ACTA MYOL 2025;XLIV:93-95;
DOI: 10.36185/2532-1900-1424

ORIGINAL ARTICLE

National diagnostic gaps for TK2 Deficiency in Italy: insights from the AIM Multicenter Survey

Michelangelo Mancuso[1], Costanza Lamperti[2], Olimpia Musumeci[3]

[1] Department of Clinical and Experimental Medicine, Neurological Institute, University of Pisa, Pisa, Italy; [2] Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy; [3] Department of Clinical and Experimental Medicine, Neurological Institute, University of Messina, Messina, Italy

Objective. Thymidine kinase 2 (TK2) deficiency is a rare mitochondrial disease with variable phenotypes and emerging treatments. Prompt diagnosis is essential to optimize patient outcomes and management. To assess the current awareness, diagnostic approaches, and readiness to include TK2 screening in Italian neuromuscular clinical practice.
Methods. A nationwide survey was distributed to AIM-affiliated clinicians. The questionnaire assessed TK2 awareness, diagnostic pathways, gene panel content, and attitudes towards screening in unresolved cases.
Results. while awareness of TK2 deficiency was almost universal, inclusion of TK2 in genetic panels varied: 85% in metabolic myopathy panels, 56% in LGMD panels. Screening for TK2 in genetically unsolved SMA, FSHD, and OPMD phenotypes was inconsistent.
Conclusions. Although awareness of TK2 deficiency is widespread, diagnostic strategies are inconsistent. Standardizing *TK2* inclusion in NGS panels and promoting differential screening are key steps toward earlier diagnosis in the view of future treatment options.

Key words: TK2, mitochondrial disease, awareness

Introduction

Thymidine kinase 2 (TK2) deficiency (TK2d) is an autosomal recessive disorder caused by mutations in the *TK2* gene, which encodes a mitochondrial matrix enzyme essential for the phosphorylation of deoxythymidine and deoxycytidine [1]. These nucleotides are critical for maintaining the mitochondrial DNA (mtDNA) pool, and their disruption leads to mtDNA depletion or multiple deletions. TK2 deficiency belongs to the group of mitochondrial DNA maintenance disorders and has a broad phenotypic spectrum that spans from severe infantile myopathy with rapid progression and early respiratory failure to milder, late-onset myopathies presenting in adulthood [2].

The clinical heterogeneity of TK2 deficiency often makes diagnosis challenging. Early-onset forms typically present with hypotonia, motor regression, and encephalopathy, while late-onset phenotypes may mimic limb-girdle muscular dystrophy (LGMD), facioscapulohumeral dystrophy (FSHD), oculopharyngeal muscular dystrophy (OPMD), or spinal muscular atrophy (SMA) [3]. Muscle MRI often reveals a suggestive muscle pattern involvement, and biopsy may show both dystrophic and neurogenic features with variable mitochondrial abnormalities [2].

In recent years, treatment options have expanded. Notably, nucleoside replacement therapy has shown promising results, especially when administered early[4], and its approval is currently under evaluation at both FDA and EMA. This therapeutic advance emphasizes the importance of timely and accurate diagnosis. However, awareness of TK2d remains variable, and the gene is not consistently included in next-generation sequencing (NGS)

Received: June 18, 2025
Accepted: Septermber 10, 2025

Correspondence
Michelangelo Mancuso
E-mail: michelangelo.mancuso@unipi.it

How to cite this article: Mancuso M, Lamperti C, Musumeci O. National diagnostic gaps for TK2 Deficiency in Italy: Insights from the AIM Multicenter Survey. Acta Myol 2025;44:93-95. https://doi.org/10.36185/2532-1900-1424

panels tailored to inherited myopathies.
To assess national awareness of TK2d, the Associazione Italiana di Miologia (AIM) launched the 'TK2 Hunter Project'. The primary objectives were to map diagnostic practices across Italian centres, evaluate clinician knowledge on TK2d, and determine the extent to which TK2d is considered in differential diagnoses of unsolved myopathies.

Methods

A 12-question survey was distributed electronically to AIM members in Italy, approximately 400, the majority of whom are affiliated with the 34 AIM clinical centers (https://miologia.org/pages/centri-clinici-associati), with additional contributors from other institutions The questionnaire explored various domains including clinical experience with TK2d, composition of NGS panels and the use of differential screening in specific phenotypes. The survey was conducted online, with 3 reminders, from January to early September 2025. Data were anonymized and aggregated.

