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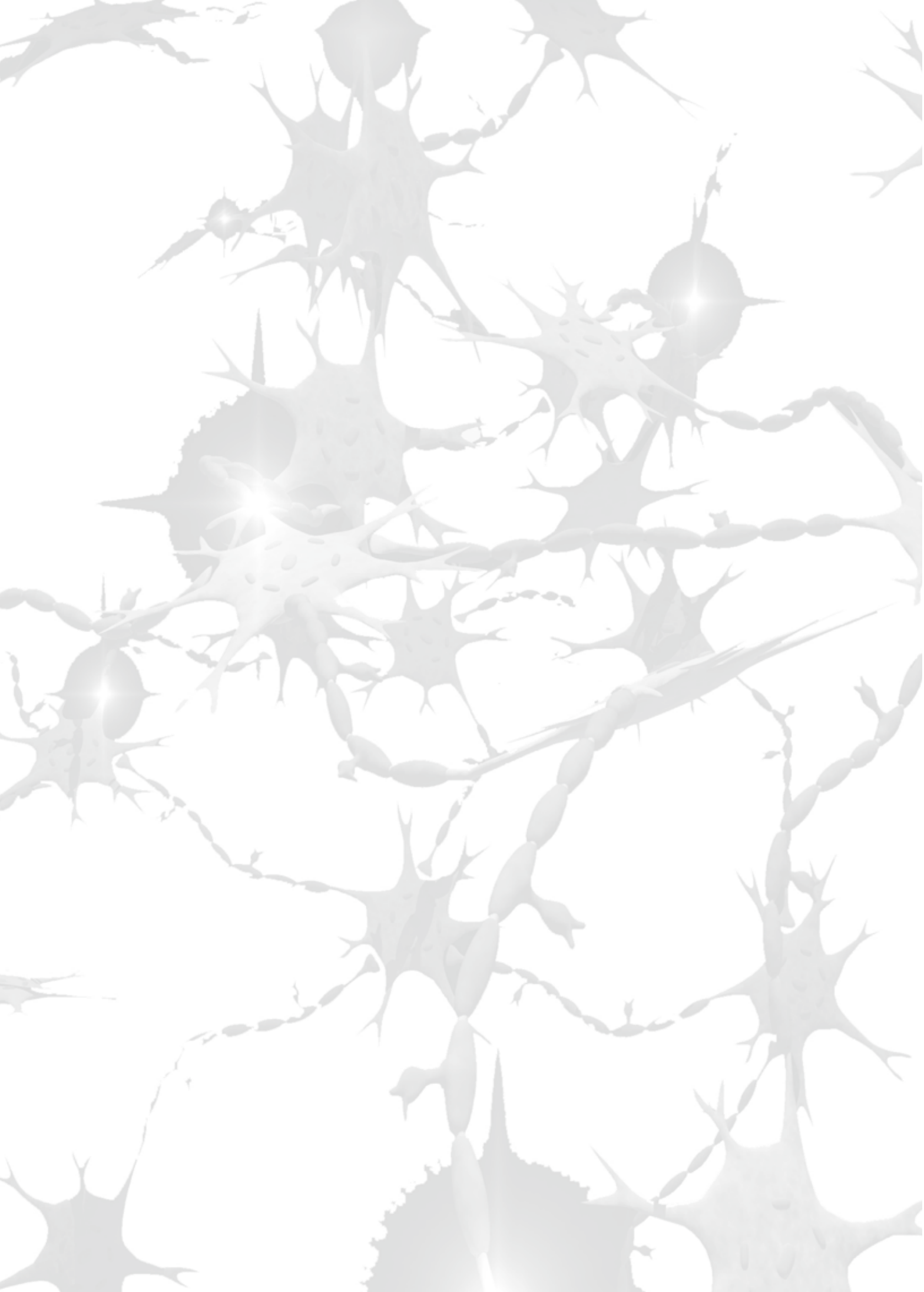
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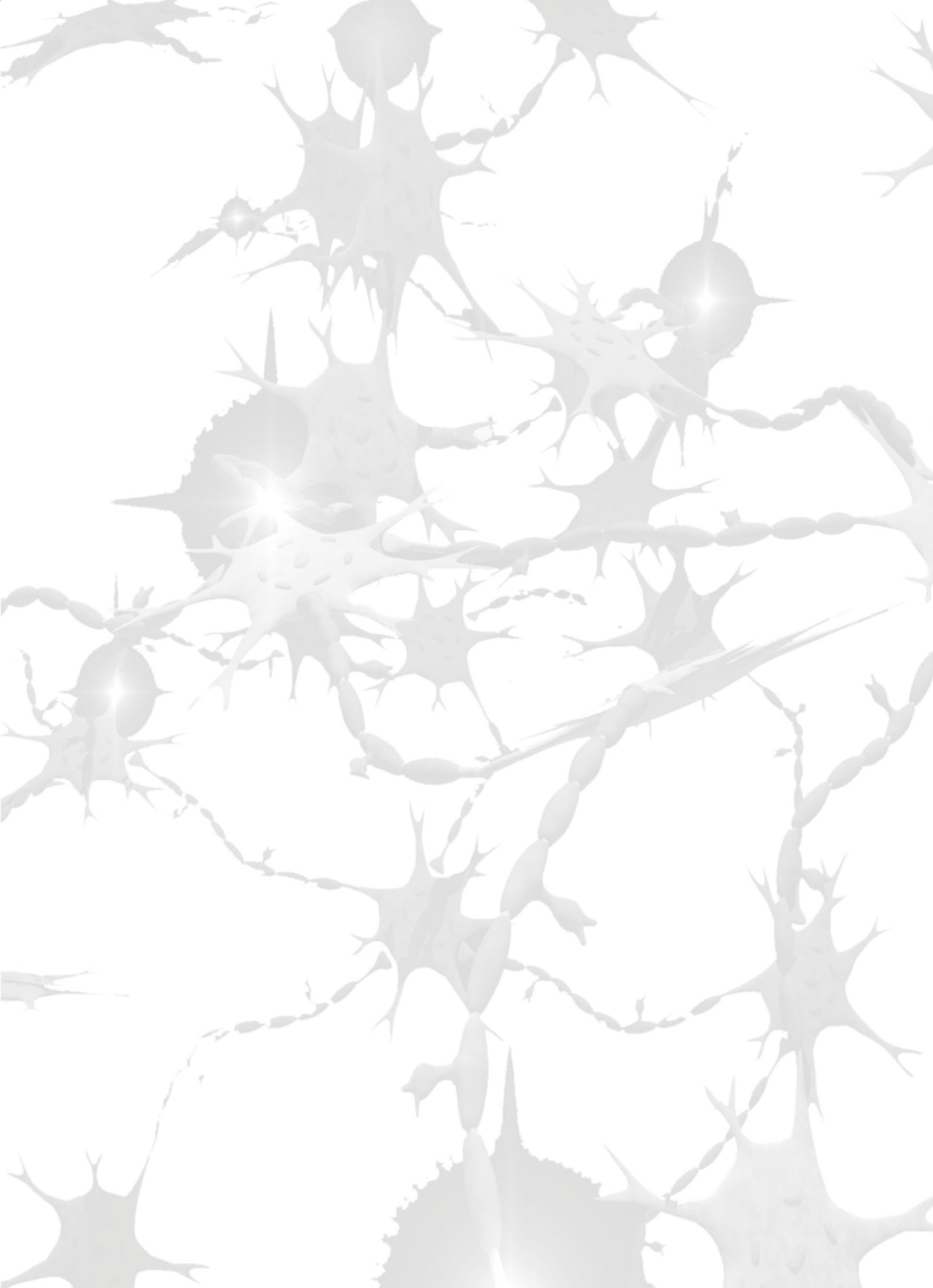
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The current issue of Archives of Neurology contains a research paper entitled “Validation of blood PRKN mRNA expression as a biomarker of PRKN – related Parkinson’s disease (PD)”, a case report of “Leykoencephalopathy in a patient with Girdle Muscular Dystrophy” and a research study of “the binocular videonystagmographic analysis of smooth pursuit, saccades and optokinetic nystagmus in healthy adults”.

In the first article, Parissis et al present an interesting case report of a patient diagnosed with muscular dystrophy associated with LAMA2 gene pathogenic variants. Interestingly enough, missense changes in the LAMA2 gene according to the genetic analysis performed, established the diagnosis of LAMA2 muscular dystrophy. This is a rare subtype of limb girdle muscular dystrophy. This finding led the authors to perform brain MRI where the characteristic leukoencephalopathic pattern of this disorder was identified thus providing good evidence of their final diagnosis. This case is a good example of performing diagnosis in the setting of precision medicine. The MRI scans performed are highly didactic.

In the second article, Siliogka et al performed a clinical study aiming to validate blood PRKN mRNA expression as a biomarker of PRKN – related Parkinson’s disease (PD). PRKN gene mutations are a major cause of early-onset, autosomal recessive PD. To this aim, the authors quantified PRKN mRNA expression using RT-PCR with newly designed primes in PBMCs isolated from 7 PRKN-related PD patients (3 biallelic, 4 heterozygous), 8 idiopathic PD patients and 7 matched healthy controls. Genetic characterisation employed Multiplex Ligation-dependent Probe Amplification and Whole Exome Sequencing. The authors concluded that peripheral PRKN mRNA quantification is a highly sensitive and specific marker for PRKN mutation carrier status in PD, including in the heterozygous state. Interestingly enough, pathogenic variants in the PRKN gene resulted in reduced expression of the respective mRNA in PBMCs both in biallelic individuals and heterozygous PD patients, thus suggesting severe suppression of PRKN mRNA levels as a common feature of PRKN-related PD. These findings are sound. However, the small sample size and the single-centre design are among the limitations of this study. Therefore, larger, multi-ethnic cohorts are warranted.

