The Journal of Laryngology & Otology
September 2001, Vol. **115,** pp. 730–732

Clinical Records

Cochlear implantation of a patient with a previously undescribed mitochondrial DNA defect

P. R. Counter, F.R.C.S., M. P. Hilton, F.R.C.S., D. Webster, M.B.A.T.T., T. Wardell, B.Sc., Ph.D.[*], R. W. Taylor, Ph.D.[*], G. Besley, Ph.D.[†], D. M. Turnbull, F.R.C.P.[*], P. J. Robinson, F.R.C.S.

Abstract
Mitochondrial DNA (mtDNA) defects are responsible for symptom complexes that are characteristically heterogeneous but are typically represented by muscle weakness and neurological deficits. One common feature of mitochondrial disease is deafness. This report details the assessment and outcome of a patient with a previously undescribed mtDNA rearrangement who underwent cochlear implantation. The patient shows a marked improvement in sentence recognition tests and recognition of environmental sounds. Patients with profound sensorineural hearing loss due to mtDNA defects should be considered as candidates for cochlear implantation when they no longer benefit from conventional hearing aids.

Key words: Cochlear Implantation; DNA; Mitochondria

Introduction

The mitochondrial genome encodes essential information for the synthesis of the mitochondrial respiratory chain, which in turn is responsible for the production of the majority of cellular adenosine triphosphate (ATP). In recent years the rapid increase in understanding of genetic disorders has led to the identification of a disease complex due to mitochondrial DNA (mtDNA) defects. The main effects of the disorder are neurological, with deafness as a symptom of many of the phenotypes. A high number of copies of the mitochondrial genome are found within cells (one to 10 genomes per mitochondrion, 100–10 000 mitochondria per cell). The normal mitochondrial genotype is termed homoplasmy. In this situation all copies of the mitochondrial genome are the same. Mutations of mtDNA create a state termed heteroplasmy, where a mixture of wild type and mutant genomes exist in a variable proportion. Disease phenotypes are expressed when the level of mutated mtDNA genomes exceeds a threshold. This threshold is variable and may depend on a number of factors including mutation type and position and tissue type. MtDNA is strictly maternally inherited. Point mutations and large scale partial duplications of the mitochondrial genome may be traced along the maternal lineage. Large scale partial deletions of mtDNA are exceptional as they are rarely, if ever, transmitted and account largely for reported sporadic cases. Over 150 mutations of the mitochondrial DNA have been described, of which 100 are rearrangements and 50 are point mutations.[1–3] There are very few reports of cochlear implantation in patients with mtDNA mutations, and these only report patients with point mutations. This is the first report of cochlear implantation in a previously undescribed mtDNA duplication.

TABLE I
AUDIOMETRIC SCORING AT PRE-IMPLANT AND AT THREE MONTHS

Measure		Score pre-implant	Score post-implant
CUNY*	– Lip reading & Aided	5%	84%
Sentence	– Aided alone	0%	57%
Tests	– Lip reading alone	4%	5%
AB word test**		N/A	61%
Environmental sound recognition		N/A	83%
Telephone interview		N/A	66%

*the CUNY sentence test is a recognition test of open set spoken sentences presented without context.
**the AB word test is a recognition test of open set spoken single syllable words, without context. Individual phonemes are scored.

From the University Department of Otolaryngology, Southmead Hospital, Bristol, the Department of Neurology[*], Medical School, Newcastle upon Tyne and the Willink Biochemical Genetics Unit[†], Royal Manchester Children's Hospital, Manchester, UK.
Accepted for publication: 8 March 2001.

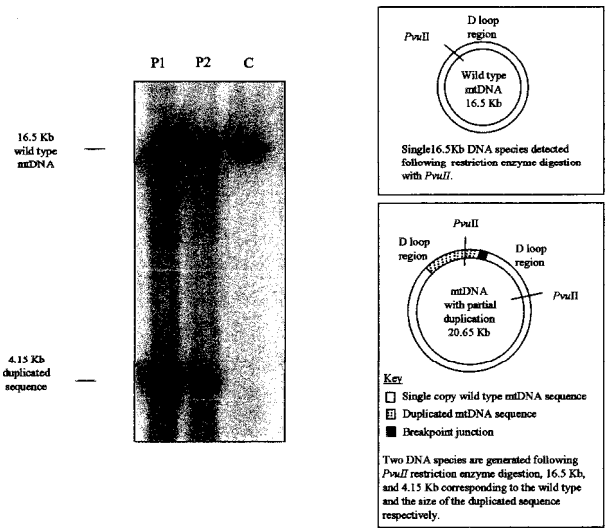

FIG. 1

Southern blot of the patient (P1), his sister (P2) and control (C). The faster migrating species (4.15 Kb) revealed corresponds to the region of mtDNA sequence duplicated in this inherited rearrangement. All family members revealed high levels of the duplication (50–60 per cent) in whole DNA extracted from leukocytes.

