Review

J. Biol. Regul. Homeost. Agents. 2022; 36(3): 445–453
https://doi.org/10.23812/j.biol.regul.homeost.agents.20223603.52

Crucial Roles of the m.1555A>G Mutation on *MT-RNR1* Gene in Hearing Loss

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Published: 20 June 2022

Hearing loss is caused by a variety of genetic and environmental factors, with inherited causes assumed to account for 50% to 60%. Mutations in mitochondrial DNA (mtDNA) 12S rRNA, especially the m.1555A>G mutation, are the most common molecular cause for nonsyndromic sensorineural hearing loss and aminoglycoside-induced deafness. The spectra of this gene varies among different ethnic populations. In current review, the m.1555A>G mutation of 12S rRNA decoding gene was compared in different populations for ethnic-specific allele frequency and their contribution to genetic hearing loss. Differences in the distribution of mutation to diverse regions of the world showed that it occurred from the ancestors of each ancestral generation and in immigrant populations at different time periods. Furthermore, we also include the functional studies of this mtDNA variation in the etiologies of aminoglycoside-induced hearing loss. Carriers of the mutation (m.1555A>G) should avoid aminoglycosides and use alternatives for antibiotic therapy to avoid the possibility of drug-induced hearing loss. Comprehensive summary of the m.1555A>G mutation can help provide scientific basis for disease diagnosis and consultation for hearing loss and develop optimal therapeutic strategies for deaf patients.

Keywords: hearing loss; mtDNA; m.1555A>G

Introduction

Hearing loss (HL) is one of the most common sensory deficits in human beings. Current studies have shown that one per 1000 newborns has congenital hearing problem [1,2]. It is estimated that approximately 50–60% of HL are caused by genetic factors [3]. Among them, non-syndromic hearing loss (NSHL) with hearing impairment as the only significant clinical feature accounts for about 70%, whereas the remaining 30% is syndromic hearing loss (SHL) accompanied by other abnormalities [4]. Genetic hearing loss of non-syndromic form can follow a pattern of autosomal recessive (DFNB), autosomal dominant (DFNA), mitochondrial inheritance and X-linked recessive [1]. NSHL is genetically very heterogeneous. To date, according to the website (https://hereditaryhearingloss.org/), about 110 genes with more than 1000 mutations and 150 loci were found to be associated with NSHL. Interestingly, the most common genes detected in NSHL are SLC26A4, GJB2, GJB3, and mtDNA 12S rRNA [5–7].

Although most cases of NSHL are caused by mutations in nuclear genes, it is clear that mitochondrial pathology is also important both in inherited and acquired hearing loss [8,9]. Of note, the first genetic defect associated with NSHL is a mitochondrial alteration detected by Prezant *et al.* [10] in 1993. Based on the website (http://www.mitomap.org, 2020), over 100 mitochondrial alterations in coding and control regions have been found to be associated with hearing loss. Several mtDNA alterations leading to NSHL have been reported, of which the *MT-RNR1* gene encoding 12S rRNA is a hot spot for variants causing NSHL. Especially, the most common mutation of mtDNA 12S rRNA is m.1555A>G, which is well known to be related to aminoglycoside-induced NSHL in individuals from different races [11–13].

The m.1555A>G mutation, an mtDNA mutation firstly identified as a cause of maternally inherited hearing impairment, has been associated with extremely variable phenotypes [14,15]. The phenotypes of the m.1555A>G mutation vary from normal hearing with same maternal lineage, moderate progressive hearing loss, and severe deafness. The incomplete penetration and varied expressivity of hearing loss related to the m.1555A>G mutation are associated with the modified nuclear genes, mitochondrial haplotype, interaction among genetic factors, and environmental factors such as aminoglycosides [16].

In this study, the evidences of genetic association between the m.1555A>G mutation in *MT-RNR1* gene and NSHL-related phenotypes were reviewed. Furthermore, functional studies of this mtDNA variation were included in the etiologies of aminoglycoside-induced hearing loss.

Table 1. The prevalence of m.1555A>G in the *MT-RNR1* gene associated with hearing disorders.

