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Multifocal ERG Defects Associated with Insufficient Long-Term Glycemic Control in Adolescents with Type 1 Diabetes

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Abstract

Purpose—To investigate the relationship between long-term glycemic control and localized neuroretinal function in adolescents with type 1 diabetes (T1D) without diabetic retinopathy (DR).

Methods—Standard (103 hexagons) and slow-flash (61 hexagons) multifocal ERGs (standard mfERG and sf mfERG) were recorded in 48 patients and 45 control subjects. Hexagons with delayed responses were identified as abnormal. Negative binomial regression analysis was conducted with the number of abnormal hexagons as the outcome variable. Glycated hemoglobin (HbA_{1c}) levels, time since diagnosis of T1D, age at diagnosis of T1D, age at testing, and sex were the covariates. Another model replacing HbA_{1c} closest to the date of testing with a 1-year average was also generated.

Results—There were more abnormal hexagons for mfOPs in patients than in control subjects ($P = 0.005$). There was no significant difference in the mean number of abnormal hexagons for standard mfERG responses between patients and control subjects ($P = 0.11$). Negative binomial regression analysis for the standard mfERG data demonstrated that a 1-unit increase in HbA_{1c} was associated with an 80% increase in the number of abnormal hexagons ($P = 0.002$), when controlling for age at testing. Analysis using the 1-year HbA_{1c} averages did not result in significant findings.

Conclusions—Poor long-term glycemic control is associated with an increase in areas of localized neuroretinal dysfunction in adolescents with T1D and no clinically visible DR. Stricter glucose control during the early stages of the disease may prevent neuroretinal dysfunction in this cohort.

Diabetic retinopathy (DR) is a chronic microvascular complication of diabetes mellitus that may result in severe visual impairment. It affects nearly all people with type 1 diabetes (T1D) after ~20 years' duration of the disease.[1] Recent data have shown a decrease in the

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prevalence of DR because of improved diabetes management and glycemic control.[2] However, the number of cases of DR is expected to increase because of the anticipated increase in the number of people diagnosed with diabetes. Whereas 440,000 children in 2006 were estimated to have T1D worldwide, 70,000 newly diagnosed cases are expected each year.[3] Therefore, DR constitutes a major health concern.

Current standards of diagnosis of DR, based on the modified Airlie House Classification,[4] primarily rely on vascular lesions visible on clinical examination. These vascular abnormalities, some of which may be sight-threatening, are clinically visible when the disease has progressed to later stages. To prevent vision loss in patients with diabetes, it is essential to establish reliable clinical markers of early-stage DR.

That DR has a major vascular component is unequivocal; however, the retina is primarily neural tissue. Studies have demonstrated neuroretinal dysfunction, including delayed and diminished oscillatory potentials (OPs), in patients with diabetes before the appearance of vascular lesions.[5] Decreased OP amplitudes have also been associated with the severity of DR[6] and are thought to predict development of proliferative DR.[7,8] Delayed multifocal OPs have been demonstrated in patients with diabetes[9–11] and to a greater extent in patients with DR.[9,10] Similarly, standard multifocal (mf)ERG studies have shown delayed implicit times in patients with diabetes that are exacerbated in patients with nonproliferative (NP)DR.[12] In patients with NPDR, localized retinal areas with delayed mfERG timing have been shown to precede the development of new vascular lesions.[13–16] Decreased amplitudes of the second-order response, which are suggested to reveal abnormalities in the circuitry involved in retinal adaptation,[17] have also been demonstrated in patients with diabetes.[18] Findings from these studies suggest that measures of localized neuroretinal function in particular could be useful biomarkers of the early changes associated with DR.

