Supplement to:

Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for the use of aminoglycosides based on *MT-RNR1* genotype

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LITERATURE REVIEW

We searched the PubMed® database (1966 to January 2020) for keywords ((RNR1 OR 12S ribosomal RNA) AND (aminoglycoside OR antibiotic OR amikacin OR gentamicin OR tobramycin OR ribostamycin OR spectinomycin OR streptomycin OR plazomicin OR apramycin OR arbekacin OR astromicin OR bekanamycin OR butirosin OR dibekacin OR fortimicin OR framycetin OR hygromycin OR isepamicin OR kanamycin OR lividomycin OR nebramycin OR neomycin OR netilmicin OR pactamycin OR paromomycin OR puromycin OR sisomicin OR verdamicin)) NOT (review[Publication Type]). This search returned 148 results.

Inclusion criteria included studies gathering primary data (i.e., no review articles or meta-analyses), studies in human subjects or cells and clear results pertaining to an association (or lack of) between genetic variants in *MT-RNR1* and toxicity to aminoglycosides. Following application of the inclusion criteria, 94 publications were reviewed and included in the evidence Table S1.

AVAILABLE GENETIC TEST OPTIONS

Commercially available genetic testing options change over time and a number of different platforms are currently available for *MT-RNR1* genotyping. Additional information about pharmacogenetic testing options can be found at the Genetic Testing Registry (https://www.ncbi.nlm.nih.gov/gtr/).

Clinical laboratories may analyze for different SNPs or other genetic variants, depending on the genotyping platform currently used by each laboratory. As such, we make no formal recommendation in relation to the optimal platform choice. Ideally, any panel design should include all variants which confer an increased risk of *MT-RNR1*-related aminoglycoside-induced hearing loss, as outlined in the main manuscript. Standard qualitative genotyping methodologies, including allele specific PCR, allele specific oligo-hybridization and by Sanger sequencing are all appropriate to discriminate between the variants and wild type mitochondrial alleles. Alternative methodologies including Pyrosequencing are able to quantify the proportions if more than one variant is present

There are many unique features inherent to mtDNA, including multiple genome copy numbers per cell, which can lead to complex heteroplasmy and threshold effects. Most modern quantitative genotyping approaches allow sensitive assessment of heteroplasmy to a pre-defined threshold. As outlined in the main manuscript, there is no clear heteroplasmy level where aminoglycoside administration becomes safe when a high risk *MT-RNR1* variant is detected. However, a lower threshold for heteroplasmy detection of approximately 20% is most likely be sufficient to detect clinically relevant *MT-RNR1* variation based on the available literature. Laboratories should be aware of their lower heteroplasmy threshold and ensure results are reported with that context.

LEVELS OF EVIDENCE

The evidence summarized in **Supplemental Table S1** is graded using a scale modified slightly from Valdes *et al*. (1)

High: Evidence includes consistent results from well-designed, well-conducted studies.

Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.

Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

STRENGTH OF RECOMMENDATIONS

CPIC's recommendations are based on weighing the evidence from a combination of preclinical functional and clinical data, as well as on some existing disease-specific consensus guidelines (2). Overall, the therapeutic recommendations are simplified to allow rapid interpretation by clinicians. CPIC uses a slight modification of a transparent and simple system for just three categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of antiretroviral agents (3):

Strong recommendation for the statement**:** The evidence is high quality and the desirable effects clearly outweigh the undesirable effects.

Moderate recommendation for the statement: There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.

Optional recommendation for the statement: The desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
No recommendation: There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time.

RESOURCES TO INCORPORATE PHARMACOGENETICS INTO AN ELECTRONIC HEALTH RECORD WITH CLINICAL DECISION SUPPORT

Clinical decision support (CDS) tools integrated within electronic health records (EHRs) can help guide clinical pharmacogenetics at the point of care (4-8). See https://cpicpgx.org/cpic-guideline-for-aminoglycosides-and-mt-rnr1/ for resources to support the adoption of CPIC guidelines within an EHR. Based on the capabilities of various EHRs and local preferences, we recognize that approaches may vary across organizations. Our intent is to synthesize foundational knowledge that provides a common starting point for incorporating the use of *MT-RNR1* genotype results to guide aminoglycoside use.