Authors	Year of publication	Sample origin	Phenotype	Detection method	Range of ages (years)	Sample size	Mutation frequency (%)	Reference
			Asia					
Fischel-Ghodsian *et al.*	1993	Chinese	Deaf after aminoglycoside exposure	Allele-specific oligonucleotide hybridization	13–21	36	2.78	[17]
Tamagawa *et al.*	1996	Japanese	Bilateral sensorineural hearing loss	PCR-RFLP	43–65	7	14.3	[18]
Pandya *et al.*	1999	Mongolia	Deafness	PCR-RFLP	NA	480	7.7	[19]
Usami *et al.*	2000	Japanese	SNHL/Profound hearing loss	PCR-RFLP	3–92/NA	319/140	3.45/10	[13]
Tono *et al.*	2001	Japanese	Post-lingual non-syndromic deafness	PCR-RFLP	14–84	68	5.88	[33]
Malik *et al.*	2003	Southeast Asian	Non-syndromic sensorineural deafness	PCR-RFLP	6–43	75	5.33	[49]
Tekin *et al.*	2003	Turkey	Pre-lingual sensorineural non-syndromic deafness	PCR-RFLP	NA	168	1.79	[36]
Noguchi *et al.*	2004	Japanese	NSHL	Sequencing	6–77	138	5.07	[34]
Li *et al.*	2005	Chinese	Aminoglycoside-induced/NSHL	Sequencing	7–17	128	13/2.9	[32]
Wu *et al.*	2007	Han Chinese	Idiopathic sensorineural hearing loss	PCR-RFLP	1–54	315	3.2	[50]
Liu *et al.*	2008	Chinese	NSHL	PCR-RFLP	7–80	290	15.5	[24]
Kato *et al.*	2010	Japanese	Suspected hereditary HL	Suspension array	1–77	373	2.9	[35]
Lu *et al.*	2010	Han Chinese	Aminoglycoside-induced and NSHL	Sequencing	1–17	1642	3.96	[37]
Ji *et al.*	2011	Chinese	NSHL	PCR-RFLP	0.3–67	473	1.63	[51]
Chen *et al.*	2011	Chinese	Hearing loss	PCR-RFLP	NA	813	11.81	[25]
Wei *et al.*	2013	Chinese	NSHL	Sequencing	2–45	658	5.93	[52]
Chai *et al.*	2013	Han Chinese	NSHL	Sequencing	NA	619	4.70	[53]
Du *et al.*	2014	Chinese (Han/Hui/Uyghur)	SNHL	Sequencing	1–39/2–28/4–22	1835/306/208	6.05/3.27/1.44	[54]
Jiang *et al.*	2015	Chinese	NSHL	Sequencing	5–36	155	3.87	[11]
Subathra *et al.*	2016	South Indian	NSHL	PCR-RFLP	6–18	729	0.69	[29]
Luo *et al.*	2017	Chinese	profound NSHL	SNP scan assay	0.7–70	535	1.12	[31]
Wu *et al.*	2018	Chinese	NSHL	PCR-RFLP	1–3	300	1.67	[55]
Xiang *et al.*	2019	Chinese	NSHL	Sequencing and Microarray	0.8–53	506	17.0	[30]

Table 1. Continued.

