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Association of polymorphisms in grainyhead-like-2 gene with the susceptibility to age-related hearing loss

A systematic review and meta-analysis

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Abstract

Objective: The grainyhead-like-2 (GRHL2) genetic variants were reported in age-related hearing impairment (ARHI) susceptibility in several case–control studies. However, their conclusions are conflicting; it is difficult to precisely assess the disease risk associated with the variants. Therefore we conduct the meta-analysis to discover the association of GRHL2 polymorphisms and the risk of ARHI.

Methods: A related literature search was conducted in on-line databases, such as Wanfang database, China National Knowledge Infrastructure (CNKI), EMBASE, Web of Science, and PubMed (updated to August 30, 2018). We use Review Manager 5.0 and Stata SE 12.0 software to reckon the odds radio (OR), 95% confidence interval (CI) and *P* value in random- or fixed-effects model according to the I2 value in the heterogeneity test.

Results: 2762 cases and 2321 controls in 5 articles were provided data to the meta-analysis. The pooled ORs (95% CI) of the rs10955255 polymorphism were 1.26 (1.05–1.50, P = .01), 1.33 (1.07–1.65, P = .01), and 1.32 (1.12–1.55, P = .0007) in the allele, homozygote and recessive model separately. Besides, a significant association was detected between rs1981361 in mixed population and the ARHI risk in the allele, heterozygote, and dominant genetic model respectively. Then subgroup analyses was performed by ethnicity, for rs10955255 meaningful associations were detected for the allele model, homozygote model, dominant model and recessive model in the Caucasian population but no relations in any of the 5 genetic models in Asian population.

Conclusion: The meta-analysis indicated that the rs10955255 polymorphism could be an important risk factor for ARHI, especially in the Caucasians. The rs1981361 polymorphism may be a risk factor for ARHI in Asians. Larger scale researches are needed to further bring the consequences up to date.

Abbreviations: ARHI = age-related hearing impairment, CI = confidence interval, CNKI = China National Knowledge Infrastructure, GRHL2 = grainyhead-like-2, HWE = Hardy–Weinberg Equilibrium, NOS = Newcastle–Ottawa scale, OR = odds radio.

Keywords: age-related hearing loss, grainyhead-like-2 gene polymorphism, meta-analysis

1. Introduction

Age-related hearing loss (AHL), also known as presbycusis is a complex and multifactorial disorder, characterized by the progressive deterioration of auditory sensitivity associated with aging.^[1] As a natural consequence of a society developing process and along with the intensifying of the population aging, the number of people with AHL in our country is accumulates and has become a severe health and social problem.^[2–4] Most of the time, hearing loss initially involves high-frequency tones, results

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in communication impairment, particularly in reverberant and/or noisy environments. Epidemiologically, some medical comorbidity related to AHL, such as social isolation, depression,^[5–8] cognitive impairment, and dementia,^[9] placing heavy burden on the society. Although AHL usually could be caused, aggravated or associated by the common risk factors such as hypertension,^[10,11] exposure to the noise, smoking, ototoxic drugs,^[12] and so on, growing number of evidences indicate genetic factors play a significant role in the etiology of AHL through their interactions with the environmental components.^[13–15] As a result, it is essential to identify the gene variants that contribute to AHL pathogenesis. The knowledge of the genetic component that influences AHL susceptibility could be crucial in creating efficient therapeutic strategies and improving disease risk's prediction, based on targeted approach.

AHL susceptibility is affected by numerous environmental and genetic factors.^[16] What cannot be overlooked is that the susceptibility to AHL among individuals is apparently various, some people are more vulnerable to AHL than others. Several genetic association studies reported a number of genes involved in neurotransmission, cell adhesion, oxidative stress, apoptosis, immunity, cell morphology, development, and so on. could be implicated in pathophysiology of age-related hearing loss, but the reasons for all individual differences still cannot be given by these genetic variations. Thus it is required to discover more AHL-associated genes and genetic polymorphisms to further enhance genetic predictive system of AHL.

