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University College London Hospitals NHS Foundation Trust

Gentamicin, Genetic Variation and Deafness in Preterm Children

Short title: MitoGent

Protocol v.1.6
9 December 2014

Sponsor: **UCL ICH**
Sponsor reference number: **09CM13**

Funding source: **Action on Hearing Loss**
Funder's reference: **RNID G47**

REC: **London - Central**
REC Reference number: **12/LO/0005**

Mitogent
Understanding deafness in preterm children

STUDY PERSONNEL AND CONTACT DETAILS

Sponsor:

UCL Institute of Child Health

Chief Investigators:

Professor Maria Bitner-Glindzicz and Dr Shamima Rahman
Clinical and Molecular Genetics Unit
UCL Institute of Child Health
30 Guilford Street
London WC1N 1EH
Phone: 020 7905 2608
Fax: 020 79052832
Email: maria.bitner@ucl.ac.uk
shamima.rahman@ucl.ac.uk

Co-investigators:

Professor Neil Marlow
Professor of Neonatal Medicine
Institute for Women's Health
86-96 Chenies Mews
London WC1E 6AU
Tel: +44 (0) 20 7679 0834 (PA: Nada Wansa)
Fax:+44 (0) 3108 2036
Email: n.marlow@ucl.ac.uk

Professor Ian Chi Kei Wong
Director and Professor of Paediatric Medicines Research, Centre for Paediatric Pharmacy Research, School of Pharmacy, London
Email: Ian.Wong@dpp.pharmacy.ac.uk

Dr William van't Hoff
Head of Clinical Research Facility & Director of Medicines for Children London & South East LRN
Email: VANTHW@gosh.nhs.uk

Audiological medicine lead:

Dr Breege MacArdle
Nuffield Hearing and Speech Centre,
Royal National Throat, Nose and Ear Hospital,
330 Gray's Inn Road, London WCIX 8DA
Email: breege.macardle@royalfree.nhs.uk

Study Statistician:

Professor Tim Cole
MRC Centre of Epidemiology for Child Health
UCL Institute of Child Health, London
Email: tim.cole@ich.ucl.ac.uk

Research Nurse:

Kathy Chant
Institute for Women's Health, University College London
Medical School building,
74 Huntley Street, London WC1E 6AU
Tel: 0207 679 6031
Email: k.chant@ucl.ac.uk

SYNOPSIS

Title	Gentamicin, Genetic Variation and Deafness in Preterm Children
Acronym	MitoGent
Chief investigators	Maria Bitner-Glindzicz, Reader, and Shamima Rahman, Reader, UCL Institute of Child Health
Objectives	1. To calculate in preterm infants <31/40 gestation the burden that the m.1555A>G mutation represents to deafness. 2. To calculate the relative odds of carrying the m.1555A>G mutation and being in the 'deaf' group versus the 'hearing' group. 3. To explore the relevance of aminoglycoside exposure to deafness in the presence of the m.1555A>G mutation.
Study configuration	Case control study
Setting	Hospital clinics
Sample size estimate	3000 babies and children surviving premature birth <31/40 gestation
Number of participants	30-60 deaf children and 5 controls per deaf baby/child (total 150-300 controls)
Eligibility criteria	Deafness in children graduating from NICUs in Greater London or matched control of deaf child.
Case Identification	Ascertainment of cases of deafness in children following admission to NICU from eSP (e-Screener Plus, which is the database of the Newborn Hearing Screening Programme), audiological services, neonatal follow up clinics, genetic services, and support groups (Action on Hearing Loss and National Deaf Children's Society, NDCS). Collection of detailed clinical and audiological data about these children. Selection of controls using eSP and SEND (Standardised Electronic Neonatal Database). Molecular testing for the m.1555A>G mutation. Review of NICU records, pathology and pharmacy records about aminoglycoside administration for cases and controls.
Duration of study	3 years (January 2013- January 2016, depending on necessary approvals)
Ascertainment period	Babies born 1 January 2009 – 31 December 2013
Outcome measures	Estimation of contribution of m.1555A>G to deafness in NICU graduates. Relative odds of carrying m.1555A>G mutation in deaf vs hearing babies of 31/40 gestation.
Statistical methods	The burden of disease attributable to m.1555A>G will be calculated as a percentage with confidence interval. We will calculate the relative odds and CI of the m.1555A>G mutation in deaf and hearing children. Conditional logistic regression will be used to evaluate the role of a range of potential causative factors for hearing loss in addition to m.1555 A>G.