Authors	Year of publication	Sample origin	Phenotype	Detection method	Range of ages (years)	Sample size	Mutation frequency (%)	Reference
			Europe					
Lehtonen *et al.*	2000	Northern Finland	SNHI	PCR-RFLP	NA	117	2.6	[56]
Kupka *et al.*	2002	Hungarian/Polish/German	NSSHI	PCR-RFLP	1–73/NA/1–60	56/125/160	1.8/2.4/0.7	[12]
Ostergaard *et al.*	2002	Denmark	NSHI	PCR-RFLP	4–67	85	2.4	[57]
del Castillo *et al.*	2003	Spain	NSHL	PCR-RFLP	NA	649 families	16	[23]
Jacobs *et al.*	2005	Southern Italy/UK	Post-lingual NSHI	Sequencing or primer extension	NA	128/80	1.56/2.5	[58]
Bravo *et al.*	2006	Spain	NSHL	PCR-RFLP	NA	54	16.67	[59]
Leveque *et al.*	2007	France	SNHI	Sequencing	NA	29 families	17.24	[22]
Berrettini *et al.*	2008	Italy	NSHL	PCR-RFLP	15–76	167	5.4	[42]
Kokotas *et al.*	2009	Greece	NSHL	PCR-RFLP	NA	478	0.42	[40]
Rydzanicz *et al.*	2010	Polish	Aminoglycoside-induced and NSHL	Sequencing	NA	250	3.6	[41]
Kokotas *et al.*	2011	Greece	Deafness	PCR-RFLP	NA	513	0.4	[39]
			America					
Li *et al.*	2004	Caucasian	NSHI	Sequencing	<19	164	0.6	[26]
Abreu-Silva *et al.*	2006	Brazil	HI	PCR-RFLP	NA	203	2	[43]
Liu *et al.*	2008	USA	NSHL	PCR-RFLP	7–80	208	1.9	[24]
Salomao *et al.*	2013	Brazil	Non-syndromic deafness	PCR-RFLP	0.3-76	78	1.3	[44]
			Africa					
Matthijs *et al.*	1996	Zaire	Non-syndromic deafness	Sequencing	NA	12 families	100	[47]
Mkaouar-Rebai *et al.*	2006	Tunisian	NSHL	PCR-RFLP	NA	100 families	1	[27]
Nahili *et al.*	2010	Morocco	NSSHL	PCR-RFLP	NA	84 families	3.6	[45]
Fassad *et al.*	2014	Egypt	NSHL	PCR-RFLP	0.1–65	97	1.3	[46]
			Mix					
Vivero *et al.*	2012	Ethnically Diverse	NSD	PCR-RFLP	0.3–80	217	0.9	[38]
Yelverton *et al.*	2013	Ethnically Diverse	Hearing loss with mitochondrial mutation variants	Sequencing	NA	86	20.9	[48]

Abbreviations: PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; NSHL, Non-syndromic Hearing Loss; NA, Not Available; SNHL, Sensorineural Hearing Loss; SNHI, Sensorineural Hearing Impairment; NSSHI, Non-syndromic Sensorineural Hearing Impairment; NSD, Non-syndromic Deafness.

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Genetic Studies of the m.1555A>G Mutation

The m.1555A>G mutation was first found in a large Arab-Israeli family [10]. Subsequently, Fischel-Ghodsian *et al.* (1993) [17], Tamagawa *et al.* (1996) [18], and Pandya *et al.* (1999) [19] reported populations with this same variation and variable degrees of HL. Recent studies showed that there are ethnic-related differences in the prevalence of the m.1555A>G mutation in HL-related phenotypes, ranging from 0 to 17.24% [20–22]. In general, the m.1555A>G variation is a frequent cause of hearing disorders in some Asian and European populations [23–25]. However, it was rare or even absent in the American and African population [26–28] (Table 1, Ref. [11–13,17–19,22–27,29–59]).

Asia

In Asia, the prevalence of HL ranges between 0.69% and 17.0%, of which the lowest frequency was observed in a South Indian population [29] while the highest frequency was observed in a Chinese population [30]. Xiang *et al.* [30] showed that a total of 86 of 506 Chinese harbored m.1555A>G (86/506), which is markedly higher than the reported 1.12%–13% frequency found in other regions of China [31,32]. Of note, the severity and age of onset of hearing loss varied in the 86 Chinese patients with the m.1555A>G mutation. Due to the geographical separation in China which has the largest population in the world, different areas may have different genetic backgrounds [60]. Recent studies also reported HL frequencies of 2.9%–14.3% in Japanese [13,18,33–35] and 1.79% in Turkish populations [36]. However, in a Chinese population, Jiang *et al.* [21] found that nobody with nonsyndromic SNHL carried the m.1555A>G mutation. In Zohour's survey of an Iranian population, this mutation was not detected in any of the studied NSHL or control samples (a frequency of 0% for each) [61]. The difference in the frequency of mutations among Asian countries explains that the complexity of the genetic epidemiology of NSHL is strongly influenced by the ethnic composition of a particular population [37,38].

Europe

In Europe, the lowest frequency of NSHL associated with m.1555A>G was observed in a Greek population [39] and the highest frequency of that was observed in a French population [22]. The prevalence of this allele varied between 0.4% and 17.24%.

