pt1/2, Family GE-184_pt3/4, and Family NA-01_pt8/9), the variants are inherited from a parent presenting with an overlapping clinical phenotype. **B** Schematic drawing of the dystroglycan protein (NP_001159400.3) showing the localization of the variants identified in our cohort. The variants are scattered across the protein. All the identified *DAG1* variants are truncating and predicted to lead to either nonsense-mediated mRNA decay (NMD) or the generation of a truncated transcript. Adapted from ref. [1] (PMID: 16410545).

indirectly affecting the glycosylation process [16]. Disrupted glycosylation impairs the binding of dystroglycan to extracellular matrix proteins, leading to muscle fiber degeneration and neuronal migration defects in the brain [9].

The wide spectrum of dystroglycanopathies ranges from severe congenital muscular dystrophies with significant brain and eye involvement, such as Walker-Warburg syndrome (WWS, MIM # 236670) and Muscle-Eye-Brain disease (MEB, MIM # 253280), to milder forms like limb-girdle muscular dystrophy with no significant cognitive impairment [17]. Affected individuals present with muscle weakness, hypotonia, and elevated CK levels, often associated with structural brain abnormalities and cognitive deficits [17]. Biallelic variants in *DAG1* can disrupt the glycosylation process, leading to a reduction in the functional glycosylated dystroglycan and subsequent muscular dystrophy [18]. Different types of variants in the *DAG1* gene and in genes involved in the glycosylation of dystroglycan have been reported, contributing to the heterogeneous dystroglycanopathies spectrum [19].

Different types of *DAG1* variants have been reported in association with *DAG1*-related dystrophy, either in homozygous or compound heterozygous status [18, 20, 21]. Although no clear genotype-phenotype correlations emerged, severe muscular and cognitive phenotypes appear to be most likely associated with truncating and more damaging missense variant [20, 21]. This supports a correlation between the degree of dystroglycan functional deficiency and the severity of clinical manifestations, suggesting a possible gene dosage effect [20, 21]. In our cohort, we identified seven novel truncating variants in *DAG1* segregating with isolated or pauci-symptomatic hyperCKemia. All these variants are very rare and are predicted to lead to a loss of function. Despite these variants are only present in heterozygous status in our patients, our observations support the idea that *DAG1* haploinsufficiency can be sufficient to affect skeletal muscle structure and function, leading to an extremely mild muscular phenotype.

DAG1 variants have been only occasionally associated with pauci-symptomatic hyperCKemia. The c.220G>A, p.(Val74Ile) and c.331G>A., p.(Asp111Asn) variants were detected in trans in a patient with a mild muscular phenotype described as asymptomatic hyperCKemia [21]. However, this subject also showed calf pseudohypertrophy, low CT intensity in lower limbs muscles, and muscular dystrophy-like appearance in muscle biopsy, suggestive of mild muscular dystrophy [21]. Furthermore, these variants are observed in homozygous state in one and three healthy controls in gnomAD for the p.(Val74Ile) and p.(Asp111Asn), respectively. They are predicted to be only

