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# KCNQ4 c.546C>G variant is associated with early-onset high-frequency hearing loss, tinnitus, and cardiovascular comorbidities in Taiwanese adults

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**KCNQ4 encodes a voltage-gated potassium channel essential for ion balance and membrane potential regulation in inner ear hair cells. Mutations in KCNQ4 are associated with late-onset, high-frequency hearing loss that progressively worsens. This study aimed to compare carriers of the KCNQ4 c.546C>G variant with non-carriers to examine the relationship between this mutation and hearing loss, tinnitus, and cardiovascular diseases. This case–control study used data from the Taiwan Precision Medicine Initiative (TPMI) at Taichung Veterans General Hospital. A total of 95 KCNQ4 c.546C>G carriers and 95 non-carriers were recalled between August 2022 and June 2023. Participants underwent pure-tone audiometry, completed the Tinnitus Handicap Inventory (THI), and provided medical histories. Chi-square and Fisher's exact tests were used to compare categorical variables, and logistic regression assessed associations between various factors, THI scores, and hearing loss. The KCNQ4 carrier group showed significant hearing loss at 4 kHz (21.3 ± 16.1 dB) and 8 kHz (26.4 ± 21.6 dB), with greater severity at higher frequencies. The proportion of hearing loss was highest at 8 kHz (49.5%), followed by 4 kHz (33.7%) and 2 kHz (21.1%). THI scores and incidence of cardiovascular diseases were also significantly higher among carriers. Factors affecting mid- and high-frequency hearing loss included the KCNQ4 variant (odds ratio [OR], 2.07) and age (OR, 1.12). After adjusting for cardiovascular disease, carriers still exhibited significant hearing loss at 4 kHz and 8 kHz. Carriers younger than 40 years had a higher risk of hearing loss at 8 kHz (OR, 4.89). Genetic testing for the KCNQ4 c.546C>G variant and annual audiometric evaluations are strongly recommended for patients under 40 years old with high-frequency hearing loss, tinnitus, and cardiovascular comorbidities.**

**Keywords** Autosomal dominant nonsyndromic hearing loss, KCNQ4 c.546C>G, High-frequency hearing loss, Tinnitus, Cardiovascular disease

Hearing loss (HL) is a prevalent global health issue, often linked to hereditary factors. In 2019, approximately 1.57 billion individuals, or 20.3% of the world's population, experienced some form of hearing loss[1]. Non-syndromic hereditary hearing impairment (HHI) accounts for a significant portion of these cases and is associated with mutations in over 150 genes[2]. HHI can be categorized by onset as pre-lingual or post-lingual with genetic factors accounting for approximately 60% of pre-lingual deafness cases[3]. Currently, 63 genes are known to contribute to autosomal dominant non-syndromic hearing loss (ADNSHL)[2,4]. Among the genes implicated in ADNSHL, mutations in MYO6 (DFNA22) and TECTA (DFNA8/12) are particularly prevalent in European populations,

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accounting for 21% and 18% of cases, respectively[5]. Other notable genes include WFS1, KCNQ4, and COCH, each contributing to the diverse genetic landscape of hereditary hearing loss[6].

The KCNQ4 gene encodes a voltage-gated potassium channel critical for maintaining ion homeostasis and regulating the membrane potential of cochlear hair cells[7]. This channel is predominantly expressed in the outer hair cells of the organ of Corti, playing a vital role in auditory transduction[8]. KCNQ4 channels facilitate the efflux of potassium ions into the perilymph, which is essential for resetting hair cell membrane potentials after depolarization[7,9]. Mutations in KCNQ4 can disrupt potassium recycling, leading to persistent depolarization and eventual degeneration of outer hair cells, resulting in progressive sensorineural hearing loss[10].

To date, numerous variants of KCNQ4 have been identified, many of which are inherited missense mutations associated with autosomal dominant hearing loss[11,12]. The ClinVar database lists several pathogenic variants, with symptoms typically beginning in early adulthood and characterized by progressive high-frequency hearing loss[12]. The c.827G>C variant is the most common and was first reported by Dutch and Japanese researchers[13,14]. While KCNQ4 mutations generally present with progressive high-frequency hearing loss, the onset and severity can vary. Previous studies have demonstrated that mutations such as c.211delC and p.G285S lead to hearing impairment starting in early adulthood, with gradual progression over time[15–18]. However, a hospital-based study in Taiwan identified a link between the c.546C>G variant of KCNQ4 and moderate-to-high-frequency hearing loss in elderly individuals, though it was limited by a small sample size and retrospective design[19].

Tinnitus, defined as the perception of sound without an external source, is a common symptom that can result from various factors, including noise exposure, aging, ototoxic medications, and hearing loss[20–22]. The relationship between KCNQ4 mutations and tinnitus has not been thoroughly investigated. Our previous study indicated that approximately 41.7% of individuals with the KCNQ4 c.546C>G variant reported tinnitus[19], suggesting a potential association that warrants further exploration.

Emerging evidence suggests that the KCNQ4 c.546C>G variant may have systemic implications beyond auditory dysfunction. Dysfunction of Kv7.4 channels can lead to increased vascular smooth muscle contraction and elevated blood pressure, indicating a potential link between KCNQ4 mutations and hypertension[23–25]. Our prior research identified a significant association between the KCNQ4 c.546C>G variant and a higher prevalence of cardiovascular conditions such as hypertension, hyperlipidemia, aortic aneurysm, lower limb fractures, and diabetic polyneuropathy in a Taiwanese population[19]. A study from China indicated that the Kv7.4 channel, encoded by KCNQ4, may exacerbate vascular inflammatory responses, promoting neointimal hyperplasia and abdominal aortic aneurysm formation[26]. This suggests that the KCNQ4 c.546C>G variant may contribute to cardiovascular risk by affecting vascular smooth muscle function, although further research is needed to elucidate the underlying mechanisms.

