North Thames
NHS Genomic Medicine Service Alliance

Aminoglycosides Induced Hearing Loss Pharmacogenetic Test: m.1555A>G

Dharmisha Chauhan: Lead Genomic Medicine Pharmacist
Veronica Chorro-Mari: Lead Antimicrobial Pharmacist

April/May 2023 – Aminoglycosides Pharmacogenetics Webinar

Please acknowledge presenters if using slides for your own presentations – thank you

Aims and objectives

- Understanding how aminoglycosides work
- Toxicity and aminoglycoside
- Genetic cause of ototoxicity
- Current available genetics tests
- Data for point of care testing – The PALOH study

NHS
North Thames
NHS Genomic Medicine Service Alliance

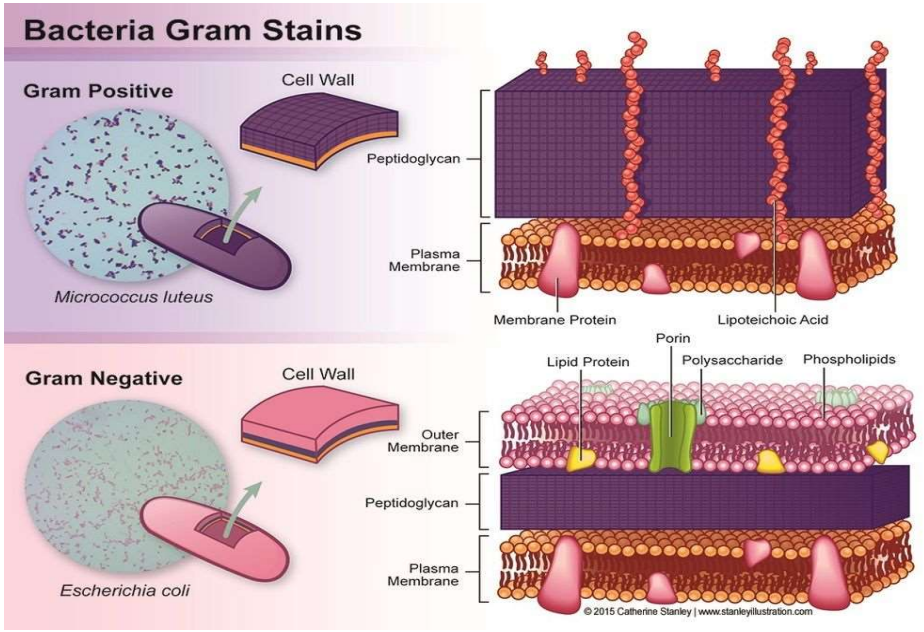
Aminoglycosides

Hosted by UCLPartners

Aminoglycosides
- *What are they?*

- Antibiotic to kill bacterial action.

- Aminoglycosides
  - Amikacin
  - Gentamicin
  - Neomycin
  - Streptomycin
  - Tobramycin

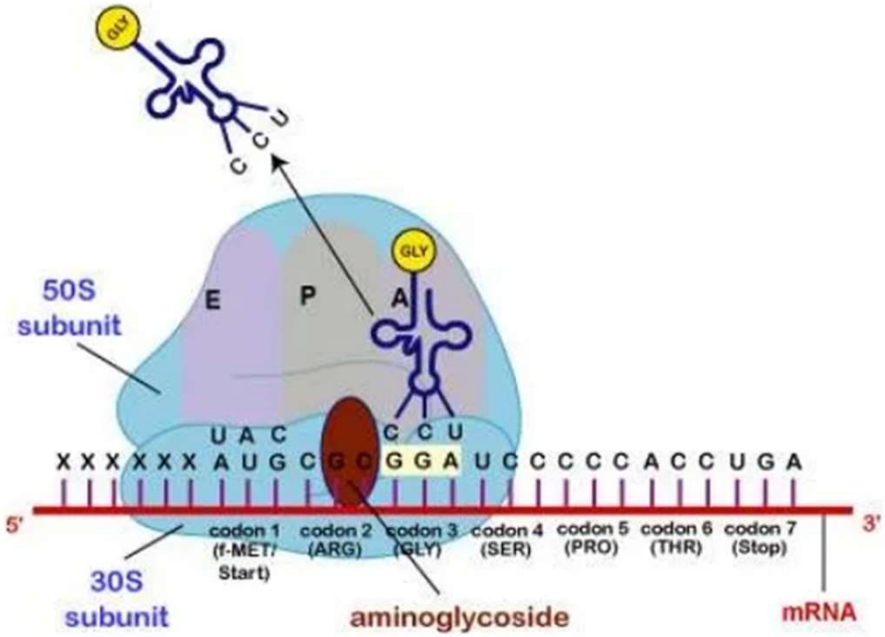


- Aminoglycosides are active against various Gram-positive and Gram-negative organisms

- Aminoglycosides widely used for treatment and prophylaxis against serious Gram-negative infections e.g., neonatal septicaemia.
  - Well known to be ototoxic – cochlea and the vestibular system (~25%)

Aminoglycosides
- *Mechanism of Action*

- Aminoglycosides **inhibit** translation of the mRNA by binding to the 30S subunit of the ribosome.
- The **irreversible binding** of the aminoglycosides to the 30S subunit of the ribosome causes the misreading of the codons along the mRNA.
- This **misreading of the codons** causes an error in the proofreading process of translation leading to improper protein expression leading to **bacterial cell death**.



Aminoglycosides
- *Side effects*

Nephrotoxicity (renal impairment)

- In most cases, aminoglycoside nephrotoxicity is reversible
- Acute Kidney Injury due to acute tubular necrosis is a relative common complication of aminoglycoside therapy
- Regular TDM and monitoring of renal function is needed
- Check drug interactions with other nephrotoxic agents (NSAIDs, furosemide, …)

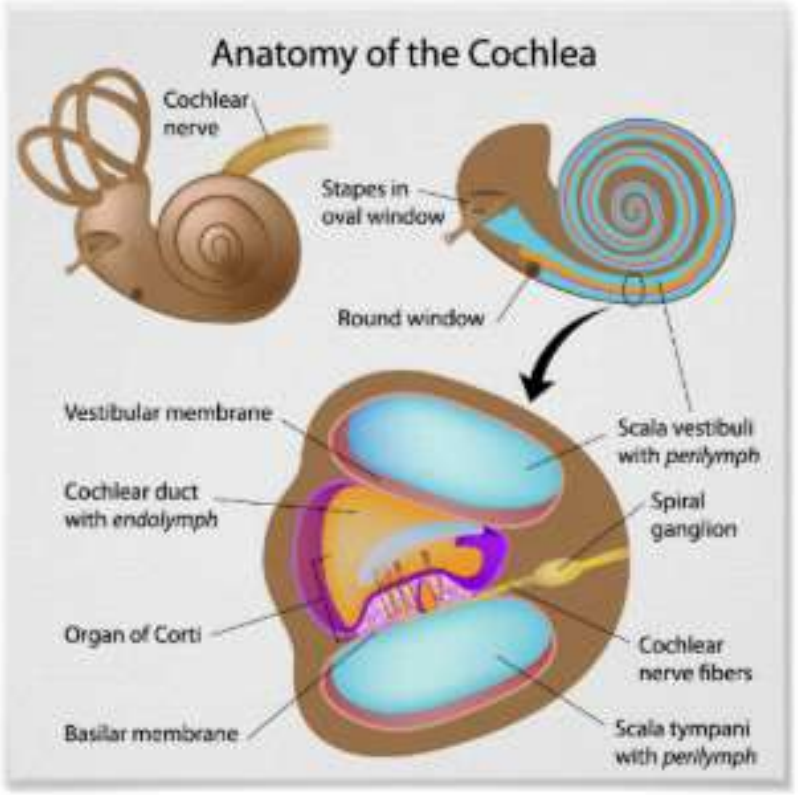
Ototoxicity (hearing impairment)

- Independent of drug concentration and suggested by any of the following:
    - New tinnitus
    - Dizziness
    - Poor balance
    - Hearing loss
    - Oscillating vision

