Gene-environment interaction between angiotensinogen and chronic exposure to occupational noise contribute to hypertension

Running Head: AGT, Noise and Hypertension

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Abbreviations

CI = confidence interval

AGT = Angiotensiongen

dBA = A-weighted decibels

IA = interaction

PY = person-years at risk

IR = incidence rate

IRR = incidence rate ratio

ABSTRACT

Objectives Previous studies on the effects of angiotensinogen (AGT) gene polymorphisms and chronic exposure to occupational noise on the risk of hypertension have mainly been cross-sectional or prevalent case-control studies, where temporality constitutes problems. The present study was to assess longitudinally both independent and joint effects of AGT gene polymorphisms and chronic exposure to occupational noise on occurrence of hypertension.

Methods We conducted a 20-year prospective cohort study of 1,301 aviation workers in Taiwan. The study population included 912 workers without hypertension at baseline. The outcome of interest was the development of hypertension during the study period. The studied determinants were three AGT genotypes (TT, TM and MM) and four exposure categories according to the levels of noise representing high (> 80 dBA), medium (80-65 dBA), low exposure (64-50 dBA), and the reference level (49-40 dBA).

Results In Poisson regression adjusting for confounders, AGT (TT vs. MM adjusted incidence rate ratio [IRR] 1.77, 95% CI 1.24-2.51) and noise exposure (high and medium combined) during 3-15 years (adjusted IRR 2.35, 95 % CI 1.42-3.88) were independent determinants of hypertension. Further, the risk of hypertension increased with noise exposure (adjusted IRR 3.73; 95% CI 1.84, 7.56) among TT homozygotes, but not among those with at least one M allele (Rothman synergy index = 1.05).

Conclusions The results evidence further the independent effects of AGT gene polymorphisms and exposure to occupational noise. Our finding also suggests that workers carrying TT variant allele have higher risk of hypertension under chronic exposure to occupational noise.

Key words: AGT, noise, hypertension, interaction, effect modification

What is known

► Findings from previous studies on the effects of angiotensinogen (AGT) gene polymorphisms and chronic exposure to occupational noise on the risk of hypertension have mainly been cross-sectional or prevalent case-control studies, where temporality constitutes problems.

What this paper adds

► Our prospective cohort study obtains further evidence on the independent effects of AGT gene polymorphisms and chronic exposure to occupational noise.

► Our finding also suggests that workers carrying TT variant allele have higher risk of hypertension under chronic exposure to occupational noise.

Policy implications

► Genotyping for AGT polymorphisms, a simple and inexpensive assay, could be a suitable biomarker identifying genetically susceptible workers in the development of hypertension under occupational noise exposure.

INTRODUCTION

In 1992, Jeunemaitre et al. first reported that angiotensinogen (AGT) M235T gene polymorphisms were associated with the risk of hypertension, yielding an odds ratio of 1.95 for TT genotype compared with the MM wide type.[1] Since then, a series of epidemiological studies found inconsistent results in different ethnic populations. Three recent critical reviews suggested that the AGT TT genotype was associated with the risk of hypertension, particularly in white and Asian subjects.[2-4]

There were limited studies concerning chronic noise exposure and the prevalence of hypertension.[5-13] A recent review of nine studies suggested a positive association between occupational noise exposure and hypertension (RR 1.14 per 5 dBA, noise range 55-116 dBA, 95% CI 1.01-1.29).[14] These previous epidemiological studies were mainly cross-sectional or case-control studies with prevalent rather than incident cases, where selection and information bias as well as establishment of temporality between exposure and outcome constitute problems. We identified only one previous longitudinal study assessing exposure prior to the onset of hypertension. It reported that exposure to noise above 85 A-weighted decibels (dBA) for more than 30 years was associated with the development of hypertension.[15] Furthermore, potential modification of the relation between noise exposure and development of hypertension by AGT gene polymorphisms (i.e., gene-environment interaction) has not been investigated.