Effectively incorporating pharmacogenetic information into an EHR to optimize drug therapy should have some key attributes. Pharmacogenetic results, an interpreted phenotype, and a concise interpretation or summary of the result must be documented in the EHR (9, 10). To incorporate a phenotype in the EHR in a standardized manner, genotype test results provided by the laboratory must be consistently translated into an interpreted phenotype (**Table 1, main manuscript**). Because clinicians must be able to easily find the information, the interpreted phenotype may be documented as a problem list entry or in a patient summary section; these phenotypes are best stored in the EHR at the "person level" rather than at the date-centric "encounter level". Additionally, results should be entered as standardized and discrete terms to facilitate using them to provide point-of-care CDS (4, 11).

Because pharmacogenetic results have lifetime implications and clinical significance, results should be placed into a section of the EHR that is accessible independent of the test result date to allow clinicians to quickly find the result at any time after it is initially placed in the EHR. To facilitate this process, CPIC is providing gene-specific information figures and tables that

include full genotype to phenotype tables, diagram(s) that illustrate how *MT-RNR1* pharmacogenetic test results could be entered into an EHR, example EHR consultation/genetic test interpretation language and widely used nomenclature systems for genes relevant to the CPIC guideline (see https://cpicpgx.org/cpic-guideline-for-aminoglycosides-and-mt-rnr1/) (12).

SUPPLEMENTAL TABLE S1. EVIDENCE LINKING *MT-RNR1* GENOTYPE WITH AMINOGLYCOSIDE-INDUCED HEARING LOSS

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
In vitro	The presence of the C1494T variant in LCLs and cybrid lines leads to reduced growth	Zhao, *et al*. 2004 (13) Zhao, *et al*. 2005 (14)	High
In vitro	The presence of the C1494T variant in LCLs leads to reduced oxygen consumption	McCandless, *et al*. 2004 (15)	High
In vitro	Aminoglycosides have increased binding to 12S rRNA with the C1494T variant as compared to WT 12S rRNA	Qian, *et al*. 2009 (16) Degtyareva, *et al*. 2017 (17)	High
In vitro	The presence of the C1494T variant in cybrid lines leads to reduced mitochondrial translation in both the presence and absence of aminoglycosides	Yu, *et al*. 2014 (18)	Moderate
In vitro	The presence of the C1494T variant in cybrid lines leads to reduced ATP production in both the presence and absence of aminoglycosides, with a greater reduction seen in the presence of aminoglycosides	Yu, *et al*. 2014 (6)	Moderate
In vitro	The presence of the C1494T variant in cybrid lines exposed to aminoglycosides leads to reductions in ATP production efficiency, mitochondrial membrane potential and an increase in cellular ROS levels	Yu, *et al*. 2014 (6)	Moderate

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
In vitro	The presence of the C1494T variant in cybrid lines leads to altered mitochondrial morphology (elongated, fused) in both the presence and absence of aminoglycosides and increased mitophagy when treated with aminoglycosides	Yu, *et al*. 2014 (6)	Weak
In vitro	The presence of the A1555G variant in fibroblasts and cybrid lines leads to inhibition of mitochondrial translation in the presence of aminoglycosides	Inoue, *et al*. 1996 (19)	High
In vitro	Aminoglycosides have increased binding to 12S rRNA with the A1555G variant as compared to WT 12S rRNA	Hamasaki, *et al*. 1997 (20) Ryu, *et al*. 2002 (21) Qian, *et al*. 2009 (16) Degtyareva, *et al*. 2017 (17)	High
In vitro	The presence of the A1555G variant in LCLs or cybrid lines leads to reduced cell growth in the presence of aminoglycosides	Guan, *et al*. 2000 (22) Giordano, *et al*. 2002 (23) Pacheu-Grau, *et al*. 2011 (24)	High
In vitro	The presence of the A1555G variant in cybrid lines does not have a significant effect on mitochondrial translation, cellular respiration or RNA expression in the presence of aminoglycosides	Giordano, *et al*. 2002 (23)	Moderate
In vitro	The presence of the A1555G variant in human cells leads to reduced quantity and activity of the OXPHOS complex IV protein in the presence of aminoglycosides	Pacheu-Grau, *et al*. 2011 (24)	High
Clinical	Variants at the 750, 884, 951, 988, 1055, 1420, 1438 or 1505 loci increase susceptibility to aminoglycoside-induced hearing loss	Conrad, *et al*. 2008 (25)	Weak

