North Thames
NHS Genomic Medicine Service Alliance

hosted by Great Ormond Street Hospital for Children NHS Foundation Trust

Best Practice Recommendations for *MT-RNR1* Testing

Pharmacogenetic Testing for Aminoglycosides Induced Ototoxicity

Version 5.0

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This document is intended for healthcare professionals/ clinical teams who prescribe, administer, screen or dispense aminoglycosides or who look after patients exposed or potentially exposed to aminoglycosides. It details recommendations for *MT-RNR1* implementation, however individual organisations will be responsible to determine the necessary governance and best implementation model for their organisation/clinical cohort. **To jump to frequently asked questions, please click here.**

Contents

Background

Aminoglycosides (amikacin, gentamicin, tobramycin, neomycin, and streptomycin) are antibiotics known to cause ototoxic adverse events, such as hearing loss and vestibular problems. Evidence suggests that carriers of clinically *MT-RNR1* mitochondrial variants linked to increased risk of aminoglycoside hearing loss such as m.1555A>G are at higher risk of aminoglycoside-induced ototoxicity, even within normal therapeutic levels.

In 2018, m.1555A>G was commissioned and placed on the National Test Directory (NTD), listed as R65 test within the rare and inherited diseases directory. The R65 NTD eligibility criteria was updated in 2022 and currently it is available for patients predisposed to risk of gram-negative infection due to other conditions (e.g., cystic fibrosis, bronchiectasis, structural or voiding genitourinary tract disorders), and to those who have experienced hearing loss following exposure to aminoglycosides and if they are found to carry this variant – to their family members.

The aim of this document is to provide best practice recommendations to support healthcare professionals who are planning to implement R65 testing within their clinical pathways. This document has information specific to the North Thames Region.

Commissioning criteria

The NTD specifies which genomic tests are commissioned by NHS England, the genomic technology used and the patient eligibility criteria. The eligibility commissioning criteria are reviewed regularly. The current NTD, accessed in September 2023, has the R65 test for ototoxicity under rare and inherited diseases. Please use this link to find the most up to date eligibility criteria for R65 NHS England » National genomic test directory.

Most of the evidence available regarding the *MT-RNR1* variants are based on studies conducted predominantly in white Caucasian populations; the frequency of different variants and their related phenotypes will vary between different ethnic groups, and this must be considered when prescribing treatment or considering toxicity. At the time of publishing this guideline, the authors are aware that a request for other variants to be tested in the same panel has been submitted to the NTD.

Implementing a new R65 pathway

When implementing the R65 test for the first time or if you are expanding to a new clinical cohort, please contact the North Thames Genomic Medicine Service Alliance (NTGMSA) dharmisha.chauhan1@nhs.net and the North Thames Genomic Laboratory Hub (NTGLH) norththamesglh@nhs.net. NTGLH can provide guidance on the current testing capacity and turnaround times. The NTGLH and NTGMSA can provide education and training, and any further pathway implementation advice if needed.

Testing Pathway

The North Thames GLH for R65 testing is based at Great Ormond Street Hospital. The test is a blood test, and a genetic test request form is required. The form and instructions for North Thames can be found in this link: genetic_test_request_form_v5.pdf (norththamesglh.nhs.uk). Clearly state that you are requesting the R65 test.

Currently the R65 test is for one variant, m.1555A>G but in the future, it may include other variants as new evidence emerges and subsequently considered for national implementation. Please check the most up to date NTD, NHS England » National genomic test directory.

Genetic testing for other genetic forms of hearing loss is available separately via the R67 monogenic hearing loss panel. R67 panel testing does not include *MT-RNR1* gene testing due to inaccuracy of mitochondrial

targets on a DNA gene panel. If R67 gene panel testing is indicated, please discuss with audiology or clinical genetics for advice before requesting.

Turnaround time (TAT)

It is important to keep in mind the TAT of this genetic test. Whilst the GLH is processing the requests as soon as possible, the report may only be visible in your Trust within 5 to 10 working days of the request. This TAT is from the time the blood is drawn from the patient to when the result is available to the requesting clinician.