In the third paper, Tzortzakakis et al aim to provide normative reference data for oculomotor parameters (optokinetic nystagmus, saccadic eye movements, and smooth pursuit eye movements) using videonystagmography in a healthy Greek cohort (31 individuals). This is a well-scheduled study providing good evidence of symmetrical oculomotor performance and age-related differences in selected parameters, thus highlighting the importance of considering age effects when interpreting oculomotor findings in healthy individuals. Taking into consideration the potential role of oculomotor parameter analysis in the early detection of neurological disorders, including neurodegenerative diseases, as well as in the differential diagnosis between central and peripheral nervous system pathologies, this study provides good evidence for both clinical and research settings. However, the current study is an initial normative dataset in a healthy adult population; continuous data collection may improve representativeness.

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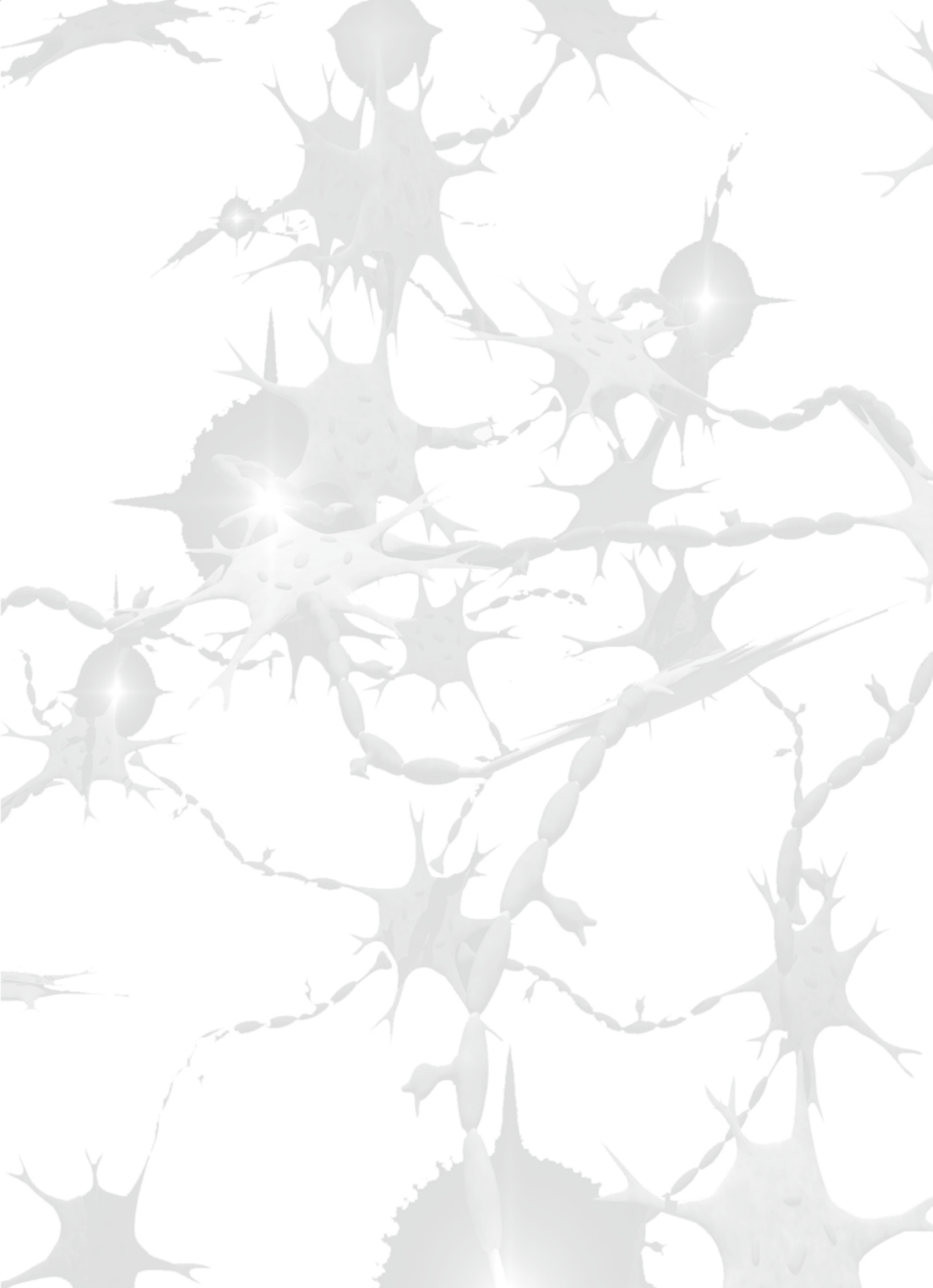
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# VALIDATION OF BLOOD PRKN MRNA EXPRESSION AS A BIOMARKER FOR PRKN-RELATED PARKINSON'S DISEASE

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## ABSTRACT

**Background:** Mutations in the *PRKN* gene, encoding the E3 ubiquitin ligase Parkin, are a major cause of early-onset, autosomal recessive Parkinson's disease (PD). Identifying carriers currently requires complex genetic testing. Prior work suggested that *PRKN* mRNA levels in peripheral blood mononuclear cells (PBMCs) may discriminate PRKN-related PD from idiopathic PD (iPD) and controls. **Objective:** To validate and expand previous findings using a partially independent cohort and a novel RT-PCR primer set, assessing the diagnostic potential of *PRKN* mRNA qualification as a screening tool for PRKN-related PD. **Methods:** PBMCs were isolated from 7 PRKN-related PD patients (3 biallelic, 4 heterozygous), 8 iPD patients and 7 matched healthy controls. *PRKN* mRNA expression was quantified using RT-PCR with newly designed primers. Genetic characterisation employed Multiplex Ligation-dependent Probe Amplification (MLPA) and Whole Exome Sequencing (WES). Statistical comparisons were performed using ANOVA with post hoc tests and diagnostic accuracy was assessed by ROC analysis. **Results:** PRKN mRNA levels were significantly reduced in PRKN-related PD compared to controls (181-fold or 99,45% lower,  $p=0,0041$ ) and iPD (74-fold or 98,7% lower,  $p=0,021$ ). Expression levels were uniformly low in both biallelic and heterozygous carriers. ROC analysis showed 100% sensitivity and specificity for distinguishing PRKN mutation carriers from non-carriers (PPV=100%, 95% CI: 72,3-100%). **Conclusion:** Peripheral PRKN mRNA quantification is a highly sensitive and specific marker for PRKN mutation carrier status in PD, including in the heterozygous state. This non-invasive assay holds promise as a rapid screening tool, but warrants larger, multicentre validation.

**Keywords:** Parkinson's disease, Parkin, PRKN, PBMCs, RT-PCR

## ΑΞΙΟΛΟΓΗΣΗ ΤΗΣ ΕΚΦΡΑΣΗΣ MRNA ΤΟΥ ΓΟΝΙΔΙΟΥ PRKN ΣΤΟ ΠΕΡΙΦΕΡΙΚΟ ΑΙΜΑ ΩΣ ΒΙΟΔΕΙΚΤΗ ΓΙΑ ΤΗ ΝΟΣΟ ΤΟΥ ΠΑΡΚΙΝΣΟΝ ΠΟΥ ΣΧΕΤΙΖΕΤΑΙ ΜΕ ΜΕΤΑΛΛΑΞΕΙΣ ΤΟΥ ΓΟΝΙΔΙΟΥ ΤΗΣ ΠΑΡΚΙΝΗΣ

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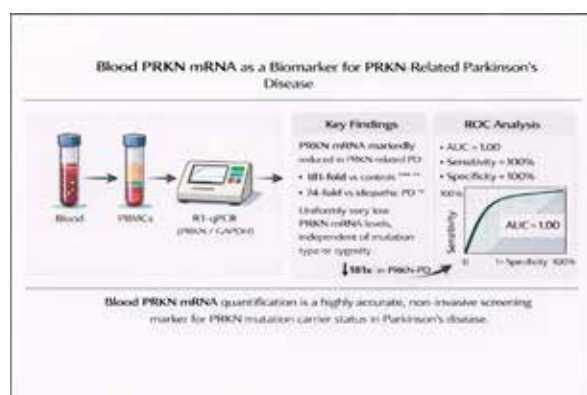
## ΠΕΡΙΛΗΨΗ

**Υπόβαθρο:** Μεταλλάξεις στο γονίδιο της παρκίνης (PRKN), που κωδικοποιεί την E3 ουβικουιτίνη-ηλιγάση παρκίνη, αποτελούν σημαντική αιτία πρώιμης έναρξης, αυτοσωμικής υπολειπόμενης νόσου του Πάρκινσον (NP). Η αναγνώριση φορέων απαιτεί σήμερα πολυπλόκο γενετικό έλεγχο. Προηγούμενες μελέτες έδειξαν ότι τα επίπεδα mRNA του PRKN σε μονοπύρνα κύτταρα περιφερικού αίματος μπορεί να διακρίνουν τη νόσο του Πάρκινσον που σχετίζεται με PRKN από την ιδιοπαθή μορφή και από υγιείς μάρτυρες. **Σκοπός:** Η επικύρωση και επέκταση προηγούμενων ευρημάτων με τη χρήση μερικώς ανεξάρτητου δείγματος και ενός νέου σετ εκκινητών RT-PCR, αξιολογώντας τη διαγνωστική αξία της ποσοτικοποίησης του mRNA του PRKN ως εργαλείου προσυμπτωματικού ελέγχου για τη νόσο που σχετίζεται με PRKN. **Μέθοδοι:** Απομονώθηκαν PBMCs από 7 ασθενείς με νόσο Πάρκινσον σχετιζόμενη με PRKN (3 με ομόζυγες μεταλλάξεις, 4 ετερόζυγοι), 8 ασθενείς με ιδιοπαθή νόσο Πάρκινσον και 7 υγιείς μάρτυρες. Η έκφραση του mRNA του PRKN