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
Clinical	The A663G variant does not increase susceptibility to aminoglycoside-induced hearing loss	Hutchin, *et al*. 1993 (26) Pandya, *et al* 1997 (27)	Moderate
Clinical	The T669C variant increases susceptibility to aminoglycoside-induced hearing loss	Leveque, *et al*. 2007 (28) Rydzanicz, *et al*. 2010 (29)	Weak
Clinical	The A747G variant increases susceptibility to aminoglycoside-induced hearing loss	Shen, *et al*. 2011 (30)	Weak
Clinical	The G786A variant increases susceptibility to aminoglycoside-induced hearing loss	Guaran, *et al*. 2013 (31)	Weak
Clinical	The A807G or A807C variants increase susceptibility to aminoglycoside-induced hearing loss	Conrad, *et al*. 2008 (25)	Weak
Clinical	The A827G variant increases susceptibility to aminoglycoside-induced hearing loss	Xing, *et al*. 2006 (32) Chaig, *et al*. (33) Conrad, *et al*. 2008 (25) Rydzanicz, *et al*. 2010 (29)	Weak
Clinical	The A839G variant increases susceptibility to aminoglycoside-induced hearing loss	Shen, *et al*. 2011 (30)	Weak
Clinical	The A896G variant increases susceptibility to aminoglycoside-induced hearing loss	Conrad, *et al*. 2008 (25)	Weak
Clinical	The A930G variant increases susceptibility to aminoglycoside-induced hearing loss	Conrad, *et al*. 2008 (13)	Weak
Clinical	The C960del and G951A variants increase susceptibility to aminoglycoside-induced vestibular dysfunction	Elstner, *et al*. 2008 (34)	Weak
Clinical	The 961delT variant does not increase aminoglycoside-induced hearing loss	Kobayashi, *et al*. 2005 (35)	Weak

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
Clinical	The T961G variant does not increase aminoglycoside-induced hearing loss	Conrad, *et al*. 2008 (25)	Weak
Clinical	The 961delT+Cn variant increases susceptibility to aminoglycoside-induced hearing loss	Bacino, *et al*. 1995 (36) Casano, *et al*. 1999 (37) Yoshida, *et al*. 2002 (38) Human, *et al*. 2010 (39)	Weak
Clinical	The 961delT+Cn variant does not increase susceptibility to aminoglycoside-induced vestibular dysfunction.	Yoshida, *et al*. 2002 (38)	Weak
Clinical	The G988A variant increases susceptibility to aminoglycoside-induced hearing loss	Rydzanicz, *et al*. 2010 (29)	Weak
Clinical	The T1095C variant increases susceptibility to aminoglycoside-induced hearing loss	Thyagarajan, *et al*. 2000 (40) Tessa, *et al*. 2001 (41) Zhao, *et al*. 2004 (42) Li, *et al*. 2005 (43) Dai, *et al*. 2006 (44) Shen, *et al*. 2011 (30)	Moderate
Clinical	The T1189C variant increases susceptibility to aminoglycoside-induced hearing loss	Meza, *et al*. 2011 (45)	Weak
Clinical	The T1243C variant increases susceptibility to aminoglycoside-induced hearing loss	Bacino, *et al*. 1995 (36)	Weak
Clinical	The G1438A and/or the G1462A variants increase susceptibility to aminoglycoside-induced hearing loss	Gurtler, *et al*. 2005 (46)	Weak

