THIRTY-THREE YEARS OF TRANSLATIONAL NEUROGENETICS IN CYPRUS

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Abstract

The Cyprus Institute of Neurology and Genetics (CING) is a bicommunal non-profit organisation formed in 1991. The mission of CING is to develop and provide high level medical and clinical laboratory services, develop and pursue advanced research, and provide education in the areas of Neurology, Genetics, Biomedical, Medical and related Sciences for the benefit of patients and society. The Neurogenetics Department developed translational neurogenetics alongside the global advances in genomic studies. We investigated neurological diseases prevalent in specific geographical regions of the island, such as Friedreich ataxia and many other neurogenetic disorders. Translational neurogenetics research studies in Cyprus attracted extensive funding by international and national funding bodies, produced a remarkable research output such as gene mapping and identification, and established a fruitful global network of collaborations.

Key words: Neurogenetics, Cyprus, rare neurological diseases, translational research

The first report of Friedreich ataxia (FRDA) in Cyprus was published thirty-three years ago [1] and constitutes the initiation of a fascinating journey of translational neurogenetics in Cyprus. During a study of multiple sclerosis, researchers found a cluster of FRDA patients in the neighbouring villages of Kathikas and Arodhes in Paphos. The authors estimated that 1-in-6 to 1-in-7 of the population of the villages carry the FRDA pathogenic variant. We further investigated these families with linkage analysis and Sanger sequencing and, in 1996, established the genetic diagnosis. The CING neurologists ascertained additional FRDA families. In the year 2000, it became apparent that 10 out of 11 patients originating from the district of Paphos had no evidence of origin from the above two villages. We thus focused on investigating whether the pathogenic variant spread outside the founder villages. We were successful in obtaining funding for an 18-month FRDA carrier screening programme. The programme aimed at:

1. Informing the population of the Paphos district about the disease, the mode of inheritance and available diagnostic options.
2. Collect samples from volunteers after informed and signed consent to estimate the FRDA carrier frequency in the district.
3. To offer further genetic counselling to the FRDA carriers.

This programme established a high frequency of FRDA carriers (1-in-12) in the overall district of Paphos [2]. It also confirmed the 1-in-7 FRDA carrier frequency in the population of Kathikas and Arodhes. These findings led to the introduction of the National Prevention Programme for Friedreich ataxia in 2010, an ongoing successful collaboration of the Ministry of Health and the CING.

Another cluster of patients with familial amyloidotic polyneuropathy (FAP) exists in Cyprus. The CING has been investigating FAP patients and their family members since 1987. All Cypriot FAP patients have a single pathogenic variant, the TTR Val30Met. We reported the prevalence and incidence of the disease at two-time points [3,4]. A dedicated clinic at CING is following up on patients. Many of them have undergone liver transplantation, and currently, Cypriot patients participate in two international clinical trials (Alnylam, since 2016 and IONIS, since 2020) through their CING neurologists.

The CING participated in several studies on gene mapping and gene identification of neuropathies through international collaborations. We mapped a distal form of spinal muscular atrophy with upper limb predominance to chromosome 7 [5]. Pathogenic variants in the Glycyl tRNA synthetase (GARS) gene are associated with this distal spinal atrophy type V and Charcot-Marie-Tooth disease type 2D [6, 7]. We mapped a novel form of distal hereditary motor neuronopathy to chromosome 9p21.1-p12 [8] that we named Jerash type dHMN (HMNJ), and recently we have reported a novel SIGMAR1 pathogenic variant that is associated with the development of this disease [9].

We participated in mapping and identifying the

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Charcot-Marie-Tooth type 4B (CMT4B) gene, encoding the myotubularin-related protein-2 [10, 11] and in identifying PDXK variants that cause polyneuropathy responsive to PLP supplementation [12]. We reported several novel pathogenic variants and functional studies in CMT disease [13, 14, 15, 16, 17, 18, 19, 20, 21].