- Toxicity is associated with prolonged use and is secondary to drug accumulation in the inner ear
- While ototoxicity can be transient in some cases, it can also be irreversible
- Regular audiometry monitoring is required if duration > 7days (check baseline audiology testing is possible)

Aminoglycoside Induced Ototoxicity (AIO)

- Most ototoxic substances are found in high concentrations in the **inner ear** and cause hearing loss by **damaging the cochlea**, in particular the **auditory hair cells and the stria vascularis.**



AIO: Potential mechanisms

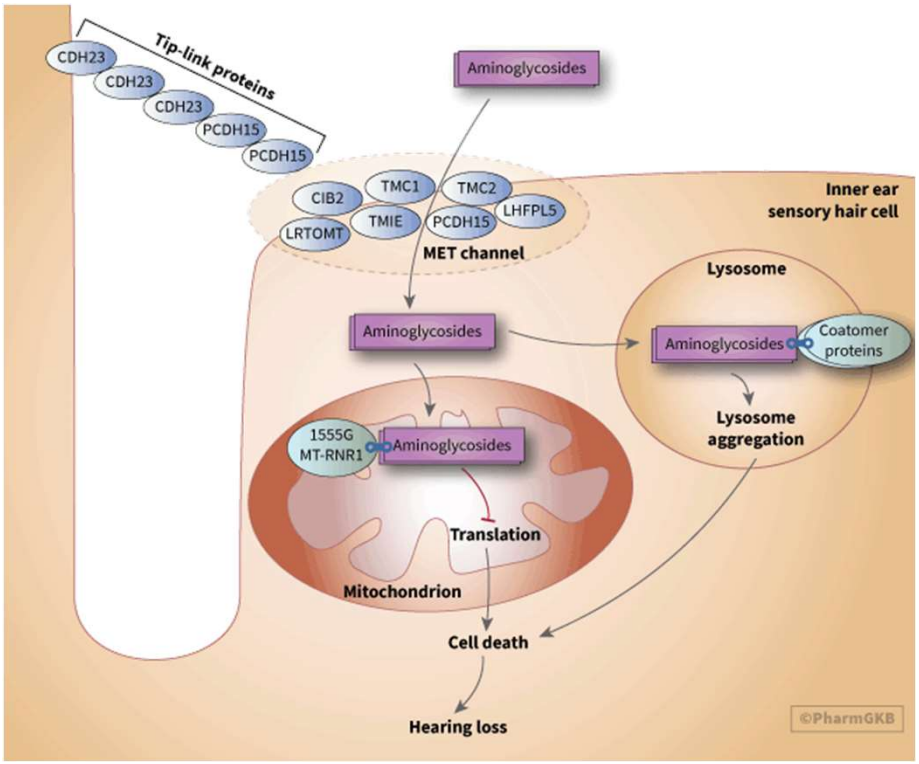
The mechanism of the *m.1555A>G* variant leading to greater toxicity has not been fully established.

One theory suggests that aminoglycosides which accumulate in high concentrations within the inner ear causes to damage to the cilia hair via:

1. Drug trafficking across endothelia and epithelial barrier

2. Aminoglycosides accumulating in lysosomes within hair cells leading to rupture leading to hair cell death

3. Aminoglycoside binding to alleles of the 12S mitochondrial ribosomal subunit, encoded by the gene *MT-RNR1* gene



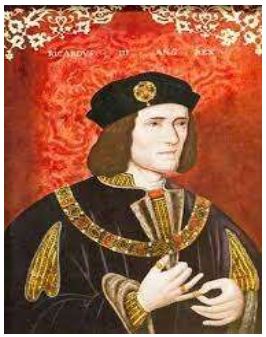
NHS
North Thames
NHS Genomic Medicine Service Alliance

Mitochondrial DNA mutation
m.1555A>G

Hosted by UCLPartners

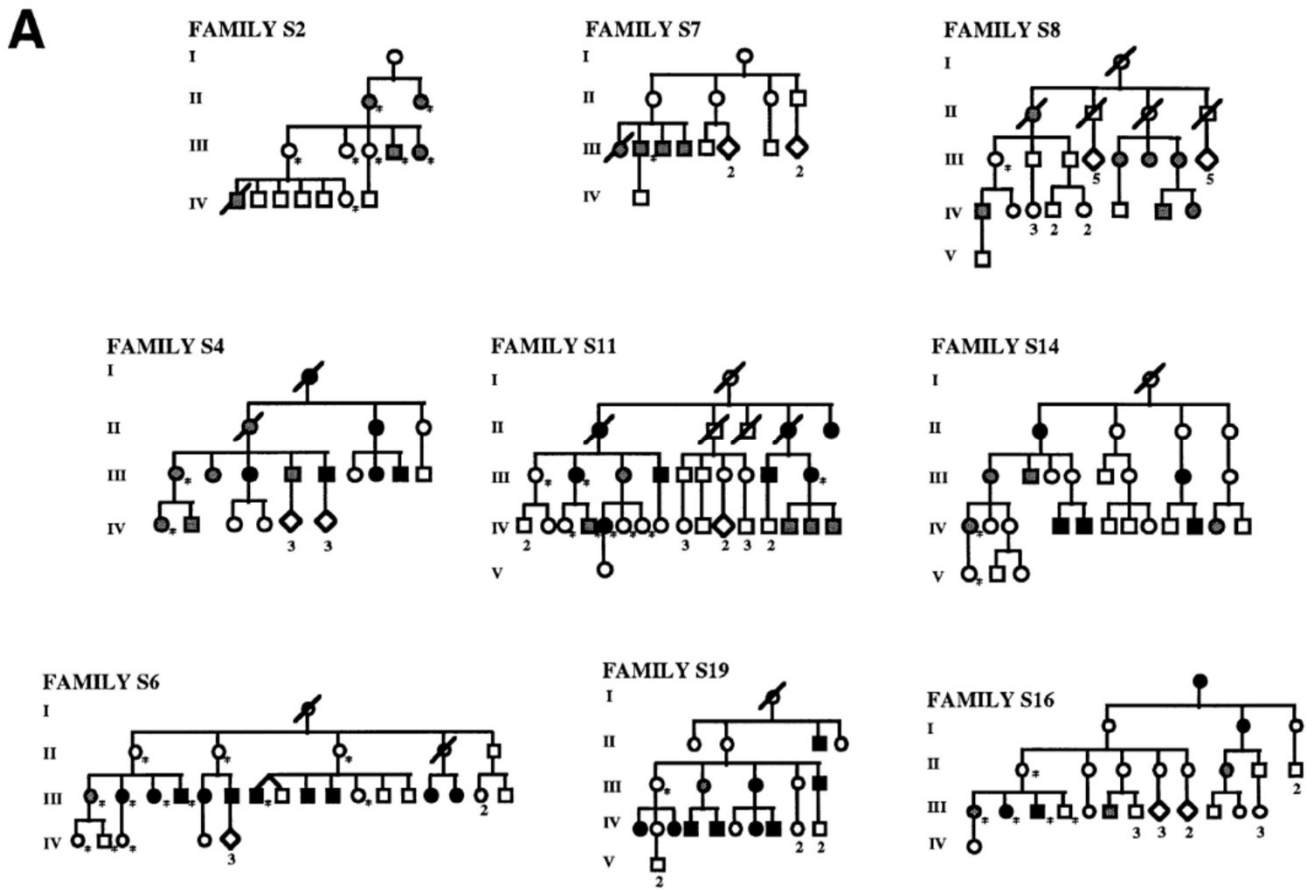
Mitochondrial DNA

- Mitochondrial DNA
- Originated from an aerobic prokaryotic cell engulfed by a anaerobic eukaryotic cell
- Explains why mitochondria have their own ribosomes
- **Mitochondria DNA inherited from mothers only**
- Mitochondria mutations inherited from mother directly to her children.