Glycated hemoglobin (HbA_{1c}) levels are an index of long-term glycemic control. Population-based studies, such as the Diabetes Control and Complications Trial (DCCT),[19,20] show that a high HbA_{1c} is a strong risk factor for increased incidence and progression of DR. The DCCT group[20] followed patients for an average of 7.4 years. Adolescent patients were assigned to intensive and conventional treatment groups. Those in the intensive treatment group, who had lower HbA_{1c} levels than those in the conventional treatment group, had a 53% decrease in the development and 70% decrease in the progression of DR. Glycemic control has been shown to be particularly impaired in adolescents with diabetes as puberty worsens metabolic control in this age group.[21–23]

The purpose of the present study was to determine whether high HbA_{1c} levels in adolescents with T1D are associated with increased localized neuroretinal dysfunction, measured using standard and slow flash (sf) mfERG paradigms, before DR is clinically detectable. To this end, we generated a negative binomial regression model with covariates including HbA_{1c} and time since diagnosis of T1D, which are known risk factors of DR. Age at testing, age at diagnosis, and sex were included, since they were collected as part of the standard protocol, and previous research has shown that they may contribute to the model.[13–15]

Methods

Subjects

Eighty-five adolescents with T1D were recruited at the Hospital for Sick Children. Inclusion criteria were duration of T1D of at least 5 years, age 12 to 20 years, and normal visual development. Participants with any DR were excluded based on analysis of seven-field, 30° stereoscopic color fundus photographs, graded according to the modified Airlie House classification,[4] by retinal specialists. Patients and control subjects with eye diseases, hemoglobinopathy, high refractive error (worse than ±5 D), and poor visual acuity (worse than 0.3 logMAR) were excluded. Also excluded were participants with neurologic or psychiatric disorders affecting visual or retinal function and those on medications with similar effects. Of the 85 patients who were recruited, 37 were excluded. These included 29 who did not attend the scheduled testing session and one who had blood glucose levels so high (>20 mM) that it was deemed unsafe to titrate to the required 4-to 10-mM range. Seven patients did not meet the inclusion criteria: Four had mild nonproliferative NPDR, the fifth had a vascular lesion of unknown origin, the sixth had high refractive error, and the seventh was taking medication with visual system side effects. Data from the remaining 48 patients were analyzed. Forty-five age-similar participants acted as control subjects. Informed consent was obtained from all participants after the procedures and possible consequences of the study were explained to them. All procedures conformed to the tenets of the Declaration of Helsinki and were approved by the Research Ethics Board at the Hospital for Sick Children.

Data Acquisition

All participants were tested at the Hospital for Sick Children. Since acute blood glucose levels are known to affect mfERG responses in patients with diabetes,[24,25] patients' blood glucose levels were measured by a registered nurse at least three times: before intake and psychophysical testing, before mfERG testing, and after mfERG testing. Glucose levels were adjusted with moderate exercise or the administration of insulin or food and maintained within 4 to 10 mM.

One eye of each participant was randomly chosen for testing. Participants had visual acuity (ETDRS, logMAR) and contrast sensitivity (Pelli-Robson) assessed. Color vision was tested using Hardy-Rand-Rittler (HRR) pseudoisochromatic plates and the Mollon-Reffin Minimalist test.[26] Thereafter, a topical corneal anesthetic (0.5% proparacaine) and dilation eye drops (2.5% phenylephrine and 1% tropicamide) were applied to the tested eye. A dilated ophthalmic examination was performed, including measurement of refractive errors, and funduscopic assessment of ocular media and posterior pole, on most of the patients. Age at diagnosis of T1D and HbA_{1c} levels of patients were obtained from the hospital database.

Multifocal Electroretinography

The standard mfERG was recorded in all participants according to ISCEV guidelines.[27] Recordings were obtained with a visual evoked potential system (VERIS Science System; Electro-Diagnostic Imaging, Inc., Redwood City, CA). A ground electrode was taped onto the forehead and a bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic

Development Laboratory, Iowa City, IA) was placed on the cornea. The untested eye was occluded. An infrared light source (if not already built into the contact lens electrode) was placed at the outer corner of the tested eye for fundus illumination. The stimulus, subtending the central retina approximately 25° in radius and consisting of an array of 103 hexagons, was displayed on a fundus eye camera, which allowed real-time monitoring of fixation. Hexagons alternated between light (200 cd · m^{-2}) and dark (0 cd · m^{-2}) frames in a pseudorandom m-sequence with a length of $2^{15} - 1$ and a base period of 13.33 ms. The 8-minute recording was divided into 16 segments for the participant's comfort. The incoming signal was filtered with an analog 10- to 300-Hz band-pass filter. Segments during which the subject was observed to lose fixation were repeated.