ποσοτικοποιήθηκε με Real time – Polymerase Chain Reaction (RT-PCR) χρησιμοποιώντας νέους εκκινητές. Ο γενετικός χαρακτηρισμός πραγματοποιήθηκε με MLPA και αλληλούχιση ολόκληρου εξώματος (WES). Οι στατιστικές συγκρίσεις έγιναν με ANOVA και δοκιμές post hoc, ενώ η διαγνωστική ακρίβεια αξιολογήθηκε με ανάλυση ROC. **Αποτελέσματα:** Τα επίπεδα mRNA του PRKN ήταν σημαντικά μειωμένα στη νόσο Πάρκινσον που σχετίζεται με PRKN σε σύγκριση με τους υγιείς μάρτυρες (181 φορές ή 99,45% χαμηλότερα,  $p=0,0041$ ) και με την ιδιοπαθή μορφή (74 φορές ή 98,7% χαμηλότερα,  $p=0,021$ ). Τα επίπεδα έκφρασης ήταν ομοιόμορφα χαμηλά τόσο σε ομόζυγα άτομα με μεταλλάξη όσο και σε ετερόζυγους φορείς. Η ανάλυση ROC έδειξε 100% ευαισθησία και ειδικότητα στη διάκριση φορέων μεταλλάξεων PRKN από μη φορείς (PPV=100%, 95% CI: 72,3–100%). **Συμπεράσματα:** Η ποσοτικοποίηση του mRNA του γονιδίου PRKN στο περιφερικό αίμα αποτελεί έναν εξαιρετικά ευαίσθητο και ειδικό δείκτη για την παρουσία μεταλλάξεων PRKN στη νόσο Πάρκινσον, ακόμη και στην ετερόζυγη κατάσταση. Η μη επεμβατική αυτή μέθοδος παρουσιάζει προοπτικές ως γρήγορο και εύχρηστο εργαλείο προσυμπτωματικού ελέγχου, αλλά απαιτείται επιβεβαίωση με μεγαλύτερες, πολυκεντρικές μελέτες.

**Λέξεις-κλειδιά:** Νόσος Πάρκινσον, Parkin, PRKN, PBMCs, RT-PCR

## Graphical Abstract



## INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1% of individuals over 60 years old.<sup>[1]</sup> Mutations in **PRKN**, encoding the mitochondrial quality –control protein Parkin, cause up to 50% of early –onset autosomal recessive PD (ARPD).<sup>[2–5]</sup> PRKN-associated PD is characterised by early onset, slow progression and marked dopaminergic responsiveness, but genetic confirmation requires advanced laboratory methods.<sup>[4,5]</sup>

Parkin acts in the PINK1/Parkin pathway to remove damaged mitochondria via mitophagy. Loss-of-function PRKN mutations reduce mitochondrial quality control, contributing to selective degeneration of substantia nigra pars compacta dopaminergic neurons.<sup>[6,7]</sup> In addition to biallelic pathogenic variants, the role of heterozygous variants as disease susceptibility factors is under investigation.<sup>[8]</sup>

We previously demonstrated that **PRKN** mRNA levels in PBMCs could accurately differentiate PRKN-related PD from iPD and controls.<sup>[9]</sup> The present study aimed to independently validate these findings using

a novel RT-PCR primer design and additional subjects with **PRKN** mutations, exploring the potential of **PRKN** mRNA quantification as a clinically feasible screening tool.

## MATERIALS AND METHODS

### Participants

These groups were enrolled at the First Dept of Neurology, Eginition University Hospital, Athens, Greece:

- PRKN-related PD (n=7, 3 biallelic, 4 heterozygous)
- Idiopathic PD (n=8)
- Healthy controls (n=7)

Groups were matched for age and sex where possible. All participants provided written informed consent. Ethical approval was obtained from the Scientific Committee of Eginition Hospital.

Genetic analysis: **PRKN** variants were identified using MLPA (Neurogenetics Unit, Eginition Hospital) and WES (Greek Genome Centre, BRFAA). Variant classification followed ACMG guidelines. Further details on the methods of genetic testing are provided by Papagiannakis et al (2024).<sup>[9]</sup>

PBMC Isolation, RNA extraction and Reverse Transcription: PBMCs were isolated by Ficoll gradient classification. Total RNA was extracted, quantified (NanoDrop), and assessed for integrity. Reverse transcription was performed using random hexamers. Further details on these methods are provided by Papagiannakis et al (2024).<sup>[9]</sup>

Quantitative RT-PCR: A novel primer pair targeting PRKN with the following sequences:

- PRKN forward primer: 5' - AAA ACC ACC AAG CCC TGT C-3'
- PRKN reverse primer: 5' TGC GGA CAC TTC ATG TGC -3'

was designed to optimise amplification efficiency. The aforementioned primer pair produced better

meting curves in the subsequent trial of RT-PCR compared to the ones used in Papagiannakis et al. (2024),<sup>[9]</sup> and therefore it was used in all subsequent analyses. GADPH served as the reference gene. Relative expression levels were calculated using the  $\Delta\Delta C_t$  method. Apart from the novel primer set, the methodology followed the lines of Papagiannakis et al (2024),<sup>[9]</sup>

Statistical Analysis: Group comparisons used one-way ANOVA with Bonferoni correction. ROC analysis determined sensitivity, specificity and predictive values.

RESULTS

Participant characteristics

No significant difference in age or sex distribution was observed between groups ( $p>0.05$ ). PRKN-related PD patients had an earlier mean age of onset ( $38,3 \pm 10,2$  years) compared to iPD ( $56,5$

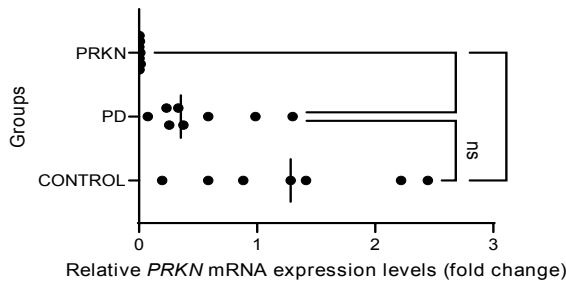


Figure 1. Relative PRKN mRNA expression (fold change) among groups (Controls, PD, PRKN).

$\pm 7,7$  years) (Table 1). The PRKN mutation types and their clinical significance are depicted in Table 2. The samples with the genetic background of c101\_102delAG;Exon 7 del (7), Exon 2 del (3) and Exon 6: c540delinsGC:p.V181Rfs\*16 (5) have already been tested with the PRKN mRNA assay in our previous study,<sup>[9]</sup> while the samples with the variants c401T>C (6), c758G>A; exon 2 deletion (4), c101\_102delAG homozygous (1) and c376C>T (2) were tested for the first time, which permits us to verify, using yet another primer set, and extend our previous findings.

Table 1. Demographic characteristics of study participants.

| Characteristics           | CT (n=7)    | iPD (n=8)   | PRKN (n=7)    |
|---------------------------|-------------|-------------|---------------|
| Mean age (years)          | 59.9 (11.6) | 59.9 (7.5)  | 46.7 (14.4)   |
| Mean age of onset         | -           | 56.5 (7.71) | 38.29 (10.19) |
| Sex (M/F)                 | 1/6         | 5/3         | 4/3           |
| Disease duration (years)  | -           | 2.88 (1.22) | 8.67 (8.03)   |
| PRKN mutations (Bial/Het) | -           | -           | 3/4           |

CT: controls; iPD: idiopathic Parkinson disease patients; PRKN: Parkin-related Parkinson’s disease patients; M: male; F: female; Bial: biallelic; Het: heterozygous; Standard Deviation in parentheses.

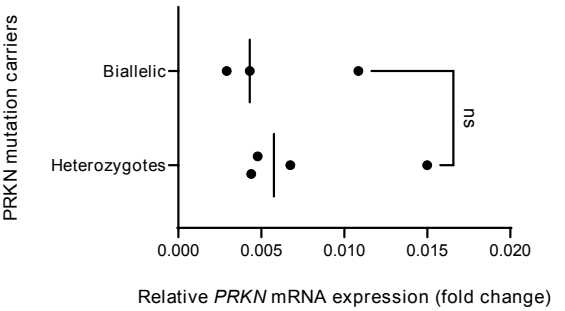


Figure 2. Relative PRKN mRNA expression levels (% fold change) among PRKN mutation carriers (biallelic, heterozygotes).

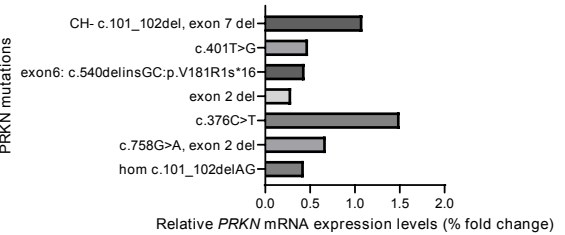


Figure 3. Relative PRKN mRNA expression levels (% fold change) among PRKN mutations carriers (regarding type of mutation).