We conducted a prospective 20-year cohort study on the relation between exposure to noise and development of hypertension in Taiwan. This design enabled us at the same time to verify an appropriate temporality between the hypothesized exposure and outcome and to eliminate the possibility that the presence of outcome would influence the assessment of exposure. We also tested the hypothesis that the joint

effect of AGT and noise exposure on the risk of hypertension is stronger than expected on the basis of their independent effects. We assumed that the presence of the AGT TT genotype increases the susceptibility of workers to the effects of noise exposure on developing hypertension.

METHODS

Study population

The source population included all aviation workers in the aircraft industry in Taiwan. At the cohort entry, informed consent forms and baseline questionnaires were distributed in June 1988. The baseline study population included a total of 1301 workers who completed the questionnaires and health check-ups. We conducted annual follow-up survey directed at all the workers of the cohort from 1988 through 2008. The follow-up time began from the first date of employment of each subject until the onset of hypertension. Completed questionnaires, health check-ups, noise exposure assessment and blood sample were obtained from 993 workers (76.3% of the baseline study population). In the present analyses, we excluded workers with hypertension discovered by the baseline health check-ups (N = 52), and those with missing information on noise exposure assessment (N = 29). Thus, the study population comprised a total of 912 workers.

Data collection

In the baseline survey, workers were asked about their personal characteristics, health, working history, lifestyle, other relevant factors and health examination. Some of the questions on cardiovascular health were adopted from the American Heart Association and Taiwan Heart Association. The follow-up survey included questions about environment identical to those of the baseline, as well as more detailed information on use of personal protection equipment (PPE). The study protocol was approved by the

Institutional Review Board of China Medical University, and it complied with the principles outlined in the Helsinki Declaration.

Definition of hypertension

The outcome of interest was the development of hypertension during the study period. The definition of hypertension was doctor-diagnosed hypertension, or the mean value of three measurements of resting systolic blood pressure (SBP) ≥ 140 mm Hg or the mean value of resting diastolic blood pressure (DBP) ≥ 90 mm Hg in the annul health check-up. The definition followed that in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[16] According to these criteria, we identified those who developed hypertension during the study period. The information on the age of onset of hypertension was employed to calculate the person-time at risk.

AGT genotyping

Genomic DNA extraction was performed using the DNA Blood Mini Kit by QIAGEN®. DNA fragments including the AGT M235T variant were amplified a 303 base pair (bp) long by allele-specific polymerase chain reaction (PCR). The forward primer sequence from +921 to +941 in exon 2 of the AGT gene is 5' GAT GCG CAC AAG GTC CTG TC 3' whereas the reverse primer sequence from +1202 to +1224 is 5' GGT GCT GTC CAC ACT GGA CCC C 3'. The reverse primer was designed to contain a base substitution T→C at the fourth last nucleotide from its 3' end. The individual PCR reaction vial contains a final volume of 20 µl solution. Two hundred nanograms of DNA samples were added to 8 µl of PCR master mix consisting of 2.0 µl of 10X Mg Free PCR Buffer, 2.0 µl of 10 µM forward primer, 2.0 µl of 10 µM reverse primer and 2.0 ml of 10 mM dNTP. An appropriate amount of sterile ultrapure water (which amounts to 20 µl) was added to each of the microfuge tube. One micro liter of

5 units/µl *Taq* DNA polymerase was added to the reaction vial only after 5 minutes of pre-denaturation process prior to performing 'hot start' PCR. The PCR was performed for 35 cycles using the Thermal Cycler (Applied Biosystems 9800®). The temperature for the initial denaturation of DNA was 95°C for 1 minute, annealing at 68°C for 1 minute and extension at 72°C for 1 minute and a final extension at 72°C for 7 minutes following the last cycle. The PCR product was subjected to *Psy*I (isoschizomere for *Tth111*I restriction enzyme) digestion for 3 hours at 37°C and electrophoresed on a 3.0% agarose gel with ethidium bromide staining. The AGT M235T polymorphism was visualized as 279 bp and 24 bp fragments for TT genotype, 303 pb fragment for MM genotype and 303 bp, 279 bp, 24 pb fragments for TM genotype.[17] All assays were performed by a laboratory worker unaware of the clinical status of individual workers, and genotype assignments were made according to two consistent experimental results. About 15% of randomly selected samples were directly sequenced, and all of them were concordant with the initial genotyping results.