Inner retinal activity was evaluated by assessing multifocal oscillatory potentials with the sf mfERG paradigm. The interval of time between focal flashes in the standard paradigm is short, so that the retinal response to the preceding flash does not fully develop before the subsequent flash is presented.[10] Thus, the higher-order effects are superimposed on the latter part of the standard mfERG first-order kernel response,[17,18,28–34] making them difficult to measure. The sf mfERG paradigm allows for the isolation of the inner retinal contribution or oscillatory content of the standard mfERG response by separating focal flashes with several dark frames.[35–39] The stimulus consisted of 61 hexagons with a base period of 79.8 ms (five dark frames and one stimulus frame). The signal was filtered with a band-pass 75- to 300-Hz digital filter.[39]

Data Analysis

Implicit time of first-order standard mfERG responses was analyzed with the template-stretching method described by Hood and Li.[40] The template wave was derived by averaging control data. Focal responses from each participant were compared with the template wave, which was independently stretched in the vertical and horizontal axes by multiplying each parameter with a stretch factor to obtain the best fit.

Sf mfERG OP responses were analyzed by using a peak-picking method in which the mean implicit time per waveform was derived from values for the three most prominent peaks.

Implicit times of mfERG responses were converted into *z*-scores to determine the number of hexagons with neuroretinal dysfunction per patient.[10] A hexagon was defined as abnormal if its associated z-score was ≥ 1.96. The total number of abnormal hexagons was determined for each patient, representing the extent of affected retina.

Negative Binomial Regression Modeling

The relationship between localized neuroretinal function and long-term glycemic control was examined using a negative binomial regression model. The influence of other patient demographic factors was also considered. Classic linear regression, such as multiple regression, is used to model data that are continuous and have a Gaussian distribution. Our chosen outcome variable, the number of abnormal hexagons for implicit time of standard mfERG responses, comprises discrete, nonnegative numbers (count data). Since the distribution is not Gaussian and the outcome comprises count data with a large number of 0 values, the negative binomial regression is the appropriate approach to modeling.[41]

The output for regression consists of parameter estimates or βs, which are an index of the amount of variation in the dependent variable explained by the associated independent variable.[42] Because the negative binomial regression is based on the logarithm of the outcome variable rather than being a linear function as in standard regression, the βs are exponentiated (e^{β}).[42] Therefore, a 1-unit increase in the independent variable multiplies the outcome variable by a factor of e^{β}, whereas the other independent variables are held constant.[42,43]

Negative binomial regression was performed on patient data using R (version 2.8.1).[44] Covariates considered for inclusion in the model were HbA_{1c} (percentage) closest to the date of testing, time since diagnosis of T1D (years), age at diagnosis (years), age at testing (years), and sex (male, 1; female, 0). The dependent variable consisted of the number of mfERG responses with abnormal implicit times. To explore further the relationship between long-term glycemic control and neuroretinal function, we generated another model replacing the HbA_{1c} values (closest to the date of testing) with the average HbA_{1c} over a period of 1 year from the date of testing. The 1-year HbA_{1c} averages were available for 39 of the 48 patients.

Age at diagnosis correlated highly with time since diagnosis and therefore was excluded from the regression analysis. A backward selection procedure was used to arrive at the final model. $P \geq 0.157$ was chosen as the criterion[45] for removing a variable from the model, as this allows selection of a model that is inclusive of useful predictors without overfitting it with too many parameters.[41,46]

Comparison of models was achieved with the likelihood ratio test. The likelihood ratio test evaluates the difference between how well one model fits the data compared with another.[42] The test was used to compare the reduced model with the full model. To test the null hypothesis that none of the covariates explained significant variability in the dependent variable, the likelihood ratio test was used to compare the presumed final model with the null model (all βs = 0).

All descriptive statistics are reported as the mean ± SD, unless stated otherwise. With the exception of the negative binomial regression modeling procedure, $P > 0.05$ was considered to be nonsignifi-cant. Patient and control group means for variables were compared by using the Mann-Whitney test, as data for one or both groups was not normally distributed.