Therefore, in this study, we aimed to explore the auditory phenotype, its relationship with tinnitus severity as measured by the Tinnitus Handicap Inventory (THI), and the incidence of cardiovascular disease in individuals with the c.546C>G variant of the KCNQ4 gene within a hospital-based adult population in Taiwan. By examining both auditory and cardiovascular parameters, we seek to provide a comprehensive understanding of the clinical implications of this genetic variant and its potential role in systemic health.

## Materials and methods

### Data source

This case–control study utilized data from the Taiwan Precision Medicine Initiative (TPMI), which collected data from a convenience sample of Taiwanese volunteers at Taichung Veterans General Hospital (TCVGH) from June 2019 to June 2020. The dataset included 32,728 adult patients aged 20 years or older (mean age 57.3 ± 15.1 years; 15,249 males, 17,479 females) with genotyping information, demographics, medical history, and biochemical reports. We identified 398 individuals harboring the c.546C>G variant in KCNQ4. All identified individuals were invited to participate in the study. Ultimately, 95 identified carriers agreed to participate. Additionally, 95 non-carriers from the TPMI were included as a control group.

### Participants

A total of 190 participants (mean age 42.5 ± 9.9 years; 40 males, 150 females) were recruited from the Taiwan Precision Medicine Initiative (TPMI) database and divided into two groups:

*Experimental group (n = 95)*

Inclusion Criteria: Carriers of the KCNQ4 c.546C>G gene variant.

Exclusion Criteria: Exclude individuals with other known hearing loss-associated gene variants (including but not limited to WFS1 c.2051C>T, SLC26A4 c.919-2A>G, SLC26A4 c.2168A>G, CEP78 c.1251 + 5G>A, GJB2 c.299_300del, and GJB2 c.235del).

Recruitment: 398 identified carriers were contacted, and 95 agreed to participate in the study.

*Control group (n = 95)*

Inclusion Criteria: Non-carriers of the KCNQ4 c.546C>G gene variant.

Exclusion Criteria: Exclude individuals carrying any known hearing loss-associated gene variants (including KCNQ4 c.546C>G and other listed variants).

Recruitment: Control participants were identified from the TPMI database, which initially included over 32,000 individuals. Individuals carrying known hearing loss-related genetic variants were excluded. From the remaining eligible population, participants matched to the experimental group by age and sex were selected and invited via telephone to participate in the study.

### Data collection
Participants provided genotyping data, demographic information (age and sex), and medical histories (ICD-9 diagnoses). During the recall phase, additional data were collected, including Pure-tone Audiometry (PTA), Tinnitus Handicap Inventory (THI) score, Body Mass Index (BMI), blood pressure measurements, family history of hearing loss, noise exposure history, lifestyle habits (smoking, alcohol consumption, exercise). Noise exposure was defined as long-term exposure to environments with noise levels above 85 dB. Cardiovascular disease history (hypertension, hyperlipidemia, diabetes mellitus) were recorded. The final cohort consisted of 95 individuals with KCNQ4 variants and 95 controls without specific hearing loss genes.

### Pure tone audiometry
Pure-tone audiometry was performed using a Grason–Stadler audiometer (Otometrics, USA). Air and bone conduction thresholds were measured at frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz, following standardized procedures. Pure-tone averages (PTAs) were calculated using thresholds of 500, 1000, 2000, and 4000 Hz. Hearing loss was classified as sensorineural hearing loss (SNHL), mixed hearing loss, or conductive hearing loss based on air and bone conduction patterns. An abnormal hearing threshold was defined as < 20 dB at any frequency. Hearing loss was categorized as sensorineural or mixed hearing loss in the ear, with high-frequency hearing loss defined as impairment in the 2000 8000 Hz range[27]. The worst hearing threshold at each frequency from the bilateral ear was recorded for the final analysis.

### Chinese-Mandarin version of the tinnitus handicap inventory
The Tinnitus Handicap Inventory (THI) is a 25-item questionnaire divided into three subscales: Functional (11 items), Emotional (9 items), and Catastrophic (5 items). Each iten has three response options: "No," "Mild," or "Severe." The total score classifies tinnitus severity into five grades: Grade 1 (0–16 points) indicates no or slight handicap; Grade 2 (18–36 points) represents mild handicap; Grade 3 (38–56 points) signifies moderate handicap requiring further assessment and treatment; Grades 4 (58–76 points) and 5 (78–100 points) denote severe to catastrophic handicap, necessitating comprehensive medical and psychological evaluation. The THI was translated into Mandarin Chinese using forward and backward translation methods[28].

### Statistical analysis
Descriptive statistics are presented as mean values and ranges. Continuous variables between the KCNQ4 and control groups were compared using the Mann–Whitney U test, while categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Univariable and multivariable logistic regression analyses were conducted to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for factors associated with abnormal hearing at 2, 4, and 8 kHz, as well as for Tinnitus Handicap Inventory (THI) grades 2–5, including the KCNQ4 c.546C>G variant and other demographic and health factors. The prevalence of abnormal hearing at these frequencies was stratified by age, sex, and cardiovascular disease status (defined as hypertension, diabetes mellitus, or hyperlipidemia), and odds ratios (ORs) were calculated. Statistical analyses were performed using SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA), with $p < 0.05$ considered statistically significant.

### Ethical consideration
This study adhered strictly to ethical standards and received approval from the Institutional Review Board (IRB) of Taichung Veterans General Hospital (TCVGH-IRB No.: CE21043A). The IRB confirm that all methods were carried out in accordance with relevant guidelines and regulations. In accordance with IRB regulations, informed consent was obtained from all participants.

## Results
### Demographics and health characteristics
As shown in Table 1, the KCNQ4 group had a significantly higher mean age compared to the control group (44.9 ± 10.3 years vs. 40.1 ± 9.0 years; $p = 0.001$). The proportion of females was significantly lower in the KCNQ4 group (71.6%, n = 68) than in the control group (86.3%, n = 82; $p = 0.013$). No significant difference in BMI was observed between the two groups (24.2 ± 4.4 vs. 23.0 ± 3.6; $p = 0.133$).