Noise exposure assessment

We focused on 794 workers who were not involved in work shift that changed their level of noise exposure during the study period. The noise exposure at the workplace was assessed according to cumulative data over the study period. We used a sound analyzer (TES-1358, TES Electronic Corp., Taipei, Taiwan), which can report 1-sec to 24-h continuous equivalent sound levels (Leq) in the range of 30-130 dBA and time-weighted-average (TWA) noise levels. The 15-min TWA Leq was collected at 332 locations (approximately 9 m^2 for each location) around the manufactory by industrial hygienists. For those measured with 15-min TWA Leq $\geq$ 65 dBA, 8-h TWA measurements were further conducted at 121 locations. Since it would be impossible to measure the subjects' individual noise levels every day, all subjects were divided into one

of the similar exposure groups on the basis of the similarity and frequency of tasks, agents and processes with which they worked as well as the ways in which they performed the tasks.[18] Each subject was assigned a specific value of noise exposure that corresponded with the 8-h TWA Leq measured in his/her workplace.

We compared the risk of hypertension in four exposure categories according to the distribution of the levels of the 8-h TWA Leq of noise exposure representing high (> 75th percentile; > 80 dBA), medium (75th to 50th percentile; 80-65 dBA), low exposure (< 50th to 25th percentile; 64-50 dBA) and the reference category (< 25th percentile; 49-40 dBA). In addition, we classified the duration of noise exposure with similar groups (3-15 years vs. > 15 years) used by Sbihi and colleagues for comparison with previous studies.[15]

Covariates

The following covariates at the baseline survey were included in the analyses: age, sex, body mass index (BMI; kg/ m^2), employment duration, cigarette smoking, alcohol consumption, daily salt intake, exercise, ~~and the~~ use of PPE, low density lipoprotein (LDL; mg/dl), high density lipoprotein (HDL; mug/dl) and triglyceride level (TG; mg/dl). Age was fitted in five indicator variables (40-44, 45-49, 50-54 and ≥ 55 years with < 40 years as the reference category) to allow non-linear adjustment. Cigarette smokers were defined as those who had smoked cigarettes ~~on~~ more than 3 days per week for at least 6 months; alcohol consumption was dichotomous as non-habitual drinker (no) and habitual drinker of at least 50 g of ethanol per day (equivalent to approximately 500 ml of wine) (yes). High salt-intake workers were defined as those who reported to ingesting food cooked with soy source for more than one meal per day for at least 6 months. Exercisers were defined in terms of hours of exercise per week. The exercise was categorized into i) < 1 hour, ii) 1-2 hours, iii) 3-4 hours, and iv) ≥ 5 hours (reference). The use of PPE included the percentage of time they wore PPE and

the types (i.e., earplugs and earmuffs) used at work.