Case report

A 44-year-old man was referred for assessment to the West of England Cochlear Implant Programme with a 20-year history of progressively worsening sensorineural hearing loss and insulin-dependent diabetes. He was profoundly deaf and was no longer gaining benefit from conventional hearing aids (Table I). After undergoing the full medical, audiological and psychosocial assessment of the programme, he was judged to be a suitable candidate for implantation. The patient's only sibling (a sister) and his mother had both developed a similar pairing of progressive sensorineural hearing loss and diabetes in their early twenties. They currently suffer with only moderate sensorineural hearing loss. There was no evidence in the family history of problems in any earlier generations.

In June 2000 the patient underwent surgery and a Med-El Combi 40+ standard electrode cochlear implant was implanted in his left ear via a posterior tympanotomy and cochleostomy. The implant package was sited above and behind his ear and recessed into the skull. The external stimulator was fitted at four weeks post-operatively and the initial programming of the implant performed. He showed an immediate positive subjective response, and formal testing at three months showed a dramatic improvement on his pre-implant sentence recognition tests (Table I). At this stage the patient reported he was typically wearing the implant for 12 or more hours per day.

Molecular genetic analysis of mtDNA

Symptoms of maternally-inherited diabetes and deafness have previously been reported associated with partial duplications of the mitochondrial genome.[4–6] The DNA samples from the family members were, therefore, investigated for this particular mtDNA defect by Southern blot.

Whole DNA was extracted from leukocytes following overnight incubation at 37°C with one per cent SDS and 2 $mgml^{-1}$ proteinase K, phenol: chloroform removal of protein and ethanol precipitation.

Two micrograms of whole DNA from each patient, and also from controls, were restriction enzyme digested with *Pvu*II. This enzyme linearizes wild-type mtDNA allowing it to be accurately sized following agarose gel electrophoresis. The digested samples were Southern blotted using standard techniques.[7] MtDNA was detected using a radiolabelled probe complementary to the displacement loop (D-loop) region.[8] This region of the mitochondrial genome contains control sequences and is therefore essential for the maintenance of rearranged genomes.

The results (Figure 1) revealed a 4.15 Kb mtDNA species in addition to the wild type (16.5 Kb). On the basis that partial mtDNA deletions are not generally detected in whole leukocyte DNA nor are they inherited,[9] and that they typically exceed approximately 6 kB,[10] it can be concluded that the additional mtDNA species corresponds to a 4.15 Kb partial duplication. In the three family members studied (the patient, his mother and sister), the level of duplicated species was estimated to be between 50 and 60 per cent.

The breakpoint junction of the duplication was determined by sequencing a polymerase chain reaction (PCR) product generated using a primer shift assay,[11] across the breakpoint. Sequencing data revealed a nine base pair repeat sequence at the breakpoint. This nine base pair sequence lies at positions 3328–3336 and 15740–15748 in the wild type mtDNA Cambridge reference sequence.[8] In the mutated genomes the intervening sequence across the D-loop and including the *Pvu*II restriction site, is duplicated in addition to the full wild-type sequence.

Discussion

The most likely pathogenesis of mitochondrially-based deafness is that the defective mitochondria are unable to provide sufficient ATP for the high energy demands of the stria vascularis and the outer hair cells. This leads to their metabolic failure and an irreversible cochlear sensorineural hearing loss.

It has been shown[12] that the point prevalence of mtDNA mutations is higher than has previously been estimated and lies at about seven per 100 000 of the population of the United Kingdom. Approximately 60 per cent of cases have an associated sensorineural hearing loss.[13] This gives a figure of around four per 100 000 of the population with a hearing loss due to mtDNA mutation, which equates to around 2500 people in the UK.

A recent review of mitochondrial inherited hearing loss[14] stated, '. . . no therapeutic interventions are known'. Since that date four authors have published reports of successful cochlear implantation of patients with point mtDNA mutations (Table II). The hearing loss associated with mtDNA mutations is cochlear[12,15] and it is unlikely that there is a significant central component in the vast majority of cases.

