Recessive Retinopathy Consequent On Mutant G Protein β Subunit 3 (*GNB3*)

AUTHORS: Gavin Arno PhD[1,2]; Graham E Holder MSc, PhD[1,2]; Christina Chakarova PhD[1,2]; Susanne Kohl PhD[3]; Nikolas Pontikos PhD[4]; Alessia Fiorentino PhD[1,2]; Vincent Plagnol MSc, PhD[4]; Michael E Cheetham PhD[1,2]; Alison J Hardcastle PhD[1,2]; Andrew R Webster MD(Res), FRCOphth[1,2]; Michel Michaelides MD(Res), FRCOphth[1,2]; for the UK Inherited Retinal Disease Consortium.

1. UCL Institute of Ophthalmology, London, United Kingdom.
2. Moorfields Eye Hospital, London, United Kingdom.
3. Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany
4. University College London Genetics Institute, London, United Kingdom

Corresponding author:
Professor Michel Michaelides
UCL Institute of Ophthalmology
11-43 Bath Street,
London EC1V 9EL
Tel: 020 7608 6864
Email: Michel.michaelides@ucl.ac.uk

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Abstract

Importance: Mutations in phototransduction and retinal signaling genes are implicated in many retinopathies. To our knowledge, *GNB3* encoding the G protein beta subunit 3 (Gβ3) has not previously been implicated in human disease.

Observations: Whole exome sequencing on a patient with distinct inherited retinal disease presenting in childhood, with a phenotype characterized by nystagmus, normal retinal examination, mild disturbance of the central macula on detailed retinal imaging, and previously undescribed ERG findings revealed a homozygous *GNB3* nonsense mutation (c.124C>T ; p.Arg42Ter).

Conclusions and relevance: Gβ3, expressed in cone photoreceptors and ON-bipolar cells, is essential in phototransduction and ON-bipolar cell signaling. Knockout of *Gnb3* in mice results in dysfunction of cone photoreceptors and ON-bipolar cells and a naturally occurring chicken mutant leads to retinal degeneration. Identification of further affected patients may allow description of the phenotypic and genotypic spectrum of disease associated with *GNB3*-retinopathy.

Introduction

Many inherited retinal diseases are associated with mutation of genes encoding proteins involved in phototransduction and consequent signaling within the retina (RetNet – http://www.sph.uth.tmc.edu/RetNet/). This report describes a human retinopathy associated with a homozygous null-mutation in the *GNB3* gene, identified using whole exome sequencing (WES). *GNB3* encodes the G protein β subunit 3 (Gβ3), involved in the signaling of mammalian cone photoreceptors and ON-bipolar cells[1]. To our knowledge,

GNB3 has not previously been implicated in human disease, although a homozygous *GNB3* mutation can cause retinal dystrophy in chickens[2].

Methods

The study protocol adhered to the tenets of the Declaration of Helsinki and received local ethics committee approval. Parental, informed written consent was provided. The proband underwent full ophthalmic examination including dilated retinal examination and color fundus photography (Topcon Great Britain Ltd, Berkshire, UK; Optos plc, Dunfermline, UK), spectral-domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF) imaging (Spectralis, Heidelberg Engineering Ltd, Heidelberg, Germany), and Goldmann visual field testing. Full field electroretinography (ERG) was performed using gold foil electrodes to incorporate the ISCEV standard responses but pattern electroretinography (PERG) was recorded using surface electrodes and a 24x30 degree field due to nystagmus.

Full details of molecular investigations are included in the online supplementary data (eMethods). The proband underwent WES as part of a collaborative study (UK Inherited Retinal Disease Consortium) of 95 probands in whom previous extensive genetic screening proved negative.

Results

The proband, a male child with several siblings (GC20578) born to Somali parents, initially presented several years earlier with horizontal nystagmus and intermittent convergent squint. Parents reported difficulties with near

vision, and no night blindness. Best corrected visual acuity (BCVA) was 20/40 (0.3LogMAR) in both eyes. A high hypermetropic refractive error was identified (+7.50DS OU) and was prescribed. Vision did not improve over time despite correction, with a small left/alternating convergent squint noted. At last follow-up, several years after his initial presentation, his BCVA wearing +6.25/-0.50x180 in both eyes was 20/40 OD, 20/100 (0.7LogMAR) OS, with no change in symptoms.