Statistical methods

We hypothesized that the empirical induction period of the noise exposure on the development of hypertension was at least 3 years. First, we estimated the incidence rate of hypertension during the 3- to 20-year study period according to three genotypes of AGT (TT, TM and MM) and four exposure categories according to levels of noise representing high (> 80 dBA), medium (80-65 dBA), low exposure (64-50 dBA), and the reference level (49-40 dBA). In the crude analysis, incidence rate ratios of the relations between exposure and outcome relations were estimated. To elaborate sources of confounding, we fitted models with different combinations of covariates and compared the effect from models with and without the covariate of interest. If the inclusion of a covariate changed the studied effect estimated by more than 10%, the corresponding covariate was kept in the final model.[19] We estimated adjusted incidence rate ratios by applying Poisson regression analysis. The incidence rate ratios were adjusted for the covariates described above. Second, we studied the joint effects of AGT and exposure to noise on the risk of hypertension. We compared the incidence rate of hypertension in four exposure categories: 1) MM genotype and exposure to noise 49-40 dBA (IR_{00}, reference category); 2) MM genotype and exposure to noise > 65 dBA (IR_{10}); 3) TT genotype and exposure to noise 49-40 dBA (IR_{01}); and 4) TT genotype and exposure to exposure to noise > 65 dBA (IR_{11}). The biological interaction (IA) of two factors was quantified by calculating the risk that is higher than expected according to the independent effects of these factors:[20]

$$IA = (IR_{11} - IR_{00}) - (IR_{10} - IR_{00}) - (IR_{01} - IR_{00}).$$

Then we used the incidence rate ratio as a measure of effect and estimated adjusted incidence rate ratios as above after adjusting for the covariates described above. To

assess the joint effect of AGT and exposure to noise, we focused on exposure to noise above 65 dBA for 3-15 years and calculated incidence rate ratios contrasting each of the three exposure categories to the reference category. Estimate for the independent effects of AGT and noise exposure and their joint effect was derived from the same Poisson regression model after adjusting for the covariates. Additionally, the Rothman synergy index and its 95% CI were calculated to investigate the joint effect of the two factors.[21] The synergy index (S) is equal to the calculation of $[IR_{11}-1]$ / $[(IR_{10}-1)+(IR_{01}-1)]$. An observed synergy index value that departs substantially from the expected additive null, i.e., synergy index $\neq$ 1, suggests an additive interaction effect. The IR values and their variance covariance matrix were then employed to calculate values for synergy index and 95% CIs.[22] SAS version 9.2 was used for all statistical analyses.

RESULTS

Study population

A total of 238 workers (26.1%) developed hypertension during the study period. The estimated incidence rate was 194 per 10 000 person-years (95% confidence interval: 95% CI 169-219). There were 48 workers (48/211 = 22.7%) without exposure to noise who developed hypertension during the study period. The estimated incidence rate was 147 per 10,000 person-years (95% confidence interval (CI): 95% CI 106-219), which was lower than that for the total population.

Table 1 shows the incidence of hypertension, and incidence rate ratio as well as 95% confidence interval for covariates. As can be seen, male workers of older age and with alcohol consumption and higher daily salt intake, higher TG and lower HDL are more likely to develop hypertension. We adjusted for these factors in the multivariate Poisson regression model.

Independent effects of AGT and noise exposure

The AGT TT genotype was significantly associated with increased risk of hypertension with an adjusted incidence rate ratio of 1.77 (95% CI 1.24-2.51) but not for the TM genotype (adjusted IRR 1.10 95% CI 0.71-1.72), as compared with that for the MM genotype (Table 2). Contrary to the combined TM and MM genotypes analysis, the effect estimated for the TT genotype had statistically significant association with increased risk of hypertension (adjusted IRR 1.69 95% CI 1.27-2.24). Furthermore, the distribution of genotypes in the study population did not deviate from the expected Hardy-Weinberg equilibrium (p = 0.21).

Table 3 presents the incidence rates for hypertension according to the three exposure indicators of noise, as well as incidence rate ratios in comparison with the reference category. The incidence of hypertension was related to the medium- (adjusted incidence rate ratio: IRR 2.32 and 95% CI 1.38-3.90) and high-exposure (IRR 2.53 95% CI 1.14-5.65) categories during 3-15 years of exposure. The risk estimate for the combined medium- and high-exposure categories was 2.35 (95% CI 1.42-3.88). The risk of developing hypertension under exposure to noise after 15 years was not related to the three exposure indicators.