King, T., Fortes, G., Balaresque, P. *et al.* Identification of the remains of King Richard III. *Nat Commun* **5**, 5631 (2014). https://doi.org/10.1038/ncomms6631

AIO pattern discovered within families – maternal side



Estivill X, et al. Familial Progressive Sensorineural Deafness Is Mainly Due to the mtDNA A1555G Mutation and Is Enhanced by Treatment with Aminoglycosides. *The American Journal of Human Genetics*. 1998;62(1): 27–35. https://doi.org/10.1086/301676.

Inheritance of mitochrondrial DNA

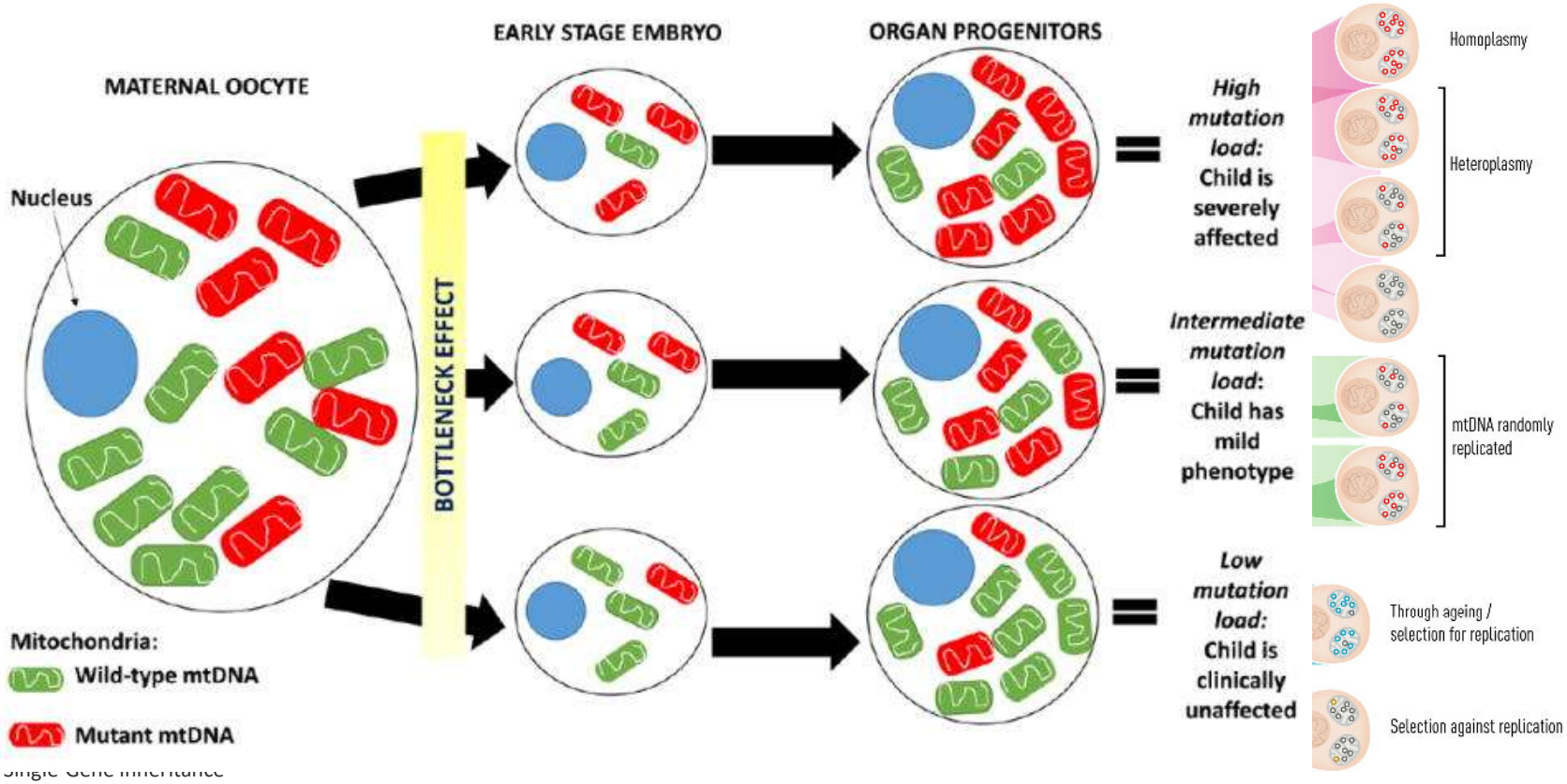
- a genomic consideration

A note of r
two or mor

In cell divis
the variant
into daugh
may conta
mutant mit

This can
mt1555A>(
hearing los

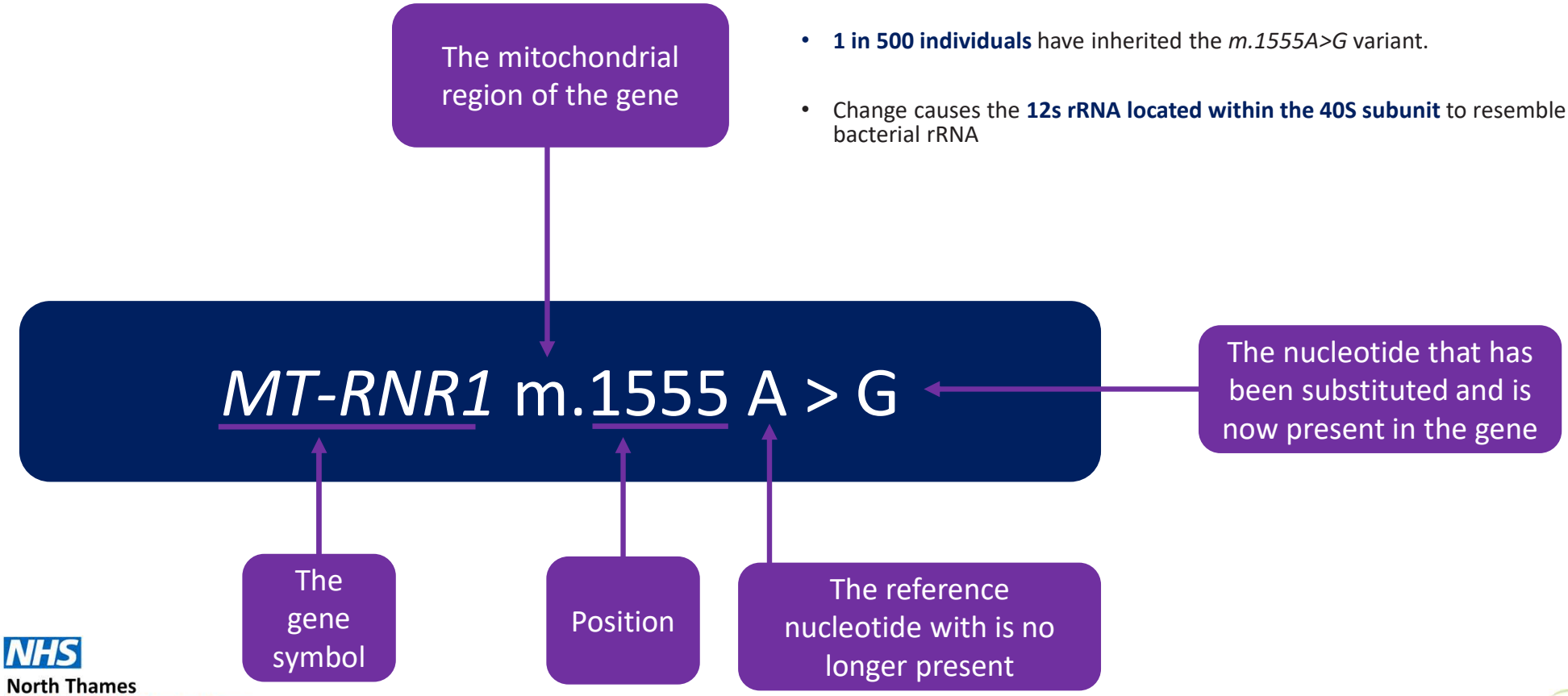
Environme



Patterns o
Robert L. Nussbaum MD, FACP, FACMG, in Thompson & Thompson Genetics in Medicine, 2016
Wei, W., & Chinnery, P. F. (2020). Inheritance of mitochondrial DNA in humans: implications for rare and common diseases. *Journal of internal medicine*, *287*(6), 634–644.
McCormick EM, Muraresku CC, Falk MJ. Mitochondrial Genomics: A complex field now coming of age. Curr Genet Med Rep. 2018 Jun;6(2):52-61

