

ПРИЛОЖЕНИЕТО НА БИОМАРКЕРИ ПРИ НАСЛЕДСТВЕНА ТРАНСТИРЕТИНОВА АМИЛОИДОЗА – ТЕКУЩ ПРОЕКТ С БЪЛГАРСКИ ПАЦИЕНТИ

З. Павлова^{1,2}, А. Орманджиева^{1,2}, Кр. Георгиев⁶, О. Асенов⁴, А. Танева⁴, Т. Чамова⁴,
И. Търнев^{4,5}, Алб. Тодорова^{1,2,3}

¹ СМДЛ Геномен център „България“, София, България

² Генетична медико-диагностична лаборатория „Геника“, София, България

³ Катедра по медицинска химия и биохимия, Медицински университет – София, София, България

⁴ Катедра по неврология, УМБАЛ Александровска, Медицински университет – София, София, България

⁵ Катедра по когнитивна наука и психология, Нов български университет, София, България

⁶ ULB Neuroscience Institute, Université libre de Bruxelles (ULB), Брюксел, Белгия

THE APPLICATION OF BIOMARKERS IN HEREDITARY TRANSTHYRETIN AMYLOIDOSIS – AN ONGOING RESEARCH PROJECT ON BULGARIAN PATIENTS

Z. Pavlova^{1,2}, A. Ormandjieva^{1,2}, C. Georgiev⁶, O. Asenov⁴, A. Taneva⁴,
T. Chamova⁴, I. Tournev^{4,5}, A. Todorova^{1,2,3}

¹ Independent Medico-Diagnostic Laboratory Genome Center „Bulgaria“,
Sofia, Bulgaria

² Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria

³ Department of Medical Chemistry and Biochemistry, Medical University – Sofia,
Sofia, Bulgaria

⁴ Department of Neurology, University hospital „Alexandrovska“,
Medical University – Sofia, Sofia, Bulgaria

⁵ Department of Cognitive Science and Psychology, New Bulgarian University,
Sofia 1618, Bulgaria

⁶ ULB Neuroscience Institute, Université libre de Bruxelles (ULB),
Brussels, Belgium

ABSTRACT

The current research project is being conducted in IMDL Genome Center Bulgaria / GMDL Genica (part of the Expert Centers for ATTR in Bulgaria¹). It aims at advancing the accurate prognosis and monitoring of the onset and progression of hereditary transthyretin amyloidosis (vATTR) in Bulgarian Glu89Gln positive asymptomatic and symptomatic polyneuropathy stage I patients on Tafamidis by investigating the biomarker potential of NfL. Furthermore, our goal is to evaluate other better-known biomarkers for cardiac affection such as hs-cTnT, NT-ProBNP and eGFR. In so doing, our multidisciplinary team aims to improve the processes of timely diagnosis and therapy of the Bulgarian vATTR patient community.

KEY WORDS: neurofilaments, hereditary transthyretin amyloidosis, vATTR, NfL

РЕЗЮМЕ

Настоящият изследователски проект се провежда в СМДЛ Геномен център България / Генетична медико-диагностична лаборатория „Геника“ (част от Експертните центрове за ATTR в България¹). Целта е да се подобри диагностицирането и проследяването на наследствената транстиретинова амилоидоза (vATTR) при български пациенти с Glu89Gln, които са асимптоматични или симптоматични с полиневропатия в първи стадий, лекувани с Тафамидис, чрез изследването на биомаркерния потенциал на NfL. В допълнение, наша цел е да се оценят на други добре известни сърдечни биомаркери като hs-cTnT, NT-ProBNP и eGFR за оценка на сърдечното засягане. По този начин нашият мултидисциплинарен екип има за цел да подобри навременното

диагностициране и терапия на българската общност от vATTR пациенти.

КЛЮЧОВИ ДУМИ: неврофиламенти, наследствената транстиретинова амилоидоза, vATTR, NfL

BACKGROUND

ATTRv is an autosomal dominant late onset genetic disease presenting as system amyloidosis affecting the peripheral and autonomic nervous systems, the heart, gastrointestinal system, etc. This is a progressive disease associated with build-up of amyloid deposits which damage the peripheral nerves and the cardiovascular system, resulting in severe peripheral neuropathy and cardiomyopathy. If untreated, vATTR is fatal within 3-10 years post symptom onset. The possibility to monitor the onset and progression of vATTR with reliable biomarkers is of particular importance for the Bulgarian medical community. vATTR is substantially more common among Bulgarian patients compared to its European prevalence, making Bulgaria an endemic region with 402 patients from 155 families identified at the time of this writing. Such endemicity requires highly reliable diagnostic tools with appropriate practical applicability. The disease is caused by pathogenic variants in the gene encoding the transthyretin protein (TTR) and the most common TTR pathogenic variant in Bulgaria is briefly called p.Glu89Gln which is exclusively found in the south-west part of the country. The Bulgarian TTR Glu89Gln positive patients manifest severe mixed (neurologic and cardiologic) phenotype with fast progression^{2,3}.