Joint effect of AGT and noise exposure

Table 4 shows the incidence rates of hypertension in four categories representing the reference, independent effects of AGT and exposure to noise, and their joint effect.

Workers who was exposed to noise above 65 dBA and carried AGT (MM and TM combined) had significantly higher risk of developing hypertension with an adjusted incidence rate ratio of 3.04 (95% CI 1.45 – 6.42), which corresponds to a 204% (3.04-1) excess risk (Table 4). The effect of noise exposure below 50 dBA among workers who carried the TT genotype elevated, but showed no statistical significance with an IRR of 1.56 (95% CI 0.86-2.83), corresponding to a 56% (1.56-1) excess risk. In workers with the TT genotype and exposure to noise above 65 dBA, the adjusted IRR of hypertension was 3.73 (95% CI 1.84-7.56), a 273% (3.73-1) excess risk, compared with workers of the reference category. The expected joint effect of additive scale was 260% (excess risks due to TT genotype + noise, 56% + 204%). Thus, the joint effect of TT genotype and noise exposure (IA) was 13% [(3.73-1)-(1.56-1)-(3.04-1) = 0.13] stronger than expected on the basis of their additive independent effects. Additionally, the Rothman synergy index (S) was 1.05 (95% CI 0.92-1.19) greater than 1, suggesting an additive interaction effect, although the confidence interval was found to be slightly wider.

DISCUSSION

Workers who carried the AGT TT genotype had over 70% increased risk of developing hypertension. Noise exposure above 65 dBA increased hypertension incidence by 135% during 3-15 years, but the effect disappeared after 15 years. Further, the results indicate that the joint effect of AGT, representing genetic susceptibility, and exposure to noise was stronger than expected on the basis of their independent effects in additive scale.

Validity of results

This prospective cohort study offered a suitable approach to assessing the role of noise on the subsequent development of hypertension at work. We were able to follow 71% of the 1,301 aviation workers for 20 years. The validity was not likely to be

compromised by losses to follow-up, because distributions of the characteristics of the study population at baseline were similar to those of the 20-year cohort (Table 5). The prospective study design eliminates selection bias, if the workers with hypertension are more likely to change diet, physical activity, alcohol or salt consumption after the first symptoms and signs of hypertension compared with healthy workers.

Important features of the Taiwan National Health Insurance scheme limit the amount of outcome misclassifications. Taiwan has an affordable public health care system, which results in easy access to medical consultation. Further, the National Health Insurance scheme covers all residents of Taiwan and provides 90% reimbursement of medications for those with hypertension fulfilling their diagnostic criteria. In application for subsidies, the diagnoses are approved centrally by the National Health Insurance Bureau, which reduces heterogeneity in diagnostic practice.

We were able to take into account most of the known potential confounders related to individual characteristics, employment history, cigarette smoking, alcohol consumption, daily salt intake, regular exercise, use of PPE, LDL, HDL and TG levels in the Poisson regression analysis where most of the known determinants were included. In addition, these important determinants were collected at baseline before the onset of hypertension, thus minimizing bias due to awareness of the disease or exposure to noise.

Synthesis with previous knowledge

We identified only one previous prospective cohort study on the relation between noise exposure and incidence of hypertension. Sbihi and colleagues conducted a cohort study of 10,872 sawmill workers in British Columbia from 1991 to 1998, where they reported an increased risk of hypertension among workers under noise exposure above 85 dBA for more than 30 years with an adjusted incidence rate ratio of 1.50.[15] In 2009, Lee and

colleagues conducted a cohort study of 532 male workers in Busan, Korea to examine the association between exposure to noise and blood pressure. After adjusting for age, BMI, smoking, alcohol consumption, and exercise, the mean values for the systolic blood pressure (SBP) was 3.8 mm-Hg higher in workers exposed to noise above 85 dBA and using both earplugs and earmuffs than in those exposed to noise < 60 dBA.[23]