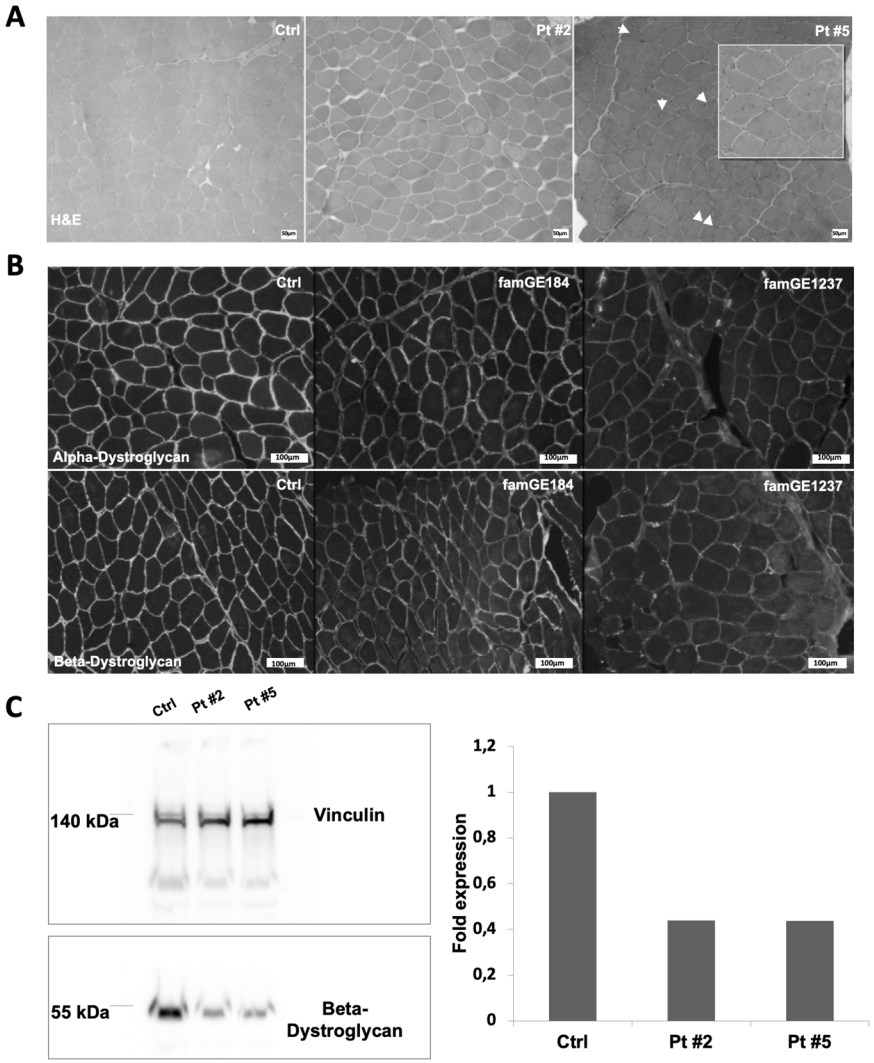

Fig. 2 Immunohistochemical findings in heterozygous *DAG1* patients. A–C Histopathology studies on muscle biopsy samples from patients #3 and #5. **A** Hematoxylin and Eosin staining shows mild nonspecific abnormalities, consisting of mild fiber size variability and the presence of internal nuclei in several muscle fibers (white arrows and highlighted in the embedded square box). **B** Immunofluorescence analysis of members of the dystrophin glycoprotein complex performed on muscle samples of patients #3 and #5 showed significantly reduced signal intensity of beta-dystroglycan protein in both subjects. The differences in the intensity between the two samples may be related to the diverse conditions in which the two pictures were taken and could be influenced by the older age at biopsy in patient #5 compared to #3. **C** Western Blot analysis probed with anti-beta-dystroglycan on muscle lysates obtained from the same biopsy samples showed a decreased protein expression, with only 43% of normalized expression in both subjects compared to the control.

moderately damaging by in silico tools and are classified as VUS according to ACMG/AMP guidelines. A large de novo 2-Mb deletion on chromosome 3 encompassing *DAG1* among other genes was reported in a subject with learning difficulties, white matter abnormalities, hyperCKemia, dyspraxia and facial hypotonia [13]. However, the only heterozygous *DAG1* variant so far associated with isolated hyperCKemia is the truncating c.931del, p.(Arg311Glyfs*72) [12]. This is a class IV variant identified in four members of a Japanese family, including the proband, two siblings, and the mother [12]. These subjects showed abnormal CK levels associated with muscular deficits most likely related to exercise intolerance [12]. Although similar manifestations were observed in some of our cases, other subjects were totally asymptomatic. Indeed, elevated CK levels were detected as incidental findings in seven out of twelve subjects (#3 and #7-12), supporting the association between *DAG1* haploinsufficiency and isolated hyperCKemia. Based on the very few patients reported, genotype-phenotype correlations are lacking in heterozygous *DAG1* patients, as the same variant may lead to either isolated or pauci-symptomatic hyperCKemia in members of the same family (Table 1) [12].