TABLE II
PREVIOUS REPORTS OF COCHLEAR IMPLANTATION IN MTDNA MUTATIONS

Paper	Genetic defect	Implant
Sue *et al.*[15]	A3243G	Not specified
Tono *et al.*[16]	A1555G	Nucleus 22 channel
Rosenthal *et al.*[17]	A3243G	Not specified
Cullington[18]	Not specified	Nucleus C124M

https://doi.org/10.1258/0022215011908766 Published online by Cambridge University Press

Cochlear implantation is ideally suited to pure cochlear failure. The authors have reported the first case of cochlear implantation in a patient with proven mtDNA duplication. The post-operative results for this patient are very encouraging, and taken along with the limited data from patients with point mtDNA translocation, suggest that cochlear implantation is an effective treatment to overcome the effects of profound sensorineural hearing loss due to mtDNA mutation when more conservative measures fail.

References

1 Chinnery PF, Turnbull DM. Mitochondrial DNA and disease. *Lancet* 1999;**354**(Suppl I):117–21
2 DiMauro S, Bonilla E, Davidson M, Hirano M, Schon EA. Mitochondria in neuromuscular disorders. *Biochim Biophys Acta* 1998;**1366**:199–210
3 Wallace DC. Mitochondrial diseases in mouse and man. *Science* 1998;**283**:1482–8
4 Dunbar DR, Moonie PA, Swingler RJ, Davidson D, Roberts R, Holt IJ. Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. *Hum Mol Genet* 1993;**2**:1619–24
5 Ballinger SW, Shoffner JM, Gebhart S, Koontz DA, Wallace DC. Mitochondrial diabetes revisited [letter]. *Nature Genet* 1994;**7**:458–9
6 Martin Negrier ML, Coquet M, Moretto BT, Lacut JY, Dupon M, Bloch B, *et al*. Partial triplication of mtDNA in maternally transmitted diabetes mellitus and deafness [letter]. *Am J Hum Genet* 1998;**63**:1227–32
7 Sambrook J, Fritsch EF, Maniatis T. Analysis and cloning of eucaryotic genomic DNA. In: Nolan C, ed. *Molecular Cloning*, 2nd Edn. USA: Cold Spring Harbour Press, 1989; 9.31–9.59
8 Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, *et al*. Sequence and organization of the human mitochondrial genome. *Nature* 1981;**290**:457–65
9 Larsson NG, Eiken HG, Boman H, Holme E, Oldfors A, Tulinius MH. Lack of transmission of deleted mtDNA from a woman with Kearns-Sayre syndrome to her child. *Am J Hum Genet* 1992;**50**:369–3
10 Holt IJ, Harding AE, Cooper JM, Schapira AH, Toscano A, Clark JB, *et al*. Mitochondrial myopathies: clinical and biochemical features of 30 patients with major deletions of muscle mitochondrial DNA. *Ann Neurol* 1989;**26**:699–708
11 Moslemi AR, Melberg A, Holme E, Oldfors A. Clonal expansion of mitochondrial DNA with multiple deletions in autosomal dominant progressive external ophthalmoplegia. *Ann Neurol* 1996;**40**:707–13
12 Chinnery PF, Johnson MA, Wadell TM, Singh-Kler R, Hayes C, Brown DT, *et al*. The epidemiology of pathogenic mitochondrial DNA mutations. *Ann Neurol* 2000;**42**:188–93
13 Gold M, Rapin I. Non-Mendelian mitochondrial inheritance as a cause of progressive genetic sensorineural hearing loss. *Int J Paediatr Otorhinolaryngol* 1994;**30**:91 104
14 Ensink RJH, Camp GV, Cremers CWRJ. Mitochondrial inherited hearing loss. *Clin Otolaryngol* 1998;**23**:3–8
15 Sue CM, Lipsell LJ, Crimmins DS, Tsang TS, Boyages SC, Presgrave CM, *et al*. Cochlear origin of hearing loss in MELAS syndrome. *Ann Neurol* 1998;**43**:350–9
16 Tono T, Ushisako Y. Cochlear implantation of a patient with profound hearing loss with the A1555G mitochondrial mutation. *Am J Otol* 1998;**19**:754–7
17 Rosenthal EZ, Kileny PR, Boerst A, Telian SA. Successful cochlear implantation in a patient with MELAS syndrome. *Am J Otol* 1999;**20**:187–90
18 Cullington HE. Cochlear implantation of a deaf blind patient with mitochondrial cytopathy. *J Laryngol Otol* 1999;**113**:353–4

Address for correspondence;
Mr Paul Counter,
Department of Otolaryngology and Head and Neck Surgery,
Freeman Hospital,
High Heaton,
Newcastle upon Tyne NE7 7DN, UK.

Mr P. Counter takes responsibility for the integrity of the content of the paper.
Competing interest: None declared