PRKN mRNA Expression: PRKN mRNA levels differed significantly between groups ( $p<0.0001$ ). Expression was markedly reduced in PRKN-related PD samples compared to both controls and iPD (Figure 1). PRKN expression levels were decreased in biallelic and heterozygous PRKN mutation carrier PD patients compared to healthy controls (181-fold or 99,45% lower) and this relation was statistically significant ( $p$ -value=0.0004). Similarly, mRNA levels were significantly decreased in PRKN-related PD compared to iPD (74-fold or 98,7% lower,  $p$ -value=0.0210). PRKN mRNA levels were slightly decreased in iPD compared to healthy controls (2,44-fold or 59% lower), but this relationship was not statistically significant ( $p$ -value=0.6497) (Table 3). PRKN mRNA levels were uniformly very low in all PRKN mutation carriers, even heterozygotes (Figures 1,2). In fact, heterozygote mRNA levels were not significantly different from biallelic carriers ( $p$ -value=0.6458). Individual values

**Table 2.** Type of mutation of *PRKN*-related PD patients and clinical significance.

| Patient | Mutation                         | Status                | Clinical Significance        | Protein        |
|---------|----------------------------------|-----------------------|------------------------------|----------------|
| 1       | c.101_102delAG                   | Homozygous            | Pathogenic                   | p.Gln34Argfs*5 |
| 2       | c.376C>T (exon 4)                | Heterozygous          | Pathogenic-likely pathogenic | p.Arg126Trp    |
| 3       | Exon 2 del                       | Heterozygous          | Pathogenic                   | -              |
| 4       | c.758G>A; exon 7 del             | Compound heterozygous | Pathogenic/likely pathogenic | p.Cys253Tyr    |
| 5       | Exon 6: c540delinsGC:pV181Rfs*16 | Heterozygous          | Uncertain significance       | p.V181Rfs*16   |
| 6       | c401T>C                          | Heterozygous          | Uncertain significance       | p.Leu.134Pro   |
| 7       | c101_102delAG; exon 7 del        | Compound Heterozygous | Pathogenic                   | p.Gln34fs      |

**Table 3.** Fold-change of expression levels and % *PRKN* expression reduction between the 3 groups.

| Groups      | Fold-change     | % <i>PRKN</i> expression | p-value      |
|-------------|-----------------|--------------------------|--------------|
| iPD vs CT   | 2,44-fold lower | 59% lower                | 0.6497 (ns)  |
| PRKN vs CT  | 74-fold lower   | 98,7% lower              | 0.0004 (***) |
| PRKN vs iPD | 181-fold lower  | 99,45% lower             | 0.0210 (*)   |

CT: controls; iPD: idiopathic Parkinson disease patients; PRKN: Parkin-related Parkinson's disease patients and their statistical significance (p-value).

for each PRKN-PD subject are presented in **Figure 3**.

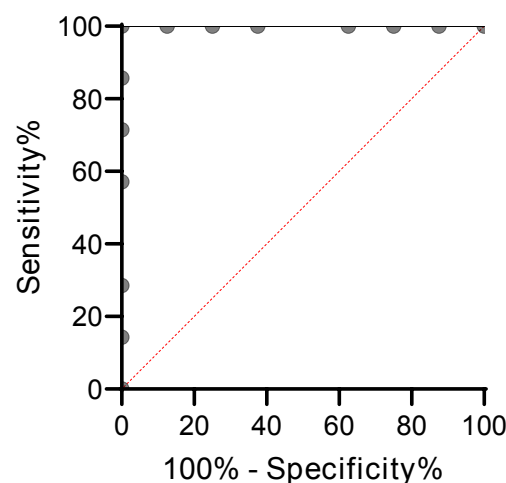
#### Diagnostic Performance

ROC analysis was performed to assess the usefulness of *PRKN*-expression levels as discriminator of the presence of *PRKN* variants in PD patients. The area under the curve was 1000 (95% confidence intervals 1.000 to 1.000), for the cut-off value of *PRKN* mRNA expression Ct=32.85, yielding a sensitivity of 100 % (64.57-100%) and a specificity of 100% (67.56-100%). In the context of PD, the positive predictive value of low *PRKN* expression levels for the presence of heterozygote or biallelic *PRKN*-variants was 100% (72.3%-100%) (**Figure 4**).

#### DISCUSSION

Our study provides new insights into the differential expression of *PRKN* mRNA in PBMCs across various groups, including healthy controls, idiopathic PD patients and PD patients carrying various *PRKN* variants. The findings underscore the critical role of *PRKN* expression in the pathophysiology of PD and its potential as a biomarker for the identification of *PRKN*-variant carriers.

A major result of our study is that pathogenic variants in the *PRKN* gene result in reduced expression of the respective mRNA in PBMCs. This result

**Figure 4.** ROC analysis.

was evident not only in biallelic individuals, but also in heterozygous PD patients. Although the role of heterozygous *PRKN* variants in the pathogenesis of PD remains controversial,<sup>[8]</sup> our results suggest that a profound *PRKN* expression impairment, beyond what would be expected from a single allele disruption, is present in most heterozygote *PRKN*-variant carriers who have already developed early-onset PD. The sig-

nificant reduction in **PRKN** mRNA expression among **PRKN** variant carriers, compared to both healthy controls and iPD, corroborates the prevailing idea that these genetic variations are strongly associated with reduced **PRKN** activity. Such reduced activity is linked to compromised mitochondrial quality control and dopaminergic neuronal death.<sup>[6,7]</sup> Interestingly, iPD patients also exhibited slightly lower **PRKN** mRNA levels compared to healthy controls. This non-significant trend may suggest a broader involvement of **PRKN** dysregulation in iPD cases or reflect heterogeneity within this subgroup. Alternatively, it might just be the result of the limited number of samples studied; indeed, in the previous study from the lab, no difference was discerned between iPD and controls regarding PBMC **PRKN** mRNA levels, although again levels in controls were slightly higher.<sup>[9]</sup> It would be important to perform such studies in a larger scale in the future.

It is striking that, in all cases with **PRKN** mutations examined, whether heterozygous or biallelic, **PRKN** mRNA levels were extremely low. This is true not only for large deletions, and the small deletion c.101\_102delAG (which creates a premature translational stop signal in the **PRKN** gene), but is also the case even for 3 single nucleotide variants (c.376C>T, c.401T>C, c.758G>A). A possible mechanism of mRNA degradation in the case of frameshift mutations produced by deletions is the process of non-sense mediated mRNA decay.

It has been suggested that heterozygous **PRKN**-mutation carriers, even when asymptomatic, carry a small degree of dopaminergic nigrostriatal dysfunction. It is possible that participants in our cohort did not have the necessary compensatory mechanisms, as they have already developed PD. The fact that in most cases in our study these mutations lead to an important reduction of **PRKN** mRNA levels close to those in biallelic carriers suggests that additional related variants may exist in the other allele, possibly in regulatory regions for **PRKN** expression. Long read sequencing of 4 heterozygote carriers in our original study, however, did not reveal any additional causal genomic alterations. Long read sequencing for the two new heterozygote samples in the current study has not been performed. It remains quite puzzling why heterozygote mutation carriers have such low **PRKN** mRNA levels, quite similar to those with biallelic mutations. An explanation, apart from a yet unidentified second genetic hit, could be that in these cases there are further epigenetic mechanisms that lead to a substantial reduction of **PRKN** mRNA levels and the resultant early disease manifestation.

These results align with the findings of our previous study.<sup>[9]</sup> In 3 samples the results of the prior study were verified using another primer pair, different from the ones in our previous work, providing an

independent verification. The primer pair evaluated in the current work gave the best melting curves and thus would appear to be the best choice at this point for assessing accurately **PRKN** mRNA levels in PBMCs. Furthermore, results of low **PRKN** mRNA levels in **PRKN** mutation carriers were extended to 4 additional samples with the following genetic background: the homozygous c.101\_102delAG mutation, the heterozygous c.376C>T mutation, the heterozygous c540delinsGC mutation in exon 6, and the heterozygous 401T>G mutation. All these samples had very low **PRKN** mRNA levels, which is especially notable for the heterozygous cases, as these 3 additional mutations had not been previously assessed.

As far as diagnostic implications are concerned, PBMCs are easily accessible blood cells, while RNA isolation, reverse transcription and quantitative PCR are rather standard laboratory methods; as such, the methodology described here holds promise as a first screening tool to assess for the presence of **PRKN** pathogenic variants. The ROC analysis we have performed shows high sensitivity and specificity for the recognition of a PD patient with a **PRKN** variant. With a ROC area under the curve of 1.00 and sensitivity and specificity both at 100%, **PRKN** mRNA levels appear to be a robust biomarker for detecting the presence of **PRKN** variants. In fact, the positive predictive value of low **PRKN** levels with this assay is 100% indicating that the current protocol may be extremely useful for screening for **PRKN**-mutation carrier status in the PD population. This finding has significant implications for clinical practice, as it offers a non-invasive and reliable method for identifying genetic subtypes of PD, which may inform personalised therapeutic strategies. To our knowledge, there are no other studies reporting an easily accessible screening test for **PRKN** mutation status. This could simplify the genetic work-up of PD patients, especially in underserved areas, with limited access to genetic testing.

Limitations of our study include a small sample size and single-centre design. Larger, multi-ethnic cohorts are needed to validate clinical applicability at a larger clinical scale.

## CONCLUSION

This report, using a new set of primers and additional cases, confirms that **PRKN** mRNA quantification in PBMCs is a promising biomarker for **PRKN** mutation status in PD. With its high diagnostic accuracy, it may become a useful screening tool for genetic testing, enabling earlier identification and, in the future, targeted management of **PRKN**-related PD. Furthermore, the present report extends on previous findings that suggest that severe suppression of **PRKN** mRNA levels is a common feature of **PRKN**-related

PD, irrespective of heterozygote or biallelic status or the particular mutation type. This intriguing biological phenomenon warrants further investigation into the underlying mechanisms regulating *PRKN* transcription.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## LEYKOENCEPHALOPATHY IN A PATIENT WITH LIMB GIRDLE MUSCULAR DYSTROPHY

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### ABSTRACT

We describe the case of a 53-year-old female patient diagnosed with muscular dystrophy associated with LAMA2 gene pathogenic variants, a rare subtype of limb girdle muscular dystrophy, and highlight the characteristic leukoencephalopathic pattern of this disorder.