Results

Demographic data and psychophysical testing results for patients and control subjects are shown in Table 1. Patients and control participants had normal scores on the HRR and Mollon-Reffin Minimalist color vision tests. There was no significant difference in contrast sensitivity and visual acuity scores between the two groups. On average, control participants were older (17.55 ± 4.22 years) than patients by ~2 years (Mann-Whitney test, $P = 0.15$). Approximately half of the patients had diabetes for ≤10 years, and all had diabetes for <15 years, with most of the patients (37/48) receiving the diagnosis before the age of 10. The average duration of time between HbA_{1c} measurements and mfERG recordings was 2.00

± 3.07 months. The duration of time between HbA_{1c} measurement and mfERG testing was more than 3 months for 7 of the 48 patients. However, their HbA_{1c} readings had remained relatively constant for 1 year before the date of mfERG testing. The change in HbA_{1c} for the patient group during the 1-year period ranged from 0.4 to 4.7 with a median of 1.2. All patients except one had HbA_{1c} levels greater than the Canadian Diabetes Association's recommended target of 7%.

The mean number of abnormal hexagons for timing of standard mfERG responses was greater for the patient group (3 ± 4.76) compared with control participants (1.38 ± 2.38); however, the difference was not statistically significant (Mann-Whitney test, $P = 0.11$; Fig. 1a). On average, patients (1.94 ± 1.90) had significantly more abnormal hexagons for timing of sf mfERG responses in comparison with control subjects (0.93 ± 1.09; Mann–Whitney test, $P = 0.005$; Fig. 1b).

Negative Binomial Regression Model Based on Patient mfERG Implicit Times

Negative binomial regression using the number of abnormal hexagons for patient standard mfERG responses yielded significant results. However, modeling using sf mfERG data did not.

Three iterations of the backward selection procedure were performed for standard mfERG data with the purpose of generating the simplest model that fit the data best. The first two models revealed that time since diagnosis ($P = 0.26$) and sex ($P = 0.85$) did not predict significantly the number of abnormal hexagons. Therefore, these covariates were excluded from further analysis. This adjustment led to a final model that included the covariates HbA_{1c} and age at testing ($P < 0.157$). The model (Table 2) showed that HbA_{1c} was the strongest predictor of the number of abnormal hexagons, followed by age at testing.

A likelihood ratio test comparing the final model to the null model was significant ($P = 0.003$), which indicated that the final model fit the data better than the null model (all βs = 0). The model demonstrated that a 1-unit increase in HbA_{1c} predicted an increase in the number of abnormal hexagons for implicit time of standard mfERG responses by a factor of 1.80 or by 80% when age at testing was held constant. A 1-year increase in age predicted a decrease in the number of abnormal hexagons by a factor of 0.77 or by 23% when HbA_{1c} was held constant.

A scatterplot of the univariate correlation between the number of abnormal hexagons for implicit time of standard mfERG responses and HbA_{1c} (Fig. 2) yielded a significant Spearman's ρ of 0.423 ($P = 0.001$).

Negative binomial regression modeling using the 1-year HbA_{1c} averages in lieu of the single HbA_{1c} values obtained closest to the date of testing did not yield any significance.

Discussion

The DCCT study (1993) emphasized the importance of tight glycemic control in reducing the development and progression of DR in patients with T1D, including specifically the adolescent cohort.[20] The present study investigated whether poor long-term glycemic

control was associated with worsening localized neuroretinal dysfunction in adolescents with T1D. The final negative binomial regression model showed that high HbA_{1c} levels were associated with an increase in areas of localized neuroretinal dysfunction in this population when controlling for age at testing, before clinical signs of DR are visible. This step is an important one toward the larger goal of identifying accurate and sensitive biomarkers for monitoring retinal integrity in patients with diabetes and in identifying those at risk of DR.

With the same model, the 1-year average HbA_{1c} data did not demonstrate significance, probably because of several factors. The model may be more sensitive to relatively short-term glycemic control over a period of about 3 months, rather than more chronic glycemic control over a period of 1 year. Also, data were available for only 39 of the 48 patients, which would have the reduced statistical power.