With the new era of next-generation massively parallel sequencing (NGS), we initiated studies with this new technological tool to diagnose rare neurological diseases. Initial studies of ataxias and spastic paraplegias enabled the identification of novel pathogenic variants in long-pending diagnosis patients and families. An example of applying various techniques in combination is the case of family 903 with spastic ataxia due to a GBA2 pathogenic variant [22]. Initial linkage analysis of the specific family back in the 1990s has mapped the disease in the family to the aprataxin (APTX) gene locus on chromosome 9p21.1. Sanger sequencing of the APTX gene and MLPA based investigation for APTX duplication/deletion excluded the probability of a pathogenic variant in this gene. Because the parents were third cousins, a common genetic background was suspected, and thus we performed genome-wide homozygosity mapping to identify common by descent chromosomal regions. We detected two areas with high homozygosity scores: a 3.1 Mb region on chromosome 5 that harbours eight protein-coding genes and a 6.49 Mb region on chromosome 9 with ninety-six protein-coding genes. We then performed whole-exome sequencing (WES), which helped exclude any pathogenic variant in the chromosome 5 candidate region and revealed possible pathogenic variants in three genes within the chromosome 9 candidate region. We confirmed the GBA2 pathogenic variant in this family with spastic ataxia with a segregation analysis of the five members of the family. We further performed biochemical studies of the GBA2 variant in lymphoblastoid cell lines derived from family members and healthy control individuals [23]. We recently performed transcriptomic characterisation of tissues from patients and healthy control individuals. We discovered more than 5000 differentially expressed genes. Subsequent pathway analyses reveal biological pathways implicated in spastic ataxia. This work is currently under review for publication (Kakouri A et al., under review).

We investigated several additional patients and families with rare neurological diseases using NGS based approaches. Although the hopes for reaching a molecular diagnosis were high at the initiation of this investigation, through our ten years of experience, the diagnostic yield is only above 30%. A combination of WES and transcriptomic analyses in the availability of patient material for RNA level investigation should enable a higher diagnostic yield. Thus, we are currently working in this direction. In addition, participation in multicentre studies within the framework of recently established European Reference Networks (ERNs) or any other international collaborative effort should improve diagnostic yields and improve the time to diagnose rare neurological disorders. The CING is committed to introducing cutting edge technological approaches both in the diagnostic and in the research sector for the benefit of patients and society.

References

[1] Dean G, Chamberlain S, Middleton L. Friedreich's ataxia in Kathikas-Arodhes, Cyprus. Lancet. 1988 Mar 12;1(8585):587. doi: 10.1016/s0140-6736(88)91378-5. PMID: 2894517.

[2] Zamba-Papanicolaou E, Koutsou P, Daiou C, Gaglia E, Georghiou A, Christodoulou K. High frequency of Friedreich's ataxia carriers in the Paphos district of Cyprus. Acta Myol. 2009 Jul;28(1):24-26. PMID: 19772192.

[3] Dardiotis E, Koutsou P, Papanicolaou EZ, Vonta I, Kladi A, Vassilopoulos D, Hadjigeorgiou G, Christodoulou K, Kyriakides T. Epidemiological, clinical and genetic study of familial amyloidotic polyneuropathy in Cyprus. Amyloid. 2009 Mar;16(1):32-37. PMID: 19291512.

[4] Andreou S, Panayiotou E, Michailidou K, Pirpa P, Hadjisavvas A, El Salloukh A, Barnes D, Antoniou A, Christodoulou K, Tanteles G, Kyriakides T. Epidemiology of ATTRV30M neuropathy in Cyprus and the modifier effect of complement C1q on the age of disease onset. Amyloid. 2018 Dec;25(4):220-226. doi: 10.1080/13506129.2018.1534731. Epub 2018 Dec 20. PMID: 30572722.