Diagnosing dystroglycanopathies can be a complex process, especially in the case of mild disorders or pauci-symptomatic hyperCKemia, which may underlie a progressive muscular disorder prompting follow-up surveillance [22, 23]. No specific guidelines have been approved for the clinical and diagnostic management of this condition [23]. However, the combination of electrophysiological data, biochemical tests, and genetic investigations may be a valuable approach [23]. The immunostaining of muscle biopsies can reveal reduced expression of glycosylated dystroglycan, while genetic testing may be helpful to identify biallelic variants in *DAG1* and related glycosylation genes [22]. NGS panels have improved diagnostic accuracy and provided insights into the genetic landscape of dystroglycanopathies, also proving advantageous in the identification of the genetic etiology in isolated hyperCKemia [23].

The recent improvement of sequencing techniques combined with the availability of new diagnostic methods and techniques has remarkably implemented the identification and detailed characterization of biochemical, clinical, and radiological phenotypes, also providing insightful hints in terms of precision medicine approaches [24, 25]. These advancements may be especially helpful in patients with only mild disease manifestations, allowing to detect disease causing variants and to comprehensively dissect mild clinical and biochemical phenotypes [24, 25]. Traditionally, Genetic disorders have been classified as monogenic or polygenic/genetically complex diseases [26, 27]. However, this dichotomy is being reconsidered in light of the emerging concept of genetically transitional disease (GTD) [28]. This novel concept implies that a certain disease or disease status lies in between the upper and lower extremes of a genetic continuum represented by monogenic and polygenic conditions, respectively [28]. In this context, the presence of a highly damaging pathogenic variant in a specific gene is necessary, but not sufficient, to cause the disorder, while the genetic background plays a pivotal role in influencing the penetrance and the expressivity [28]. In line with this revolutionary concept, our observations support *DAG1*-related disorder as a GTD, in which the phenotypic heterogeneity reflects the genetic diversity of affected individuals. Indeed, the presence of additional variants in the genetic background of *DAG1* subjects might modulate the expression and penetrance of the clinical phenotype. Future studies will be crucial to investigate and potentially identify genetic and epigenetic factors able to modify penetrance and expressivity in dystroglycanopathies.

The presence of milder clinical manifestations in patients harboring heterozygous variants in genes associated with recessive disorders has been reported in a number of autosomal and X-linked recessive conditions. These include either neuromuscular and non-neuromuscular disorders (Table 2). For example, hyperCKemia can be a biochemical marker in carriers of disease-causing variants in *RYR1* (MIM * 180901) or *SGCA* (MIM * 600119), associated with a recessive congenital myopathy (MIM # 255320) and a limb-girdle muscular dystrophy (MIM # 608099), respectively. Similarly, hyperCKemia can be a marker in female carriers harboring heterozygous rearrangements in the *DMD* gene (MIM * 300377), associated with Duchenne muscular dystrophy (MIM # 310200). Similarly, beyond neuromuscular disorders, the presence of elevated Hemoglobin A2 (HbA2) and very long chain fatty acid (VLCFA) levels can be reliable biochemical marker of heterozygous beta-thalassemia and *ABCD1*-related adrenoleukodystrophy (Table 2) [29].

The identification of heterozygous loss-of-function variants in *DAG1* in subjects with isolated or pauci-symptomatic hyperCKemia may be also relevant in terms of genetic counseling. Although it is a rare circumstance, children born to parents who are asymptomatic *DAG1* carriers have a 25% chance of inheriting both *DAG1* variants and develop the full dystrophy phenotype. However, the identification of elevate serum CK levels may serve as a biochemical marker for a possible *DAG1* carrier status, prompting a genetic investigation to search for loss-of-function variants in the *DAG1* gene. Although subjects with asymptomatic hyperCKemia are not routinely screened by genetic testing, our study supports NGS as a first-tier diagnostic test in these patients. Indeed, the identification of a *DAG1* variant in a subject with hyperCKemia may be helpful to guide follow-up clinical management and implement prenatal genetic testing strategies. Additionally, this information can be particularly relevant for the genetic counseling of families in which CK testing is employed as a method for the early identification of a muscular disorder, targeting the pre-symptomatic diagnosis of Duchenne muscular dystrophy or other muscular dystrophies [30, 31]. In these families, the absence of a potentially disease-causing variant in genes associated with muscular dystrophies and the presence of a

Table 2. Examples of mild clinical phenotypes in carriers of disease-causing variants.

