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Relative Genetic and Environmental Contributions to Variations in Human Retinal Electrical Responses Quantified in a Twin Study

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Purpose: To estimate heritability of parameters of human retinal electrophysiology and to explore which parameters change with age.

Design: Prospective, classic twin study.

Participants: Adult monozygotic and dizygotic twin pairs recruited from the TwinsUK cohort.

Methods: Electroretinogram responses were recorded using conductive fiber electrodes in response to stimuli incorporating standards set by the International Society for the Clinical Electrophysiology of Vision. These parameters were extracted; in addition, photopic negative-response (PhNR; originating from retinal ganglion cells) and i-wave components were extracted from responses to the photopic single flash. Parameter values were averaged from both eyes.

Main Outcome Measures: Mean values were calculated for the cohort. Correlation coefficients with age were calculated (averaging parameters from both twins from each pair). Coefficients of intrapair correlation were calculated for monozygotic and dizygotic twins. Age-adjusted heritability estimates were derived using standard maximum likelihood structural equation twin modeling.

Results: Responses were recorded from 210 participants in total (59 monozygotic and 46 dizygotic twin pairs). Ninety-three percent were women. Mean age for the cohort was 62.4 years (standard deviation, 11.4 years). In general, response amplitudes correlated negatively, and implicit times positively, with age. Correlations were statistically significant ($P < 0.05$) and moderate or strong (coefficient, >0.35) for the following parameters: scotopic standard and bright-flash a-wave implicit times, photopic 30-Hz flicker and single-flash b-wave implicit times, and PhNR and i-wave implicit times. Intrapair correlations were higher for monozygotic than dizygotic twins, suggesting important genetic influences. Age-adjusted estimates of heritability were significant for all parameters (except scotopic dim-flash b-wave implicit time), ranging from 0.34 to 0.85. Highest estimates were for photopic single-flash a-wave and b-wave amplitudes (0.84 and 0.85, respectively).

Conclusions: This study explored heritability of retinal electrophysiologic parameters and included measurements reflecting ganglion cell function. Most parameters showed significant heritability, indicating that genetic factors are important, determining up to 85% of the variance in some cone system response parameters. Scotopic responses tended to show lower heritability (possibly relating to greater rod system susceptibility to environmental factors). Future studies can explore the identity of these genetic factors, improving our understanding of how they shape retinal function. *Ophthalmology 2017;■:1–11 © 2017 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

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Impairments in function of retinal cell populations are an important cause of global visual impairment: Diabetes and inflammatory diseases frequently affect inner retinal neurons; age-related macular degeneration and inherited dystrophies affect outer retinal cells (photoreceptors, retinal pigment epithelium, and transmission to bipolar cells); and glaucoma, an important cause of sight loss worldwide, is a disease of retinal ganglion cells. In addition, myopia, whose prevalence is increasing worldwide, is now understood to be driven largely by retinal mechanisms.[1] Our ability to image retinal anatomic features in vivo is advancing rapidly, with high-resolution imaging of retinal architecture widely available. This gives important information on structure, but does not always correlate completely with cellular function. Electroretinography, which is used less widely, allows objective, quantitative, noninvasive assessment of retinal function and can identify dysfunction before cell loss. In addition, because the synaptic pathways in the retina share features with excitatory and inhibitory pathways elsewhere in the brain, retinal

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electrophysiologic recordings can yield insights into pathogenetic mechanisms in neurologic conditions ranging from migraine[2,3] to schizophrenia[4] and attention deficit–hyperactivity disorder.[5]

Studies in monozygotic and dizygotic twin pairs allow quantification of relative genetic and environmental contributions to variance in phenotypic traits. If the correlation of a phenotypic trait (or concordance of a disease) between monozygotic twins is higher than that between dizygotic twins, then the heritability (the proportion of the variance attributable to genetic factors) can be calculated (this is termed a *classic twin study*). Previous studies have shown that genetic factors make an important contribution to variance in retinal structure: macular thickness, as assessed by optical coherence tomography, has been estimated to have 81% to 85% heritability[6]; macular pigment density and patterns, as determined by 2-wavelength autofluorescence imaging, also are significantly heritable.[7–9] However, there have been no large studies to date that directly explore heritability of electrophysiologic parameters of retinal function. A previous study in 42 twin pairs assessed aspects of visual function psychophysically, finding that processes involved in scotopic thresholds and adaptation may be more affected by environmental factors.[10] Such psychophysical measurements relate to conscious perception, which is the culmination of layers of retinal and higher neuronal processing. This study aimed to quantify relative genetic and environmental contributions to visual function at the level of retinal cell signaling by measuring parameters of retinal electrophysiology in a significantly larger twin cohort. Correlations with age also were explored.