STUDY PROTOCOL

Purpose

Our aim is to assess the contribution of m.1555A>G to deafness in <31/40 gestation babies, who are very likely to have received multiple courses of aminoglycoside antibiotics. This study will help to formulate national policy on genetic testing for the mitochondrial m.1555A>G mutation in the context of aminoglycoside usage in NICU.

Background

The prevalence of deafness in children in the UK is 1 per 1000 children at birth, with a similar number becoming deaf before maturity [1]. Causes of deafness can be broadly divided into 'genetic' and 'environmental', each thought to contribute to around half of all cases. Environmental causes include congenital and postnatal infections, ototoxic medication, trauma and factors surrounding preterm delivery. Genetic causes are also highly heterogeneous (Hereditary Hearing Loss Homepage http://webh01.ua.ac.be/hhh/).

However among preterm and very low birthweight babies the prevalence of sensorineural hearing loss is ten times that of the normal paediatric population [2]. Aetiological factors are complex and are likely to include hyperbilirubinaemia, hypoxia, drug-induced ototoxicity, noise and infection, particularly cytomegalovirus.

Although advances in neonatal intensive care have resulted in remarkable improvements in outcomes, particularly survival of the most preterm babies [3], follow-up studies have shown a high rate of disability among survivors [4;5]. Deafness occurs in about 1-2% of this group of preterm and low birthweight survivors, and is often combined with other disabilities such as cerebral palsy, intellectual and visual impairment [2].

Aminoglycosides are routinely used in hospitals for first-line treatment of suspected or proven Gram-negative infection particularly on neonatal intensive care units (NICU) where Gram-negative infections can prove fatal within hours. Their low-cost, synergistic effect with beta-lactam antibiotics and low levels of resistant organisms make them highly attractive for this purpose. We estimate that 20-24,000 babies are treated with aminoglycoside antibiotics on NICUs per year in the UK, based on the numbers of babies admitted to NICUs, local prescribing policies, and usage within London networks. These antibiotics are well-known to be ototoxic and nephrotoxic, and so levels are monitored to maintain drug concentration within a narrow therapeutic range to help prevent these complications.

However individuals with a mutation of mitochondrial DNA, m.1555A>G, suffer from catastrophic, rapidly-progressive, profound and permanent hearing loss as a result of an idiosyncratic reaction if they are given aminoglycoside antibiotics, even when drug levels are within normal limits [6]. It is thought that risk of deafness after exposure to aminoglycosides is lifelong in those with m.1555A>G. m.1555A>G is a mutation of mitochondrial DNA which means that it is maternally transmitted from a woman to all of her offspring.

Our experience of patients seen in the genetic deafness clinics at Great Ormond Street and the Royal National Throat Nose and Ear Hospitals suggested to us that this mutation might be more common than was previously suspected from studies of its contribution to deafness. Our study of the prevalence and penetrance of this mutation in a large birth cohort of children (the Avon Longitudinal Study of Parents and Children cohort, ALSPAC) showed that the prevalence is around 1 in 500 children and that at 9 years (the age at which the children last had their hearing tested) their hearing is normal, i.e. these children

cannot be distinguished from other children except by genetic testing [7]. None of the children with the mutation had a history of NICU admission and it is possible that none had ever been exposed to aminoglycosides or other ototoxic drugs.