**Keywords:** Limb girdle muscular dystrophy,  $\alpha$ 2-laminin, leukoencephalopathy

## ΛΕΥΚΟΕΓΚΕΦΑΛΟΠΑΘΕΙΑ ΣΕ ΑΣΘΕΝΗ ΜΕ ΖΩΝΙΑΙΑ ΜΥΪΚΗ ΔΥΣΤΡΟΦΙΑ

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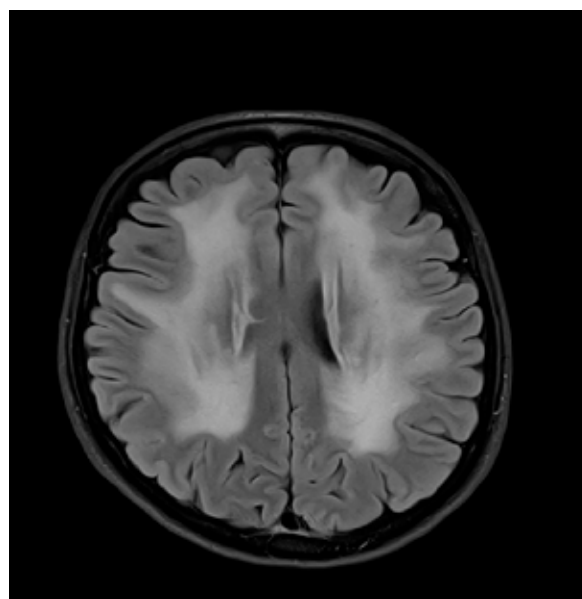
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### Περίληψη

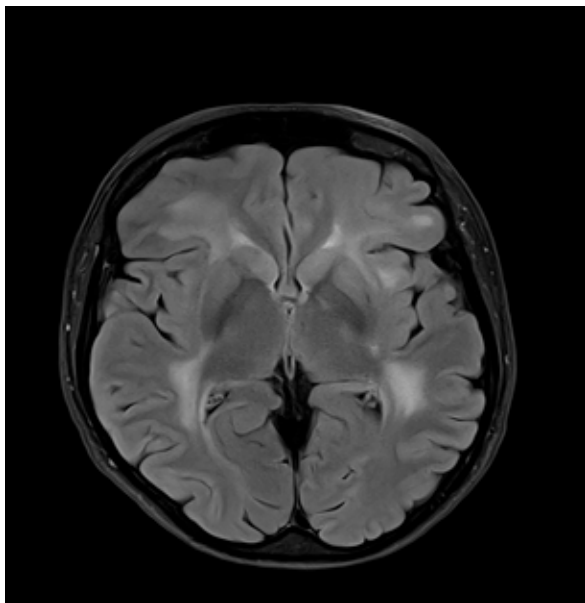
Περιγράφουμε την περίπτωση μιας ασθενούς 53 ετών με διάγνωση μυϊκής δυστροφίας σχετιζόμενης με παθογόνες παραλλαγές στο γονίδιο της LAMA2, ενός σπανίου υποτύπου ζωνιαίας μυϊκής δυστροφίας, και αναδεικνύουμε το λευκοεγκεφαλοπαθητικό πρότυπο που χαρακτηρίζει την διαταραχή.

**Λέξεις-κλειδιά:** Ζωνιαία μυϊκή δυστροφία,  $\alpha$ 2-λαμινίνη, λευκοεγκεφαλοπάθεια

Το ιστορικό της ασθενούς αρχίζει στην παιδική ηλικία με βάδισμα στα δάκτυλα λόγω ρικνώσεων των ποδοκνημικών. Η νόσος εμφανίσε βραδεία πορεία με ανάπτυξη κεντρομελικής μυϊκής αδυναμίας με έμφαση στον τραπεζοειδή-υπακάνθιο (3/5 κατά MRC) και μέσο γλουτιαίο-τετρακέφαλο (2/5 κατά MRC) αμφοτεροπλευρώς. Διαπιστώνεται, επιπλέον, πτερυγοειδής ωμοπλάτη, στροφο-σκολίωση και σκαφοειδής θώρακας. Η παρουσία σταθερής αύξησης της CPK (550-1200 U/l) και τα ευρήματα του νευροφυσιολογικού ελέγχου τεκμηρίωσαν μυοπαθητική διαταραχή, ενώ η ανάλυση του κωδικοποιητικού γονιδιώματος αποκάλυψε 2 παρανοηματικές (missense) αλλαγές στο γονίδιο της  $\alpha$ 2-λαμινίνης (LAMA2), c.2960G>T [p.Cys987Phe] και c.3833G>T [p.Gly1278Val], θέτοντας την διάγνωση της σχετιζόμενης με το LAMA2 μυϊκής δυστροφίας (LAMA2-MD). Έγινε σύσταση για διενέργεια MRI εγκεφάλου, όπου αναδείχθηκε εκτεταμένη και συμμετρική λευκοεγκεφαλοπάθεια (Εικόνα 1).



**Εικόνα 1Α.** Διάχυτη και συμμετρική αύξηση του MR σήματος στην περικολιδιακή και εν τω βάθει λευκή ουσία υπερσκληνίδια με διάσωση των U ινών. Εγκάρσια τομή FLAIR



**Εικόνα 1B.** Περιορισμένης έκτασης συρρέουσες εστίες παθολογικού σήματος στο επίπεδο των βασικών γαγγλίων.

Εγκάρσια τομή FLAIR

Το φαινοτυπικό φάσμα της LAMA2-MD περιλαμβάνει την συγγενή μυϊκή δυστροφία (Congenital muscular dystrophy type 1A, MDC1A) και, σπανιότερα, ηπιότερες περιπτώσεις με μεταγενέστερη ηλικία έναρξης και εικόνα ζωνιαίας μυϊκής δυστροφίας (LGMD R23), όπως στην ασθενή μας <sup>[1]</sup>. Σταθερό

απεικονιστικό εύρημα της LAMA2-MD αποτελεί η προσβολή της λευκής ουσίας του εγκεφάλου. Παθογενετικά θεωρείται ότι η ανεπάρκεια της α2-λαμινίνης σχετίζεται με δυσλειτουργία του αιματο-εγκεφαλικού φραγμού με αποτέλεσμα διαταραχή στην ομοιόσταση του ύδατος<sup>[1][2]</sup>. Εκτός της LAMA2-MD, λευκοεγκεφαλοπάθεια περιγράφεται στην μυοτονική δυστροφία τύπου 1, το σύνδρομο Kearns-Sayre, το σύνδρομο MNGIE (Mitochondrial NeuroGastroIntestinal Encephalomyopathy) και άλλες σπανιότερες μυοπάθειες.

#### ΔΗΛΩΣΗ ΣΥΜΦΕΡΟΝΤΩΝ

Οι συγγραφείς δεν αναφέρουν κάποια σύγκρουση συμφερόντων.

#### ΒΙΒΛΙΟΓΡΑΦΙΑ

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# SMOOTH PURSUIT, SACCADDES, AND OPTOKINETIC NYSTAGMUS IN HEALTHY ADULTS: BINOCULAR VIDEONYSTAGMOGRAPHIC ANALYSIS

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## ABSTRACT

**Background:** Objective assessment of oculomotor function provides valuable information for the evaluation of central and peripheral nervous system integrity. Videonystagmography (VNG) allows quantitative analysis of eye movements and facilitates the establishment of normative reference values in healthy populations. **Methods:** Oculomotor function was assessed in 31 healthy adults (mean age: 53.6 years, range: 19–79 years) with no history of neurological disease using videonystagmography. Optokinetic nystagmus, saccadic eye movements, and smooth pursuit eye movements were recorded. Participants were divided into two age groups (<50 and ≥50 years) to investigate age-related effects. Parameters analysed included gain, latency, velocity, accuracy, and peak velocity (velocity/amplitude ratio), depending on the type of eye movement. **Results:** Optokinetic nystagmus gain showed symmetrical responses between movement directions and decreased significantly with increasing stimulus velocity, while no significant association with age was observed. Saccadic eye movements demonstrated symmetrical performance in both horizontal and vertical planes. Saccadic latency increased significantly with age, whereas mean velocity, peak velocity, and accuracy were not significantly affected. Smooth pursuit gain decreased with increasing target motion frequency and age, particularly at higher frequencies, with horizontal smooth pursuit exhibiting higher gain values than vertical pursuit. **Conclusions:** The present study provides normative reference data for oculomotor parameters in a healthy Greek cohort. The findings also indicate that age-related effects on oculomotor function are parameter-specific, predominantly affecting timing-related and dynamic performance measures, while basic execution-related parameters remain relatively preserved. Ultimately, the data highlight the utility of modern binocular videonystagmography in precisely assessing oculomotor function, aiding in the differentiation between physiological aging and pathological conditions.

**Keywords:** saccades, smooth pursuit, optokinetic nystagmus

## ΔΙΟΦΘΑΛΜΗ ΒΙΝΤΕΟΝΥΣΤΑΓΜΟΓΡΑΦΙΚΗ ΑΝΑΛΥΣΗ ΛΕΙΑΣ ΠΑΡΑΚΟΛΟΥΘΗΣΗΣ, ΣΑΚΚΑΔΙΚΩΝ ΚΙΝΗΣΕΩΝ ΚΑΙ ΟΠΤΙΚΟΚΙΝΗΤΙΚΟΥ ΝΥΣΤΑΓΜΟΥ ΣΕ ΥΓΙΕΙΣ ΕΝΗΛΙΚΕΣ

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## Περίληψη

**Εισαγωγή:** Η αντικειμενική αξιολόγηση της οφθαλμοκινητικότητας παρέχει σημαντικές πληροφορίες για την εκτίμηση της ακεραιότητας τόσο του κεντρικού όσο και του περιφερικού νευρικού συστήματος. Η βιντεονυσταγμογραφία (VNG) επιτρέπει την καταγραφή και ανάλυση των οφθαλμικών κινήσεων διευκολύνοντας τον προσδιορισμό φυσιολογικών τιμών αναφοράς στον υγιή πληθυσμό. **Μεθοδολογία:** Μελετήθηκαν οι οφθαλμικές κινήσεις 31 υγιών ενηλίκων (μέση ηλικία: 53,6 έτη, εύρος: 19–79 έτη) με ελεύθερο ιστορικό νευρολογικής νόσου, με τη χρήση βιντεονυσταγμογραφικού συστήματος. Συγκεκριμένα καταγράφηκαν ο οπτοκινητικός νυσταγμός, οι σακκαδικές κινήσεις και η λεία κίνηση παρακολούθησης. Οι συμμετέχοντες χωρίστηκαν σε δύο ηλικιακές ομάδες (<50 και ≥50 ετών) για τη διερεύνηση της επίδρασης της ηλικίας. Οι παράμετροι που αναλύθηκαν περιλάμβαναν το gain, τη λανθάνουσα περίοδο, τη μέση και τη μέγιστη ταχύτητα,