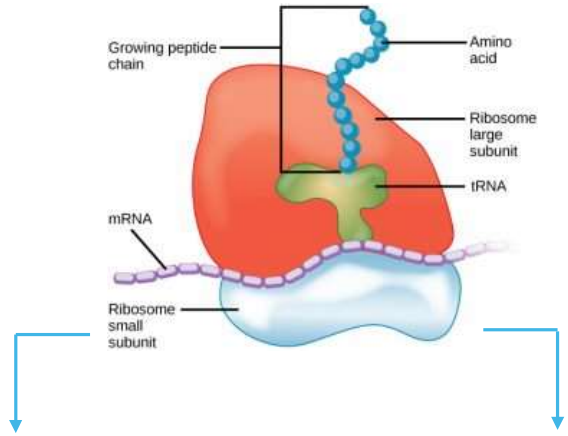
MT-RNR1 Gene

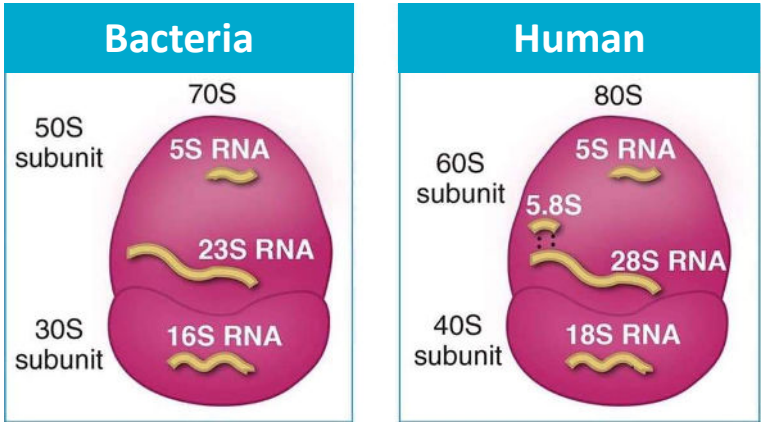
- Susceptibility to ototoxicity has been linked to ***MT-RNR1* gene.**
- Encoded within the **circular mitochondrial DNA = 37 genes** and is only inherited via the maternal germline.
- **1 in 500 individuals** have inherited the *m.1555A>G* variant.
- Change causes the **12s rRNA located within the 40S subunit** to resemble bacterial rRNA



Mutation in the mitochondrial 12s ribosome

- Aminoglycosides cause bactericidal effects through inhibiting protein synthesis to the **16s rRNA of the bacterial** 30s ribosome.
- The 12s rRNA in humans is encoded by ***MT-RNR1* gene** and is a homologue to bacterial 16s rRNA
- The ***m.1555A>G*** mutation in the mitochondrial 12s subunit pre-dispose individuals to aminoglycoside induced hearing loss as it now **resembles the bacterial 16s rRNA subunit** - allowing the aminoglycosides to bind more easily.
- Individuals who have the *m.1555A>G* mutation are exquisitely sensitive to rapid-onset hearing loss (bilateral) after receiving aminoglycosides **at normal therapeutic levels.**





7th Jan 2021: MHRA Alert

In 2020, we conducted a safety review following concerns received about the impact of mitochondrial mutations on the risk of ototoxicity with aminoglycosides. We identified several published **epidemiological studies** showing an increased risk of deafness in patients with the m.1555A>G mutation who were given aminoglycosides. There have also been reported **cases of deafness** in m.1555A>G patients with aminoglycoside use **within the recommended serum level**s. Some cases were associated with a **maternal history of deafness** or mitochondrial mutations or both.

The m.1555A>G mutation is the most common mitochondrial DNA (mtDNA) mutation, with an estimated **prevalence of 0.2% in the general population**. The mutation is associated with sensorineural deafness and occurs in families with maternally transmitted deafness.

Clinicians should follow local guidelines on mitochondrial mutation screening in patients with a maternal history of deafness or mitochondrial mutations or both and who require aminoglycoside therapy.

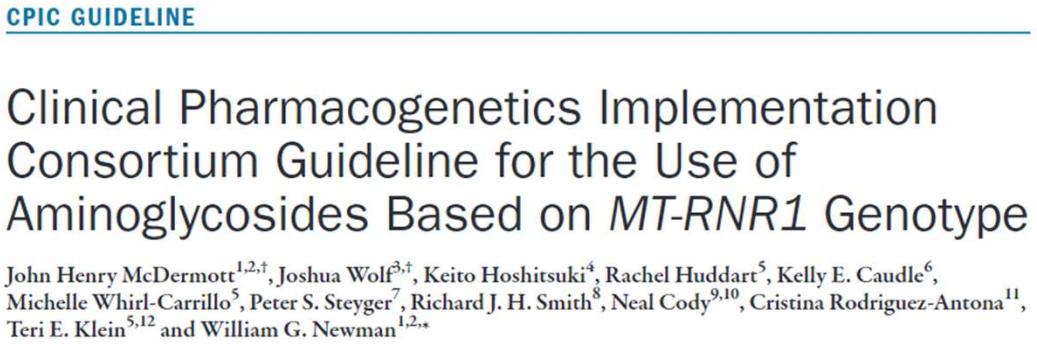
Genetic screening may be **especially appropriate in patients requiring recurrent or long-term aminoglycoside** therapy where the risk of ototoxicity is increased.

CPIC Guidelines

March 2021: Clinical Pharmacogenetics Implementation Consortium Guideline for the Use of Aminoglycosides Based on MT-RNR1 Genotype

CPIC is an international consortium of individual volunteers and a small dedicated staff who are interested in facilitating use of pharmacogenetic tests for patient care

CPIC®
Clinical Pharmacogenetics Implementation Consortium


CPIC GUIDELINE

Clinical Pharmacogenetics Implementation Consortium Guideline for the Use of Aminoglycosides Based on *MT-RNR1* Genotype

John Henry McDermott[1,2,†], Joshua Wolf[3,†], Keito Hoshitsuki[4], Rachel Huddart[5], Kelly E. Caudle[6], Michelle Whirl-Carrillo[5], Peter S. Steyger[7], Richard J. H. Smith[8], Neal Cody[9,10], Cristina Rodriguez-Antona[11], Teri E. Klein[5,12] and William G. Newman[1,2,*]