However it is also possible that some of the ALSPAC children had been exposed to aminoglycosides but had preserved hearing (ie. non-penetrance of the mutation even in the presence of aminoglycosides). According to the literature, penetrance of m.1555A>G in the presence of aminoglycosides is close to 100% over an individual's lifetime [8]. However such data need to be recognised as being extremely biased. If a person is given a standard treatment and shows no ill effect, they are unlikely to ever undergo genetic testing and would not be ascertained as 'non-penetrant'. This study may provide an indication that penetrance of the mutation following aminoglycoside exposure may not be complete if we find fewer cases than expected among the deaf NICU survivors, or cases with m.1555A>G among hearing survivors who have clearly been exposed to aminoglycosides.

This study aims to determine the burden that m.1555A>G represents to deafness in preterm babies. It will also inform the argument as to whether genetic testing before aminoglycoside administration to identify those with the mutation, and subsequent use of an alternative antibiotic, is worthwhile and cost-effective in these babies who are likely to receive multiple courses of aminoglycosides. An alternative way of avoiding aminoglycoside-induced deafness would be to change antibiotic policy so that aminoglycosides are no longer first-line drugs. Counter-arguments to this include cost, because aminoglycosides are cheap, and significant concern about high levels of resistance to other antibiotics. Therefore it is important not to deny aminoglycoside treatment to babies at risk of life-threatening infection on the basis of unsubstantiated risk of deafness. **This study will help to provide evidence of the true risk**.

Aim:

We will determine the contribution of gentamicin-induced m.1555A>G-associated deafness among children discharged from neonatal intensive care units in Greater London in the at-risk population of babies born before 31 completed weeks of gestation, and the relative odds of carrying the mutation and being in the 'deaf' versus 'hearing' group.

Hypothesis:

We hypothesise that m.1555A>G makes a significant contribution to deafness among survivors of neonatal intensive care even when aminoglycoside levels have been in the therapeutic range.

Objectives:

1. To calculate in preterm infants <31/40 gestation the burden that the m.1555A>G mutation represents to deafness.
2. To calculate the relative odds of carrying the m.1555A>G mutation and being in the 'deaf' group versus the 'hearing' group.
3. To explore the relevance of aminoglycoside exposure to deafness in the presence of the m.1555A>G mutation.

Study Design:

This is a case-control study.

Inclusion Criteria for Cases: Babies of <31 weeks completed gestation with hearing loss, treated on NICUs in the Greater London region between 1st January 2009 and 31st December 2013.

Exclusion Criteria for Cases: None[1].

Inclusion Criteria for Controls: Babies of <31 weeks completed gestation treated on NICUs in the Greater London region between 1st January 2009 and 31st December 2013, who have no documented hearing loss.

Exclusion Criteria for Controls: Missing data on drug exposure (no drug chart or discharge summary) [1].

1. Identification of children with hearing loss among NICU graduates

We will use multiple methods of ascertainment to identify children with hearing loss who were previously admitted to a neonatal unit:

a. From eSP the database of the Newborn Hearing Screening Programme
b. Records of hearing assessment centres throughout London
c. Records of neonatal unit follow up services from each of the 5 neonatal networks
d. Universal Neonatal Hearing Screening Records in London maternity hospitals
e. Patients presenting to the Genetic Deafness clinic at Great Ormond Street Hospital
f. By approaching parents directly using the support services and newsletters of the Royal National Institute for Deaf People (RNID) and the National Deaf Children's Society
g. By advertising via the study internet website

The research nurse(s) will identify duplicates and check admission details against NICU records. Following confirmed case identification, contact will be with parents directly for those contacting the study office through the 'Action On Hearing Loss', NCDS or website and via a letter from the local paediatrician/ neonatologist , audiological physician, or occasionally through the GP, who provides/provided care for those identified via other routes. Given the small number of children and likely motivation we will endeavour to visit or telephone each index family to explain and discuss the study with them before study entry. A few subjects, presenting to the Genetic Deafness clinic at Great Ormond Street Hospital (we anticipate less than 10 families over 3 years) and at the clinic at University College London Hospital may also be recruited there, including discussion, consent and taking of samples