Dilated retinal examination and color fundus photography was unremarkable (Figure 1). SD-OCT and FAF imaging were suggestive of bilateral central macular disturbance given both the smaller size and less reduction in FAF of the macular hypoautofluorescent area than would be expected, and less foveal cone outer segment lengthening associated with mild inner segment ellipsoid (ISe) layer interruption centrally on SD-OCT (Figure 1). Goldmann visual field testing revealed relatively intact isopters to the larger, brighter targets, with mild constriction to smaller, dimmer targets – to a greater extent in the right eye than left (Figure 1). ERGs showed a reduced rod specific (DA 0.01) response; an electronegative bright flash dark-adapted ERG (DA 10.0) with normal amplitude but delayed a-wave; a profoundly delayed 30Hz flicker ERG of subnormal amplitude; and a markedly delayed and reduced single flash photopic ERG (LA 3.0) of markedly altered waveform (Figure 2). PERG was bilaterally profoundly subnormal.

WES revealed a likely homozygous nonsense mutation in *GNB3* (Chr12: 6952161C>T ; NM_002075.3: c.124C>T ; p.Arg42Ter) (see eResults and

eTable 1). This was confirmed by bi-directional Sanger sequencing and is expected to behave as a true null; only one allele was noted in the ExAC database (MAF=0.000009). The premature termination codon occurs in exon 4 of 11 and the truncated mRNA transcript is likely to be subject to nonsense mediated decay. However should a transcript survive, the protein is terminated before the first of seven WD40 consensus sequences and would be rendered non-functional.

Subsequent Sanger sequencing of *GNB3* coding exons or whole genome sequencing (WGS) revealed no further mutations in 13 patients exhibiting electrophysiological similarity to enhanced S-cone syndrome (ESCS), 16 patients with congenital stationary night blindness (CSNB), and 213 other retinopathy patients (189 complete and incomplete achromatopsia, 9 stationary cone dysfunction syndromes, 15 cone dystrophy) (details on request). Polymorphisms and rare sequence variants observed are summarised in eTable 2.

Discussion

Gβ3 is expressed in cones and ON-bipolar cells in the mammalian retina,[3–5] where it forms the heterotrimeric G-protein second messenger of the metabotropic receptor cone-opsin and mGlu6R in cones and ON-bipolar cells respectively.

Knockout of *Gnb3* (*Gnb3*$^{-/-}$) in mice results in ERG abnormalities exhibiting as a partial loss of ON-bipolar cell sensitivity and downregulation of signal

cascade protein expression including mGluR6, Gαo□□Gγ13 and Trpm1[1]. Furthermore, absence of Gβ3 in mouse cones leads to reduced expression of the Gαt2□and Gγt2 subunits and corresponding loss of cone response and sensitivity[6]. The murine knockout photopic ERGs appear not to show the profound delay present in this patient, and thus do not faithfully model the human disease, but the retinal structure and photopic ERGs in the mouse differ markedly from those in human and extrapolation from a mouse knockout model to human disease should always be made with caution.

A naturally occuring homozygous chicken mutation of Gβ3, p.D153del, abolishes protein function and leads to a retinopathy with a globe enlargement (RGE) phenotype and complete visual loss[2,5,7,8]. However Gβ3 in chicken is expressed in both rods and cones in addition to ON-bipolar cells, thereby differing from the mammalian retina[5].

The delayed ERG bright flash a-wave is compatible with loss of rod photoreceptor sensitivity, with the electronegative waveform suggesting additional dysfunction occurring post-phototransduction. However, although there is a negative ERG waveform, the marked a-wave delay is not a feature of CSNB and the patient also denies night blindness.

ERG data in the cone system are more challenging. Selective loss of the ON-pathway but preservation of OFF- pathway function associated with CSNB gives pathognomonic findings[9], including that the LA 3.0 ERG a-wave commences normally, but has a broadened trough with sharply rising b-wave

showing marginal delay and reduced b:a ratio. The b-wave is of higher amplitude than the a-wave. The flicker ERG shows minimal peak-time shift and amplitude change, but with some broadening of the trough. When there is additional OFF- pathway involvement the findings are far more abnormal. Both photopic a- and b-wave are profoundly reduced, and are of equivalent amplitude, and the flicker ERG shows a characteristic triphasic waveform with profound delay and amplitude reduction[9].