Neurofilaments (NFs) comprise a group of polypeptide proteins consisting of 3 subcomponents: light (NfL), medium (NfM), and heavy (NfH). NFs are found in very large quantities along the axons of neurons in both the central and peripheral nervous system. NFs play a key role in multiple processes such as facilitation of axonal growth and proliferation, maintenance of axonal structural integrity, axonal myelination, and action potential propagation⁴⁻⁶.

The ubiquity of NFs in axons explains the commonly reported elevated release of NFs following axonal damage due to neurodegenerative processes or physical injury⁷. However, the accuracy of NfL has never been studied in more detail in a TTR mutation specific cohort.

In recent years, major advances have been made with respect to the assessment of NfL in CSF and blood.

The application of the Proximity Extension Assay (PEA)⁸, offered by Olink (www.olink.com) which presents a combination of DNA-based and antibody-based techniques to provide precise protein biomarker detection (as shown in APOLLO trial⁹). This suggests that the application of this technology would allow for accessible investigation of NfL and their biomarker potential in Bulgarian vATTR patients.

Given the mixed clinical manifestation of vATTR in Bulgaria^{2,3},

another approach to achieving an improvement in early diagnostics and monitoring of vATTR is to identify cardiac biomarkers which can be reliably assessed and used for making inferences about a given patients' condition and their likely prognosis. Two proteins which have shown such potential are Cardiac Troponin T (cTnT) and the N-terminal Pro-Brain Natriuretic Peptide (NT-ProBNP). Moderate but consistent elevation of cTnT has been reported in ATTR patients with cardiac symptoms¹⁰ and NT-ProBNP elevation has also been consistently identified in ATTR patient samples^{11,12}. There is newly developed high-sensitivity cTnT assay which is currently used for assessment of cardiac dysfunction. Another biomarker which is known to be informative for disease progression of vATTR is the estimated glomerular filtration rate (eGFR)¹³. Based on the aforementioned biomarkers several staging systems have been proposed for ATTR cardiac amyloidosis^{13,14}.

OBJECTIVES

The objectives of the current ongoing project include:

1. To estimate the magnitude of association between the levels of NfL and cardiac biomarkers with vATTR disease progression in symptomatic Glu89Gln vATTR stage 1 polyneuropathy (PN) patients (Modified Neuropathy Impairment Score +7 (mNIS+7)) compared to Asymptomatic Glu89Gln carriers.
2. Determine the mean NfL value in Glu89Gln stage 1 PN symptomatic patients and Glu89Gln asymptomatic carriers.
3. Determine the mean hs-cTnT, NT-ProBNP and eGFR value in Glu89Gln stage 1 PN symptomatic patients and Glu89Gln asymptomatic carriers.
4. Determine the relative difference in the NfL concentration in blood between Glu89Gln stage 1 PN symptomatic patients and Glu89Gln asymptomatic carriers.

HYPOTHESES AND END-POINTS

In plasma samples, NfL concentration is expected to be higher in symptomatic vATTR patients due to the pronounced axonal damage that results in the severe peripheral polyneuropathy.

NfL is hypothesized also to be positively correlated with cardiomyopathy in patient groups with mixed phenotype.

The null hypothesis to be tested is that NfL levels are not different between symptomatic patients and asymptomatic carriers of the Glu89Gln variant, and NfL levels are not correlated with NT-ProBNP in asymptomatic carriers of the Glu89Gln positive carriers or ATTRv patients with Glu89Gln variant with Stage 1 PN independent of CM status.

SAMPLE INCLUSION AND EXCLUSION CRITERIA

A preliminary Power analysis revealed that a total sample size of 106 participants is required for detection of medium effect size of $f = 0.22$. Hence, we will aim to recruit 53 participants per each of our 2 groups.

Participants will only be included in the symptomatic vATTR group if:

- they have been diagnosed with vATTR Glu89Gln based on genetic testing
- they exhibit peripheral neuropathy (Stage I) and cardiomyopathy, diagnosed by a licensed physician
- they have not been diagnosed with any additional chronic conditions which may affect the cardiovascular system and the peripheral nervous system.
- they are being treated with Tafamidis 20 mg

Participants will only be included in the asymptomatic vATTR group if:

- they have been confirmed carriers of the Glu89Gln variant in the TTR gene based on genetic testing
- they do not exhibit peripheral neuropathy or cardiomyopathy yet
- they have not been diagnosed with any additional chronic

conditions which may affect the cardiovascular system and the peripheral nervous system.