καθώς και την ακρίβεια, αναλόγως του είδους της οφθαλμικής κίνησης. **Αποτελέσματα:** Ο οπτοκινητικός νυσταγμός παρουσίασε συμμετρική απόκριση μεταξύ των κατευθύνσεων κίνησης και μείωση του gain με την αύξηση της ταχύτητας του ερεθίσματος, χωρίς στατιστικά σημαντική συσχέτιση με την ηλικία. Οι σακκαδικές κινήσεις εμφάνισαν συμμετρική συμπεριφορά στο οριζόντιο και στο κάθετο επίπεδο, ενώ η λανθάνουσα περίοδος παρουσίαζε αύξηση με την αύξηση της ηλικίας. Αντίθετα, η μέση και η μέγιστη ταχύτητα, καθώς και η ακρίβεια, δεν επηρεάστηκαν σημαντικά από την ηλικία. Στις λείες κινήσεις παρακολούθησης παρατηρήθηκε μείωση του gain με την αύξηση της συχνότητας κίνησης του στόχου και της ηλικίας, κυρίως στο οριζόντιο επίπεδο. **Συμπεράσματα/συζήτηση:** Τα αποτελέσματα της μελέτης δείχνουν ότι οι ηλικιακές επιδράσεις στην οφθαλμοκινητικότητα είναι εξαρτώμενες από το είδος της παραμέτρου και του συστήματος ελέγχου, επηρεάζοντας κυρίως χρονικές και δυναμικές παραμέτρους, ενώ οι βασικοί μηχανισμοί εκτέλεσης των κινήσεων διατηρούνται. Η παρούσα εργασία παρέχει φυσιολογικές τιμές αναφοράς για παραμέτρους οφθαλμοκινητικότητας σε υγιή ενήλικο πληθυσμό και υποστηρίζει τη χρησιμότητα της βιντεονυσταγμογραφίας στην κλινική αξιολόγηση και στη διάκριση μεταξύ φυσιολογικής γήρανσης και παθολογικών καταστάσεων.

**Λέξεις-κλειδιά:** Σακκαδικές κινήσεις, λεία κίνηση παρακολούθησης, οπτοκινητικός νυσταγμός

## INTRODUCTION

Oculomotor function is fundamental for maintaining clear, stable, and high-resolution vision, allowing accurate projection of a visual target onto the macula and, more specifically, the fovea. The transfer and stabilisation of the retinal image at the desired location are achieved through specialised eye movements, which are controlled by complex cortical, subcortical, cerebellar, and brainstem neural networks. In humans, eye movements are predominantly binocular and conjugate, with the visual axes of both eyes moving in the same direction<sup>[1]</sup>. Different types of eye movements are subserved by distinct neural circuits within the central nervous system, all of which converge on a common final pathway downstream of the three ocular motor nuclei to innervate the extraocular muscles. The detailed functional neuroanatomy of these circuits has been extensively elucidated over recent decades, providing an excellent tool for the clinical neurologist in diagnostic practice. The present study focuses on three major types of conjugate eye movements: saccades, smooth pursuit, and optokinetic nystagmus, which constitute key indicators of the functional integrity of the oculomotor system.

Saccadic eye movements are rapid, brief eye movements that shift the visual axis from one target to another within the visual field. They represent the fastest voluntary movements of the human body and are characterised by high velocities, short duration, and a distinct latency period.<sup>[1,3]</sup> The principal central nervous system structures involved in the execution of saccadic eye movements include<sup>[2,3]</sup>:

- Frontal Eye Fields (FEF) and other structures of the cortex
- Dorsal vermis and the fastigial nucleus of the cerebellum
- Paramedian Pontine Reticular Formation, nucleus Prepositus Hypoglossi, and Medial Ves-

tibular Nucleus, for the horizontal saccades in the pons

- rostral interstitial nucleus of the Median Longitudinal Fasciculus, and Interstitial nucleus of Cajal for the vertical saccades in the midbrain.

Their evaluation is based on specific parameters<sup>[4]</sup>:

- 1) latency: the time required for sensory processing and motor planning, until the initiation of motion.
- 2) mean and peak velocity: in degrees/sec. Considering that saccadic eye movement velocity is varying, both mean and peak velocity are employed for its assessment.
- 3) accuracy, defined as the degree of correspondence between the final eye position and the target location.

**Smooth pursuit** eye movements enable continuous tracking of slowly moving targets, maintaining the retinal image on the fovea throughout motion. Unlike saccades, smooth pursuit is characterised by continuous and smooth changes in eye position. The principal central nervous system structures involved in the execution of smooth pursuit movements include<sup>[5]</sup>:

- Mainly parts of the visual cortex
- Floculus and parafloculus in the cerebellum
- the Dorsolateral Pontine Nuclei and the Medial Vestibular Nuclei.

The primary parameter used to assess smooth pursuit function is gain, defined as the ratio of eye velocity to target velocity, which ideally approaches unity.<sup>[5]</sup>

Optokinetic nystagmus is a physiological reflex elicited by large-field visual motion stimuli that create the perception of environmental movement. It consists of an alternating slow phase, during which the eyes follow the moving visual pattern, and a fast resetting phase in the opposite direction.<sup>[6]</sup> The main brain structures involved in optokinetic nystagmus are:

- The Nucleus of the Optic Tract
- The vestibular nuclei in the pons
- The flocculus in the cerebellum.

Analysis of the slow phase, commonly expressed by the gain parameter, is widely used to assess the functional interaction between visual and vestibular mechanisms.<sup>[6]</sup>

Videonystagmography (VNG) has become an established, non-invasive method for recording and quantitatively analysing eye movements. Modern VNG systems allow precise measurement of multiple oculomotor parameters, like those that were mentioned before, and facilitate the development of normative databases, which are essential for the interpretation of pathological findings.

The aim of the present study is to quantitatively analyse saccadic eye movements, smooth pursuit, and optokinetic nystagmus in a healthy adult population using videonystagmography, as well as to investigate the effects of age and movement direction on selected parameters. The results of this study are intended to contribute to the establishment of reliable normative reference values for future comparisons with non-healthy populations.

## MATERIALS AND METHODS

The present study was approved by the Ethics and Research Committee of Eginition Hospital.

### Participants

Thirty-one healthy adults participated in the study, with no history of central nervous system disorders or other conditions that could affect oculomotor function. The sample included 16 females and 15 males, aged between 19 and 79 years. For the analysis of age-related effects, participants were divided into two age groups: younger than 50 years and 50 years or older.

### Equipment

Eye movement recordings were obtained using the VisualEyes™ 525b videonystagmography system (Interacoustics, Denmark), which is based on videonystagmography (VNG) technology. Specialised goggles equipped with integrated infrared cameras were used to continuously record eye position and movement. Data were recorded, processed, and stored using the manufacturer's dedicated software. For selected analyses of saccadic eye movements, data were exported in ASCII format and analysed offline using custom-written laboratory software in MATLAB R2021b (MathWorks, Natick, MA, USA). Eye velocity was computed by digitally differentiating the displacement signal while accounting for the sampling rate of 100 Hz. The resulting time series was then smoothed using a tenth-order Butterworth low-pass filter with a cutoff frequency of 40 Hz, followed by

an eight-sample, first-order Savitzky–Golay filter.

### Experimental procedure

Participants were examined in a seated position while wearing the VNG goggles and were instructed to visually follow stimuli presented on a screen positioned directly in front of them (**Figure 1**). All tests were performed according to standardised protocols and included recordings of saccadic eye movements, smooth pursuit eye movements, and optokinetic nystagmus.



**Figure 1.** Experimental setup for videonystagmographic recording of eye movements. Participants were seated facing the recording screen while wearing VNG goggles (here, facing a moving visual pattern for optokinetic nystagmus examination).

### Saccadic eye movements

Saccadic eye movements were recorded in both the horizontal and the vertical plane. Participants were instructed to shift their gaze between sequential visual targets appearing at predefined positions on the screen.

The following parameters were analysed:

- Mean latency (ms)
- Mean velocity (°/s)
- Accuracy (%)
- Peak velocity, estimated using the velocity/amplitude (vel/amp) ratio, defined as the ratio of peak velocity to saccade amplitude.

Analyses were performed separately for each movement direction (rightward/leftward and upward/downward).

### Smooth pursuit eye movements

Smooth pursuit eye movements were recorded in both the horizontal and the vertical plane. Participants were instructed to follow a visual target moving in a sinusoidal pattern at predefined frequencies.

The analysed parameters were:

- Gain, defined as the ratio of eye velocity to target velocity

- Symmetry, calculated from the difference in gain values between opposite movement directions and expressed as a percentage.

In the horizontal plane, five target motion frequencies were examined (0.1, 0.2, 0.3, 0.4, and 0.5 Hz), whereas in the vertical plane three frequencies were analysed (0.1, 0.2, and 0.3 Hz). Vertical smooth pursuit analysis was performed in 30 participants.

### Optokinetic nystagmus

Optokinetic nystagmus was recorded in the horizontal plane. Participants were exposed to moving visual patterns covering a large portion of the visual field.

The analysed parameter was:

- Gain, defined as the ratio of the mean eye velocity during the slow phase of optokinetic nystagmus to the velocity of the moving visual stimulus

Recordings were performed at two different stimulus velocities.

### Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 21.0). Data are presented as mean  $\pm$  standard deviation. The choice of statistical tests was based on the distribution of the variables. Normal reference ranges were determined using either  $\pm 2$  SD from the mean or the 5th–95th percentile range, depending on data distribution. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Participant characteristics

A total of 31 adults with no history of neurological disease were included in the study. Of these, 16 were female (51.6%) and 15 were male (48.4%). Participants' age ranged from 19 to 79 years, with a mean age of 53.6 years (SD: 17.9). Thirteen participants (41.9%) belonged to the younger age group (<50 years), while 18 participants (58.1%) were aged 50 years or older.