Example reports from NT GLH

Two R65 example reports can be found within the Appendix 1 for variant detected or variant not detected.

Consent considerations and information to patients/parents

Verbal consent for genetic testing should be acceptable and must be documented within the patient's clinical record and when the conversation with the parent/patient occurred. If a signed paper consent form is used, this should be uploaded in the electronic records (if these are the main records). Parent/patient should be advised that despite this being a genetic test, the aim is to provide an extra step of safety when prescribing aminoglycosides. Additionally, general practitioners and parents/patients must be informed that a negative result would not exclude the possibility of ototoxicity as aminoglycosides can cause ototoxicity via other pathways or due to variants present which are not currently tested on the NHS.

Communication pathway to other healthcare settings

Once the patient has been tested the result must be made available for all healthcare professionals to access and it must be easily accessible to view. The results, whether positive or negative, must be documented and relayed to the patient's general practitioner and to other clinical units where the patient receives regular care. You can also consider having a section in your electronic prescribing or system for laboratory results for 'pharmacogenomics'. If this is not possible or cannot be set up, though not an allergy, it may be worth adding the result to the allergy/intolerance status. Whichever method you use for recording; it must also ensure that a duplicate test is not requested, as patients only require this test to be carried out once.

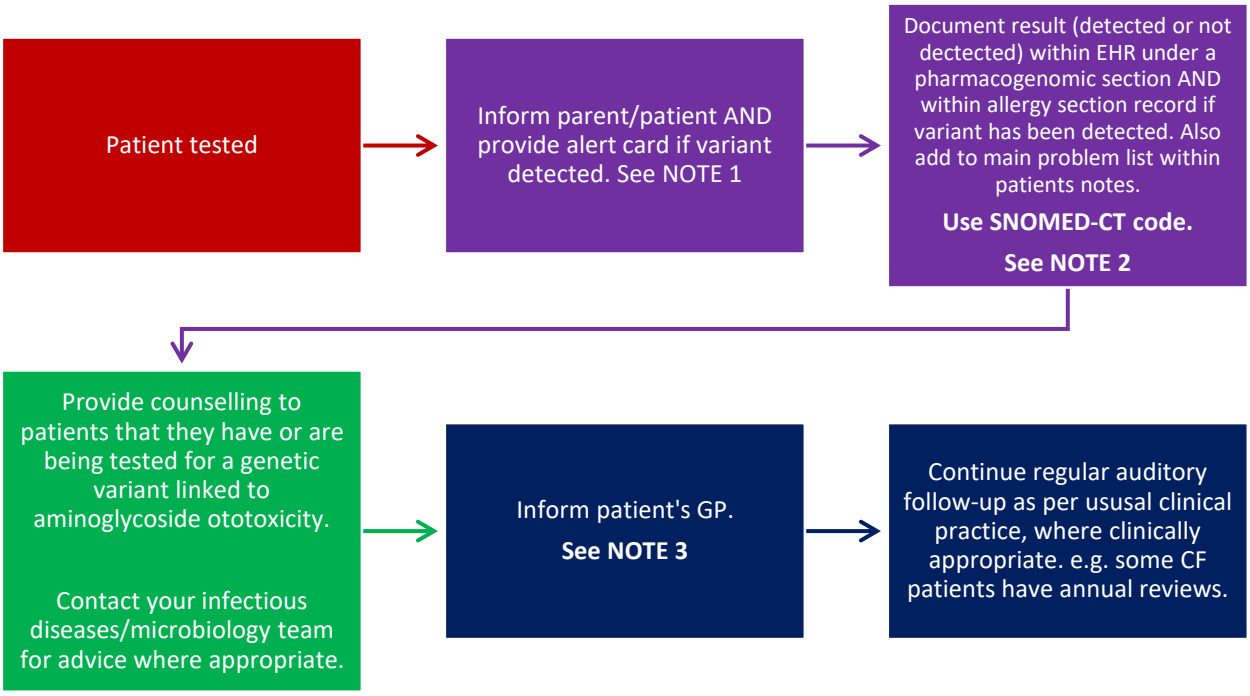

Figure 2. Communication pathway for sharing patient results, including clinical consideration (green box).