The International Society for the Clinical Electrophysiology of Vision (ISCEV) sets standards for full-field electroretinography,[11] allowing assessment of generalized retinal function. These recordings permit distinction between diseases affecting rod and cone systems and also between disease processes affecting transduction in the photoreceptors and abnormalities of inner retinal processing. In the present study, more than 100 healthy twin pairs were recruited to undergo the full ISCEV electroretinography protocol. The parameters measured, as recommended by ISCEV, were a-wave and b-wave amplitudes and peak times for all flash stimuli, as well as amplitude and peak time of the photopic 30-Hz flicker. Figure 1 shows example traces, with these parameters labeled, as well as the likely cell populations from which the labeled components are thought to arise.

A later negative component of photopic (light-adapted) flash electroretinography, occurring 65 to 75 ms after flash delivery, was identified 17 years ago as likely to be arising from retinal ganglion cells; it was abolished by experimentally induced glaucoma in macaques.[12] It was termed the *photopic negative response* (PhNR), and more than 100 publications since have explored features of this component, in particular its possible usefulness in evaluating dysfunction of the retinal ganglion cells in assessment of glaucoma. In view of the potential future clinical importance of this parameter, we identified the component in recordings from our twin participants (Fig 1) and explored its heritability in addition to the ISCEV parameters listed above. An additional electroretinography component just preceding the PhNR, the i-wave, which may originate from the OFF pathway distal to retinal ganglion cells,[13] also was identified and investigated.

Methods

Participants

Participants were recruited from the TwinsUK cohort, based at St. Thomas' Hospital, London. This cohort comprises approximately 12 000 adult twins (83% women) from the United Kingdom who have volunteered to participate in research studies.[14] Both members of each twin pair attended together, and recordings were performed consecutively, first on one twin and then the fellow twin. In the case of 1 pair, the 2 twins attended on separate days, but recordings were performed at the same time of day. Participants were asked about any eye conditions before recording. Pupils were dilated pharmacologically with mydriatic drops (1.0% tropicamide and, in most cases, 2.5% phenylephrine). Both members of each twin pair were given the same dilating drops (i.e., if one twin received only tropicamide, then so did the other twin).

Stimuli

Stimuli were delivered, and responses recorded, using the Diagnosys Colordome with Espion software (Diagnosys, Lowell, MA). Stimuli corresponded to the ISCEV standard for full-field electroretinography.[11] Participants underwent a minimum of 20 minutes of dark adaptation before the delivery of scotopic flash stimuli (white flashes, delivering 0.01, 3.0, and 10.0 cd s/m^2 photopic light). Participants underwent a minimum of 10 minutes of light adaptation (to the ISCEV white adapting background of 30 cd s/m^2 photopic light) before the delivery of photopic stimuli, which included the 30-Hz flicker and the photopic single flash (both 3.0 cd s/m^2 photopic light). Stimuli were presented repeatedly and responses were averaged. After the scotopic stimuli described previously, and before the ISCEV light adaptation period, additional flash stimuli were presented both in the dark and on a rod-saturating blue background to explore additional parameters of photoreceptor function (analysis not described here). Also, during the light adaptation period, additional photopic flicker and flash stimuli (corresponding to ISCEV standard photopic stimuli) were delivered. The responses to the flash stimuli delivered throughout this period were used to extract the PhNR component.

Recording

Electroretinography recordings were made from both eyes using a conductive fiber electrode (DTL-PLUS electrode; Unimed Electrode Supplies Limited, Farnham, Surrey, United Kingdom) placed consistently in the lower conjunctival fornix. Because electrode position can affect amplitude of electroretinography responses (Tariq A, et al., *Invest Ophthalmol Vis Sci.* 55[13], 2014; ARVO E-Abstract 5121), the location was checked regularly during recordings, and if necessary, the electrode was repositioned into the fornix. The indifferent electrode was placed at the temple and a ground electrode was placed on the forehead. These were skin-surface electrodes (24-mm disposable ground electrodes; Unimed Electrode Supplies Limited), placed after cleaning of the skin with alcohol wipes.