NHS
North Thames
NHS Genomic Medicine Service Alliance

Hosted by UCLPartners

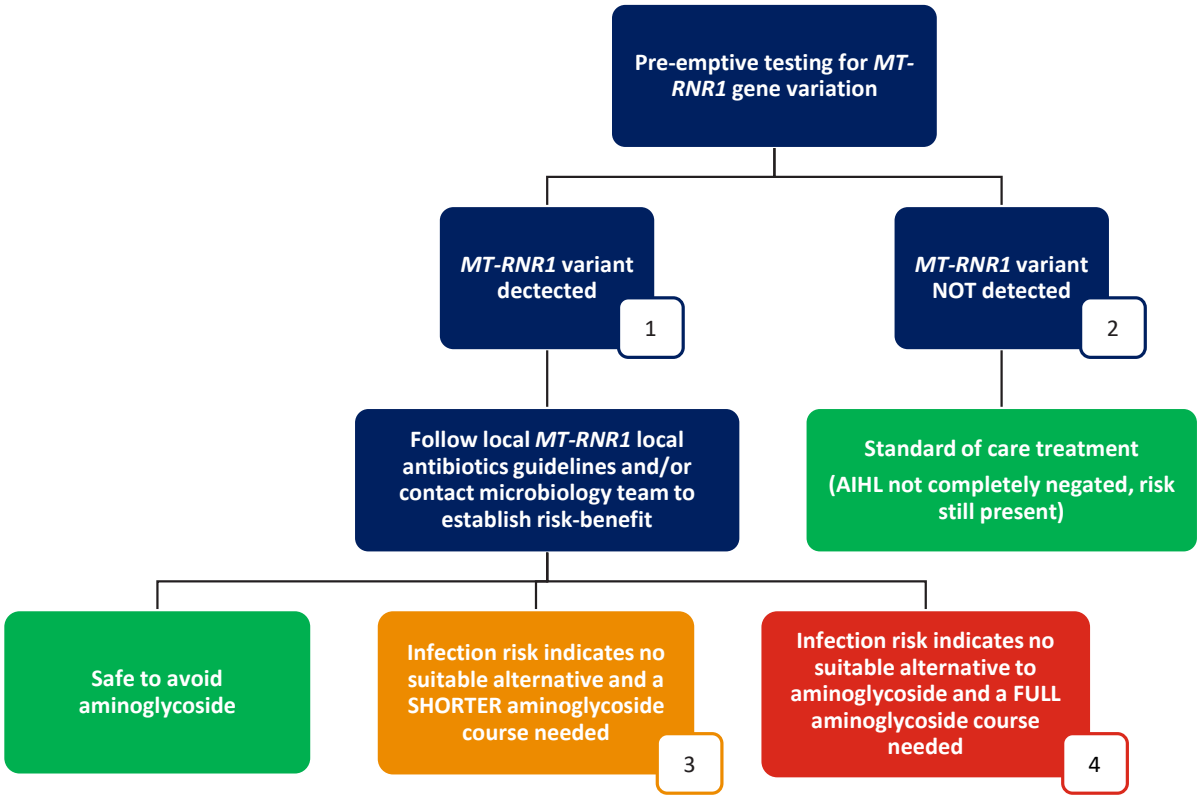
CPIC guideline – *quick summary*

1. If a high-risk MT-RNR1 variant has been detected the individual is at high risk of developing hearing loss due to aminoglycoside treatment. It is recommended that alternative antibiotic treatment should be prescribed. Risk benefit discussion.

2. If a MT-RNR1 variant has not been detected: this does not mean that there are no other pre-disposing risks for aminoglycosides induced hearing loss.

3. Check maternal history of hearing loss or vestibular problems. Consider prescribing oral N-acetylcysteine whilst on aminoglycoside, calculate dose of aminoglycoside according to weight, creatinine clearance and follow trust guidelines for therapeutic drug monitoring as part of standard safety monitoring.

4. As per point 3 above.



NHS
North Thames
NHS Genomic Medicine Service Alliance

NHS Commissioning of Genetic Tests

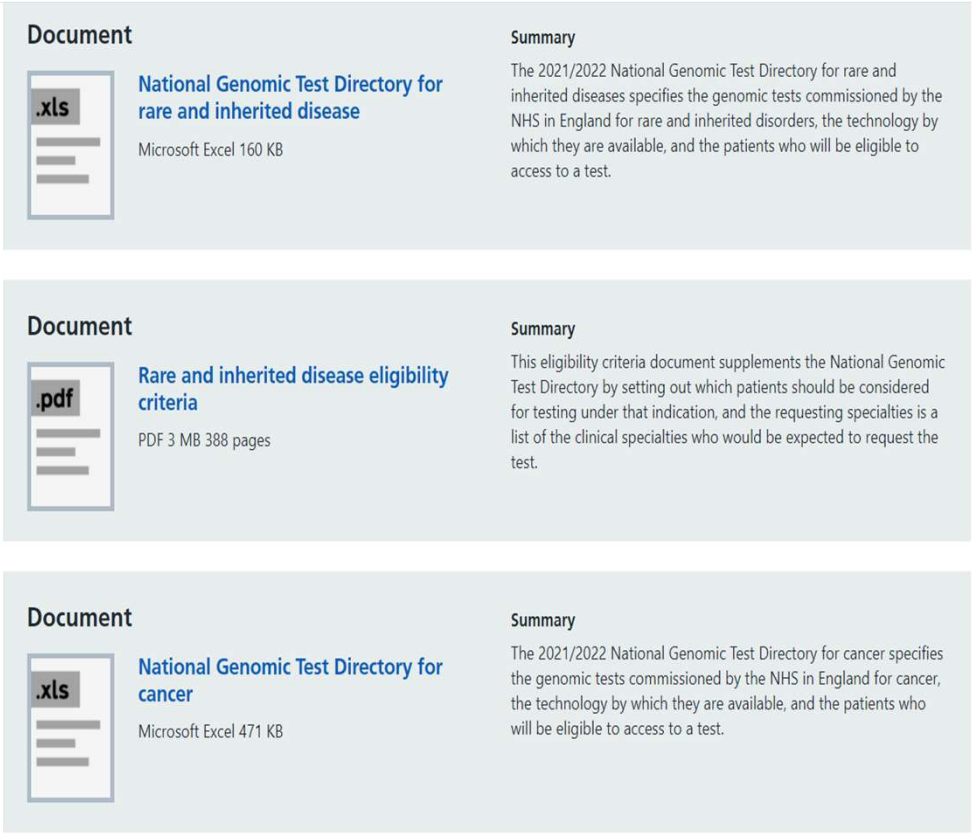
Hosted by UCLPartners

The National Genomic Test Directory

What is it?

Aims

- To ensure the directory reflects latest technological developments and scientific advances
- To support **fair and equitable** access to genomic testing
- To ensure **best value** is achieved for the NHS
- To improve our understanding of clinical utility of genomic tests and the **implications of testing** on patients and the **clinical pathway.**



m.1555A>G NTD Inclusion Criteria

Rare Diseases Test Directory

R65 Aminoglycoside exposure posing risk to hearing Testing Criteria

Significant exposure to aminoglycosides posing risk of ototoxicity. This indication would be relevant to:

1. individuals with a predisposition to gram negative infections for example due to known respiratory disease (e.g., bronchiectasis, cystic fibrosis) or due to structural or voiding genitourinary tract disorders,

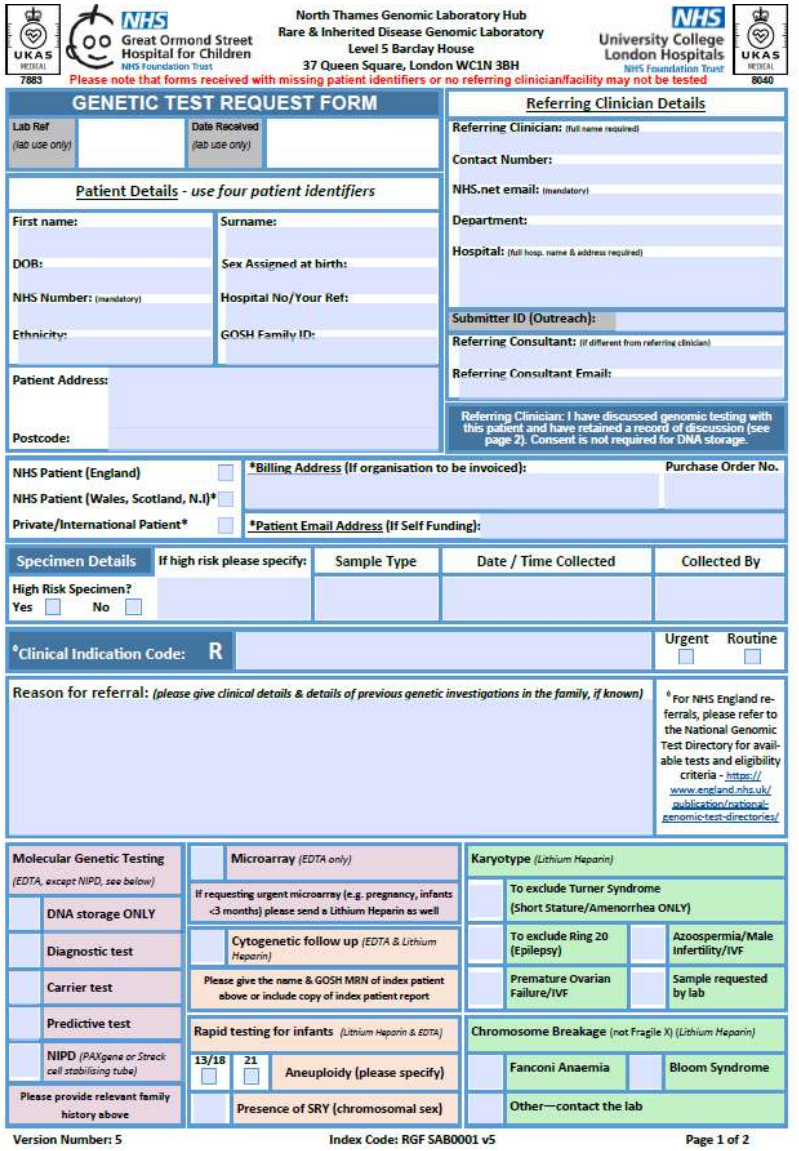
2. OR individuals with hearing loss who have been exposed to aminoglycosides