### Hearing test results
As detailed in Table 1, significant differences were observed in the hearing thresholds between the KCNQ4 and control groups at multiple frequencies.

0.25 kHz: 11.1 ± 7.2 dB vs. 8.6 ± 5.3 dB ($p = 0.007$)
0.5 kHz: 12.8 ± 6.6 dB vs. 10.6 ± 5.7 dB ($p = 0.028$)
2 kHz: 16.5 ± 10.7 dB vs. 12.5 ± 6.4 dB ($p = 0.030$)
4 kHz: 21.3 ± 16.1 dB vs. 14.6 ± 8.8 dB ($p = 0.009$)
8 kHz: 26.4 ± 21.6 dB vs. 16.6 ± 13.9 dB ($p = 0.001$)

The pure-tone average (PTA) was significantly higher in the KCNQ4 group (16.5 ± 8.8 dB) compared to the control group (12.8 ± 5.8 dB; $p = 0.002$). Abnormal hearing at 2, 4, and 8 kHz was more prevalent among KCNQ4 carriers (54.7%, n = 52) than among controls (29.5%, n = 28; $p < 0.001$).

### Tinnitus handicap inventory (THI)
As shown in Table 1, the mean THI score was significantly higher in the KCNQ4 group (9.5 ± 16.7) compared to the control group (2.2 ± 7.0; $p < 0.001$), indicating a greater tinnitus burden among carriers.

| | Total (n = 190) | | GENE | | | | |
|---|---|---|---|---|---|---|---|
| | | | KCNQ4 (n = 95) | | Control (n = 95) | | |
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | *p* value |
| Age | 42.5 | ± 9.9 | 44.9 | ± 10.3 | 40.1 | ± 9.0 | 0.001** |
| Female, n (%) | 150 | (78.9%) | 68 | (71.6%) | 82 | (86.3%) | 0.013* |
| BMI (kg/m$^2$) | 23.6 | ± 4.0 | 24.2 | ± 4.4 | 23.0 | ± 3.6 | 0.133 |
| Hearing (dB) | | | | | | | |
| 0.25 kHz | 9.8 | ± 6.4 | 11.1 | ± 7.2 | 8.6 | ± 5.3 | 0.007** |
| 0.5 kHz | 11.7 | ± 6.3 | 12.8 | ± 6.6 | 10.6 | ± 5.7 | 0.028* |
| 1 kHz | 14.4 | ± 7.8 | 15.4 | ± 8.6 | 13.4 | ± 6.8 | 0.127 |
| 2 kHz | 14.5 | ± 9.0 | 16.5 | ± 10.7 | 12.5 | ± 6.4 | 0.030* |
| 4 kHz | 18.0 | ± 13.4 | 21.3 | ± 16.1 | 14.6 | ± 8.8 | 0.009** |
| 8 kHz | 21.5 | ± 18.7 | 26.4 | ± 21.6 | 16.6 | ± 13.9 | 0.001** |
| Pure-tone threshold average(dB) | 14.7 | ± 7.7 | 16.5 | ± 8.8 | 12.8 | ± 5.8 | 0.002** |
| Abnormal_Hearing 2 k/4 k/8 kHz, n (%) | 80 | (42.1%) | 52 | (54.7%) | 28 | (29.5%) | < 0.001** |
| 2 kHz | 26 | (13.7%) | 20 | (21.1%) | 6 | (6.3%) | 0.003** |
| 4 kHz | 46 | (24.2%) | 32 | (33.7%) | 14 | (14.7%) | 0.002** |
| 8 kHz | 70 | (36.8%) | 47 | (49.5%) | 23 | (24.2%) | < 0.001** |
| Tinnitus handicap inventory | 5.9 | ± 13.3 | 9.5 | ± 16.7 | 2.2 | ± 7.0 | < 0.001** |
| Tinnitus handicap inventory, n (%) | | | | | | | 0.002** |
| Grade 1 | 166 | (87.4%) | 76 | (80.0%) | 90 | (94.7%) | |
| Grade 2–5 | 24 | (12.6%) | 19 | (20.0%) | 5 | (5.3%) | |
| Cardiovascular disease, n (%) | 40 | (21.1%) | 30 | (31.6%) | 10 | (10.5%) | < 0.001** |
| Hypertension | 24 | (12.6%) | 20 | (21.1%) | 4 | (4.2%) | < 0.001** |
| DM | 8 | (4.2%) | 7 | (7.4%) | 1 | (1.1%) | 0.065 |
| Hyperlipidemia | 23 | (12.1%) | 16 | (16.8%) | 7 | (7.4%) | 0.045* |
| SBP ≥ 140 or DBP ≥ 90, n (%) | 18 | (9.5%) | 15 | (15.8%) | 3 | (3.2%) | 0.003** |
| Family history of hearing loss, n (%) | 79 | (41.6%) | 49 | (51.6%) | 30 | (31.6%) | 0.005** |
| Noise exposure, n (%) | 13 | (6.8%) | 11 | (11.6%) | 2 | (2.1%) | 0.010* |
| Habits, n (%) | | | | | | | |
| Smoking | 15 | (7.9%) | 11 | (11.6%) | 4 | (4.2%) | 0.060 |
| Drinking | 14 | (7.4%) | 9 | (9.5%) | 5 | (5.3%) | 0.267 |
| Exercise | 84 | (44.2%) | 45 | (47.4%) | 39 | (41.1%) | 0.381 |

**Table 1**. Comparison of demographic, health, and hearing characteristics between KCNQ4 and control groups. Mann–Whitney U test. Chi-Square test. Fisher's Exact test. *$p < 0.5$, **$p < 0.01$. Body Mass Inde, BMI. Diabetes mellitus, DM. Systolic blood pressure, SBP.