[5] Christodoulou K, Kyriakides T, Hristova AH, Georgiou DM, Kalaydjieva L, Yshpekova B, Ivanova T, Weber JL, Middleton LT. Mapping of a distal form of spinal muscular atrophy with upper limb predominance to chromosome 7p. Hum Mol Genet. 1995 Sep;4(9):1629-32. PMID: 8541851.

[6] Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin SQ, Jordanova A, Kremensky I, Christodoulou K, Middleton LT, Sivakumar K, Ionasescu V, Vance JM, Goldfarb LG, Fischbeck KH, Green ED. Glycyl tRNA Synthetase Mutations in Charcot-Marie-Tooth Disease Type 2D and Distal Spinal Muscular Atrophy Type V. Am J Hum Genet 2003 May;72(5):1293-1299. PMID: 12690580.

[7] Sivakumar K, Kyriakides T, Puls I, Nicholson GA, Funalot B, Antonellis A, Ellsworth RE, Sambuughin N, Christodoulou K, Beggs JL, Zamba-Papanicolaou E, Ionasescu V, Dalakas

MC, Green ED, Fischbeck KH, Goldfarb LG. Phenotypic spectrum of disorders associated with glycyl-tRNA synthetase mutations. Brain. 2005 Oct;128(Pt 10):2304-2314. PMID: 16014653.

[8] Christodoulou K, Zamba E, Tsingis M, Mubaidin A, Horani K, Abu-Sheik S, El-Khateeb M, Kyriacou K, Kyriakides T, Al-Qudah AK, Middleton LT. A novel form of distal hereditary motor neuronopathy maps to chromosome 9p21.1-p12. Ann Neurol 2000 Dec;48(6):877-884. PMID: 11117544.

[9] Ververis A, Dajani R, Koutsou P, Aloqaily A, Nelson-Williams C, Loring E, Arafat A, Mubaidin AF, Horany K, Bader MB, Al-Baho Y, Ali B, Muhtaseb A, DeSpenza T Jr, Al-Qudah AA, Middleton LT, Zamba-Papanicolaou E, Lifton R, Christodoulou K. Distal hereditary motor neuronopathy of the Jerash type is caused by a novel SIGMAR1 c.500A>T missense mutation. J Med Genet. 2020 Mar;57(3):178-186. doi: 10.1136/jmedgenet-2019-106108. Epub 2019 Sep 11. PMID: 31511340; PMCID: PMC7042970.

[10] Bolino A, Levy ER, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou RK, Hausmanowa-Petrusewicz I, Mandich P, Gambardella A, Quattrone A, Devoto M, Monaco AP. Genetic Refinement and Physical Mapping of the CMT4B Gene on Chromosome 11q22. Genomics. 2000 Jan 15;63(2):271-278. PMID: 10673338.

[11] Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou K, Hausmanowa-Petrusewicz I, Mandich P, Schenone A, Gambardella A, Bono F, Quattrone A, Devoto M, Monaco AP. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. Nat Genet. 2000 May 25(1):17-19. PMID: 10802647.

[12] Chelban V, Wilson MP, Warman Chardon J, Vandrovcova J, Zanetti MN, Zamba-Papanicolaou E, Efthymiou S, Pope S, Conte MR, Abis G, Liu YT, Tribollet E, Haridy NA, Botía JA, Ryten M, Nicolaou P, Minaidou A, Christodoulou K, Kernohan KD, Eaton A, Osmond M, Ito Y, Bourque P, Jepson JEC, Bello O, Bremner F, Cordivari C, Reilly MM, Foiani M, Heslegrave A, Zetterberg H, Heales SJR, Wood NW, Rothman JE, Boycott KM, Mills PB, Clayton PT, Houlden H; Care4Rare Canada Consortium; SYNaPS Study Group. PDXK mutations cause polyneuropathy responsive to PLP supplementation. Ann Neurol. 2019 Aug;86(2):225-240. doi:10.1002/ana.25524. PMID: 31187503.