The ERG data in our patient differ from the aforementioned findings associated with CSNB, including the profound photopic a-wave delay, possibly indicating cone photoreceptor sensitivity loss, but with additional waveform simplification and no evidence of the features expected in pure ON-pathway dysfunction (Figure 2). OFF-pathway involvement is not entirely excluded electrophysiologically, but would not be anticipated based on Gβ3 expression data. The peak times and waveforms resemble those usually associated with S-cone function, but the flicker ERG of higher amplitude than the LA 3.0 a-wave suggests this response cannot exclusively be arising in S-cones; however, the initial ERGs impression could be that of an atypical ESCS.

In conclusion, this report describes a distinct inherited retinal disease presenting in childhood, with a phenotype characterized by nystagmus, normal retinal examination, mild disturbance on detailed retinal imaging, and previously undescribed ERG findings, associated with recessive null *GNB3* mutations. Whilst there has been no progression to date in this patient, longer

follow-up would be needed to have greater insight regarding progression. Moreover the identification of further affected patients may allow description of the phenotypic and genotypic spectrum of disease associated with *GNB3*-retinopathy.

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Group information

The UK Inherited Retinal Disease Consortium includes Graeme Black, Georgina Hall, Stuart Ingram, Rachel Gillespie, Simon Ramsden, Forbes Manson, Panagiotis Sergouniotis, Andrew Webster, Alison Hardcastle, Michel Michaelides, Vincent Plagnol, Michael Cheetham, Gavin Arno, Alessia Fiorentino, Shomi Bhattacharya, Anthony Moore, Chris Inglehearn, Carmel Toomes, Manir Ali, Martin McKibbin, James Poulter, Emma Lord, Andrea Nemeth, Susan Downes, Jing Yu, Stefano Lise, and Veronica van Heyningen.

Conflict of Interest: The authors have no proprietary or commercial interest in any materials discussed in this article. No conflicting relationship exists for any author

Authorship: GA, GH and MM had full access to all of the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis. Contributions: GA, ARW, AJH, MEC and MM designed the study; all authors acquired, analyzed or interpreted data; GA, GH, ARW and MM drafted the manuscript; all authors critically revised the manuscript for important intellectual content.

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References

1. Dhingra A, Ramakrishnan H, Neinstein A, et al. Gβ3 is required for normal light ON responses and synaptic maintenance. *J Neurosci*. 2012;32(33):11343-55. doi:10.1523/JNEUROSCI.1436-12.2012.

2. Tummala H, Ali M, Getty P, et al. Mutation in the guanine nucleotide-binding protein beta-3 causes retinal degeneration and embryonic mortality in chickens. *Invest Ophthalmol Vis Sci*. 2006;47(11):4714-8. doi:10.1167/iovs.06-0292.

3. Peng YW, Robishaw JD, Levine MA, Yau KW. Retinal rods and cones have distinct G protein beta and gamma subunits. *Proc Natl Acad Sci U S A*. 1992;89(22):10882-6.

4. Lee RH, Lieberman BS, Yamane HK, Bok D, Fung BK. A third form of the G protein beta subunit. 1. Immunochemical identification and localization to cone photoreceptors. *J Biol Chem*. 1992;267(34):24776-81.

5. Ritchey ER, Bongini RE, Code KA, Zelinka C, Petersen-Jones S, Fischer AJ. The pattern of expression of guanine nucleotide-binding protein beta3 in the retina is conserved across vertebrate species. *Neuroscience*. 2010;169(3):1376-91. doi:10.1016/j.neuroscience.2010.05.081.

6. Nikonov SS, Lyubarsky A, Fina ME, et al. Cones respond to light in the absence of transducin β subunit. *J Neurosci*. 2013;33(12):5182-94. doi:10.1523/JNEUROSCI.5204-12.2013.

7. Ritchey ER, Zelinka C, Tang J, et al. Vision-guided ocular growth in a mutant chicken model with diminished visual acuity. *Exp Eye Res*. 2012;102:59-69. doi:10.1016/j.exer.2012.07.001.

8. Montiani-Ferreira F, Li T, Kiupel M, et al. Clinical features of the retinopathy, globe enlarged (rge) chick phenotype. *Vision Res*. 2003;43(19):2009-18.