## Cardiovascular disease

Table 1 also indicates that cardiovascular disease was more prevalent among carriers (31.6%) than controls (10.5%; $p < 0.001$), particularly:

Hypertension: 21.1% (n = 20) of carriers vs. 4.2% (n = 4) of controls ($p < 0.001$).

Hyperlipidemia: 16.8% (n = 16) carriers vs. 7.4% (n = 7) controls ($p = 0.045$).

Diabetes Mellitus: No significant difference between groups.

## Family history and noise exposure

As shown in Table 1, a higher proportion of KCNQ4 carriers reported a family history of hearing loss (51.6%, n = 49) compared to controls (31.6%, n = 30; $p = 0.005$). Noise exposure was also more frequently reported in the KCNQ4 group (11.6%, n = 11) than in controls (2.1%, n = 2; $p = 0.010$).

## Logistic regression analysis

The associations between clinical variables and abnormal hearing are summarized in Table 2.

*Univariable analysis*

Abnormal Hearing at 2–8 kHz: The KCNQ4 variant was significantly associated (odds ratio [OR] = 2.89; 95% confidence interval [CI]: 1.59–5.26; $p < 0.001$).

Age: Each additional year increased the odds of abnormal hearing (OR = 1.14; 95% CI 1.09–1.18; $p < 0.001$).

Cardiovascular Disease: Associated with abnormal hearing (OR = 3.80; 95% CI 1.81–7.98; $p < 0.001$).

Hypertension and Hyperlipidemia: Hypertension (odds ratio [OR] = 3.19; $p = 0.012$) and hyperlipidemia (OR = 6.10; $p = 0.001$) were significant risk factors.

| | Simple model | | | Multiple model | | |
|---|---|---|---|---|---|---|
| | OR | (95%CI) | *p* value | OR | (95%CI) | *p* value |
| KCNQ4 vs Control | 2.89 | (1.59–5.26) | < 0.001** | 2.07 | (1.02–4.20) | 0.043* |
| Age | 1.14 | (1.09–1.18) | < 0.001** | 1.12 | (1.07–1.17) | < 0.001** |
| Female | 0.67 | (0.33–1.34) | 0.257 | | | |
| BMI (kg/m$^2$) | 1.05 | (0.98–1.13) | 0.146 | | | |
| Cardiovascular disease | 3.80 | (1.81–7.98) | < 0.001** | | | |
| Hypertension | 3.19 | (1.29–7.87) | 0.012* | 0.75 | (0.23–2.41) | 0.630 |
| DM | 4.38 | (0.86–22.29) | 0.075 | | | |
| Hyperlipidemia | 6.10 | (2.16–17.24) | 0.001** | 2.60 | (0.78–8.64) | 0.119 |
| SBP ≥ 140 or DBP ≥ 90 | 4.07 | (1.39–11.95) | 0.010* | | | |
| Family history | 1.52 | (0.85–2.73) | 0.159 | | | |
| Noise exposure | 1.19 | (0.39–3.70) | 0.760 | | | |
| Habits | | | | | | |
| Smoking | 1.63 | (0.57–4.71) | 0.363 | | | |
| Drinking | 1.41 | (0.47–4.20) | 0.536 | | | |
| Exercise | 0.97 | (0.54–1.73) | 0.913 | | | |

**Table 2**. Logistic regression analysis of factors associated with abnormal hearing at 2 k/4 k/8 kHz. Logistic regression. *$p < 0.05$, **$p < 0.01$. BMI, Body Mass Index; DM, Diabetes mellitus; SBP, Systolic blood pressure.

| | Simple model | | | Multiple model | | |
|---|---|---|---|---|---|---|
| | OR | (95%CI) | *p* value | OR | (95%CI) | *p* value |
| KCNQ4 vs Control | 4.50 | (1.60–12.62) | 0.004** | 3.20 | (1.02–10.06) | 0.046* |
| Age | 1.06 | (1.02–1.11) | 0.008** | 1.02 | (0.97–1.08) | 0.391 |
| Female | 0.77 | (0.28–2.10) | 0.613 | | | |
| BMI (kg/m$^2$) | 1.04 | (0.94–1.15) | 0.443 | | | |
| Cardiovascular disease | 3.24 | (1.31–7.99) | 0.011* | 1.13 | (0.34–3.80) | 0.841 |
| Hypertension | 2.74 | (0.96–7.80) | 0.059 | | | |
| DM | 2.42 | (0.46–12.77) | 0.296 | | | |
| Hyperlipidemia | 3.86 | (1.39–10.71) | 0.009** | | | |
| SBP ≥ 140 or DBP ≥ 90 | 0.38 | (0.05–3.00) | 0.360 | | | |
| Family history | 1.00 | (0.42–2.39) | 0.993 | | | |
| Noise exposure | 7.57 | (2.29–25.00) | 0.001** | 3.24 | (0.72–14.66) | 0.126 |
| Habits | | | | | | |
| Smoking | 16.00 | (5.01–51.07) | < 0.001** | 5.19 | (1.14–23.72) | 0.034* |
| Drinking | 6.58 | (2.05–21.11) | 0.002** | 2.83 | (0.55–14.49) | 0.211 |
| Exercise | 0.59 | (0.24–1.46) | 0.255 | | | |

**Table 3**. The logistic regression analysis for the Tinnitus Handicap Inventory (THI) grades 2–5. Logistic regression. *$p < 0.05$, **$p < 0.01$. BMI, Body mass index; DM, Diabetes mellitus; SBP, Systolic blood pressure.

*Multivariable analysis*

After adjusting for age and cardiovascular disease:

KCNQ4 Variant: Remained significantly associated with abnormal hearing (OR = 2.07; 95% CI 1.02–4.20; $p = 0.043$).

Age: Continued to be significant (OR = 1.12; 95% CI 1.07–1.17; $p < 0.001$).