Notes for figure 2:

1. Alert card should explain that the patient has been identified to carry the *MT-RNR1* variant and aminoglycosides should be avoided where clinically possible. This card can be presented if needed e.g., ED departments.
2. SNOMED-CT codes for R65 test are available for m.1555A>G variant only and can be searched as:
   - Mitochondrial 1555 A to G mutation OR Mitochondrial 1555A>G
   - **Using the SNOMED-CT codes allows this information to be transferred to the patient's summary care records.** Please note SNOMED-CT codes can be inputted as positive or negative finding. If the patient has a negative finding, **this must also be recorded**, and the parent/patient informed.
3. Inform GP that patient has had R65 testing and provide the result, even if it is a negative finding. A suggested wording for the letter to the GP could read: *"MT-RNR1 testing shows no evidence of the m.1555A>G variant in .... This means ... is unlikely to have an increased risk to develop hearing loss due to m.1555A>G aminoglycoside toxicity. We are currently unable to test for other such variants".*

Clinical considerations

- In the rare event that an aminoglycoside is needed in the presence of an *MT-RNR1* variant, the clinical reasoning must be clearly documented and discussed with the patient/parent and with your infectious disease/microbiology team. Guidance on this clinical scenario can be found within clinical Pharmacogenetics Implementation Consortium (CPIC) guideline, McDermott et al (2021) CPIC® Guideline for Aminoglycosides and MT-RNR1 – CPIC (cpicpgx.org) (1).
- A negative *MT-RNR1* result would not eliminate the risk of aminoglycoside induced hearing loss. This is because there are other, more common, mechanisms in which aminoglycosides can cause hearing loss. Other risk factors include prematurity, renal impairment, severe inflammatory response syndrome, prolonged exposure, and high plasma concentrations.
- If an individual with a positive *MT-RNR1* results has previously received aminoglycosides and not developed hearing loss, this does not exclude them from developing it with subsequent doses.
- Prior to *MT-RNR1* testing, within certain clinical scenarios where long term/recurrent aminoglycoside treatment is needed, some clinicians opted to co-administer N-acetylcysteine (NAC) with aminoglycosides. NAC acts as an otoprotective agent and clinicians may want to consider the utility of NAC within an *MT-RNR1* testing pathway e.g., delay in receiving a result. The use of NAC is an unlicensed indication and would require local approval.
- It should be noted that vaccines contain trace amounts of aminoglycosides, however it is recommended that an individual with an *MT-RNR1* variant detected, should receive their vaccine schedule as normal. Full details on this can be found within this paper by McDermott et al (2023) MT-RNR1 Genotype Should Not Affect Childhood Vaccination—Unintended Consequences of Guidelines | Genetics and Genomics | JAMA Pediatrics | JAMA Network (2).
- In addition to consent and pre-test *MT-RNR1* counselling, you may also need to consider post-test counselling and testing, especially for maternal relatives and siblings. A paper by Rigobello et al (2023), discusses key questions and pathways to consider after a positive *MT-RNR1* variant has been detected (3). **Please contact your local clinical genetics team and the North Thames GLH team who will be able to provide guidance, if you decide that additional testing for family members is required within your pathway.**

Frequently Asked Questions

How do I start developing an MT-RNR1 testing pathway for my patient cohort?

1. When implementing R65 read through the CPIC® Guideline for Aminoglycosides and MT-RNR1 – CPIC (cpicpgx.org) and ensure local guidelines reflect the evidence presented within the CPIC guidelines, where clinically appropriate. You may wish to conduct your own literature search, but this is a good place to start.