The Research Nurse will obtain informed consent for participation in the study, will collect information about family history and perinatal care from the parents using a standardised form and questionnaire and will request a saliva/buccal sample for molecular testing for the m.1555A>G mutation. This will be conducted mostly by telephone or in the patient's home, however this could also be at University College London Hospital or Great Ormond Street Hospital. Audiological data will be obtained with parental consent from the local hearing services.

The study will be publicised through the following routes to maximise interest and ascertainment:

a. Study website
b. Newsletters and weblinks from the 'Action On Hearing Loss' and NDCS websites (MB-G)
c. Circulation of audiological physicians (BA)

[1] Where there is no drug summary available for cases we assume that there are multiple alternative sources of obtaining this information. For controls, we will exclude cases without drug exposure details.

d. Thames Perinatal Group (NM)
e. Discussion with follow up leads (NM)
f. Dissemination via MCRN events and Extended Neonatal Network (MB-G, WvH)
g. British Association of Perinatal Medicine newsletter (NM)

2. Recruitment of control children
For each deaf index child identified, we will use eSP and NDAU (Neonatal Data Assessment Unit) to identify 6 controls, matched for gestational week, sex and NICU on which the baby was treated (this will allow calculation of narrow confidence intervals for the odds ratios and also allow for recruitment failure). The consent that is obtained for Newborn Hearing Screening includes consent to screen and consent to national audit.

NDAU is a web-based 'opt-out' database containing basic demographic and clinical information about all babies admitted to neonatal units in London and most of the South of England. Controls must have data on drug exposure in order to be recruited to the study (ie. a drug chart or discharge summary available for examination).

Professor Marlow will write to the parents of the control children via their local neonatologist to request participation in the study. Parents are asked to return the reply sheet and indicate if they would prefer us not to contact them. For those that do not respond we will contact them to check they have received the study information. If the parents agree, the Research Nurse will take informed consent for collation of audiological and other clinical data about the baby/child and will ask for a saliva/buccal sample for molecular testing for the m.1555A>G mutation. This will be conducted mostly by telephone or in the patient's home, however this could also be at University College London Hospital or Great Ormond Street Hospital.

Parents of both children with hearing loss (cases) and children with normal hearing (controls) will be offered a £10 shopping voucher to say thank you for their time and any inconvenience.

3. Clinical data set
Clinical data will be abstracted from the NICU records for each child using a standardised clinical record form. Prescription records, hospital pharmacy records and microbiology records will be examined, to determine aminoglycoside exposure – dose, duration and number of courses and the blood concentrations taken during treatment. Clinical data collection will be as described in Appendix 1.

4. **Hearing – audiological assessment**

Audiological records will be examined to define the severity and pattern of hearing loss of cases. Further audiological testing will be performed if clinically indicated. Family history of hearing loss will also be obtained at interview during the consent and recruitment process.

5. Molecular analysis

Saliva/buccal swabs will be collected from the cases with hearing loss and also from the matched controls with normal hearing following informed consent by the Research Nurse. These will be taken directly to UCL Institute of Child Health or sent directly by post. Only the unique study number and date of birth will be used as identifiers. DNA will be extracted from the samples, in order to test for the m.1555A>G mutation. Dr Bitner-Glindzicz will be the custodian of the samples. Mutation analysis will be outsourced to KBiosciences who have performed this assay for us previously. All samples will be coded and no personal information will be sent with the samples.