Gene	MIM *	Disease	MIM #	Inheritance	Biomarker	Clinical features	PMIDs
ABCD1	300371	Adrenoleukodystrophy	300100	XLR	Abnormal pattern of VLCFA	-	26718981
CTP2	600650	Carnitine palmitoyltransferase II deficiency, myopathic, stress-induced	255110	AR	Residual CPT activity	Myalgia, muscle weakness, rhabdomyolysis	15622536, 23184072
DMD	300377	Muscular dystrophy, Duchenne type	310200	XLR	HyperCKemia	Mild muscle weakness, calf hypertrophy	26718981
EMD	300384	Emery-Dreifuss muscular dystrophy 1, X-linked	310300	XLR	-	Heart involvement, cardiac symptoms later in life	25454731, 31718017
GJB1	304040	Charcot-Marie-Tooth disease, X-linked dominant, 1	302800	XLD	Abnormal nerve conduction velocities	Mild neuropathy, absent tendon reflexes	21254193
PLP1	300401	Pelizaeus-Merzbacher disease	312080	XLR	-	White matter abnormalities at MRI, mild neurological signs (spasticity, ataxia, cognitive impairment)	31137068, 23771846
RYR1	180901	Congenital myopathy 1B, autosomal recessive	255320	AR	HyperCKemia	Exertional myalgia, unexplained rhabdomyolysis, mild weakness	35428369
SGCA	600119	Muscular dystrophy, limb-girdle, autosomal recessive 3	608099	AR	HyperCKemia	Mild muscle weakness, exercise intolerance	14595658

AD autosomal dominant, *AR* autosomal recessive, *CPT* Carnitine palmitoyltransferase, *VLCFA* very long chain fatty acid, *XLD* X-linked dominant, *XLR* X-linked recessive.

heterozygous truncating variant in *DAG1* may reveal helpful to increase the confidence in a diagnosis of *DAG1*-related hyperCKemia rather than a severe muscular disorder. Aside from the diagnostic relevance, this may be particularly beneficial for these families, largely reducing the remaining apprehensions regarding the possible presence of disease-causing variants in introns or other non-coding regions of neuromuscular genes [32].

Our study has limitations. First, the patients enrolled in our study were investigated through targeted panel sequencing, using panels designed to identify genetic variants in genes specifically involved in neuromuscular disorders. Although every effort is made to keep these panels up to date, it is plausible that recently discovered disease genes are not included yet in the gene lists, especially in the era of rapid gene discovery. Thus, it is not possible to exclude that the subjects reported harbor variants in other neuromuscular genes, possibly influencing the expressivity of the observed phenotype. A second related limitation is the lack of a thorough genetic information in all patients but #1 and #2, who underwent exome sequencing. More specifically, although the phenotypes of the reported subjects hardly suggest an involvement of extra-neuromuscular genes, this circumstance cannot be excluded due to the lack of information from extensive exome or genome sequencing.

In conclusion, our findings support the association of heterozygous loss-of-function variants in *DAG1* with a mild emerging muscular phenotype characterized by isolated or pauci-symptomatic hyerCKemia. This condition can affect different members within a family and remain long undiagnosed due to the mild clinical presentation, with a risk of leading to a full dystrophy phenotype in the offspring of unaware carrier parents. Our study suggests that NGS panels may be helpful to identify the underlying genetic cause of hyperCKemia, allowing to improve clinical surveillance and genetic counseling. The report of further pauci-symptomatic hyperCKemia cohorts and the identification of novel causative genes will be essential to further implement our knowledge on this intriguing condition.