2. Engage with key stakeholders, for example:
    - Lead pharmacists for the clinical area the test is being considered in e.g., paediatric pharmacist.
    - Chief Pharmacist
    - Antimicrobial pharmacist
    - Antimicrobial consultant /Medical Microbiologist
    - Laboratory lead manager
    - Pathologists
    - Audiologists
    - DTC team members and/or other governance leads within your institution.

This list is not exhaustive and based on the patient cohort being tested, additional clinical and non-clinical team members may need to be involved. **It is advised that for an efficient end to end testing pathway, member(s) of the laboratory team need to be present.** Your laboratory team member will also be able to liaise with the North Thames Genomic Laboratory Hub team members.

*****Before implementation you must contact the North Thames GLH** norththamesglh@nhs.net **to assess testing capacity and update to testing turnaround times*****

3. Set out your ideal clinical pathway based on the clinical cohort you would like to test.
4. Ensure the relevant safety documents and SOPs are in place as per your local governance measures e.g., patient alert cards, patient information leaflets, documentation of results within the patient electronic records, informing GPs and other external healthcare providers, and consideration of audiology referrals where clinically appropriate.
5. As best practice, the new R65 testing pathway should be presented to your drugs and therapeutics committee and other clinical governance committees/leads as per your local clinical practice service approval pathways.

What are the Expected / Recommended roles and responsibilities of healthcare professionals with the pathway?

The table below provides the expected roles and responsibilities for healthcare professionals within the pathway. The list is not exhaustive, and you may wish to change who will be responsible for each part of the pathway based upon the clinical cohort you are testing. Please use this as a starting point. If you have any further questions, please contact the GMSA team.

Consultants	• Establish what pathway will be adopted for a clinical unit(s). • Consider new variants may be identified in the future and they may be commissioned for testing. • Identified patients with a detected variant, must be discussed with the MDT for awareness and incorporate this information on the main problems list for future letters, as an allergy/intolerance, GP and patient/carer.

	• Ensure antimicrobial guidelines contain alternative antibiotics and liaise with antimicrobial team members.

Registrars/FY1/FY2 doctors, other prescribers	• Ensure no aminoglycoside will be prescribed without checking allergy/intolerance section and/or consulting patient/carer if aware of any maternal history of deafness.
	• To check with patient / patient record if patient has been on aminoglycosides previously.
	  ○ If so and ototoxicity problems identified or
	  ○ if not but going to prescribe aminoglycosides for the first time, check if there is any maternal history of deafness prior prescribing aminoglycosides for the first time. Contact Consultant.

Nurse/Clinical nurse specialist	Keep a record of all tests done, technique used and variant tested.
Pharmacist	Pharmacist(s) responsible for a given clinical area/unit, to confirm with microbiology and consultant of best alternative antibiotic treatment(s) and update guidelines with sensitivities, as clinically needed.
GPs	Update records received with type of test and variant studied and if identified, mark visible as a problem e.g., allergy/intolerance tab and patient problem list.

Where should the result be kept and how should it be shared?

The ideal place for the R65 test result would be within the patient's allergy/intolerance box (this way all clinicians would be able to see the results). It will also require an addition to the main problems list within the patient's notes as well as using the SNOMED code, since this will ensure that the result is transferred to the patient's summary care records. Finally, the patient's GP's and other clinical teams caring for the patient, especially if accessing care outside of your institution should be informed.

Alternatively, you can create a pharmacogenetic section in your local electronic health record system. As best practice both positive and negative results should be recorded. Within your trust you may use a different approach for sharing the test results, however the results must be placed where it is visible to all healthcare professionals.

Do we need an alert card?

Patients may access healthcare outside of your institution e.g., emergency departments. Therefore, an alert card may be helpful to inform other clinicians that aminoglycosides are contraindicated. However, it is noted that this is not the most ideal method to share information and your institution may decide to use an alternative method.

Do we need to consider written consent?