STUDY DESIGN

This is a case-control study in which the plasma and serum levels of the biomarkers listed below will be measured once every 3 months during a period of 12 months for a total of 5 measurement per patient (Figure 1).

- Neurofilament light chain (NfL)
- High-sensitivity cardiac Troponin-T (hs-cTnT)
- N-terminal-pro hormone BNP (NT-ProBNP)
- Estimated glomerular filtration rate (eGFR)

Patients who will be part of the current study will have genetically proven vATTR disease, asymptomatic or symptomatic. The tests will be carried out in IMDL Genome Center Bulgaria / GMDL Genica. Patients sign an informed consent form and receive an information leaflet with a brief description of the project, including patient inclusion and exclusion criteria. All information obtained in connection with this study that may identify the subject will remain confidential within the framework of Bulgarian and international laws.

The study will use a one-way analysis of variance (ANOVA) to compare the two mean NfL values from the two independent (unrelated) groups of patients (asymptomatic vATTR compared with early-stage familial amyloid polyneuropathy patients on tafamidis therapy). Regarding the other biomarkers (hs-cTnT, NT-ProBNP and eGFR), they will be measured over the same period of time in the same patients as the analysis will be part of the second step of the project.

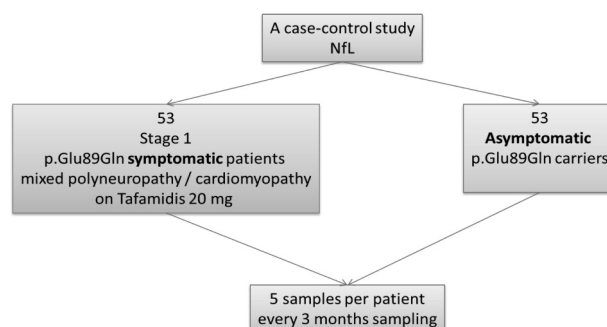


Fig. 1 Study design

CONCLUSION

vATTR is a more common disease in Bulgaria compared to Europe, which makes Bulgaria an endemic region. Such endemicity requires reliable diagnostics with practical applicability. The relatively low cost and minimal invasiveness of NfL detection in blood plasma/serum make this method a good candidate for broad-based diagnosis, prognosis and follow-up of vATTR.

The study will have a global contribution due to the still little data available to determine the significance of the studied biomarkers. The study will have a local contribution to the different patient groups of the endemic regions and the need to define new criteria and standards for the follow-up of the disease carriers and new criteria and standards for the follow-up of the disease progression in the patients with transthyretin amyloidosis.

Acknowledgements: The financial support for the current study from Alnylam Pharmaceuticals Inc is highly appreciated.

REFERENCE LIST

1. Nakov, R., Sarafov, S., Gospodinova, M. et al. Transthyretin amyloidosis: Testing strategies and model for center of excellence support. Clin Chim Acta Int J Clin Chem. 2020, 509, 228-234.

2. Chamova, T., Gospodinova, M., Asenov, O. et al. Seven Years of Selective Genetic Screening Program and Follow-Up of Asymptomatic Carriers With Hereditary Transthyretin Amyloidosis in Bulgaria. *Front Neurol.* 2022, 13, 844595.
3. Gospodinova, M., Kinova, E., Simova, I. et al. Diagnostic algorithm in transthyretin amyloidosis with cardiomyopathy. *Bulg Cardiol.* 2020, 26, 5-20.
4. Eyer, J., Peterson, A. Neurofilament-deficient axons and perikaryal aggregates in viable transgenic mice expressing a neurofilament-beta-galactosidase fusion protein. *Neuron.* 1994, 12, 2, 389-405.
5. Yum, S.W., Zhang, J., Mo, K., Li, J., Scherer, S.S. A Novel Recessive NEFL Mutation Causes a Severe, Early-Onset Axonal Neuropathy. *Ann Neurol.* 2009, 66, 6, 759-770.
6. Yuan, A., Sasaki, T., Kumar, A. et al. Peripherin is a subunit of peripheral nerve neurofilaments: implications for differential vulnerability of CNS and peripheral nervous system axons. *J Neurosci Off J Soc Neurosci.* 2012, 32, 25, 8501-8508.
7. Khalil, M., Teunissen, C.E., Otto, M. et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol.* 2018, 14, 10, 577-589.
8. Lundberg, M., Eriksson, A., Tran, B., Assarsson, E., Fredriksson, S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res.* 2011, 39, 15, 102.
9. Tica, S., Aldinc, E., Polydefkis, M. et al. Treatment response and neurofilament light chain levels with long-term patisiran in hereditary transthyretin-mediated amyloidosis with polyneuropathy: 24-month results of an open-label extension study. *Amyloid Int J Exp Clin Investig Off J Int Soc Amyloidosis.* Published online July 20, 2023, 1-11.
10. Takashio, S., Yamamuro, M., Izumiya, Y. et al. Diagnostic utility of cardiac troponin T level in patients with cardiac amyloidosis. *ESC Heart Fail.* 2017, 5, 1, 27-35.
11. Castiglione, V., Aimo, A., Vergaro, G., et al. Biomarkers for the diagnosis and management of heart failure. *Heart Fail Rev.* 2022, 27, 2.
12. Lehrke, M., Becker, A., Greif, M. et al. Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol.* 2009, 161, 2, 339-344.
13. Gillmore, J.D., Damy, T., Fontana, M. et al. A new staging system for cardiac transthyretin amyloidosis. *Eur Heart J.* 2018, 39, 30, 2799-2806.
14. Grogan, M., Scott, C.G., Kyle, R.A. et al. Natural History of Wild-Type Transthyretin Cardiac Amyloidosis and Risk Stratification Using a Novel Staging System. *J Am Coll Cardiol.* 2016, 68, 10, 1014-1020.