- *Any patient with family history (maternal side) would be eligible to test - referrals may come through via the audiologist pathway.*
- *Reflex testing upon confirmed diagnosis of cystic fibrosis – April 2023*

Genetic referral forms

- **North Thames:** Healthcare Professionals – North Thames Genomic Laboratory Hub (norththamesglh.nhs.uk)
- **South East:** Documents and forms – South East Genomics
- **South West**: SWGLH Requesting a genomic test for Rare Disease | North Bristol NHS Trust (nbt.nhs.uk)
- **East:** Test order forms index page | East Genomics
- **Central and South:** Core Genomic testing | Birmingham Women's and Children's (bwc.nhs.uk)
- **North East**: Genomic Testing - North East and Yorkshire Genomic Medicine Service North East and Yorkshire Genomic Medicine Service (ney-genomics.org.uk)
- **North West**: Documents and Forms - Manchester University NHS Foundation Trust (mft.nhs.uk)

Example from NTGMSA



North Thames Genomic Laboratory Hub
Rare & Inherited Disease Genomic Laboratory
Level 5 Barclay House
37 Queen Square, London WC1N 3BH

Great Ormond Street Hospital for Children NHS Foundation Trust
University College London Hospitals NHS Foundation Trust

UKAS MEDICAL 7883 | UKAS MEDICAL 8040

Please note that forms received with missing patient identifiers or no referring clinician/facility may not be tested

GENETIC TEST REQUEST FORM

Lab Ref (lab use only) | Date Received (lab use only)

Patient Details - *use four patient identifiers*

First name: | Surname:
DOB: | Sex Assigned at birth:
NHS Number: (mandatory) | Hospital No/Your Ref:
Ethnicity: | GOSH Family ID:
Patient Address:
Postcode:

Referring Clinician Details

Referring Clinician: (full name required)
Contact Number:
NHS.net email: (mandatory)
Department:
Hospital: (full hosp. name & address required)
Submitter ID (Outreach):
Referring Consultant: (if different from referring clinician)
Referring Consultant Email:

Referring Clinician: I have discussed genomic testing with this patient and have retained a record of discussion (see page 2). Consent is not required for DNA storage.

NHS Patient (England)
NHS Patient (Wales, Scotland, N.I)*
Private/International Patient*

*Billing Address (If organisation to be invoiced): | Purchase Order No.
*Patient Email Address (If Self Funding):

Specimen Details	If high risk please specify:	Sample Type	Date / Time Collected	Collected By
High Risk Specimen? Yes ☐ No ☐				

*Clinical Indication Code: R | Urgent ☐ Routine ☐

Reason for referral: *(please give clinical details & details of previous genetic investigations in the family, if known)*

*For NHS England referrals, please refer to the National Genomic Test Directory for available tests and eligibility criteria - https://www.england.nhs.uk/publication/national-genomic-test-directories/

Molecular Genetic Testing *(EDTA, except NIPD, see below)*
- ☐ DNA storage ONLY
- ☐ Diagnostic test
- ☐ Carrier test
- ☐ Predictive test
- ☐ NIPD *(PAXgene or Streck cell stabilising tube)*

Please provide relevant family history above

- ☐ Microarray *(EDTA only)*

If requesting urgent microarray (e.g. pregnancy, infants <3 months) please send a Lithium Heparin as well

- ☐ Cytogenetic follow up *(EDTA & Lithium Heparin)*

Please give the name & GOSH MRN of index patient above or include copy of index patient report

Rapid testing for infants *(Lithium Heparin & EDTA)*
- 13/18 ☐ 21 ☐ Aneuploidy (please specify)
- ☐ Presence of SRY (chromosomal sex)

Karyotype *(Lithium Heparin)*
- ☐ To exclude Turner Syndrome (Short Stature/Amenorrhea ONLY)
- ☐ To exclude Ring 20 (Epilepsy)
- ☐ Azoospermia/Male Infertility/IVF
- ☐ Premature Ovarian Failure/IVF
- ☐ Sample requested by lab

Chromosome Breakage (not Fragile X) *(Lithium Heparin)*
- ☐ Fanconi Anaemia
- ☐ Bloom Syndrome
- ☐ Other—contact the lab

Version Number: 5 | Index Code: RGF SAB0001 v5 | Page 1 of 2

NHS
North Thames
NHS Genomic Medicine Service Alliance

Considerations

- *On discussion with the GLH*

- Turnaround time is variable within regions 10 days, can be to up to 6 weeks in some regions.
- For individuals in which prolonged/recurrent aminoglycosides prescribed.
- If adult who has been treated with an aminoglycoside and no hearing loss = do not test.
- Adults where suspected hearing could be due to aminoglycoside, ***preferable*** to refer to audiologist first before requesting test.
- Established cystic fibrosis and bronchiectasis patients with no audiology problems, no need to test.

NHS
North Thames
NHS Genomic Medicine Service Alliance

Point of Care Testing
(NOT an NHS commissioned test)

Hosted by UCLPartners

POC – bedside test

Pilot Study

News

NHS develops world-first bedside genetic test to prevent babies going deaf

2 April 2022

Children and young people | Genomics

A world-first genetic test that could save the hearing of hundreds of babies each year has been developed and successfully piloted in the NHS.

Taking just 25 minutes, the bedside machine identifies whether a critically ill baby admitted to intensive care has a gene that could result in permanent hearing loss if they are treated with a common emergency antibiotic.

The new swab test technique would replace a test that traditionally took several days and could save the hearing of 180 babies in England alone every year.

People admitted to intensive care are usually given an antibiotic called Gentamicin within 60 minutes. While Gentamicin is used to safely treat about 100,000 babies a year, one in 500 babies carry the gene that can make it cause permanent hearing loss.

Developed in Manchester, the new test means that babies found to have the genetic variant can be given an alternative antibiotic within the 'golden hour.'

It is expected the test could save the NHS £5 million every year by reducing the need for other interventions, such as cochlear implants.

The simple genetic test for newborns that can prevent profound deafness and save the NHS millions every year

Genedrive IS the world's first genetic bedside test to be used in an emergency setting

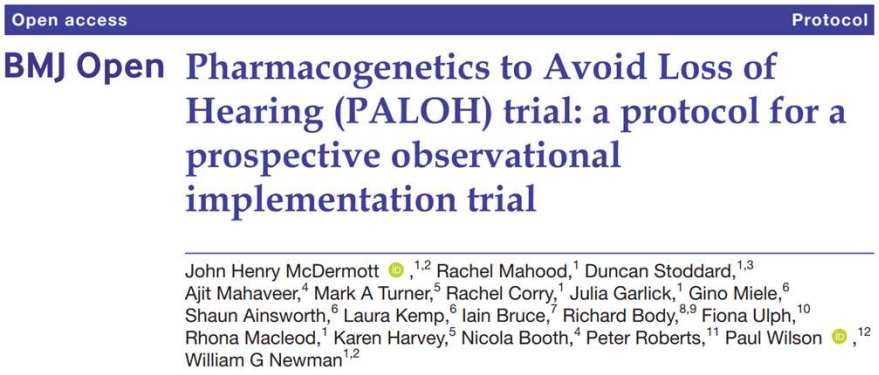
Pharmacogenetics to Avoid Loss of Hearing (PALOH) Study