Our results suggest that noise exposure above 65 dBA during 3-15 years is an important indicator of relevant exposure rather than after 15 years per se. Noise exposure after 15 years is associated with reduced risk of hypertension, possibly because of chance, insufficient power, or healthy worker effect which might mitigate the association over 15 years. The pathological mechanism of hypertension related to noise exposure is not well understood. One possible cause suggested is that noise has the potential to trigger a physical stress response by activating the sympathetic nervous system, leading to an arousal of the neuroendocrine system.[24] The release of stress hormones results in various acute hemodynamic and metabolic effects such as increased blood pressure, aggregation of thrombocytes, and release of free fatty acids into the blood stream.[25] It is possible that long-term noise exposure leads to chronic dysregulation of the stress mechanism and thus increases the risk of hypertension.

It has been reported that the AGT TT genotype is an important determinant of hypertension in white and Asian workers.[2-4] We also found the AGT TT genotype to be the main factor for development of hypertension. The possible mechanism was that a higher throughput in the rennin-angiotensin system might increase blood pressure by the actions of angiotensin II on sodium reabsorption in the kidneys and by vessel constriction. The results also show that the joint effect of AGT gene polymorphisms, representing genetic susceptibility, and high level of noise exposure was stronger than expected on the basis of their independent effects in additive scale. The observed

gene-environment interaction between AGT gene polymorphisms and high level of noise exposure could help us understand the aetiology of hypertension in occupational setting. The present results support the role of AGT gene in controlling the rennin-angiotensin system for the development of hypertension. Diverse ability of noise exposure on development of hypertension depended on variations of AGT polymorphisms. In this study, noise exposure was assessed according to group rather than individual level using personal noise dosimeter. Moreover, other genes may be also involved. Hence, long-term prospective study with personal exposure assessment is warranted to explore the relevant role of other genes in determining genetic susceptibility to hypertension.

CONCLUSIONS

Our results are consistent with the hypothesis that AGT gene polymorphisms constitute a strong determinant of hypertension. The results further evidence that noise exposure during 3-15 years increases the risk of developing hypertension. Previous knowledge of the relationship between AGT gene polymorphisms and chronic exposure to occupational noise and the risk of hypertension comes mainly from cross-sectional studies with establishment of casual relationship being the most significant threat to validity. In the present prospective cohort study, we were able to avoid the threat to validity. Our finding also supports a role of gene-environment interaction on the occurrence of hypertension; i.e., workers carrying the AGT TT genotype and exposed to high level of noise are more susceptible to develop hypertension. Our results suggest that genotyping for AGT polymorphisms, a simple and inexpensive assay, could be a suitable biomarker identifying genetically susceptible workers in the development of hypertension under occupational noise exposure.

Competing interests: None.

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Table 1. Baseline Population Characteristics