Only verbal consent will be required as this is considered as a safety test, however you may decide to obtain written consent for your patient cohort. Please follow your internal consent governance procedures.

Do we need a patient information leaflet?

As best practice it is advisable to provide your patient/parent or carer with a patient information leaflet to explain why the test has been taken and what a positive result will mean for them. Information on what this can mean for other family members, especially on the maternal side and for siblings should also be explained. Appendix 2 can be used as a model to adapt for your own institution.

What to do if new NHSE commissioned variants are added to the national test directory for the R65 test?

Currently the R65 test only tests for one variant, m.1555A>G. In the future other variants may be added as more evidence is obtained. If the National Test Directory is updated with new commissioned variants, a priority testing pathway can be implemented e.g., all CF patients who have not had m.1555A>G detected to be re-tested first. However, the Genomic Medicine Service, which consists of the GLH and GMSA teams will provide support and guidance to clinical teams if and when this does occur.

Future developments

- Point of care testing: There is a new technology available called Genedrive, a point of care test for detecting m.1555A>G variants after a buccal swab, which provides a result within 26 minutes. This technology has been trialled within the neonatal setting only and the outcomes can be found here: Pharmacogenetics to Avoid Loss of Hearing (PALOH) trial: a protocol for a prospective observational implementation trial | BMJ Open (3).
- This technology has been reviewed by NICE through the Early Values Assessment pathway, which explained that the Genedrive MT-RNR1 technology can be used, on the proviso that new evidence is generated. NICE have detailed the additional evidence they require. NICE Early Values Assessment is not a full NICE technology appraisal, but a pathway to review promising innovations. If a technology is deemed to have a positive impact and recommended, this does not mean it will be automatically commissioned by NHS England. In this case, this NICE assessment is NOT commissioned, and local implementation would require a local business case and/or research funding.

Education and training

Webinar: link to the North Thames GMSA *MT-RNR1* educational webinar can be found here: https://www.norththamesglh.nhs.uk/aminoglycosides-pharmacogenetic-testing-webinar/

GeNotes: Genomic Education Programme have a new resource called GeNotes for pharmacogenetic testing to support education at the point of care In the Clinic (hee.nhs.uk). The GeNotes resources for *MT-RNR1* and aminoglycosides are available can be found here:

- Presentation: Patient requiring aminoglycoside antibiotics — In the Clinic (hee.nhs.uk)
- Results: Patient with a known MT-RNR1 genotype requiring aminoglycoside antibiotics — In the Clinic (hee.nhs.uk)

References

1. McDermott JH, Wolf J, Hoshitsuki K, Huddart R, Caudle KE, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for the Use of Aminoglycosides Based on MT-RNR1 Genotype. *Clinical Pharmacology & Therapeutics*. 2022;111(2): 366–372. https://doi.org/10.1002/cpt.2309.
2. McDermott JH, Wolf J, Newman WG. MT-RNR1 Genotype Should Not Affect Childhood Vaccination—Unintended Consequences of Guidelines. *JAMA Pediatrics*. 2023;177(11). https://doi.org/10.1001/jamapediatrics.2023.3263.
3. Rigobello R, Shaw J, Ilg D, Zimmerman R, Edelmann L, Kornreich R, et al. Clinical Pharmacogenomic MT-RNR1 Screening for Aminoglycoside-Induced Ototoxicity and the Post-Test Counseling Conundrum. *Clinical Pharmacology & Therapeutics*. 2023;114(2): 262–265. https://doi.org/10.1002/cpt.2910.
4. McDermott JH, Mahaveer A, James RA, Booth N, Turner M, Harvey KE, et al. Rapid Point-of-Care Genotyping to Avoid Aminoglycoside-Induced Ototoxicity in Neonatal Intensive Care. *JAMA Pediatrics*. 2022;176(5): 486–492. https://doi.org/10.1001/jamapediatrics.2022.0187.