Адрес за кореспонденция:

Проф. Албена Тодорова, дбн

Адрес: ул. „Ами Буге“ №84, ет. 2, София 1612,

email: todorova_albena@abv.bg

Correspondence:

Albena Todorova,

84 „Ами Буге“ str., Sofia 1612, Bulgaria

email: todorova_albena@abv.bg

MELAS СИНДРОМ (МИТОХОНДРИАЛНА ЕНЦЕФАЛОМИОПАТИЯ, ЛАКТАТНА АЦИДОЗА И ИНСУЛТИ) – ПРЕДСТАВЯНЕ НА КЛИНИЧЕН СЛУЧАЙ

Т. Ангелов^{1,2}, Т. Чамова^{1,2}, С. Черникова², А. Еленкова³, С. Михайлова⁴, И. Търнев^{1,2,5}

¹ Катедра по неврология, Медицински факултет, Медицински университет – София

² Клиника по нервни болести, УМБАЛ „Александровска“, гр. София

³ УСБАЛЕ „Акад. Иван Пенчев“, Катедра по ендокринология, Медицински университет – София

⁴ Клиника по клинична имунология с банка за стволови клетки, УМБАЛ „Александровска“, Медицински университет – София

⁵ Департамент по когнитивна наука и психология, Нов български университет, София

MELAS SYNDROME (MITOCHONDRIAL ENCEPHALOMYOPATHY, LACTIC ACIDOSIS AND STROKES) - PRESENTATION OF A CASE REPORT

T. Angelov^{1,2}, T. Chamova^{1,2}, S. Cherninkova², A. Elenkova³, S. Mihaylova⁴, I. Tournev^{1,2,5}

¹ Department of Neurology, Faculty of Medicine, Medical University of Sofia

² Clinic of Nervous Diseases, University Hospital „Alexandrovskaya“

³ USHATE „Acad. Ivan Penchev“, Department of Endocrinology, Medical University of Sofia

⁴ Department of Clinical Immunology, University Hospital „Alexandrovskaya“, Medical University of Sofia

⁵ Department of Cognitive Science and Psychology, New Bulgarian University, Sofia

ABSTRACT

MELAS is a mitochondrial disease presenting with encephalomyopathy, lactic acidosis, and stroke-like episodes. Its onset is in the first two decades, with variable clinical symptoms, most frequently from the nervous system. The clinical and imaging findings in MELAS are topical and do not correspond to a vascular territory, more commonly are in the posterior cranial fossa, may be reversible

but often are relapsing and gradually grow adjacent to the present ones over weeks to months, accompanied by epileptic seizures, neurological dysfunction and cortical atrophy. Establishing heteroplasmy of the mutation and multisystem involvement is mandatory for the diagnosis.

In this publication, we present a 33-year-old female (MPS) with a A3243G mutation in the mitochondrial genome with 31.6% heteroplasmy in blood and 74% heteroplasmy in urinary sediment epithelial cells, corresponding to MELAS syndrome. The clinical onset was at the age of 22, and due to a misinterpretation of the MRI findings as a tumor formation, she underwent left temporal resection two years later, without improvement of complaints. The neurological examination was consistent with quadripyramidal, pancerebellar, epileptic and cognitive syndromes, and bilateral sensorineural hearing loss. Symptomatic therapy with Levetiracetam, vitamins and nutrients was administered.

KEY WORDS: MELAS, case report

РЕЗЮМЕ

MELAS е митохондриално заболяване, съчетаващо енцефало-миопатия, лактатна ацидоза и инсулто-подобни епизоди. Началото му е в първите две десетилетия от живота, с вари-