	No	New cases	person-years at risk	IR (95%CI) (per 10000 PY)	IRR (95%CI)
Total	912	238	12258	194.16 (169.49-218.82)	
Age (year)					
< 40	109	12	1053	113.93 (49.47-178.40)	R
40-44	288	55	3880	141.74 (104.28-179.20)	1.24 (0.67-2.32)
45-49	332	102	4626	220.46 (177.67-263.24)	1.94 (1.06-3.52)
50-54	138	47	2010	233.82 (166.97-300.24)	2.05 (1.09-3.87)
≥ 55	45	22	687	319.96 (186.26-453.66)	2.81 (1.39-5.67)
Sex					
Male	706	209	9548	218.88 (189.21-248.56)	2.05 (1.39-3.02)
Female	206	29	2709	107.02 (68.07-145.97)	R
Smoking					
No	785	197	10408	189.27 (162.84-215.71)	R
Yes	127	41	1849	221.62 (153.78-289.46)	1.17 (0.84-1.64)
Alcohol consumption					
No	808	197	10840	181.73 (156.35-207.10)	R
Yes	104	41	1417	289.22 (200.69-377.74)	1.59 (1.14-2.23)
BMI (kg/m^2)					
Normal	528	91	7085	128.43 (102.04-154.81)	R
Fat (> 24)	384	148	5172	286.14 (240.04-332.24)	2.21 (1.70-2.87)
HDL (mg/dl)					
Normal	555	134	7643	175.31 (145.63-204.99)	R
Low Male < 40 Female < 50	357	104	4614	225.37 (182.06-268.69)	1.29 (1.00-1.66)
LDL (mg/dl)					
Normal	767	197	10290	191.44 (164.71-218.17)	R
High (> 150)	145	41	1967	208.37 (144.59-272.15)	1.09 (0.78-1.52)
TG (mg/dl)					
Normal	456	75	6158	121.79 (94.226-149.35)	R
high(> 100)	456	163	6099	267.21 (226.19-308.24)	2.19 (1.67-2.88)
Daily salt intake					
Normal	757	184	10278	179.01 (153.14-204.87)	R
High	155	51	1979	257.69 (186.96-328.41)	1.43 (1.05-1.95)
Hours of exercise per week					
≥ 5	189	41	2569	159.57 (110.73-208.42)	R
3-4	126	35	1839	190.28 (127.24-253.32)	1.19 (0.76-1.87)
1-2	169	47	2262	207.74 (148.35-268.13)	1.30 (0.86-1.98)
< 1	428	115	5586	205.84 (168.22-243.46)	1.29 (0.90-1.84)

Table 2. Independent Effect of AGT Gene on ~~the~~ Incidence of Hypertension.

M235T	No	New cases	PY	IR (95%CI) (per 10000 PY)	IRR (95%C.I)	Adjusted* IRR (95%CI)
Total	794	205	12082	169.66 (146.44-192.89)		
TT	375	120	5662	211.94 (174.02-249.86)	1.82 (1.29-2.56)	1.77 (1.24-2.51)
TM	162	40	2555	156.50 (108.00-205.00)	1.34 (0.88-2.06)	1.10 (0.71-1.72)
MM	257	45	3864	116.45(99.17-158.45)	R	R
TT	375	120	5662	211.94 (174.02-249.86)	1.60 (1.21-2.11)	1.69 (1.27-2.24)
TM+MM	419	85	6420	132.38 (104.24-160.53)	R	R

*Poisson regression controlling for age, sex, BMI, LDL, HDL, TG, daily salt intake, PPE used, and alcohol consumption.

Table 3. Independent Effect of Noise Exposure on ~~the~~ Incidence of Hypertension.

Noise	No	New cases	PY	IR (95%CI) (per 10000 PY)	IRR (95%C.I)	Adjusted* IRR (95%CI)
Total	794	205	12082	169.66 (146.44-192.89)		
Reference group (40-40 dBA)	211	48	3257	147.35 (105.67-189.04)	R	R
Low (50-64 dBA)						
3-15 years	162	41	1491	274.85 (190.72-358.98)	1.85 (1.22-2.82)	1.71 (1.11-2.63)
> 15 years	162	46	3291	139.77 (99.38-180.17)	0.95 (0.63-1.42)	0.83 (0.54-1.27)
Medium (65-80 dBA)						
3-15 years	90	35	939	372.65 (249.19-496.11)	2.53 (1.64-3.91)	2.32 (1.38-3.90)
> 15 years	88	17	1796	94.62 (49.64-139.59)	0.64 (0.37-1.12)	0.51 (0.28-0.95)
High (81-102 dBA)						
3-15 years	27	9	261	344.56 (119.45-569.68)	2.34 (1.15-4.77)	2.53 (1.14-5.65)
> 15 years	54	9	1038	86.70 (30.06-143.34)	0.59 (0.29-1.20)	0.56 (0.26-1.23)
Medium + high (65-80 dBA)						
3-15 years	117	44	1200	366.54 (258.24-474.85)	2.49 (1.65-3.74)	2.35 (1.42-3.88)
> 15 years	142	26	2834	91.72 (56.46-126.97)	0.62 (0.39-1.00)	0.53 (0.30-0.92)

*Poisson regression controlling for age, sex, BMI, LDL, HDL, TG, daily salt intake, PPE used, and alcohol consumption.