Appendix 1 – Example of Pharmacogenomic reports

Example 1: m.1555A>G variant detected (patient name and details all fictional)

Lab G80034

Consultant Audiologist Hospital: EQA Trust	Patient Name: **ADAMS, Logan** Patient DOB: **15/7/2020** Patient Gender: **Male** NHS Number: MRN: **Not provided** Family Number: **ID is not set.** External Pat ID: **GENQA 2021AID_01**

REFERRAL REASON:

Hearing Loss - m.1555A.G

Diagnostic testing for the m.1555A>G variant in Logan ADAMS who has bilateral congenital severe sensorineural hearing loss. Logan received high levels of gentamycin in the perinatal period. He has no known family history of hearing loss.

Result Summary

This result confirms a diagnosis of hearing loss due to m.1555A>G aminoglycoside toxicity.

Result

The m.1555A>G mitochondrial variant has been detected in Logan ADAMS and confirmed by Sanger sequence analysis. Results are consistent with homoplasmy for this mitochondrial variant.

Recommended action

Logan ADAMS' maternal relatives can be tested for the presence of this variant if required. Please refer to their local clinical genetics department.

Reported by: Clinical Scientist	Date: 09/07/21
Authorised by: Senior Clinical Scientist	Date: 18/08/21

Technical Information

NB: Results depend on samples being labelled correctly and, where relevant, family relationships being as indicated.

Some mitochondrial variants have been associated with deafness, the most commonly reported being m.1555A>G. The homoplasmic variant m.1555A>G in the mitochondrial MT-RNR1 (12S rRNA) gene has been associated with aminoglycoside-induced and non-syndromic sensorineural deafness (Estivill X et al , Am J Hum Genet 62(1): 27-35, 1998; Prezant TR et al, Nat Genet 4 (3): 289-294, 1993). This variant has been detected in families with maternally transmitted deafness and seems to have an age dependent penetrance for deafness, which is enhanced by treatment with aminoglycosides. The variant removes a AlwI26I site that can be detected by restriction analysis. Note: it is possible that gain/loss of restriction sites could be introduced by other variants in the PCR fragment. Sanger sequence analysis is therefore used to confirm the genotype and rule out variants that create alternate restriction patterns.

Sanger sequence analysis has a sensitivity of approximately 99% (Ellard et al Genet Test & Mol Biomark 13,3 381-386 (2009)).

Mitochondrial genome reference sequence: NC_012920.1

DNA specimen ID 21RG-160G0163 from Blood, Venous Collected 27/5/2021 00:00 Received 9/6/2021 16:44 Authorised 18/8/2021 10:19 by
Priority Routine

Lab Comments

Consultant Audiologist
GenQA

Example 2: Variant not detected (patient name and details all fictional)

Lab G80034

Consultant Audiologist Hospital: EQA Trust	Patient Name: **HALSALL, Zachary** Patient DOB: **27/9/1982** Patient Gender: **Male** NHS Number: MRN: **Not provided** Family Number: **ID is not set.** External Pat ID: **GENQA 2021AID_02**

REFERRAL REASON:
Hearing Loss - m.1555A.G
Diagnostic testing for the m.1555A>G variant in Zachary HALSALL who has bilateral high frequency late onset hearing loss.

Result Summary
Cause of deafness not determined.
This result does not exclude a single gene cause for the hearing loss in this patient.

Result
Testing shows no evidence of the m.1555A>G variant in Zachary HALSALL.

Recommended action
No further testing has been requested; if further action is considered appropriate the referring consultant should contact the laboratory.