The results from the present study give support to multivariate predictive models generated by investigators in several studies that demonstrated that standard mfERG implicit times predict development of future DR in patients with existing DR at baseline.[13,15,16] The same group found a moderate correlation between HbA_{1c} and mfERG implicit times in adolescents with T1D (Bronson-Castain K, et al. *IOVS* 2008;49:ARVO E-Abstract 2757).[47,48] Earlier, Klemp et al.[25] also demonstrated a correlation between HbA_{1c} and mfERG implicit times in patients with T1D without DR. There are several characteristics, however, that distinguish our study. First, to the best of our knowledge, our sample size is the largest among other studies of localized neuroretinal function in patients with diabetes. Also, previous studies have correlated HbA_{1c} with the mfERG implicit times averaged across the entire array of retinal patches; thus, no spatial information remains. Our study correlated HbA_{1c} with the number of abnormal hexagons. Therefore, we show a correlation between HbA_{1c} and the extent of abnormal retina.

In addition, blood glucose levels were monitored and maintained within 4 to 10 mM throughout the testing session. This method minimized the impact of acute changes in blood glucose levels, which have been shown to affect standard mfERG responses.[24,25] The 4 to 10 mM range was broad enough to ensure patient safety and allowed the blood glucose levels to be adjusted within a reasonable amount of time. Although it is likely that blood glucose levels may have changed slightly during the electrophysiological testing, the glucose levels were adjusted in consultation with the nurse, such that any changes would be minor and still within the prescribed range.

The lack of a significant difference in the mean number of abnormal hexagons for implicit time of standard mfERG responses (Fig. 1a) between patient and control groups is contrary to findings in other studies that demonstrated delayed implicit time of standard mfERG responses in patients with diabetes.[12,25] The degree of variation in the data, which was greater in the patient group, provides one explanation for the lack of significance. This variation in the patient data, however, made it more amenable to a modeling approach. Another explanation may be our use of *z*-scores in the analysis. Although the use of *z*-scores may have reduced our sensitivity to small changes in implicit time, incorporating this with the spatial information available from the mfERG recordings would have introduced the

statistical problem of multiple testing. This problem would also have had the effect of reducing sensitivity.

The finding of a greater number of abnormal hexagons on average for the timing of mfOP responses in comparison with control subjects is consistent with findings from other studies. Several full-field ERG studies have demonstrated decreased amplitudes and delayed timings of OPs in patients before DR is clinically visible.[5,6,8,49–53] More recently, sf mfERG studies in diabetic eyes demonstrated localized implicit time delays in mfOPs.[9–11] The significant difference in the number of abnormal hexagons between patient and control groups in our study is associated with a tight distribution with low variability in data from both groups (Fig. 1b), which does not make it conducive to modeling. The significantly delayed responses in the patient group, however, may be attributable to retinal dysfunction as part of the disease mechanism of diabetes. Studies have demonstrated a loss of ganglion cells in rats with induced diabetes early in the course of the disease.[54–56] Loss of inner retinal neurons, including bipolar and amacrine cells has also been demonstrated.[55]

The value of the subject's sex as a predictive covariate has been uncertain. Previous multivariate predictive models generated by others did not find sex to be a significant predictor of DR,[13,15,16] consistent with our results. Although some studies have implicated time since diagnosis or the duration of diabetes to be a strong risk factor for DR,[1,57,58] it was not found to be significantly associated with neuroretinal function in this study. A possible explanation is that studies have found that the number of years after puberty significantly affect the risk of developing DR as opposed to the years before onset of puberty.[59–64] Since our model was focused on adolescents, the number of postpubertal years may not be high enough to show an effect. The model also demonstrates that a 1-year increase in age is associated with a decrease in the number of abnormal hexagons by 23% when controlling for HbA_{1c}. In an older population, natural aging has an effect on standard mfERG responses.[65] In our adolescent population, however, we found no correlation between the ages of control participants and the number of abnormal hexagons.

It is interesting to note that, although HbA_{1c} is the most widely used index of glycemic control and is strongly associated with the complications of diabetes,[19,20,66] it alone may not provide complete information about a patient's metabolic state. It has been suggested that variability in blood glucose levels may also be associated with complications of diabetes.[67–69] However, given that studies have demonstrated conflicting results[70–73] and that there is no agreement on the optimal measure of blood glucose variability,[74] HbA_{1c} was chosen as the best measure of glycemic control for use in the present study.