Results

Out of approximately 400 AIM members invited to participate, 46 clinicians (about 12%) fully completed the survey. While some respondents were not affiliated with the AIM clinical centers, the sample nonetheless included representatives from 30 of the 34 AIM centers (89%). This distribution supports the representativeness of the findings across the Italian network of clinicians with expertise in neuromuscular diseases. The Table I summarizes the results (available in more detail as supplementary file).
Survey respondents were broadly distributed across Italy, with 30% affiliated to hospitals in Northern Italy, 27% in Central Italy, and 26% in Southern Italy and the Islands. The majority (67%) reported a background in adult neurology, and 70% (32 out of 46) indicated that their institution has a dedicated centre for mitochondrial diseases. Awareness of TK2d was almost universal among respondents (96%), and 26% (12 out of 46) reported having diagnosed or followed at least one patient with confirmed TK2-related mitochondrial disease.
Despite high awareness, the integration of *TK2* gene into routine diagnostic panels varied. Specifically, 85% of respondents indicated that *TK2* is included in their institution's NGS panel for metabolic myopathies (82% in paediatric centers), while only 56% reported its inclusion in panels for LGMD (percentage raise to 73% in paediatric centers). This discrepancy highlights a potential gap in panel design, particularly for adult-onset or atypical presentations of TK2d.
When asked about their diagnostic approach in genetically unsolved phenotypes, clinician responses were variable. For patients with an FSHD-like phenotype who tested negative for D4Z4 contractions, only 57% of respondents (even less, 18%, in paediatric centres), indicated they would consider *TK2* screening, despite the phenotypic overlapping. A similar proportion (50%) reported considering *TK2* in cases of SMA-like phenotypes where SMN1 testing was negative and in 52% in OPMD-like presentations with negative genetic test. Notably, none of the participating paediatric centres reported screening for TK2d in patients presenting with SMA-like phenotypes who tested negative for SMN1, and only 9% did so in cases with OPMD-like presentations. These data suggest that, while TK2d is increasingly recognized as a possible diagnosis in overlapping phenotypes, it is still not systematically included in follow-up testing after initial negative results.
Importantly, most clinicians (89%) reported proceeding with broader genetic investigations such as clinical or whole-exome sequencing (WES), or whole-genome sequencing (WGS)when initial targeted panels fail to identify a diagnosis. This suggests a general trend toward comprehensive genomic testing, which is essential for detecting rare disorders like TK2d, especially in atypical or late-onset cases. Of note, 32% of respondents reported the use of WES or WGS in unresolved cases, guided by the clinical phenotype rather than applied systematically; therefore, the recognition of TK2d clinical features remains a major challenge, as is similarly observed with other ultra-rare monogenic disorders.

Table I. Please add the caption

Question	Result (n = 46)	Paediatric centres only (n = 11)
Mitochondrial disease centres YES	70%	64%
Awareness of TK2 deficiency YES	96%	82%
Experience with TK2 patients YES	26%	9%
Genetic Panel Composition		
Panel Type	Includes TK2 gene	
Metabolic myopathy panels	85%	82%
LGMD panels	56%	73%
Differential Screening Practices		
Phenotype (genetically unsolved)	Screened for TK2	
FSHD	57%	18%
SMA	50%	0%
OPMD	52%	9%

Discussion

The results of the TK2 Hunter Project survey provide an informative picture of current diagnostic attitudes and practices regarding TK2d across Italian neuromuscular centres. Several important themes emerge from the data, each highlighting strengths as well as areas for improvement in the approach to this rare but increasingly treatable condition.
The survey reveals widespread awareness of TK2d among clinicians, a significant achievement given the rarity of the disease. However, this awareness does not consistently drive in a diagnostic action. Despite the known phenotypic overlap, fewer than half of the respondents routinely screen for *TK2* variants in patients with unsolved FSHD-like or OPMD-like phenotypes. This finding suggests that while clinicians are conceptually familiar with the disease, its full phenotypic variability and diagnostic relevance across various neuromuscular diseases may still be underrecognized. Another critical finding is the inconsistent inclusion of *TK2* in commonly used NGS panels. While 85% of respondents report its presence in metabolic myopathy panels, only 56% include it in LGMD panels, even though TK2d may

presents with limb-girdle weakness, particularly in adult-onset cases. This gap may reflect historical classification biases, where TK2d was viewed as a paediatric mitochondrial disorder. As the disease spectrum has broadened, panel design should include the *TK2* gene. This is also true considering that event though a large majority (89%) of clinician's report using clinical exome, whole-exome or whole-genome sequencing in cases where targeted panels fail to yield a diagnosis. However, in 11% of cases comprehensive genomic testing is not implemented, and in 32% its use in unresolved cases is guided by the clinical phenotype rather than applied systematically.