### Optokinetic nystagmus

For optokinetic nystagmus, the gain of the slow phase was analysed in the horizontal plane at two stimulus velocities (20°/s and 40°/s) and for both directions. Mean gain values and standard deviations for the total sample, as well as for the two age groups (<50 and  $\geq 50$  years), were calculated and the proposed normal reference ranges for gain, based on data distribution, are shown in **Table 1**.

Comparison of mean gain values between the two movement directions (leftward vs. rightward) revealed no statistically significant differences at either

20°/s ( $p = 0.40$ ) or 40°/s ( $p = 0.78$ ). In contrast, comparison between stimulus velocities demonstrated significantly lower gain values at the higher velocity (40°/s) compared with 20°/s for both directions ( $p < 0.001$ ). Correlation analysis between age and gain showed a negative association across all recording conditions; however, none of the correlations reached statistical significance ( $p > 0.05$ ).

**Table 1.** Normal ranges of gain.

|                                        | Gain (20°/s) left-ward | Gain (40°/s) left-ward | Gain (20°/s) right-ward | Gain (40°/s) right-ward |
|----------------------------------------|------------------------|------------------------|-------------------------|-------------------------|
| <b>Total sample (n=31)</b>             | 60-100                 | 35-102                 | 55-108                  | 33-106                  |
| <b>Age &lt;50 (n=13)</b>               | 61 - 100               | 34 - 108               | 56 - 112                | 39 - 112                |
| <b>Age <math>\geq 50</math> (n=15)</b> | 50-113                 | 36-100                 | 53-106                  | 30-100                  |

Gain: eye velocity to target velocity ratio.

### Saccadic eye movements

Mean values and standard deviations for all parameters for the total sample, as well as for the two age groups (<50 and  $\geq 50$  years) were calculated and

### Horizontal saccades

For horizontal saccadic eye movements, the parameters latency, mean velocity, peak velocity (estimated using the velocity/amplitude (vel/amp) ratio), and accuracy were analysed for both directional movements. the proposed normal reference ranges are shown in **Table 2**.

Comparison between the two movement directions (rightward vs. leftward) revealed no statistically significant differences for any of the examined parameters ( $p > 0.05$ ). Comparison between age groups demonstrated significantly longer latency values in participants aged  $\geq 50$  years compared with those aged <50 years ( $p < 0.05$ ). No statistically significant differences between age groups were observed for mean velocity, peak velocity (vel/amp), or accuracy ( $p > 0.05$ ). Correlation analysis showed a significant positive association between age and saccadic latency ( $p < 0.05$ ), whereas no statistically significant correlations were found between age and the remaining parameters.

### Vertical saccades

For vertical saccadic eye movements, the same parameters (latency, mean velocity, peak velocity/

amplitude, and accuracy) were analysed for upward and downward movements. The normal reference ranges are shown in **Table 2**. In **Figure 2A**, the representation of saccades in a graph during the examination is shown.

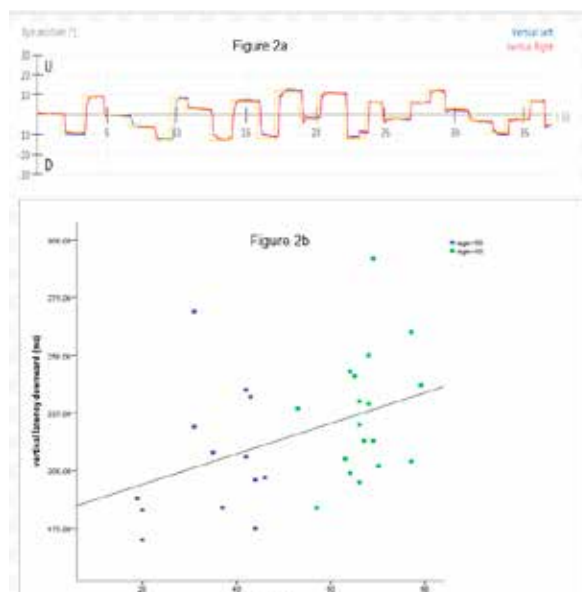
Comparison between upward and downward saccades revealed no statistically significant differences for any of the examined parameters ( $p > 0.05$ ). Com-

parison between age groups showed significantly increased latency values for vertical saccades in participants aged  $\geq 50$  years compared with those aged  $< 50$  years ( $p < 0.05$ ) (**Figure 2B**). No statistically significant differences between age groups were observed for mean velocity, peak velocity, or accuracy.

**Table 2.** Normal ranges.

|                                        | Lat leftward (ms) | Lat rightward (ms) | Vel Leftward (°/s) | Vel rightward (°/s) | Ac. leftward (%) | Ac. rightward (%) | Vel/amp horizontal |
|----------------------------------------|-------------------|--------------------|--------------------|---------------------|------------------|-------------------|--------------------|
| <b>Total sample (n=31)</b>             | 153-260           | 188-239            | 305-409            | 274-432             | 79               | 77                | 23-41              |
| <b>Age &lt;50 (n=13)</b>               | 155 – 230         | 165 – 232          | 300-445            | 282-412             | 77               | 75                | 23-42              |
| <b>Age <math>\geq 50</math> (n=18)</b> | 163-271           | 190-255            | 270-449            | 268-445             | 82               | 78                | 23-40              |
|                                        | Lat downward (ms) | Lat upward (ms)    | Vel downward (°/s) | Vel upward (°/s)    | Ac. downward (%) | Ac. upward (%)    | Vel/amp vertical   |
| <b>Total sample (n=31)</b>             | 159-273           | 163-266            | 189-393            | 192-372             | 68               | 63                | 23-42              |
| <b>Age &lt;50 (n=13)</b>               | 149-260           | 160-240            | 187-400            | 204-365             | 70               | 77                | 23-42              |
| <b>Age <math>\geq 50</math> (n=18)</b> | 171-278           | 176-275            | 188-390            | 182-380             | 69               | 59                | 21-44              |

Lat: latency; vel: mean velocity; ac: accuracy; Vel/amp: peak velocity to saccade amplitude. For the accuracy variable, only the lower limit of the normal range is reported.



**Figure 2.** (A) Diagram illustrating saccadic movements on the vertical plane. On the x axis (t) is time, and on the y axis is the amplitude (position) of the movement. The yellow line represents the movement of the visual stimulus, while the blue and red lines represent the movements of the left and right eye, respectively. (B) Association between age (x-axis, years) and saccadic latency (y-axis, ms) for vertical, downward, and saccadic eye movements.

### Smooth pursuit eye movements

Mean values and standard deviations for gain and symmetry for the total sample, as well as for the two age groups ( $< 50$  and  $\geq 50$  years) were calculated for both horizontal and vertical plane of movement.

### Horizontal smooth pursuit

For horizontal smooth pursuit eye movements, the parameters gain and symmetry were analysed at five target motion frequencies (0.1, 0.2, 0.3, 0.4, and 0.5 Hz). The proposed normal reference ranges are shown in **Tables 3** and **4**.

Comparison between the two movement directions (rightward vs. leftward) revealed no statistically significant differences for any of the examined frequencies ( $p > 0.05$ ).

Comparison between age groups demonstrated

significantly lower gain values in participants aged  $\geq 50$  years compared with those aged  $< 50$  years at the higher target motion frequencies (0.4 and 0.5 Hz;  $p < 0.05$ , Figure 3B). No statistically significant differences between age groups were observed at the lower frequencies (0.1–0.3 Hz;  $p > 0.05$ ). For the symmetry parameter, no statistically significant differences were found either between movement directions or between age groups at any of the examined frequencies ( $p > 0.05$ ).

#### Vertical smooth pursuit

For vertical smooth pursuit eye movements, the

( $p > 0.05$ ).

same parameters (gain and symmetry) were analysed at three target motion frequencies (0.1, 0.2, and 0.3 Hz). In **Figure 3A**, there is a graph depiction of smooth pursuit movement on the vertical plane during the recording. The normal reference ranges are shown in **Tables 3** and **4**.

Comparison between upward and downward movements revealed no statistically significant differences in gain or symmetry at any of the examined frequencies ( $p > 0.05$ ). Comparison between age groups did not reveal any statistically significant differences in gain or symmetry values for vertical smooth pursuit at any of the examined frequencies