DATA AVAILABILITY

All data described in this study are provided within the article and Supplementary Material. Raw sequencing data and de-identified clinical data are available from the corresponding authors upon request.

REFERENCES

1. Barresi R, Campbell KP. Dystroglycan: from biosynthesis to pathogenesis of human disease. J Cell Sci. 2006;119:199–207.
2. Ervasti JM, Campbell KP. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol. 1993;122:809–23.
3. Gee SH, Montanaro F, Lindenbaum MH, Carbonetto S. Dystroglycan-alpha, a dystrophin-associated glycoprotein, is a functional agrin receptor. Cell. 1994;77:675–86.
4. Henry MD, Campbell KP. Dystroglycan inside and out. Curr Opin Cell Biol. 1998;10:602–7.
5. Cohn RD, Henry MD, Michele DE, Barresi R, Saito F, Moore SA, et al. Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. Cell. 2002;110:639–48.
6. Jacobson C, Côté PD, Rossi SG, Rotundo RL, Carbonetto S. The dystroglycan complex is necessary for stabilization of acetylcholine receptor clusters at neuromuscular junctions and formation of the synaptic basement membrane. J Cell Biol. 2001;152:435–50.
7. Hohenester E, Yurchenco PD. Laminins in basement membrane assembly. Cell Adh Migr. 2013;7:56–63.
8. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. Curr Opin Genet Dev. 2002;12:349–61.
9. Barresi R, Michele DE, Kanagawa M, Harper HA, Dovico SA, Satz JS, et al. LARGE can functionally bypass alpha-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. Nat Med. 2004;10:696–703.
10. Moore SA, Saito F, Chen J, Michele DE, Henry MD, Messing A, et al. Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature. 2002;418:422–5.
11. Kyriakides T, Angelini C, Vilchez J, Hilton-Jones D. European Federation of the Neurological Societies guidelines on the diagnostic approach to paucisymptomatic or asymptomatic hyperCKemia. Muscle Nerve. 2020;61:E14–E15.
12. Fan L, Miura S, Shimojo T, Sugino H, Fujioka R, Shibata H. A novel 1-bp deletion variant in DAG1 in Japanese familial asymptomatic hyper-CK-emia. Hum Genome Var. 2022;9:4.
13. Frost AR, Böhm SV, Sewduth RN, Josifova D, Ogilvie CM, Izatt L, et al. Heterozygous deletion of a 2-Mb region including the dystroglycan gene in a patient with mild myopathy, facial hypotonia, oral-motor dyspraxia and white matter abnormalities. Eur J Hum Genet. 2010;18:852–5.
14. Scala M, Nishikawa M, Ito H, Tabata H, Khan T, Accogli A, et al. Variant-specific changes in RAC3 function disrupt corticogenesis in neurodevelopmental phenotypes. Brain. 2002;145:3308–27.
15. Godfrey C, Clement E, Mein R, Brockington M, Smith J, Talim B, et al. Refining genotype phenotype correlations in muscular dystrophies with defective glycosylation of dystroglycan. Brain. 2007;130:2725–35.
16. Sparks S, Quijano-Ro, S, Harper A, et al. Congenital muscular dystrophy overview. In: Adam MP, Ardinger HH, Pagon RA, et al. editors. GeneReviews® Seattle (WA): University of Washington, Seattle; 2015. https://doi.org/10.1016/j.ajpath.2015.08.010.
17. Godfrey C, Foley AR, Clement E, Muntoni F. Dystroglycanopathies: coming into focus. Curr Pediatr Rep. 2013;1:166–75.
18. Hara Y, Balci-Hayta B, Yoshida-Moriguchi T, Kanagawa M, Beltrán-Valero de Bernabé D, Gündeşli H, et al. A dystroglycan mutation associated with limb-girdle muscular dystrophy. N Engl J Med. 2011;364:939–46.
19. Song D, Dai Y, Chen X, Fu X, Chang X, Wang N, et al. Genetic variations and clinical spectrum of dystroglycanopathy in a large cohort of Chinese patients. Clin Genet. 2021;99:384–95.
