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LETTER TO THE JOURNAL

# No Maternally Inherited Diabetes and Deafness Mutations in a Sample of 193 Tasmanian Diabetics with Glaucoma

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We tested the hypothesis that the presence of the mitochondrial 3243 A-G mutation responsible for maternally inherited diabetes and deafness (MIDD)[1] may partly explain the association between glaucoma and diabetes mellitus. The 3243 A-G mutation has been estimated to be responsible for 1–3% of the non-insulin-dependent diabetes mellitus cases in Northern Europe[2] and approximately 1% in Japan.[3] We tested 193 DNA samples collected from Tasmanians with both diseases recruited through the Glaucoma Inheritance Study in Tasmania.[4] No cases with the 3243 A-G mutation were detected.

Glaucoma is more common in individuals who also have diabetes. In the USA, the rate of glaucoma in patients with diabetes is double that in patients without diabetes (8.0% vs. 4.1%).[5] Similarly, glaucoma prevalence in Australia was shown to be higher in people with diabetes than in those without diabetes (5.5% vs. 2.8%).[6] Diabetes was present in 13.0% of the individuals with glaucoma compared with 6.9% of those without glaucoma. Although early diagnosis of glaucoma in patients with diabetes having regular eye examinations has been proposed as the reason for this finding, over half the individuals in the Blue Mountain Eye Study (BMES) were diagnosed with glaucoma before they were diagnosed with diabetes. To date, the association of glaucoma with diabetes has not been fully explained.

Mitochondrial dysfunction is a major cause of optic neuropathy. Mutations in proteins that localize to the mitochondria are responsible for Leber Hereditory Optic Neuropathy (LHON),[7] autosomal dominant optic atrophy 1,[8,9] the optic atrophy of familial dystonia with visual failure and striatal lucencies,[10] and Wolfram syndrome.[11] Since glaucoma is also an optic neuropathy, we sought to determine whether the MIDD mutation might be associated with coincident cases of diabetes and glaucoma.

A total of 193 individuals with diabetes mellitus (18 type 1 and 175 type 2) aged between 39 and 93 years in January 1996, 99 (51%) of whom were female, were identified from 2000 cases of primary open-angle glaucoma (POAG) in the Glaucoma Inheritance Study in Tasmania (GIST) and from families with glaucoma referred to the study from throughout Australia.[4] The ages of the female patients were distributed around a median of 71 years with an inter-quartile range of 61–74 years and minimum and maximum ages of 41 and 93 years, respectively. The median and inter-quartile range of the male patients were 70 years and 63–75 years, respectively, with a minimum and maximum of 39 and 92 years, respectively. Index cases and available family members were examined for signs of glaucoma. Written informed consent was obtained, and genetic studies on these patients were approved by the ethics committees of The Royal Victorian Eye and Ear Hospital and The Royal Hobart

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Hospital. This study was conducted in accordance with the Declaration of Helsinki and subsequent revisions.

The following protocol was performed on all family members: interview, Goldmann applanation tonometry, anterior segment slit-lamp biomicroscopy, gonioscopy, optic disc examination and optic disc stereophotography, automated perimetry using the 24-2 program (Humphrey, San Leandro, CA, USA), and venipuncture. Optic disc appearance was graded according to the GIST scoring protocol by two glaucoma specialists. Automated perimetry was graded as normal or abnormal using the GIST field score and the Glaucoma Hemi-field Test.[12]

A mass spectrometry (MS)-based mutation detection technique (Sequenom, San Diego, CA, USA) was used to detect Single Nucleotide Polymorphism (SNP)-dependent single-base differences in amplification products.[13] PCR amplification was performed using 5′-ACGTTGGATGTATTATACCCACACCCA CCC-3′ and 5′-ACGTTGGATGAGGAATTGAACCTCTGAC TG-3′ primers. Base extension was performed from a 5′-GGTTT GTTAAGATGGCAG-3′ extension primer and a mixture of three dideoxynucleotide triphosphates and one deoxynucleotide trisphosphate which allowed only one nucleotide to be added before chain termination unless the SNP was complementary to the single deoxynucleotide species in which case two nucleotides would be added. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was performed using a Sequenom 200 K analysis platform and the results were analyzed using the Sequenom MassARRAY RT software.

A DNA sample which had previously been demonstrated to harbor the 3243 A-G mutation using a HaeIII restriction fragment length polymorphism[14] was used as a positive control for the MS-based analysis. Of the amplification products analyzed, 38.6% represented 3243 A-G mutants. This confirmed the validity of the technique. The 3243 A-G mutation was demonstrated in none of the samples from GIST patients.

The population frequencies compatible with observing no mutations in a sample of 193 were calculated using the binomial distribution. The highest population frequency of the mutation where zero mutations would be observed in at least 5% of samplings is 1.54%. Using the binomial distribution, the 95% confidence interval for the rate of mutations in the population on the basis of the results of this experiment is 0–1.54%.

The mutation detection technique employed here gave a strongly positive result for the positive control. This technique was developed to allow high throughput at low cost per sample. In situations where large-scale testing for the 3243 A-G mutation is required and where the facilities are available, this would be a worthwhile approach to consider.

The results of this study are open to a number of interpretations. It is possible that the patients in fact had 3243 A-G mutations that were not detected. This would be possible if the heteroplasmy of the 3243 A-G mutation was below the detection level of the technique. These patients may have had a very low initial heteroplasmy for the mutation or it may have been low because of the nature of the DNA sample and the age of the patients at the time of collection. It is known that the heteroplasmy of the 3243 A-G mutation in circulating leukocytes diminishes with age,[15,16] that this reduction of heteroplasmy is faster than in other accessible tissues. Our samples were mostly derived from leukocytes because blood samples allow a large amount of DNA to be collected with minimal invasiveness. This is essential in large-scale genetic studies.

The Sequenom company claims that an SNP with a 5% frequency *of the total DNA targets* can be detected. Our unpublished data suggest an even higher sensitivity of 3% of amplimers carrying the SNP. Thus, the sensitivity of this technique is very high. Except possibly through a dominant negative effect, it would be difficult to explain how a copy number of mutated genes below the detection of our technique could be pathogenic.

It is more likely that there were no patients in our sample who carried the 3243 A-G mutation. The 95% confidence interval for 3243 A-G mutations in Tasmanian diabetic patients with glaucoma is 0–1.5%. The upper levels of this confidence interval are consistent with published estimates of the contribution of mutation to the population frequency of diabetes. However, lower population frequencies would be even more likely to result in the sample frequency observed. Moreover, our sample represents a significant proportion of all Tasmanians with diabetes and glaucoma. This presents two possibilities: either the rate of 3243 A-G mutations in Tasmanian diabetic patients is lower than has been suggested by other work or, less likely, the 3243 A-G mutation is protective against glaucoma.

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