Reported by: Clinical Scientist	Date: 09/07/21
Authorised by: Senior Clinical Scientist	Date: 18/08/21

Technical Information

NB: Results depend on samples being labelled correctly and, where relevant, family relationships being as indicated.
Some mitochondrial variants have been associated with deafness, the most commonly reported being m.1555A>G. The homoplasmic variant m.1555A>G in the mitochondrial MT-RNR1 (12S rRNA) gene has been associated with aminoglycoside-induced and non-syndromic sensorineural deafness (Estivill X et al , Am J Hum Genet 62(1): 27-35, 1998; Prezant TR et al, Nat Genet 4 (3): 289-294, 1993). This variant has been detected in families with maternally transmitted deafness and seems to have an age dependent penetrance for deafness, which is enhanced by treatment with aminoglycosides. The variant removes a Alw26I site that can be detected by restriction analysis. Note: it is possible that gain/loss of restriction sites could be introduced by other variants in the PCR fragment. Sanger sequence analysis is therefore used to confirm the genotype and rule out variants that create alternate restriction patterns.
Sanger sequence analysis has a sensitivity of approximately 99% (Ellard et al Genet Test & Mol Biomark 13,3 381-386 (2009)).
Mitochondrial genome reference sequence: NC_012920.1

DNA specimen ID 21RG-160G0167 from Blood, Venous Collected 31/5/2021 00:00 Received 9/6/2021 16:46 Authorised 18/8/2021 10:20 by
Priority Routine

Lab Comments
Consultant Audiologist
GenQA

Appendix 2- Example of a PIL Drafted by South West GMSA for CF paediatric patients.

Note: Thanks to Bristol Royal Hospital for Children and South West GMSA.

This draft has NOT undergone any public and patient involvement.

Testing for potential increased risk of hearing loss with aminoglycoside antibiotics – patient information leaflet

What is a MT-RNR1 test and why do I need it?

What is the MT-RNR1 test?

The MT-RNR1 test can help to check if you are at increased risk of experiencing permanent hearing loss with certain types of antibiotics, called aminoglycosides. The ones most commonly used in Cystic Fibrosis are called tobramycin and amikacin.

What are aminoglycoside antibiotics?

Aminoglycosides are a group of antibiotics used to treat certain infections and include the antibiotics amikacin, gentamicin, streptomycin, neomycin and tobramycin.

Why is the MT-RNR1 test needed?

Some people have a greater chance of developing hearing loss with aminoglycoside antibiotics than others, which may be explained by their DNA. The MT-RNR1 test is a genetic test which will look at your DNA, to assess if there are any changes to the MT-RNR1 gene.

Around 1 in 500 people have a change in their MT-RNR1 gene which means they are more likely to experience hearing loss with aminoglycoside antibiotics. The test result will help your doctors choose the best antibiotic for you.

It is important to know that aminoglycosides might still cause permanent hearing loss in people who do not have the MT-RNR1 gene change. This is because there are other, more common, ways in which aminoglycosides cause hearing loss, which won't be detected by this test. This is the reason that you will have blood tests performed when receiving aminoglycosides to make sure that the dose given is not too high.

Who needs a MT-RNR1 test?

The MT-RNR1 test is suitable for the following groups of people;

- People with long term conditions (such as CF) which mean they are very likely to need aminoglycoside antibiotics in the future
- People who have had treatment with aminoglycoside antibiotics in the past and now have hearing loss

The MT-RNR1 test must be done well in advance of you needing aminoglycoside antibiotics and is not suitable for situations where antibiotics need to be started quickly to treat an infection.

What is involved and what happens after the test?

The MT-RNR1 test is a simple blood test and your clinical team will let you know your result. If your test indicates that you are at increased risk of hearing loss with aminoglycoside antibiotics, it will be recorded in your medical notes. It is also important to mention this to healthcare professionals in the future when you are receiving treatment. The MT-RNR1 gene change also runs in families and so your doctor may ask you for details of other members of your family and may give you an appointment to discuss this with the genetics service. The result can take up to 6 weeks to come back and will be given to you by your CF team.

Important things to remember

It is important to remember that changes in your DNA are only one potential cause of being at risk of hearing loss. You may still experience hearing loss with aminoglycosides, even if your MT-RNR1 test results are normal. It's also important to continue to attend any appointments to monitor your hearing after treatment with aminoglycosides, even if you have a normal test result. You should talk to your clinical team if you have any questions about MT-RNR1 testing or medication side effects.