Cardiovascular Disease: No longer significant when adjusted for other factors.

## Association with tinnitus severity

Associations between variables and tinnitus severity (THI grades 2–5) are shown in Table 3.

*Univariable analysis*

The KCNQ4 Variant: Significantly associated with higher THI grades (grades 2–5) (OR = 4.50; 95% CI 1.60–12.62; $p = 0.004$).

Age: Increased odds of higher THI grades (OR = 1.06; 95% CI 1.02–1.11; $p = 0.008$).

Cardiovascular Disease: Associated with a higher THI grade (OR = 3.24; $p = 0.011$).

Hyperlipidemia was a significant risk factor (OR = 3.86; $p = 0.009$).

Noise exposure showed a strong association (OR = 7.57; $p = 0.001$).

Smoking was strongly associated with a higher THI grade (OR = 16.00; $p < 0.001$).
Drinking: A significant association was observed (OR = 6.58, $p = 0.002$).

*Multivariable analysis*
KCNQ4 Variant: Remained significant (OR = 3.20; 95% CI 1.02–10.06; $p = 0.046$).
Smoking continued to have a significant effect (odds ratio [OR] = 5.19, $p = 0.034$).
Age, cardiovascular disease, noise exposure, and drinking: Associations were not significant after adjustment.

## Prevalence of abnormal hearing stratified by age, sex, and cardiovascular disease

As illustrated in Fig. 1, the overall prevalence of abnormal hearing was higher in the KCNQ4 group (54.7% vs. 29.5%; OR = 2.89; $p < 0.001$).

*Age stratification*
< 40 Years: carriers had a higher abnormal hearing prevalence (30.3% vs. 11.1%; OR = 3.48; $p = 0.030$).
40–50 Years: No significant difference (46.4% vs. 47.8%; OR = 0.95; $p = 0.921$).
≥ 50 Years: Higher prevalence in carriers (85.3% vs. 61.1%; OR = 3.69; $p = 0.056$).

*Sex stratification*
Females: Higher incidence of abnormal hearing in carriers (51.5% vs. 30.5%; OR = 2.42; $p = 0.010$).
Males: A significantly higher number of carriers (63.0% vs. 23.1%; OR = 5.67; $p = 0.024$).

*Cardiovascular disease*
Without Cardiovascular Disease: Carriers had higher prevalence (44.6% vs. 28.2%; OR = 2.05; $p = 0.039$).
The incidence of cardiovascular disease was significantly higher in carriers than in non-carriers (76.7% vs. 40.0%; OR = 4.93; $p = 0.040$).

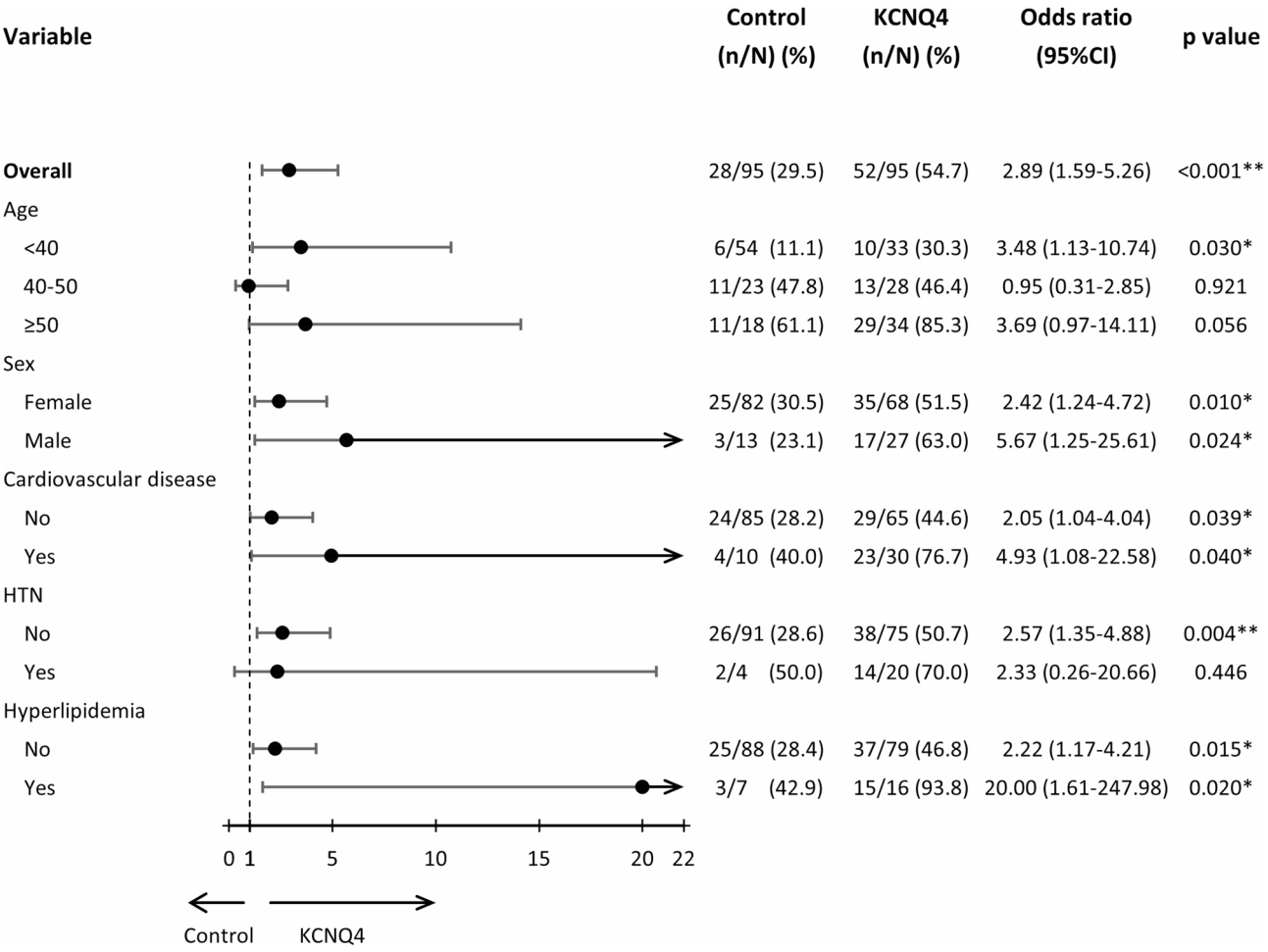


**Fig. 1**. Prevalence of abnormal hearing at 2 kHz, 4 kHz, or 8 kHz stratified by age, sex, and cardiovascular disease status.