Cases with the mutation will be confirmed by sequence analysis of DNA extracted from a second sample, which will be requested and consented by the Research Nurse. This second sample will be re-

tested in the Regional Genetic Centre Laboratory at Great Ormond Street Hospital where this test is routinely performed in the diagnostic laboratory. Families which are confirmed to have m.1555A>G will be invited for genetic counselling (with MB-G) through the Research Nurse and other family members will also be offered diagnostic testing under quality assured conditions. The mothers of all children confirmed to have the m.1555A>G mutation will be offered a genetic clinic appointment, where their mutation status will be established according to good clinical practice. This should be no more than 5 or 6 index cases over 3 years.

DNA samples will be used only for deafness research in this project. If any samples remain at the end of the project they will be stored in the Clinical Molecular Genetics Research Laboratory (authorized access only) at the UCL ICH (authorized access only) under the custodianship of Dr Maria Bitner-Glindzicz. Dr Bitner-Glindzicz will not hold personal information linked to the samples. The samples may be used for further deafness research subsequently in a project which has been reviewed and approved by a Research Ethics Committee.

6. Data collation and management

Data will be entered into an SPSS database designed for the study using SPSS Data Entry (SPSS Inc) by the Research Nurses. Data will be double entered using the automated error detection system in SPSS. The database will be password protected and maintained on a secure password protected server with regular back up. On the database children will be identified only by a unique study number. Records of parent contacts and other identifiers will be securely stored in a separate secure database.

7. Sample Size and Statistical analysis

Approximately 120,000 births occur in London annually (600,000 in 5 years), of which 1% have birthweight <1501g [9]. This gives a likely population of babies <31w gestation of 3-6,000 over 5 years. The prevalence of hearing loss in this population is 1-2% [2] resulting in 30-120 children with hearing loss over the 5-year study period.

In such a cohort of 3-6,000 we would expect 4-10 children to carry the mutation, given a mutation frequency of 1 in 526 (95%CI 1 in 357 to 1 in 770) [7].

Burden of Disease:

The burden of disease will be calculated as the proportion (and 95%CI) of deaf cases that have the mutation, using the method of Wilson. The analysis will be done initially including all cases, and repeated excluding those a) with less than one dose of aminoglycosides, and b) with less than two such doses.

Relative risk of deafness for those with the m.1555A>G mutation:

The odds ratio of children with the m.1555A>G mutation being deaf will be calculated using Fisher's exact test. Table 1 shows how the odds ratio varies according to the numbers of hearing controls with the mutation, assuming 30 deaf cases, 4 of them with the mutation, and 150 controls (5 controls per case). In practice all three numbers are likely to be bigger than this.

Number of cases with m.1555A>G (out of 30 total)	Number of controls with m.1555A>G (out of 150 total)	Odds ratio	95% CI		P-value
			lower	upper	
4	0	∞	3.5	∞	0.0007

4	1	22.3	2.1	1130	0.003
4	2	11.1	1.5	129	0.008
4	3	7.4	1.2	54	0.02

The analysis will be done initially including all cases and controls, and repeated excluding those a) with less than one dose of aminoglycosides, and b) with less than two such doses.

8. Study written and electronic records

- Each participant will be assigned a study identity code number, for use on all paper records, other study documents and the electronic database. The documents and database will also use the date of birth as a second identifier.
- All written records will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Study Number, to permit identification of all participants enrolled in the study, for the purposes of later follow-up.
- All paper forms will be completed using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

9. Quality assurance & audit

Insurance and indemnity

- Insurance and indemnity for clinical study participants and study staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but study participants may have recourse through the NHS complaints procedures.
- UCL has separate Insurance (Employers Liability Policy) to cover potential legal liability of the sponsor(s) or employer(s) for harm to participants arising from the design of the research

Conduct of the Study

- UCL Partners adhere to the Research Governance Framework, and all investigators have been trained in research governance. MB-G is the Designated Individual for the Human Tissue Act at the UCL Institute of Child Health.
- Although, it is not a legal requirement for an epidemiological study to comply with Good Clinical Practice (GCP), the MitoGent study will be conducted according to the principles of GCP when applicable and Good Pharmaco-epidemiology Practice (GPP) to ensure the quality of the study [10,11].
- The results will be the reported according STROBE statement (STrengthening the Reporting of OBservational studies in Epidemiology) [12]