Modeling results involving HbA_{1c} closest to the date of mfERG testing supported the study's hypothesis and led to the conclusion that poor long-term glycemic control is associated with an increase in areas of neuroretinal dysfunction in patients with diabetes before DR is clinically visible. In summary, this study's findings highlight the importance of maintaining good glycemic control in patients with diabetes. The findings suggest that intensive diabetes management early in the disease process may prevent neuroretinal dysfunction in adolescents with T1D without clinically evident DR.

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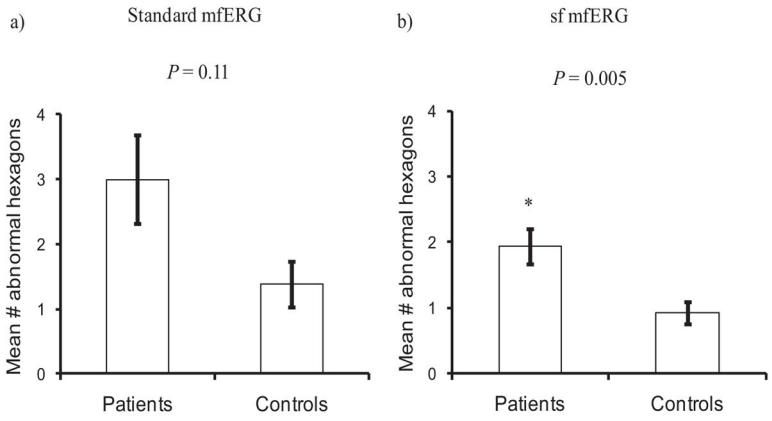

Figure 1.

Comparison of the mean number of abnormal hexagons for timing in standard mfERG (**a**) and sf mfERG (**b**) responses between patient and control groups. Error bars, SEM.

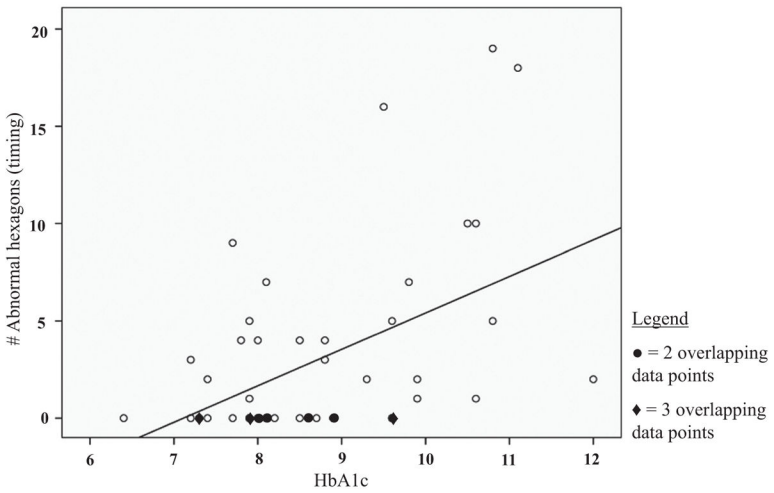

Figure 2.

Univariate correlation between HbA_{1c} and the number of abnormal hexagons for implicit time in standard mfERG responses.

Table 1

Demographic Data and Psychophysical Test Results, with Ranges, for Patients and Control Subjects

	Patients (*n* = 48)	Controls (*n* = 45)
Age at testing, y	15.66 ± 1.76 (11.95 to 18.25)	17.55 ± 4.22 (12.15 to 27.05)
Sex, male/female	23/25	16/29
Age at diagnosis, y	6.24 ± 3.54 (1.41 to 13.12)	—
Time since diagnosis, y	9.40 ± 3.17 (4.90 to 14.2)	—
HbA_{1c}, %	8.71 ± 1.24 (6.4 to 12.0)	—
Visual acuity, logMAR	0.00 ± 0.10 (−0.28 to 0.22)	−0.03 ± 0.12 (−0.24 to 0.26)
Contrast sensitivity	1.71 ± 0.10 (1.60 to 1.95)	1.74 ± 0.13 (1.6 to 2.2)

Table 2

Description and Results for the Final Model Including HbA_{1c} and Age at Testing as Covariates

	β	e^{β}	CI (95%)	*P*
Intercept (β_0)	−0.36	0.70	0.00–98.9	0.89
HbA_{1c}	0.59	1.80	1.25–2.62	0.002
Age at testing	−0.26	0.77	0.59–1.00	0.054