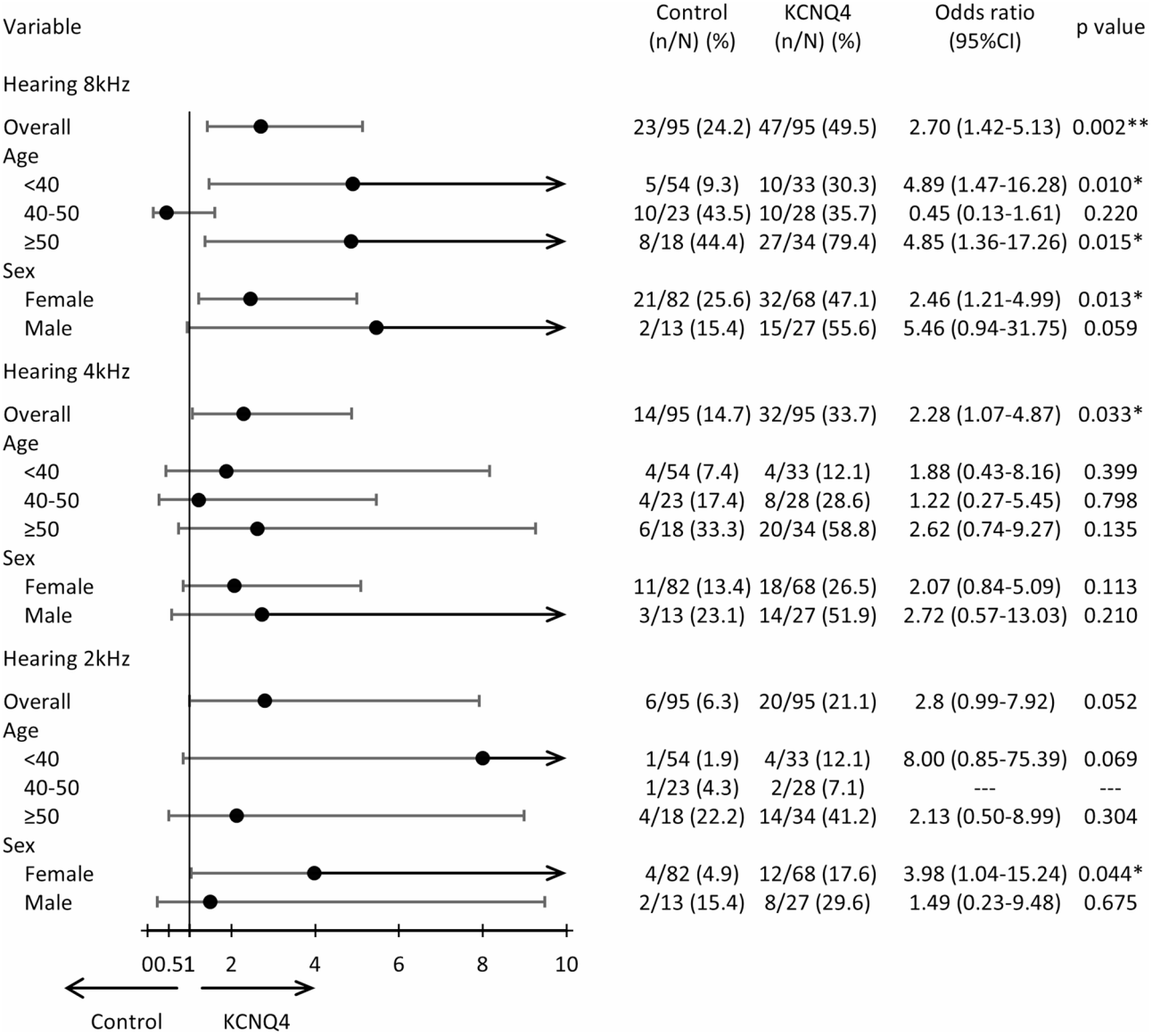


**Fig. 2**. The prevalence of abnormal hearing at 2 kHz, 4 kHz, and 8 kHz stratified by age and sex.

## Frequency-specific hearing loss

Figure 2 presents the prevalence of abnormal hearing at 2, 4, and 8 kHz.

*8 kHz*

Overall, carriers had a higher prevalence (49.5% vs. 24.2%; OR = 2.70; $p = 0.002$).

< 40 Years: Significantly higher in carriers (30.3% vs. 9.3%; OR = 4.89; $p = 0.010$).

≥ 50 Years: Higher prevalence (79.4% vs. 44.4%; OR = 4.85; $p = 0.015$).

Females: Increased abnormal hearing in carriers (47.1% vs. 25.6%; OR = 2.46; $p = 0.013$).

*4 kHz*

Overall, carriers had a higher prevalence (33.7% vs. 14.7%; OR = 2.28; $p = 0.033$).

Age and Sex Stratification: Trends toward higher prevalence in carriers, but not statistically significant.

*2 kHz*

Overall, carriers had a higher prevalence (21.1% vs. 6.3%; OR = 2.80; $p = 0.052$).

Females: significantly higher prevalence in carriers (17.6% vs. 4.9%; OR = 3.98; $p = 0.044$).