[13] Georgiou DM, Zidar J, Korosec M, Middleton LT, Kyriakides T, Christodoulou K. A novel NF-L mutation Pro22Ser is associated with CMT2 in a large Slovenian family. Neurogenetics 2002 Oct;4(2):93-96. PMID: 12481988.

[14] Kleopa AK, Georgiou DM, Nicolaou P, Koutsou P, Papathanasiou E, Kyriakides T, Christodoulou K. A novel PMP22 mutation Ser22Phe in a family with HNPP and CMT1A phenotypes. Neurogenetics. 2004 Sep;5(3):171-175. PMID: 15205993.

[15] Georgiou DM, Nicolaou P, Chitayat D, Koutsou P, Babul-Hirji R, Vajar J, Murphy J, Christodoulou K. A novel GDAP1 mutation 439delA is associated with autosomal recessive CMT disease. Can J Neurol Sci 2006 Aug;33(3): 311-316. PMID: 17001820.

[16] Kleopa K, Zamba-Papanicolaou E, Nicolaou P, Georgiou DM, Kyriakides T, Christodoulou K. Phenotypic and cellular expression of two novel Connexin32 mutations causing CMTX, Neurology 2006 66: 396-402. PMID: 16476939.

[17] Butinar D, Starr A, Zidar J, Koutsou P, Christodoulou K. Auditory nerve is affected in one of two different point mutations of the neurofilament light (NF-L) gene. Clin Neurophysiol 2008 Feb;119(2):367-75. Epub 2007 Nov 26. PMID: 18023247.

[18] Nicolaou P, Zamba-Papanicolaou E, Koutsou P, Kleopa KA, Georghiou A, Hadjigeorgiou G, Papadimitriou A, Kyriakides T, Christodoulou K. Charcot-Marie-Tooth Disease in Cyprus: Epidemiological, Clinical and Genetic Characteristics. Neuroepidemiology 2010 Jun 23;35(3):171-177. PMID: 20571287.

[19] Nicolaou P, Cianchetti C, Minaidou A, Marrosu G, Zamba-Papanicolaou E, Middleton L, Christodoulou K. A novel LRSAM1 mutation is associated with autosomal dominant axonal Charcot-Marie-Tooth disease. Eur J Hum Genet. 2013 Feb;21(2):190-4. doi: 10.1038/ejhg.2012.146. Epub 2012 Jul 11. PMID:22781092.

[20] Minaidou A, Nicolaou P, Christodoulou K. LRSAM1 depletion affects neuroblastoma SH-SY5Y cell growth and morphology: The LRSAM1 c.2047-1G>A loss-of-function variant fails to rescue the phenotype. Cell J. 2018 Oct;20(3):340-347. doi: 10.22074/cellj.2018.5352. Epub 2018 May 15. PMID: 29845787.

[21] Minaidou A, Nicolaou P, Christodoulou K. Deregulation of LRSAM1 expression impairs the levels of TSG101, UBE2N, VPS28, MDM2 and EGFR. PLoS One. 2019 Feb 6;14(2):e0211814. doi: 10.1371/journal.pone.0211814. eCollection 2019. PMID: 30726272.

[22] Votsi C, Zamba-Papanicolaou E, Middleton LT, Pantzaris M, Christodoulou K. A novel GBA2 gene missense mutation in spastic ataxia. Ann Hum Genet. 2014 Jan;78(1):13-22. doi:

10.1111/ahg.12045. Epub 2013 Nov 20. PMID:24252062.

[23] Malekkou A, Samarani M, Drousiotou A, Votsi C, Sonnino S, Pantzaris M, Chiricozzi E, Zamba-Papanicolaou E, Aureli M, Loberto N, Christodoulou K. Biochemical characterization of the GBA2 c.1780G>C missense mutation in lymphoblastoid cells from patients with spastic ataxia. Int J Mol Sci. 2018 Oct 10;19(10). pii: E3099. doi: 10.3390/ijms19103099. PMID: 30308956.