Prospective observational implementation trial based on

- Ototoxicity after aminoglycoside prevalence of **1 in 500**
- Unacceptable delay in the acute setting in current genetic testing for the m.1555A>G variant

Methods

- A healthcare professional performs a buccal swab in the baby's inner cheek whilst doing routine care and places the sample in a collection tube containing lysis buffer.
- This system was integrated into the clinical pathways at two large UK **neonatal centers** – Manchester and Liverpool

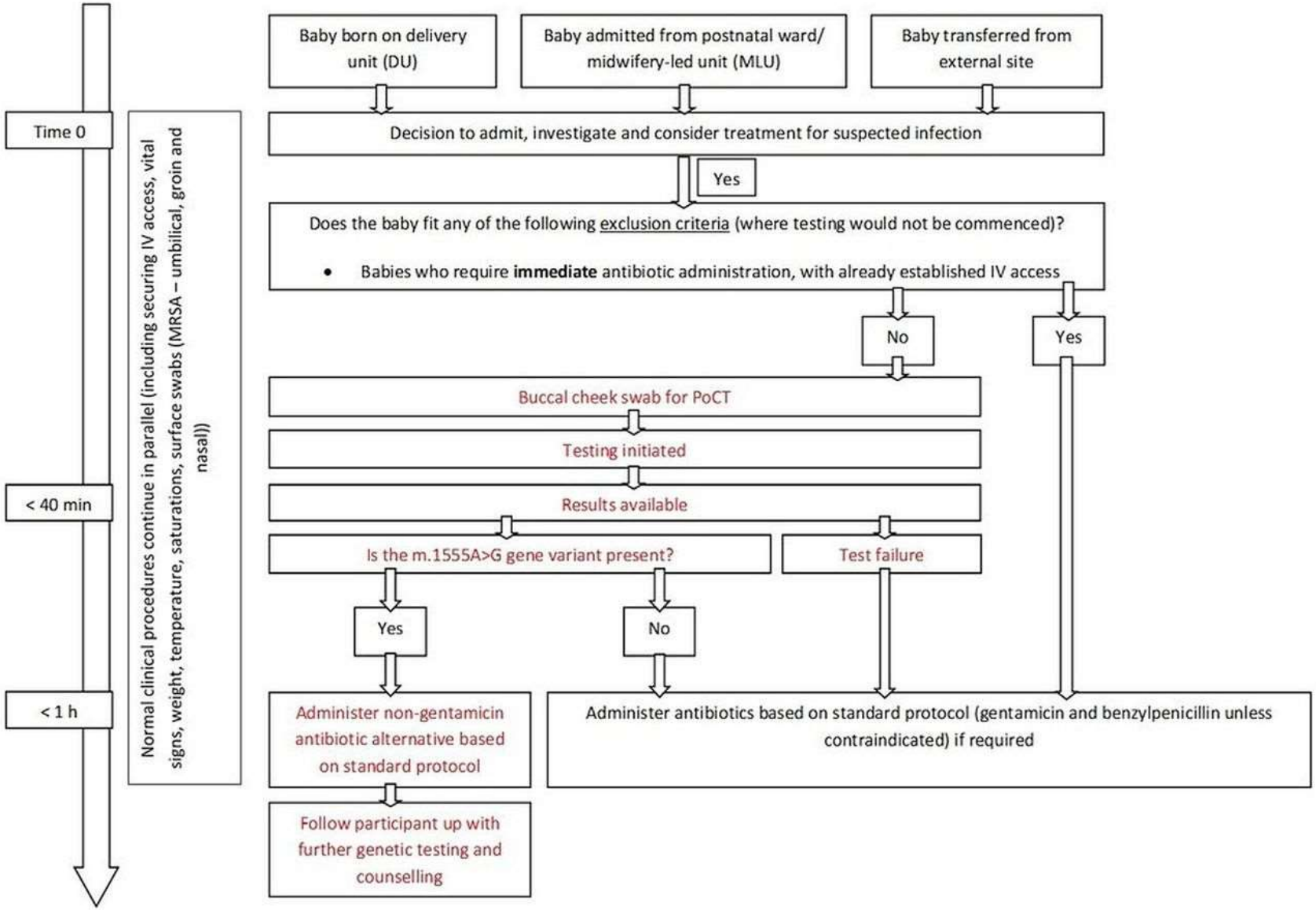

Open access Protocol

BMJ Open **Pharmacogenetics to Avoid Loss of Hearing (PALOH) trial: a protocol for a prospective observational implementation trial**

John Henry McDermott ,[1,2] Rachel Mahood,[1] Duncan Stoddard,[1,3] Ajit Mahaveer,[4] Mark A Turner,[5] Rachel Corry,[1] Julia Garlick,[1] Gino Miele,[6] Shaun Ainsworth,[6] Laura Kemp,[6] Iain Bruce,[7] Richard Body,[8,9] Fiona Ulph,[10] Rhona Macleod,[1] Karen Harvey,[5] Nicola Booth,[4] Peter Roberts,[11] Paul Wilson ,[12] William G Newman[1,2]

NHS
North Thames
NHS Genomic Medicine Service Alliance

Hosted by UCLPartners

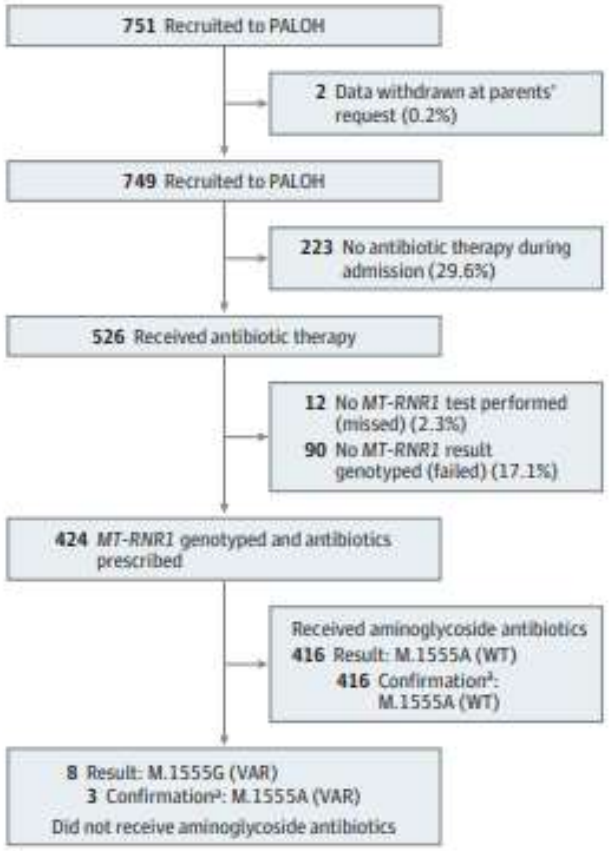


NHS
North Thames
NHS Genomic Medicin

ed by UCLPartners

PALOH Study- Results

Figure 1. Study Recruitment and Testing Metrics



Range age: 2.5 days old (0-198 days)
Mean GD: 37 weeks

Three babies with the m.1555A>G variant were identified and confirmed by Sanger sequencing-→ **0.4% prevalence vs 0.2%**

There were 5 false-positive results. There were no false-negative results → **the assay had a real-world analytical sensitivity of 100%, a specificity of 99.2% and an accuracy of 99.2%.**

The GeneDrive POCT can detect the m.1555A>G variant in **26 min from buccal swab**

PALOH Study- Limitations

- Initially the recruitment was set up for 2 NICUs, but one large hospital was the main recruiter due
- These were hospitals regularly exposed to new technologies implementation and this facilitated delivery of the trial
- Information about ancestry or gender for prevalence considerations not identified although different ethnics groups included for testing
- Variation of antibiotic practices
- The utility and cost effectiveness of m.1555A>G testing will be context dependent
- Consideration: POC policy is normally responsibility of lab/micro department

NICE Early Values Assessment *(NB: not a TA or HST)*

1 Recommendations

1.1 The Genedrive MT-RNR1 ID Kit can be used ***while further evidence is generated as an option*** for detecting the genetic variant m.1555A>G to guide antibiotic (aminoglycoside) use and prevent hearing loss in newborns who are being considered for treatment with aminoglycosides.