## Discussion

This study demonstrates that the KCNQ4 c.546C>G variant is significantly associated with an increased risk of high-frequency sensorineural hearing loss, particularly at 8 kHz, and more severe tinnitus, as measured by

the Tinnitus Handicap Inventory (THI). These associations persisted after adjusting for age and cardiovascular factors, suggesting an independent effect of the KCNQ4 variant on auditory function. Additionally, carriers exhibited a higher prevalence of cardiovascular disease, indicating a potential link that warrants further investigation. To our knowledge, this is the first case–control recall study using pure-tone audiometry (PTA) and THI to assess individuals with the KCNQ4 c.546C>G mutation with a focus on its clinical implications. Building upon previous research involving 12 carriers over 65 years old using retrospective audiogram reviews[19], we expanded the cohort to include 95 carriers with a mean age of 44.9 years, predominantly female (71.6%).

By conducting audiometric assessments, our study provided a more precise evaluation of hearing function and allowed for a direct comparison of the prevalence and severity of hearing loss. Clinically, carriers of the KCNQ4 c.546C>G mutation exhibited significantly higher odds of sensorineural hearing loss at key frequencies of 2, 4, and 8 kHz, independent of age, sex, or cardiovascular risk factors such as hypertension and hyperlipidemia. Notably, this mutation was associated with elevated hearing thresholds at higher frequencies, indicating early onset high-frequency hearing loss. Female carriers showed increased odds of hearing impairment at 2 and 8 kHz. Multivariate logistic regression confirmed significant associations between the KCNQ4 mutation and abnormal audiometric thresholds, as well as elevated THI scores, reflecting a greater burden of tinnitus among mutation carriers.

In our study, individuals carrying the KCNQ4 c.546C>G mutation exhibited significant high-frequency sensorineural hearing loss, with mean thresholds at 4 kHz (21.3 ± 16.1 dB) and 8 kHz (26.4 ± 21.6 dB) exceeding the clinical threshold for hearing impairment (> 20 dB). The severity and prevalence of hearing loss increased with frequency, affecting 21.1%, 33.7% at 4 kHz, and 49.5% at 2, 4, and 8 kHz, respectively, indicating a more pronounced deterioration at higher frequencies. While our cross-sectional design precludes confirmation of a progressive pattern, stratified analysis revealed that participants under 40 years old with the KCNQ4 mutation had significantly higher odds of high-frequency hearing impairment (odds ratio [OR]: 3.48; 95% confidence interval [CI]: 1.13–10.74; $p = 0.03$), aligning with previous findings that hearing loss in KCNQ4-related cases initiates at high frequencies[16].

Hearing loss is influenced by multiple factors, including genetic predisposition, environmental noise exposure, and the natural aging process. The contribution of each factor differs across age groups. In individuals under 40, hearing loss is more likely to be driven by genetic factors[29]. In the 40–50 age group, occupational and environmental exposures—particularly noise—may exert a more pronounced influence, potentially amplifying the effects of genetic susceptibility[30]. In contrast, for individuals over 50, age-related hearing decline (presbycusis) becomes the dominant factor[31]. These overlapping yet age-dependent etiologies may explain the differential patterns observed among the age groups in our study.

This contrasts with earlier studies, such as the Japanese research[16,32], which reported diagnoses typically between ages 5 and 15 and progression to severe impairment by age 50, without focusing on the specific c.546C>G mutation or including a control group. Our study, with a larger sample size and a control cohort, specifically highlights the impact of the KCNQ4 c.546C>G mutation in adults around 45 years of age, suggesting that mutant KCNQ4 protein accumulation may overwhelm cellular protein quality control mechanisms during aging, leading to cytotoxicity[18]. Clinically, these findings underscore the importance of early audiological screening for high-frequency hearing loss in KCNQ4 mutation carriers, even before the age 40. Recognizing that 51.6% of carriers reported a family history of hearing loss, genetic counseling and hearing assessments for family members are imperative for early detection and intervention. Additionally, our study expanded previous research by quantitatively analyzing tinnitus severity using the Tinnitus Handicap Inventory (THI), revealing that while 80% of KCNQ4 carriers did not experience significant tinnitus handicap, those affected may benefit from early psychological support due to the association of mild tinnitus with depression[33]. Overall, our findings highlight the need for proactive clinical management of hearing loss and tinnitus in KCNQ4 c.546C>G mutation carriers, differentiating our study from by focusing on genetic analysis, including a control group, and emphasizing clinical applications.

Noise exposure was reported more frequently in the KCNQ4 variant group (11.6%) than in the control group (2.1%, $p = 0.010$), suggesting potential environmental contributions to hearing impairment in genetically susceptible individuals. However, noise exposure did not emerge as a significant independent predictor of abnormal hearing at 2 k/4 k/8 kHz in multivariable logistic regression analysis (OR = 1.19, $p = 0.760$), indicating that its effect may be modest after adjusting for age and other covariates. Furthermore, due to limitations in our dataset, we were unable to systematically evaluate the relationship between noise exposure, occupation type, and sex. As such, while the difference in noise exposure prevalence may suggest an additive risk, particularly among KCNQ4 carriers, this observation should be interpreted with caution. Future studies with detailed occupational histories and objective noise exposure measures are warranted to clarify the interaction between genetic susceptibility and environmental auditory stressors.

Previous studies have suggested that the KCNQ4 c.546C>G variant may increase the risk of cardiovascular and systemic conditions such as aortic aneurysms, lower limb fractures, and diabetic polyneuropathy[19]. In the current study, we observed a significant association between the KCNQ4 c.546C>G mutation and the prevalence of hypertension and hyperlipidemia, suggesting that KCNQ4 mutations may have systemic implications beyond auditory dysfunction.