The implications of delayed or missed diagnosis are now more significant than ever, given that nucleoside therapy has shown strong efficacy, and its approval in TK2d is now under evaluation. Several studies have demonstrated improved survival, motor function, and respiratory outcomes with timely initiation of treatment[3–5]. The findings of this survey underscore a potential risk that patients may remain undiagnosed or misdiagnosed due to outdated panels, limited differential screening, or restricted access to broader testing.

The strengths of this study include its nationwide reach and focus on practical diagnostic questions relevant to emerging therapies. Limitations include potential response bias, as clinicians more familiar with TK2 may have been more likely to complete the survey, and the few complete responses obtained.

The AIM "TK2 Hunter Project" survey provides valuable insights into national diagnostic practices for TK2d. While awareness is high, gaps persist in screening strategies and genetic panel content suggesting a low diagnostic yield. Standardization and education are needed to ensure prompt diagnosis and, hopefully soon, access to therapies. TK2 gene should be included in all myopathy-related gene panels and should be considered in patients with negative genetic testing for other common neuromuscular diseases such as SMA, FSHD or OPMD. In parallel, continued professional education might reinforce the importance of early recognition and broaden the differential diagnostic mindset among neuromuscular specialists.

Supplemetary material

Supplementary data to this article can be found online at https://www.actamyologica.it/article/view/1424/837.

Acknowledgments Supplemetary data

We are grateful to the AIM members for their valuable contributions in responding to. MM, OM and CL are grateful to the European Reference Network ERN EURO-NMD as representatives for the Italian HCP partners.

Funding

M. Mancuso receives support from the European Union within the framework of the "EU4Health programme 2021-2027". http://ec.europa.eu/health/ern, from EJPRD project GENOMIT 01GM1920A, the Italian Ministry of Health (RF-2021-12373111), and from the European Union - Next-GenerationEU - National Recovery and Resilience Plan (NRRP) – Mission 4 component 2, investiment N. 1.1, Call PRIN 2022 D.D. 104 02-02-2022 – (2022B9WY4A) CUP N. D57G23000430008.

C Lamperti received research grant from and is supported by E- Rare project GENOMIT (01GM1920B), Italian Ministry of Health, MitoMyOmics, PNRR-MR1-2022-12376617 and Mitosign, RF- 2021-12373111.

M Musumeci received support from Call PRIN 2022 D.D. 104 02-02-2022 – (2022B9WY4A)J53D23010870006 and PNRR-MR1-2023-12377223_THERAPY4ALLCUP H43C24000470006.

Conflict of interests statement

None.

Authors' contributions

M.M.: study concept or design; analysis or interpretation of data, drafting the manuscript. C.L. and O.M. interpretation of data and editing of the manuscript

Ethical consideration

The AIM Board approved the TK2 Hunter Project. The administered survey included a data protection statement specifying: "For data protection purposes, this survey collects participants' names and institutional affiliations. No information that could link individual respondents to specific answers will be disclosed in the final report. By proceeding with the questionnaire, participants indicate their consent." All respondents confirmed agreement with this data protection clause.

References

1 Mandel H, Szargel R, Labay V,, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. Nat Genet. 2001;29:337–41. https://doi.org/10.1038/ng746

2 Ceballos F, Serrano-Lorenzo P, Bermejo-Guerrero, et al. Clinical and genetic analysis of patients with TK2 deficiency. Neurol Genet. 2024;10:e200138. https://doi.org/10.1212/NXG.0000000000200138

3 Berardo A, Domínguez-González C, Engelstad K, et al. Advances in thymidine kinase 2 deficiency: clinical aspects, translational progress, and emerging therapies. J Neuromuscul Dis. 2022;9:225-35. https://doi.org/10.3233/JND-210786

4 Domínguez-González C, Madruga-Garrido M, Mavillard F, et al. Deoxynucleoside therapy for thymidine kinase 2-deficient myopathy. Ann Neurol. 2019;86:293-303. https://doi.org/10.1002/ana.25506

5 Bermejo-Guerrero L, Rivero-García M, Martín-Hernández E, et al. Remarkable clinical improvement with oral nucleoside treatment in a patient with adult-onset TK2 deficiency: a case report. Mitochondrion. 2024;76:101879. https://doi.org/10.1016/j.mito.2024.101879