Table 4. Joint Effect of AGT Gene and Noise Exposure on ~~the~~ Incidence of Hypertension.

	No	New case	PY	IR (95%CI) (per 10000)	IRR (95%C.I)	Adjusted* IRR (95%CI)
MM+ TM						
Low exposure (40-49 dBA)	117	23	1783	128.94 (76.24-181.63)	R	R
Medium +High exposure (65-102 dBA)	59	21	613	342.34 (195.92-488.77)	2.66 (1.47-4.80)	3.04 (1.45-6.42)
TT						
Low exposure (40-49 dBA)	94	25	1473	169.64 (103.14-236.14)	1.32 (0.75-2.32)	1.56 (0.86-2.83)
Medium+ High exposure (65-102 dBA)	58	23	586	391.81 (231.70-551.98)	3.04 (1.71-5.42)	3.73 (1.84-7.56)
Rothman synergy index				1.05 (0.92-1.19)		

*Poisson regression controlling for age, sex, BMI, LDL, HDL, TG, daily salt intake, PPE used, and alcohol consumption.

Table 5. Personal Characteristics of Baseline Study Population that Lost To Follow-up and the 20-year Cohort.

Characteristics	Loss to follow-up	20-year cohort	Baseline
	N (%)	N (%)	N (%)
Number	389 (100 %)	912 (100 %)	1301 (100%)
Age(year)			
< 40	64 (0.17)	109 (0.12)	196 (0.15)
41-45	135 (0.35)	288 (0.32)	457 (0.35)
46-50	136 (0.35)	332 (0.36)	139 (0.34)
51-55	40 (0.10)	138 (0.15)	159 (0.12)
> 55	14 (0.04)	45 (0.05)	50 (0.04)
Sex			
Male	323 (0.83)	706 (0.77)	1029 (0.79)
Female	66 (0.17)	206 (0.23)	272 (0.21)
Smoking			
No	329 (0.85)	785 (0.86)	1121 (0.86)
Yes	60 (0.15)	127 (0.14)	180 (0.14)
Alcohol consumption			
No	341 (0.88)	808 (0.89)	1154 (0.89)
Yes	48 (0.12)	104 (0.11)	143 (0.11)
BMI (kg/m^2)			
Normal	208 (0.53)	528 (0.58)	736 (0.57)
Fat (> 24)	181 (0.46)	384 (0.42)	565 (0.43)
HDL (mg/dl)			
Normal	231 (0.60)	555 (0.61)	784 (0.60)
low			
Male < 40	158 (0.41)	357 (0.39)	517 (0.40)
Female < 50			
LDL (mg/dl)			
Normal	321 (0.83)	767 (0.84)	1088 (0.84)
High (> 150)	68 (0.17)	145 (0.16)	213 (0.16)
TG (mg/dl)			
Normal	153 (0.40)	456 (0.50)	609 (0.47)
High (> 100)	236 (0.60)	456 (0.50)	692 (0.53)
Daily salt intake			
Normal	328 (0.84)	757 (0.83)	1085 (0.83)
High	61 (0.16)	155 (0.17)	216 (0.17)
Hours of exercise per week			
≥ 5	129 (0.33)	189 (0.21)	384 (0.30)
3-4	42 (0.11)	126 (0.14)	148 (0.11)
1-2	54 (0.14)	169 (0.19)	218 (0.17)
< 1	164 (0.42)	428 (0.47)	551 (0.42)