An animal study reported that the voltage-dependent potassium channels (Kv7.4) encoded by KCNQ4 contribute to β-adrenergic receptor-mediated vasodilation of renal arterial smooth muscle[34]. Downregulation of Kv7.4 channels may be critical in the development of hypertension[34]. We speculate that alterations in Kv7.4 channel function due to the KCNQ4 c.546C>G mutation may lead to hypertension and poor glycemic control in patients with diabetes. The accumulation of mutant KCNQ4 proteins may overwhelm the cellular protein quality control system during aging, causing cytotoxic effects in vascular tissues[35]. Blood pressure in women may increase during menopause. Pre-menopausal women have greater coronary and cerebral blood flow and

a lower incidence of adverse cardiovascular events compared to men, but this protective effect diminishes after menopause[36]. Given that our KCNQ4 cohort was predominantly female, with a mean age of 44.9 years, the onset of menopause may have contributed to the increased prevalence of hypertension. Experimental results indicate that KCNQ4 does not form heteromeric channels with KCNQ1 or KCNQ2 in the cochlea when these potassium channel subunits are co-expressed in Xenopus oocytes[37], suggesting that the effects of the KCNQ4 mutation are likely due to alterations in homomeric KCNQ4 channels, rather than interactions with other KCNQ subunits.

In our study, we observed a higher prevalence of hyperlipidemia among KCNQ4 c.546C>G mutation carriers than among controls. While KCNQ4 mutations are known to cause auditory dysfunction, their association with hyperlipidemia has not been extensively explored. Previous research has focused on other KCNQ genes, such as KCNQ1, which are associated with cardiovascular conditions and metabolic disorders such as type 2 diabetes mellitus[38,39]. The increased prevalence of hyperlipidemia in KCNQ4 mutation carriers suggests that this genetic variant has a broader physiological impact. One hypothesis is that KCNQ4 mutations affect vascular smooth muscle function due to altered potassium channel activity, potentially contributing to dyslipidemia and hypertension. Gao et al.[35] proposed that the accumulation of mutant KCNQ4 proteins may overwhelm the cellular protein quality control systems during aging, leading to cytotoxic effects in both auditory and vascular tissues.

Currently, no evidence links KCNQ4 to type 2 diabetes mellitus (DM); however, KCNQ1 has been associated with type 2 DM, although its role in molecular pathogenesis remains unclear. Studies involving the Japanese, Singaporean, Danish, and Chinese Han populations have demonstrated an association between KCNQ1 and type 2 DM[40,41]. Mutations in all five KCNQ genes (KCNQ1–KCNQ5) have been implicated in various human diseases, including long QT syndrome and type 2 DM (KCNQ1)[42], epileptic encephalopathy (KCNQ2/3)[43,44], deafness (KCNQ4)[15], and complex central nervous system symptoms, including intellectual disability and epileptic encephalopathy (KCNQ5)[45,46].

In our study, the control group exhibited a notable prevalence of hearing impairment despite being younger and predominantly female, with 31.6% of reporting a family history of hearing loss, potentially indicating higher health awareness. Age was a critical factor, as the KCNQ4 mutation group was older, and advancing age is known to affect hearing acuity, tinnitus severity, and cardiovascular health[47]. KCNQ4 mutation carriers had significantly higher odds of abnormal hearing at 2, 4, and 8 kHz (OR: 2.07; 95% CI 1.02–4.20; $p = 0.043$), suggesting the mutation contributes to auditory dysfunction beyond age-related effects. Hypertension and hyperlipidemia, which are prevalent among carriers, may also contribute to hearing loss[48]. Additionally, carriers had increased odds of higher Tinnitus Handicap Inventory grades (OR: 3.20; 95% CI 1.02–10.06; $p = 0.046$), with smoking significantly associated with tinnitus severity (OR: 5.19; 95% CI 1.14–23.72; $p = 0.034$). Ion channels are promising therapeutic targets, and potassium channel openers have been developed; thus, KCNQ4 a potential target for pharmacological intervention to prevent progressive hearing loss and treat tinnitus[49].

## Strengths

The strengths of this study include the large sample size of KCNQ gene mutation carriers, which enhances statistical power, and the use of audiometric assessments. These features provide comprehensive and up-to-date data on participants' hearing profiles.

## Limitations

This study has several limitations. Potential selection bias may exist due to age and sex differences between groups. The control group was younger and predominantly female. This sex imbalance, however, reflects the demographic characteristics of the TPMI database, which mainly consists of patients from rheumatology and immunology clinics where female patients are overrepresented. As such, the observed female predominance is likely attributable to the recruitment source rather than selection bias or true differences in variant prevalence. Cardiovascular diseases were identified using ICD-9 codes, which may have resulted in misclassifications. Additionally, the limited exploration of family medical histories, along with insufficient historical data on smoking, alcohol consumption, and physical activity, restricts our ability to fully assess their impact on auditory health. Future prospective studies with more representative control groups are needed to validate these findings and assess the progression of hearing impairment over time.

## Conclusions

In conclusion, this cross-sectional study demonstrated that KCNQ4 c.546C>G carriers experienced significant high-frequency hearing loss before the age of 40. Moreover, a higher rate of comorbid cardiovascular disease was observed in KCNQ4 c. 546C>G carriers. Hearing loss in these carriers was more pronounced at higher frequencies, and the tinnitus score was worse compared to the control group. Clinically, patients with early-onset, severe high-frequency hearing loss, tinnitus, and coexisting cardiovascular disease should be recommended for further KCNQ4 gene testing and regular audiological follow-up.

## Data availability

The raw data supporting the findings of this study are available in the supplementary files provided with this article.

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## Author contributions
KLW and TGC contributed equally to this work as co-first authors, responsible for manuscript preparation and conducting the research. YMC was responsible for the research logic, ICC for database organization, and CYH for statistical analysis and figure preparation. TTY, the corresponding author, supervised the research and was responsible for writing and revising the manuscript.

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## Competing interests
The authors declare no competing interests.

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