The m.1555A>G mutation was found in maternally inherited NSHL families as well as in some patients suffered from hearing loss after the administration of aminoglycosides. While the fact that none of the 35 syndromic cases harbor the m.1555A>G mtDNA mutation, Kokotas *et al.* [39,40] reported a frequency of 0.4% which was similar to other European populations reported. In addition, Rydzanicz *et al.* [41] analyzed the entire sequence of 12S rRNA gene in 250 Polish patients with aminoglycoside-induced hearing loss and NSHL. The incidence of m.1555A>G mutation was estimated to be 3.6%, within the range previously reported for Europeans. Additionally, nine unrelated cases positive for m.1555A>G were also identified [12,42]. To further study the impact of m.1555A>G mutation in the Greek population, Kokotas *et al.* [39,40] expanded their research from 106 to 478 unrelated individuals diagnosed with either pre-lingual or post-lingual sensorineural, bilateral, non-syndromic, and hearing impairment of any degree. They observed two patients with the mutation, indicating that m.1555A>G mutation may be rather uncommon among Greeks. In fact, these studies are difficult to interpret, due to difference in the patient's selection criteria (familial or sporadic cases), with a higher rate generally reported in familial cases [22,23]. A true different prevalence or ascertainment might be explanations for this variable frequency.

America

In America, the frequency of the m.1555A>G mutation was investigated to be ranging from 0.6% to 2% [24,26,43,44]. Li *et al.* [26] conducted a retrospective database review and subsequent molecular analysis of 164 pediatric subjects with sporadic non-syndromic deafness at the Center for Hearing and Deafness Research (CHDR) at the Cincinnati Children's Hospital Medical Center (CCHMC). They showed that the frequency of the m.1555A>G mutation was 0.6% and affected subject was present in homoplasmy. By contrast, Liu *et al.* [24] demonstrated that four (2%) deaf probands from the USA carries the m.1555A>G mutation, while all the deaf patients from China were observed to carry this variation. Similarly, in study made in San Paulo, Brazil, 2% of the individuals with NSHL have the m.1555A>G mutation [43]. Additionally, among southern Brazilians, Salomao *et al.* [44] reported a frequency of 1.3% in people with NSHL with negative result for the 167delT, 35delG, and 235delC mutations in the gap junction protein beta 2 gene (GJB2). However, the m.1555A>G mutation was not found in another Brazilian study with 27 deaf subjects [62]. In another population, 15% of the hearing loss patients who had received antibiotics containing amino-group, have the m.1555A>G mutation [63].

Africa

In Africa, the m.1555A>G variant is observed in around 1%–3% of HL [27,45,46]. The allele frequencies in those populations were derived by screening both pre-lingual and post-lingual hearing impairment with or without exposure of aminoglycoside antibiotic. Mkaouar-Rebai *et al.* [27] described the first Tunisian family with NSHL carrying the m.1555A>G mutation among 100 families tested. This mutation was observed to occur both in patient with congenital hearing loss (V.3 and V.4) and in individuals with normal hearing (V.1 and V.2). The phenotypic vari-

ability reported in patients with Tunisian pedigree implied that the nuclear modifier genes are involved in the development of deafness. Thereafter, the m.1555A>G mutation was first described in Moroccan and Egyptians patient with NSHL. It should be noted that the 1555 A to G mutation was found in all deaf persons as well as their siblings tested in Matthijs's study. Additionally, this mutation was homoplasmic in all maternally related members [47]. It is likely that all the families involved originated from a small village in Zaire, in which deafness affected the maternal lineage for several generations, hinting at mtDNA mutations.

Mix

Vivero *et al.* [38] screened 217 ethnically diverse probands for mtDNA mutation, in which 117 were whites of European ancestry, 70 were whites Hispanic/Latinos, 16 were African Americans, 11 were Asians, two were of Middle Eastern descent and one Portuguese. Of the total probands screened, two was observed to have the m.1555A>G mutation (2/217). The first one was a white female with 80-year-old suffered from progressive HL which occurred at the age of 40, and the second one was a Hispanic woman with a history of childhood aminoglycoside exposure. Despite mtDNA mutation rates were higher than expected in some Asian populations, none of the 11 Asian patients tested in the study of Vivero *et al.* [38] carried the m.1555A>G mutation. In addition, Samanich *et al.* [64] did not identify any of 109 predominantly simplex African American (AA) and Caribbean Hispanic (CH) individual with the m.1555A>G mutation. However, in the largest cohort of 86 patients described in an American population possessing mitochondrial mutations, 18 cases were m.1555A>G [48]. Subjects with this mutation had the highest family history and the most severe hearing loss. Regarding to ethnicity, Western European, Asian and Hispanic decent were the main subjects, while no Eastern European proband carries the m.1555A>G mutation.