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
Clinical	The C1494T variant increases susceptibility to aminoglycoside-induced hearing loss	Zhao, *et al*. 2004 (13) Wang, *et al*. 2006 (47) Rodriguez-Ballesteros, *et al*. 2006 (48) Han, *et al*. 2007 (49) Yuan, *et al*. 2007 (50) Chen, *et al*. 2007 (51) Conrad, *et al*. 2008 (25) Zhu, *et al*. 2009 (52) Lu, *et al*. 2010 (53) Johnson, *et al*. 2010 (54) Shen, *et al*. 2011 (30) Ding, *et al*. 2016 (55) Ding, *et al*. 2017 (56)	High
Clinical	The T1520C variant increases susceptibility to aminoglycoside-induced hearing loss	Bacino, *et al*. 1995 (36)	Weak
Clinical	The A1555G variant increases susceptibility to aminoglycoside-induced hearing loss	Prezant, *et al*. 1993 (57) Fischel-Ghodsian, *et al* 1993 (58) Hutchin, *et al*. 1993 (26) Pandya, *et al*. 1997 (27) el-Schahawi, *et al*. 1997 (59) Usami, *et al*. 1997 (60) Fischel-Ghodsian, *et al*. 1997 (61) Gardner, *et al*. 1997 (62) Estivill, *et al*. 1998 (63) Casano, *et al*. 1998 (64) Tono, *et al*. 1998 (65) Shohat, *et al*. 1999 (66)	High

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
		Usami, *et al*. 2000 (67) Nye, *et al*. 2000 (68) Astengo, *et al*. 1997 (69) Kupka, *et al*. 2002 (70) Campos, *et al*. 2002 (71) Yamasoba, *et al*. 2002 (72) del Castillo, *et al*. 2003 (73) Li, *et al*. 2004 (74) Noguchi, *et al*. 2004 (75) Young, *et al*. 2005 (76) Li, *et al*. 2005 (43) Kobayashi, *et al*. 2005 (35) Zhao, *et al*. 2005 (77) Dai, *et al*. 2006 (78) Ballana, *et al*. 2006 (79) Kouzaki, *et al*. 2007 (80) Young, *et al*. 2006 (81) Tang, *et al*. 2007 (82) Leveque, *et al*. 2007 (28) Liao, *et al*. 2007 (83) Ballana, *et al*. 2008 (84) Chen, *et al*. 2008 (85) Konings, *et al*. 2008 (86) Wang, *et al*. 2008 (87) Conrad, *et al*. 2008 (25) Ding, *et al*. 2009 (88) Lu, *et al*. 2010 (89) Kokotas, *et al*. 2009 (90) Lu, *et al*. 2010 (53) Kato, *et al*. 2010 (91)	

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of Evidence
		Rydzanicz, *et al*. 2010 (29) Johnson, *et al*. 2010 (54) Ji, *et al*. 2011 (92) Shen, *et al*. 2011 (30) Men, *et al*. 2011 (93) Shen, *et al*. 2012 (94) Chen, *et al*. 2013 (95) Skou, *et al*. 2014 (96) Al-Malky, *et al*. 2014 (97) Gopel, *et al*. 2014 (98) Iwanicka-Pronicka, *et al*. 2015 (99) Liu, *et al*. 2015 (100) Ding, *et al*. 2016 (55) Abusamra, *et al*. 2016 (101) Wu, *et al*. 2018 (102)	
Clinical	The A1555G variant increases susceptibility to aminoglycoside-induced tinnitus	Tono, *et al*. 1998 (65)	Weak
Clinical	An increased variant load of A1555G increases severity of hearing loss	del Castillo, *et al*. 2003 (73) Ballana, *et al*. 2008 (84) Shen, *et al*. 2012 (94)	Weak
Clinical	The A1555G variant increases susceptibility to aminoglycoside-induced vestibular dysfunction	Usami, *et al*. 1997 (60) Noguchi, *et al*. 2004 (75) Leveque, *et al*. 2007 (28)	Weak
Clinical	The C1556T variant increases susceptibility to aminoglycoside-induced tinnitus	Tanimoto, *et al*. 2004 (103)	Weak

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