9. Zeitz C, Robson AG, Audo I. Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. *Prog Retin Eye Res*. 2015;45:58-110. doi:10.1016/j.preteyeres.2014.09.001.

Figure legends

Figure 1: Fundus imaging, SD-OCT, FAF and Goldmann visual fields. CP: Colour fundus photography, OCT: SD-OCT showing mild central ISe interruption, WF: Wide field imaging, FAF: showing less reduction than expected of the central macular hypoautofluorescent area, GVF: Goldmann Visual Fields

Figure 2: Full field ERGs and pattern ERGs (PERG). DA: dark-adapted; LA: light-adapted; the numbers refer to the stimulus strength in cd.s.m-2 as recommended by the International Society for Clinical Electrophysiology of Vision. See text for full details.

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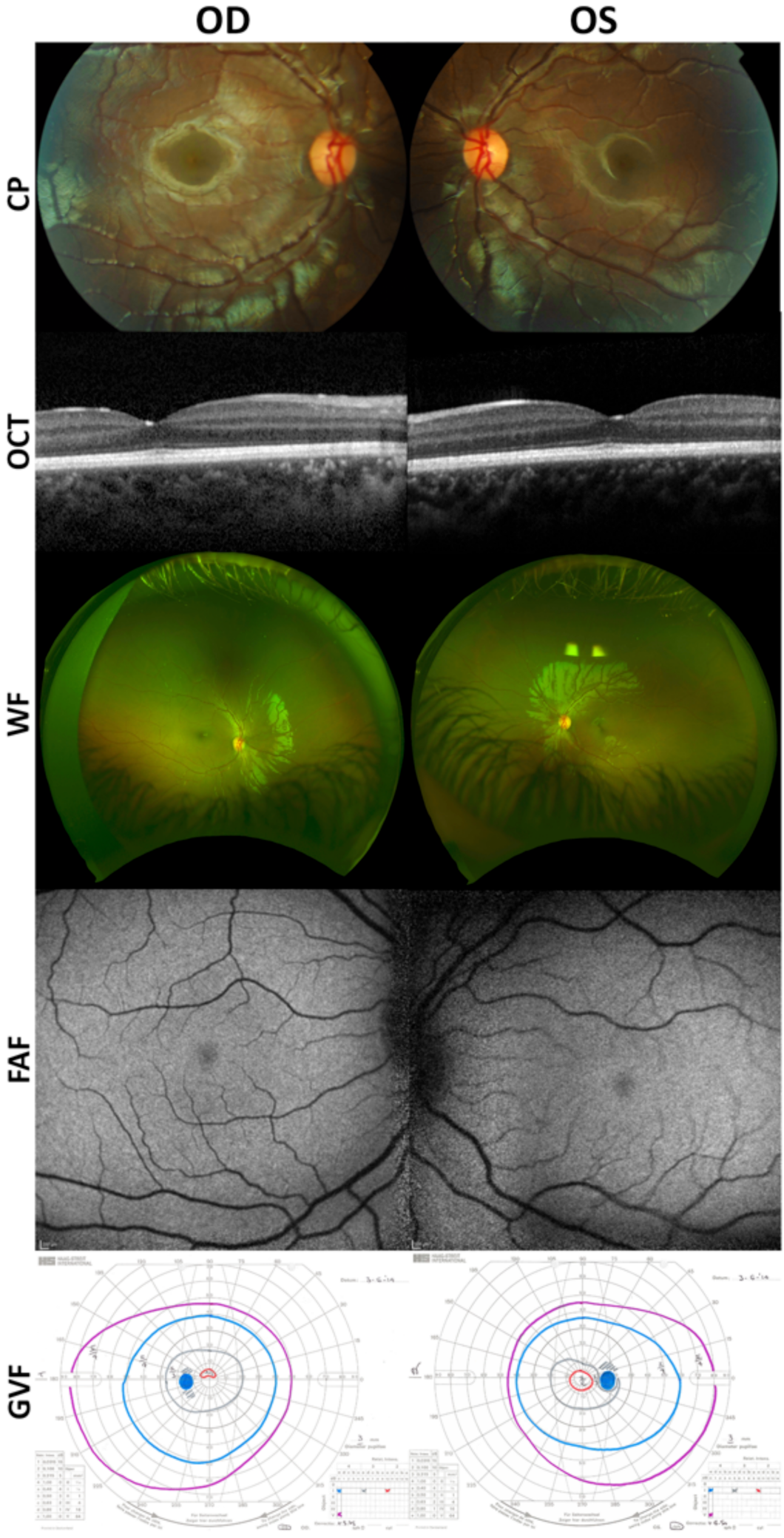
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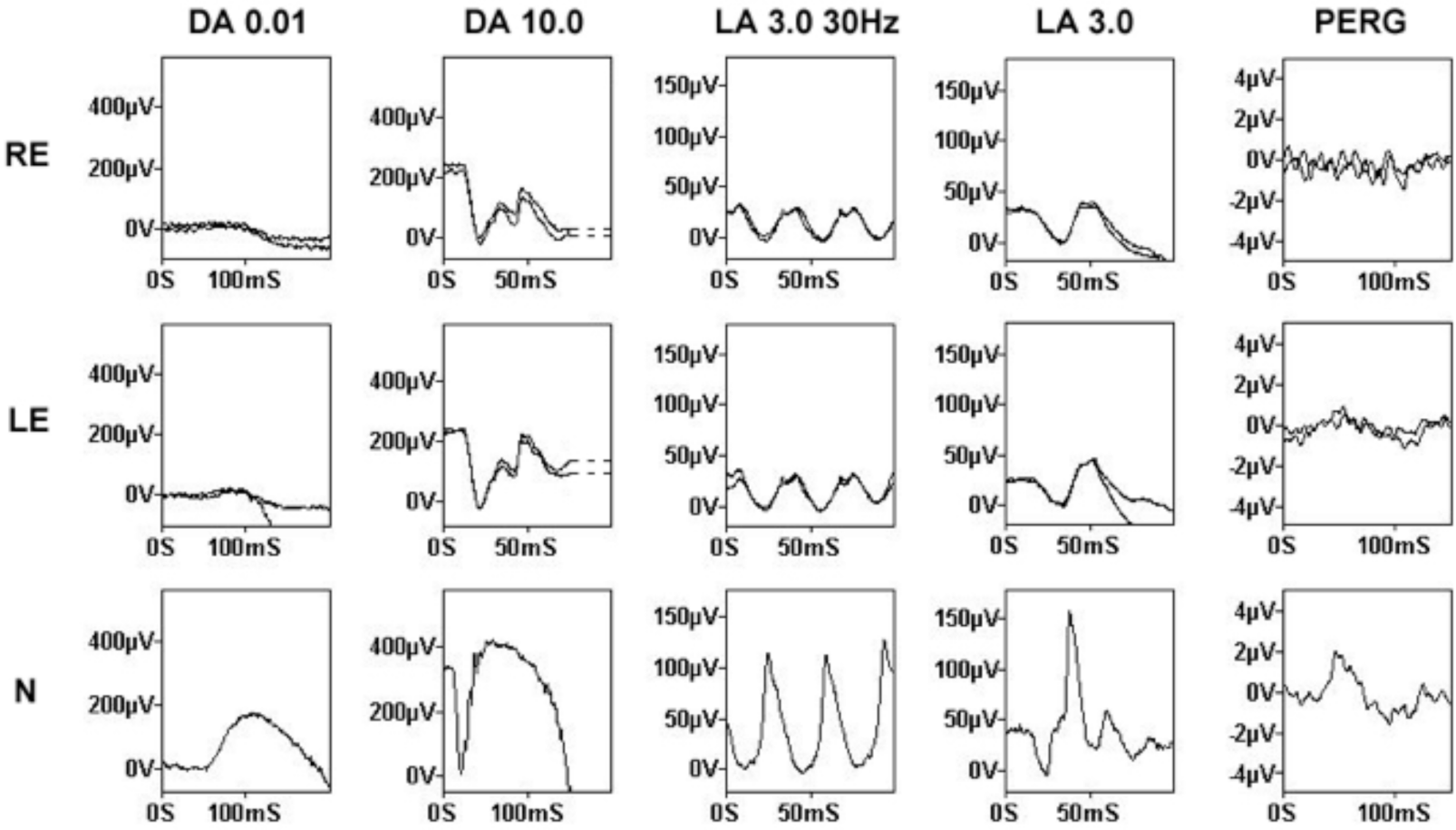
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