1.2 Healthcare professionals should tell parents about the possible implications of positive test results for their baby and their family at an appropriate time, and give support and information.

1.3 ***Positive results should be confirmed by laboratory testing***.

1.4 ***The recommendation is conditional on further evidence*** (see the section on evidence generation recommendations) being generated on:

- how the test affects time to antibiotics
- how the test result affects antibiotic prescribing decisions
- the technical performance and accuracy of the test.

NICE: Genedrive MT-RNR1 ID Kit for detecting a genetic variant to guide antibiotic use and prevent hearing loss in babies: early value assessment

- The Genedrive System screen shows the results.
  - Up to 1,000 results can be stored in the system and data can be transferred using Wi-Fi and LAN connections if needed.
- Operating conditions of 16°C to 30°C.
  - The MT-RNR1 test kits can be stored at temperatures of between 2°C and 30°C
- **Key uncertainties** around the evidence or technology are a lack of published studies assessing Genedrive MT-RNR1 ID test and its comparison to current clinical practice.
  - *One expert said it could be complex to implement the test and recommended a formal economic analysis.*
- Genedrive MT-RNR1 ID kit costs around £100. The cost per test used in the economic model (incorporating additional cost components such as purchase of the Genedrive machine, printing costs, control tests, warranty and staff costs) was £130.08.

Can genomics reduce cochlear implants requests?

- Currently around 1,260 people per annum in England have cochlear implants.
- The total number of people treated is estimated to increase to 2,790 by 2023/24.
- Of the 32,654 deaf school children in England, 2184 had implants, 7% of the total (CRIDE data).
- The cost of a cochlear implant surgery is estimated £60K per patient

1 (nice.org.uk) Cochlear implants and deaf pupils – BATODANNUAL-REPORT-2018-2019-Final.pdf (mft.nhs.uk)

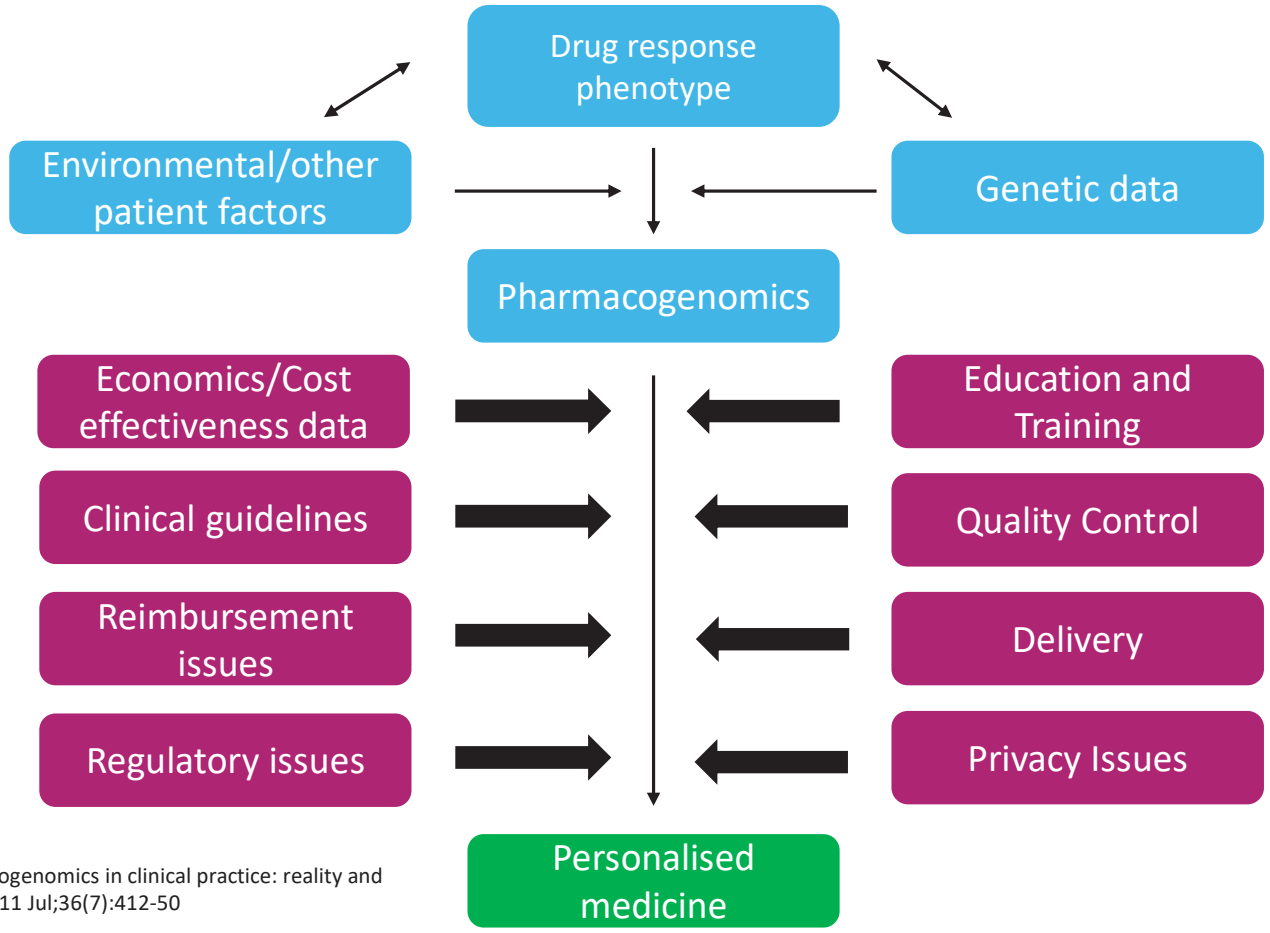
Picture source: University of Illinois Hospital

NHS
North Thames
NHS Genomic Medicine Service Alliance

Considerations for Aminoglycoside PGx testing

Hosted by UCLPartners

Key considerations when thinking about genomic medicine



Ventola CL. Pharmacogenomics in clinical practice: reality and expectations. P T. 2011 Jul;36(7):412-50

GMS Alliance and Key Considerations

- **Turnaround time** and the need to treat with antibiotics as POC test not eligible in all populations.
- **Other variants reported**; *m.1095T>C* and *m.1494C>T* – also associated with AIHL
- **Guidelines** – National approach required
- **Education and Training**: Family history, genetic counselling
- **DTCs** – Do they know how to reviewing the technology vs costs and clinical evidence? Validity and reliability?
- **Interoperability and confidentiality**– where would the test be stored? How to other NHS/private healthcare centres know an individual has been tested?
- **Population tested** e.g., ancestry, is data inclusion to the region we serve?
- **Research – prospective data collection, especially with novel technology**

Embedding Pharmacy Expertise

National Pharmacy Genomics team embedded in NHSE Genomics Unit and linked to Office of the Chief Pharmaceutical Officer

GMS Alliance Pharmacy Leads
Four pillars of practice – clinical practice, leadership, education and research

NHS North West GMSA
Jessica Keen
Jessica.Keen@mft.nhs.uk

NHS North East and Yorkshire GMSA
Emma Groves
emma.groves3@nhs.net

NHS Central and South GMSA
Hayley Wickens
hayley.wickens1@nhs.net

NHS East GMSA
Paul Selby
paul.selby@nhs.net

NHS South West GMSA
Rachel Palmer
rachel.palmer6@nhs.net

NHS North Thames GMSA
Dharmisha Chauhan
dharmisha.chauhan1@nhs.net

NHS South East GMSA
Nisha Shaunak nisha.shaunak@gstt.nhs.uk &
Lucy Galloway lucy.galloway@stgeorges.nhs.uk

NHS
North Thames
NHS Genomic Medicine Service Alliance

Thank You.

Dharmisha.Chauhan1@nhs.net
Veronica.Chorro-Mari@nhs.net