**Table 3.** Normal values for gain in the horizontal and vertical plane.

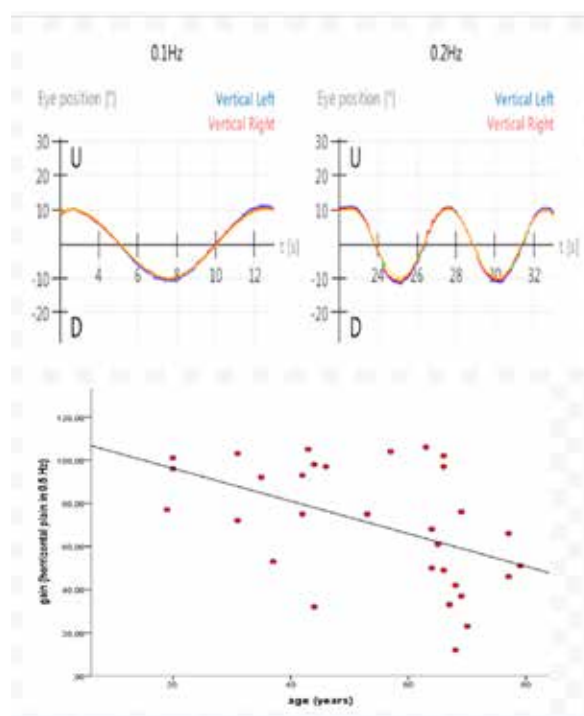
|                                       | Gain left-ward 0.1Hz | Gain rightward 0.1Hz | Gain left-ward 0.2Hz | Gain rightward 0.2Hz | Gain left-ward 0.3Hz | Gain rightward 0.3Hz | Gain left-ward 0.4Hz | Gain rightward 0.4Hz | Gain left-ward 0.5Hz | Gain rightward 0.5Hz |
|---------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| <b>Total sample (n=31)</b>            | 50-102               | 61-106               | 54-105               | 52-118               | 42-103               | 40-107               | 42-113               | 33-122               | 35-105               | 30-123               |
| <b>Age&lt;50 (n=13)</b>               | 62-101               | 80-110               | 66-103               | 60-108               | 70-112               | 72-118               | 65-108               | 61-112               | 45-104               | 47-121               |
| <b>Age<math>\geq</math>50 (n=18)</b>  | 47-117               | 42-119               | 46-116               | 50-110               | 34-118               | 30-123               | 33-110               | 22-123               | 20-113               | 19-119               |
|                                       | Gain upward 0.1Hz    | Gain down-ward 0.1Hz | Gain up-ward 0.2Hz   | Gain down-ward 0.2Hz | Gain up-ward 0.3Hz   | Gain down-ward 0.3Hz |                      |                      |                      |                      |
| <b>Total sample (n=30)</b>            | 74 $\pm$ 21          | 75 $\pm$ 22          | 81 $\pm$ 21          | 75 $\pm$ 21          | 78 $\pm$ 24          | 66 $\pm$ 25          |                      |                      |                      |                      |
| <b>Age &lt;50 (n=13)</b>              | 78 $\pm$ 24          | 80 $\pm$ 22          | 80 $\pm$ 25          | 82 $\pm$ 22          | 82 $\pm$ 23          | 74 $\pm$ 24          |                      |                      |                      |                      |
| <b>Age <math>\geq</math>50 (n=17)</b> | 71 $\pm$ 18          | 71 $\pm$ 22          | 82 $\pm$ 19          | 70 $\pm$ 22          | 74 $\pm$ 25          | 60 $\pm$ 25          |                      |                      |                      |                      |

Gain: eye velocity to target velocity ratio.

**Table 4.** Normal values for symmetry in the horizontal and vertical plane.

|                            | Symmetry 0.1Hz (hor) (%) | Symmetry 0.2Hz (hor) (%) | Symmetry (hor) 0.3Hz (%) | Symmetry (hor) 0.4Hz (%) | Symmetry (hor) 0.5Hz (%) | Symmetry 0.1Hz (ver) (%) | Symmetry 0.2Hz (ver) (%) | Symmetry 0.3Hz (ver) (%) |
|----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <b>Total sample (n=31)</b> | (-14)-11                 | (-6.5)-14                | (-18)-18                 | (-24)-24                 | (-34)-51                 | 1.5±14                   | (-4)±28                  | (-13)±30                 |
| <b>Age&lt;50 (n=13)</b>    | (-18)-3.8                | (-7)-9                   | (-18)-13                 | (-17)-17                 | (-33)-31                 | 2.3±13                   | 6.7±27                   | (-7.8)±22                |
| <b>Age≥50 (n=18)</b>       | (-13)-17                 | (-11)-21                 | (-35)-35                 | (-29)-30                 | (-66)-68                 | 1±15                     | -12±27                   | (-16)±32                 |

Symmetry: (gain difference between directions / sum of gains)%; hor: horizontal; ver: vertical.



**Figure 3. (A)** Graph illustrating smooth pursuit movement on the vertical plane in two different frequencies of the moving target (0.1 Hz, 0.2 Hz). The x-axis corresponds to time, and the y-axis to the amplitude (position) of the movement (U=up, D=down). The yellow line corresponds to the visual stimulus, while the blue and red lines correspond to the left and right eye, respectively. As time progresses, the frequency of the movement also increases. **(B)** Inverse association between age (x-axis, in years) and smooth pursuit gain (y-axis) for horizontal smooth pursuit at a target motion frequency of 0.5 Hz.

## DISCUSSION

### Summary of the study

Modern neurophysiological techniques for recording

eye movements<sup>[7,8]</sup> with unprecedented precision have gradually entered neurological practice and are yielding insights into a variety of diseases accompanied by peripheral<sup>[9-13]</sup> or central<sup>[14-21]</sup> eye movement disorders. Advances in methodology, together with the introduction of new hardware and analysis software, necessitate the continuous updating of normative values, as reliance on older data obtained with previous setups may be imprecise. The present study investigated oculomotor function in a healthy adult population using videonystagmography, focusing on optokinetic nystagmus, saccadic eye movements, and smooth pursuit eye movements. Overall, the results demonstrated symmetrical oculomotor performance and revealed age-related differences in selected parameters, highlighting the importance of considering age effects when interpreting oculomotor findings in healthy individuals.

### Optokinetic nystagmus

In the present study, the analysis of optokinetic nystagmus focused on the slow phase of the response in the horizontal plane, using the gain parameter. The results demonstrated symmetrical responses between the two movement directions (leftward and rightward), a finding consistent with normal optokinetic system function. In contrast, a clear reduction in gain was observed with increasing stimulus velocity, a pattern that has been consistently reported in previous studies and is commonly attributed to limitations in optokinetic processing at higher velocities.

With regard to the effect of age, although participants younger than 50 years exhibited higher gain values across all recording conditions, these differences did not reach statistical significance. Similarly, correlation analysis revealed a tendency toward decreasing gain with increasing age, without achieving statistical

significance. These findings are in line with previous reports showing age-related reductions in optokinetic gain, although the age at which such reductions become evident varies considerably across studies.

Specifically, Ura et al.<sup>[22]</sup> reported a significant reduction in gain when comparing age groups of 30 and 80 years, whereas Baloh et al.<sup>[23]</sup> described reduced slow-phase velocities of optokinetic nystagmus in individuals aged 75 years and older. A potential limitation of the present study is the small number of participants older than 70 years, which may have limited the ability to detect statistically significant differences in the oldest age groups.

### ***Saccadic eye movements***

In the present study, saccadic eye movements were analysed by examining latency, mean velocity, accuracy, and peak velocity, the latter estimated using the velocity/amplitude (vel/amp) ratio, separately for horizontal and vertical saccades.

With respect to horizontal saccades, no statistically significant differences were observed between the two movement directions for any of the examined parameters. This finding is consistent with previous studies, in which directional asymmetries are not expected in healthy populations.<sup>[24,25]</sup> Similar results have been reported in the literature, although some studies, such as that of Vergilino-Perez et al., have described higher peak velocities toward the dominant eye direction, without corresponding differences in saccadic latency.<sup>[26]</sup>

Age emerged as a significant factor influencing horizontal saccadic latency, with increased latency values observed in older participants. This finding corroborates previous reports demonstrating a positive association between age and saccadic latency, commonly attributed to age-related changes in the neural mechanisms underlying saccade planning and initiation. In contrast, mean velocity and peak velocity were not significantly affected by age. This observation is in agreement with studies suggesting preservation of the core neural mechanisms responsible for saccade execution with advancing age,<sup>[27-29]</sup> although the literature remains inconclusive regarding age-related changes in peak velocity.<sup>[27,30]</sup>

A similar pattern was observed for vertical saccades. Saccadic latency increased significantly with age for both upward and downward movements, whereas mean and peak velocity did not show a clear age-related effect. These findings further support the notion that aging predominantly affects the initiation and planning stages of saccadic eye movements, irrespective of movement plane.

### ***Smooth pursuit eye movements***

In the analysis of smooth pursuit eye movements, the parameters gain and accuracy were examined

separately in the horizontal and vertical planes.

With regard to horizontal smooth pursuit, a progressive reduction in gain was observed with increasing target motion frequency, particularly at frequencies  $\geq 0.3$  Hz, accompanied by increased variability of the recorded values. No statistically significant differences were detected between the two movement directions (leftward and rightward), a finding consistent with the expected symmetrical functioning of the smooth pursuit system in healthy individuals. In contrast, age emerged as a significant factor affecting performance, with younger participants exhibiting higher gain values at medium and high frequencies. Similar age-related effects have been reported in previous studies, in which the impact of aging becomes more evident under conditions of increased pursuit demands.<sup>[31,32]</sup>

For vertical smooth pursuit eye movements, the results were less homogeneous. Differences in gain between upward and downward movements were observed only under specific high-frequency conditions. This finding is in agreement with the existing literature, which reports inconsistent results regarding directional differences in vertical smooth pursuit. While some studies have found no differences between upward and downward movements,<sup>[33]</sup> others have reported higher gain either for upward or for downward smooth pursuit.<sup>[34,35]</sup>

In contrast to horizontal smooth pursuit, no clear association between age and gain was demonstrated for vertical smooth pursuit eye movements. The absence of statistically significant age-related effects may be related to the limited number of studies that have systematically investigated vertical smooth pursuit across different age groups, as well as to the greater complexity of the neural mechanisms involved in the control of vertical pursuit.

### **LIMITATIONS**

The main limitation of the present study is the relatively small sample size, which may restrict the generalisability of the findings, particularly in older age groups. Nevertheless, the study provides an initial normative dataset in a healthy adult population, which can be further expanded through continuous data collection to improve representativeness.

In addition, increased variability was observed in certain parameters, such as saccadic accuracy and smooth pursuit gain at higher stimulus velocities. This variability may not be solely attributable to sample size but could also reflect factors related to participant compliance, including fatigue and reduced attention during testing. Such factors are particularly relevant in studies involving healthy individuals, in whom motivation and alertness may vary across testing sessions.

## CLINICAL IMPLICATIONS AND CONCLUSIONS

The recording and analysis of eye movements using videonystagmography provide access to quantitative parameters, such as timing, velocity, and accuracy, which are often beyond the sensitivity of routine clinical observation. The present study contributes to the establishment of normative reference data for oculomotor parameters in a healthy adult population, offering a valuable baseline for the assessment of patients with suspected oculomotor dysfunction.

Furthermore, the ability to analyse oculomotor parameters in relation to demographic factors, such as age and sex, highlights the utility of videonystagmography in both clinical and research settings. Consequently, this method may support early detection of neurological disorders, including neurodegenerative diseases, in which oculomotor function may be impaired, as well as aid in the differential diagnosis between central and peripheral nervous system pathologies.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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