20. Geis T, Marquard K, Rödl T, Reihle C, Schirmer S, von Kalle T, et al. Homozygous dystroglycan mutation associated with a novel muscle-eye-brain disease-like phenotype with multicystic leukodystrophy. Neurogenetics. 2013;14:205–13.
21. Dong M, Noguchi S, Endo Y, Hayashi YK, Yoshida S, Nonaka I, et al. DAG1 mutations associated with asymptomatic hyperCKemia and hypoglycosylation of α-dystroglycan. Neurology. 2015;84:273–9.
22. Kanagawa M. Dystroglycanopathy: from Elucidation of molecular and pathological mechanisms to development of treatment methods. Int J Mol Sci. 2021;22:13162.
23. Gemelli C, Traverso M, Trevisan L, Fabbri S, Scarsi E, Carlini B, et al. An integrated approach to the evaluation of patients with asymptomatic or minimally symptomatic hyperCKemia. Muscle Nerve. 2022;65:96–104.
24. Scala M, Bianchi A, Bisulli F, Coppola A, Elia M, Trivisano M, et al. Advances in genetic testing and optimization of clinical management in children and adults with epilepsy. Expert Rev Neurother. 2020;2:251–69.
25. Boycott KM, Hartley T, Biesecker LG, Gibbs RA, Innes AM, Riess O, et al. A diagnosis for all rare genetic diseases: the horizon and the next frontiers. Cell. 2019;177:32–37.
26. Kaplan J. Genomics and medicine: hopes and challenges. Gene Ther. 2002;9:658–61.
27. Muse ED, Chen SF, Torkamani A. Monogenic and polygenic models of coronary artery disease. Curr Cardiol Rep. 2021;23:107.
28. Yao Q, Gorevic P, Shen B, Gibson G. Genetically transitional disease: a new concept in genomic medicine. Trends Genet. 2023;39:98–108.
29. Colaco S, Nadkarni A. Borderline HbA2 levels: dilemma in diagnosis of beta-thalassemia carriers. Mutat Res Rev Mutat Res. 2021;788:108387.
30. de Freitas Nakata KC, da Silva Pereira PP, Salgado Riveros B. Creatine kinase test diagnostic accuracy in neonatal screening for duchenne muscular dystrophy: a systematic review. Clin Biochem. 2021;98:1–9.
31. Park S, Maloney B, Caggana M, Tavakoli NP. Creatine kinase-MM concentration in dried blood spots from newborns and implications for newborn screening for Duchenne muscular dystrophy. Muscle Nerve. 2022;65:652–8.
32. Nicolau S, Milone M, Liewluck T. Guidelines for genetic testing of muscle and neuromuscular junction disorders. Muscle Nerve. 2021;64:255–69.

ACKNOWLEDGEMENTS

We would like to thank the patients and their families for their consent and valuable support to our work.

AUTHOR CONTRIBUTIONS

Conceptualization: MS. Data curation: MT, SB, MS. Formal analysis: MT, SB, MDD, CP, MI, SC, MG, FZ, RB, AF, AT, EP, LP, VN, MI, CB, CF, MS. Methodology: MT, SB, MS. Writing—original draft: MT, SB, MS. Writing—review & editing: CB, CF, MS. Supervision: FZ and MS.

FUNDING

No specific funding.

COMPETING INTERESTS

The authors have no competing interests.

ETHICS DECLARATION

This study adheres to the principles in the Declaration of Helsinki. The study was reviewed by IRCCS Istituto Giannina Gaslini Review Board (IRB) (Comitato Etico della Regione Liguria, protocol 163/2018) protocol. Written informed consent was obtained from all participants including consent for publication of photographs as required by the IRB. Consent forms are archived and available upon request.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41431-023-01516-4.

Correspondence and requests for materials should be addressed to Federico Zara, Chiara Fiorillo or Marcello Scala.