10.Data Management and analysis

- Access to all study documents is limited to the study personnel (see below) excepting that the CRF and all source documents will be made be available at all times for review by the Principal Investigators and Sponsor's designee.
- All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The Assessment procedures will only collect the minimum required information for the purposes of the trial. All records will be held securely, in a locked room, and a locked cabinet. Access to the information will be limited to the trial staff and investigators and any relevant regulatory authorities. Computer held data including the study database will be held securely and

password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

- Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.
- Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

11.Feedback to Parents

We will give all participants generic feedback from the study outlining the overall findings when it is completed. In addition, we will specifically request in the application to the Research Ethics Application that we are able to share the results of our molecular analyses in the cases where m.1555A>G has been detected, since these babies and children are already symptomatic – they have been ascertained because they are deaf. We feel it is ethical that participants who have m.1555A>G are made aware of the potential risks of having aminoglycosides to themselves and to other maternal relatives who can also be tested through Regional Genetics Services. This will be done by one of the PIs (MB-G) in the context of the Genetic Counselling clinic.

12.Management of non-responders

Families will be approached via a single invitation letter and two follow up letters requesting response to the study office.

13.Timeline

0-3 months	ethics approval and publicise study to all neonatal consultants in the networks, and establish working relationship with Neonatal clinic coordinators
3-32 months	identification of 30-60 deaf babies and 5 matched controls for each deaf baby; consent from carers; review of audiological records; DNA sample collection (saliva/buccal swab); collation of data on aminoglycosides exposure for cases and matched controls
32-36 months	genotyping of samples and collation of genotype data and calculation of odds ratio of m.1555A>G genotype in deaf vs controls; analysis, write up and dissemination.

14.Dissemination

We will discuss results from this study with DH, so that timely decisions can be made about national antibiotic policy in relation to the m.1555A>G mutation can be made by NICE (eg universal screening of pregnant mothers, point of care testing for babies admitted to NICU, or change in antibiotic policy away from first line usage of aminoglycosides).

We will publicise our findings through publication in international scientific peer reviewed journals and by presentation at national and international scientific meetings and to patient support groups. We will co-ordinate any press releases with 'Action On Hearing Loss' and the Great Ormond Street Hospital for Children press office. We will be pleased to present the findings of the study at academic meetings, relevant to our specialties.

15. Statement of confidentiality

- Individual participant medical or personal information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.

- Participant confidentiality will be further ensured by utilising identification code numbers to correspond to all data in computer files. Data generated as a result of this study will be available for inspection on request by the participating physicians, representatives of UCL Partners, the REC, local R&D Departments.

16. Study Management

- The study is overseen by the Study Investigators group,
    - Professor Bitner-Glindzicz (CI)
    - Dr Rahman (CI)
    - Professor Marlow
    - Professor Wong
    - Dr van't Hoff
    - Professor Cole
    - Dr MacArdle
- The research nurse(s) will be employed through the Institute for Women's Health with Professor Neil Marlow responsible for their role on the study, supervising their patient contact, and Sharon Barrett for nursing management issues.
- The research nurse will be responsible for liaison with the Neonatal networks; recruitment and consenting of cases and controls; collation of clinical, audiology, pharmacy and microbiology data; and collection of saliva/buccal samples.
- The Principal Investigators will lead the write up and dissemination.
- The study will be overseen by a study management group comprising the Investigators named in 'Study Personnel', the Research Nurse(s), Dr Sharon Barrett (MCRN Network Manager) which will meet monthly in the initial stages of the project.
- This group is responsible to the overarching Advisory Group, which will meet annually and also include the study statistician, a lay member, and representation invited from 'Action On Hearing Loss'.

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