Functional Studies of the m.1555A>G Mutation

The m.1555A>G mutation, which located in domain of mitochondrial 12S rRNA with a high degree of conservation, may affect its secondary structure. Bacterial studies have shown that this domain of the molecule is part of the aminoacyl site in which mRNA is decoded and lies at the ribosomal subunit interface [65–67]. Specifically, a new pair of C-G bases appears in the human 12S rRNA gene due to the m.1555A>G mutation, making it similar to the corresponding region of the *Escherichia coli* 16S rRNA gene [68]. While aminoglycoside-linkage results in protein translation errors by binding to this decoding region and subsequently in bacterial death, this mutation increases susceptibility to the effects of antibiotics on translational fidelity. This may explain the aminoglycoside-induced hearing loss in individuals who have this mutation [32,68].

Based on the accumulating genetic evidence as summarized above, we hypothesize that deafness inherited families carrying the m.1555A>G mutation have something wrong with the mitochondrion. In addition, researchers demonstrated that the use of aminoglycoside antibiotics is capable of inducing or aggravating hearing loss, and deafness varies in severity and age of onset among subjects without aminoglycoside exposure [69–73]. Patients carrying the m.1555A>G mutation can show various phenotypic variation [74–76]. For instance, some Chinese pedigrees with this mutation showed vary low penetrance of hearing loss [75–77], while others showed the contrary [78]. It may be indued by nuclear modifier genes as well as many other environmental factors. But what is really the effect of aminoglycoside antibiotics on hearing loss persons carrying the m.1555A>G mutation?

Aminoglycoside antibiotic are common clinical drugs which were often used to treat gram-negative bacterial infections that do not respond to conventional antibiotics. They may exert their effects by binding directly to the base pairs C1409-G1491 at the A-site of bacterial 16S rRNA, which serves as a crucial part of the decoding site. This interaction could result in premature termination of protein synthesis or protein mistranslation [79–81]. As a matter of fact, mitochondria ribosome of eukaryotes are similar to bacterial ribosomes. The A-to-G substitution in the aminoglycoside binding site in 16S rRNA in mammalian mitochondrial ribosomes may lead to the significantly reduced toxicity of aminoglycosides in eukaryotic cells. The A nucleotide at position 1555 in the 12S rRNA gene located in human mitochondria is similar to position 1491 in the 16S rRNA gene located in wild-type *E. coli* [82]. When 1555A was mutated to G, the secondary structure of 12S rRNA was similar to the corresponding region of 16S rRNA in *E. coli*. Therefore, it was presumed that this newly formed G-C pair gives rise to an aminoglycoside binding site (Fig. 1). In fact, Guan *et al.* [14] reported that the m.1555A>G mutation changes the binding property of aminoglycoside at the A-site of rRNA and results in a conformational change in 12S rRNA.

Overall, mutation in the mitochondrial 12S rRNA (e.g., m.1555A>G) induces defects in synthesis of mitochondrial protein, and aminoglycoside which concentrated selectively in the cochlea and vestibular exacerbate these defects. The translational defect result in the apoptosis of hair cells in the cochlea and vestibular system. However, the human cochlea has only about 5000 hair cells and do not have the ability to repair itself. Genetic defects may cause abnormal hair cells at birth, resulting in deafness-related phenotypes. In addition, mutations in nuclear-encoded modifier genes (e.g., MTO1, GTPBP3, TRMU) and many other environmental factors including aging, noise, and so on are capable of aggravating the phenotype of hearing impairment.

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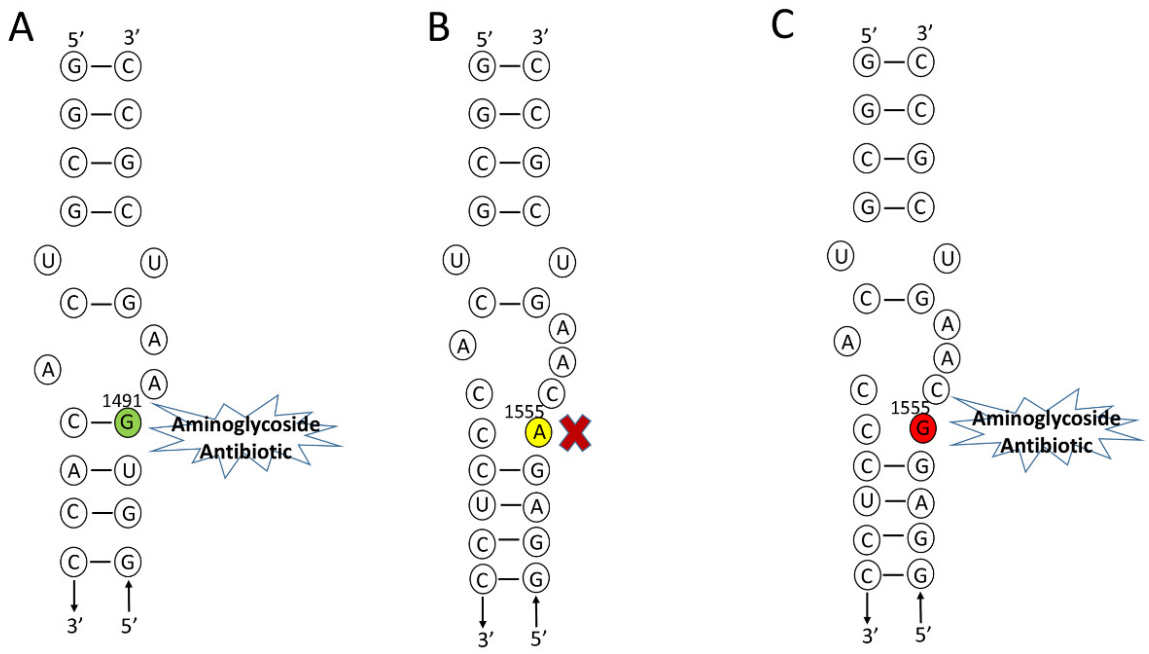

Fig. 1. Conformational changes of human mitochondrial 12S rRNA predicted by the m.1555A>G mutation. (A) Part of secondary structure of *E. coli* 16S rRNA with A-site. (B) The corresponding region of human mitochondrial 12S rRNA (wild-type). (C) 12S rRNA carrying the m.1555A>G mutation (mutant-type).

Conclusions

In current study, we presented several lines of evidences that support the m.1555A>G mutation on *MT-RNR1* gene to be associated with hearing impairment. A multitude of genetic studies analyzing various hearing loss-associated phenotypes have implicated crucial roles of the m.1555A>G mutation. Differences in the distribution of mutations in distinct regions of the world suggests that it occurred in each generation of ancestors and in different periods of immigrant populations. Despite inconsistent results, it remains important to study the genetics of hearing loss in different populations. Differences in genetic structure and allele frequencies across ethnic groups can help determine the exact role of this mutation around the world. Furthermore, the m.1555A>G mutation in the 12S rRNA gene is a molecular mechanism of aminoglycoside ototoxicity-related deafness. To mitigate the negative effect of aminoglycoside, we are capable of predicting individual's risk of ototoxicity by evaluating their pedigree or screening their 12S rRNA gene before use of these drugs. Carriers of the m.1555A>G mutation should avoid exposure to aminoglycoside and should be treated with alternative antibiotic to avoid the possibility of drug-induced hearing loss. It should be noted that mtDNA deafness-causing mutations (e.g., m.1555A>G) are not the only major risk factors; other genetic or environmental factors may be work together to cause sensorineural hearing disorders.

Author Contributions

LW—contributed to the idea, literature search, data acquisition and manuscript writing.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research was financially supported by the Natural Science Foundation of Zhejiang Province (grant number: LBY21H040001).

Conflict of Interest

The author declares no conflict of interest.

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