Recent researches illustrated that the t grainyhead-like 2 (GRHL2) gene, also known as TFCP2L3, expressing in diverse epithelial tissues, is expressed in cochlear duct that lines different cell populations, including hair cells, the lateral wall spiral ligament, SGNs and supporting cells.^[16] Their deficiencies often cause progressive dominant hearing loss in a zebrafish model of DFNA28.^[17] GRHL2 gene is part of the grainyhead family, which is crucial for several developmental processes in Drosophila, most prominently during embryonic stages. Carrying mutations of GRHL2 gene in flies is often lethal in embryonic stage. GRHL2 takes part in the maintenance and differentiation of epithelial cells throughout life. The most reasonable pathological explanation for late-onset hearing impairment is damaged epithelial cell integrity.^[17]

What is notable is that some studies suggested an intimate connection between some SNPs in GRHL2 genes, including rs10955255, and rs2127034, and AHL susceptibility, while no positive consequence for rs10955255 was detected in Lin's study. Due to the limitations of sample scale, research design, ethnicity, and numerous other factors, even for each SNP site, the outcome in different studies is various, while meta-analysis exhibits an advantage in getting over the limitations and evaluating the sources of Heterogeneity. At present, there are many studies conducted to evaluate the association between the role of GRHL2 gene polymorphism and AHL susceptibility. However, the published results are inconsistent and controversial. Here, a meta-analysis including subgroup analysis was executed based on all eligible studies to assess the association of all known GRHL2 gene polymorphisms and susceptibility to AHL in aging people.

2. Material and methods

2.1. Search strategy

The systematic review and meta-analysis were developed and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklists (Supplementary Table 1). Ethical approval was unnecessary in this study because it was a meta-analysis analyzing existing articles and did not need handle individual patient data. Literature review about the associations between the polymorphisms of GRHL2 and AHL was performed in:^[1] Wanfang database;^[2] CNKI;^[3] EMBASE;^[4] Web of Science, and^[5] PubMed. Keyword combinations, such as "GRHL2," "grainyhead like transcription factor 2," "Age-related hearing impairment," "Age-Related Hearing loss," and "Presbycusis" were imported as Medical Subject Headings (MeSH terms) or Entry Terms. All studies we searched were reported before August 26, 2018. Other articles that list in the reference lists of the retrieved literatures were likewise manually checked. The detailed steps of the literature review are shown in Figure 1. Inclusion and exclusion criteria. We selected studies according to the following inclusion and exclusion criteria in the meta-analysis: inclusion criteria:^[1] investigating the relationship between GRHL2 gene polymorphisms and susceptibility to AHL.^[2] have sufficient genotype frequencies for calculating odds ratios (ORs);^[3] different independent sample sets in 1 article were included respectively;^[4] case–control or cohort studies;^[5] published in English or Chinese. Exclusion criteria:^[1] duplicated studies;^[2] reviews or cases;^[3] not about gene polymorphisms;^[4] unavailable data.

2.2. Data extraction strategy and quality assessment

Original data were extracted from the included studies by 2 investigators (BH and HS) independently. The following data were collected from the included publications: the first author, year of publication, journal, sample size of cases and controls, study population demographics (country, ethnicity, gender, and age) of AHL and controls, methods of genotyping, genotype distributions and relevant SNP polymorphisms, and Hardy-Weinberg Equilibrium (HWE) in control group. Providing that the information for meta-analysis were lacking or simply expressed graphically, we attempted to contact the authors to get more information first, and where a reply was not got, then we gauged data from the diagrams by digital ruler package or exclude. We also used the Newcastle-Ottawa scale (NOS) to assess each included study's quality. Studies that scored over 7 were considered as high-quality studies. Discrepancies were resolved by coming to an agreement after a serious discussion.

2.3. Statistical methods

We measured the SNP sites mentioned in 2 or more studies in the meta-analysis. The OR, 95% confidence interval (CI), and P value were reckoned for each study by using genotype comparison, which included these 5 gene models in total: the allele (B vs A), homozygote (BB vs AA), heterozygote (AB vs AA), dominant (BB+AB vs AA), and recessive (BB vs AB+AA) models. The purpose of this comparison is to reduce the chance of the first type of error. P value of <.05 was considered statistically significant association between AHL risks and GRHL2 gene polymorphisms. The Q-statistic test and I-square statistic were used to measure heterogeneity among the studies. A fixed-effect model by the Mantel-Haenszel method was used when I2 values <50% or P value of heterogeneity >.10, Otherwise, a randomeffect model by the Mantel-Haenszel method was applied. Subgroup analyses were stratified by ethnicity (Asian or Caucasian) and research quality to investigate the potential



Table 1		
Summary of included studies.		

Author Vear				Sample s	size (F/M)	Age	(year)		
Author	Year	Country	Ethnicity studied	Case	Control	Case	Control	Genotype method	NOS score
Zhou	2015	China (mainland)	Asian (Chinese)	224	225	61.92	57.88	PCR (MassARRAY)	6
Lin	2011	China (Taiwan)	Asian (Chinese)	310 (137/173)	308 (163/145)	56.6 ± 8.95	57.4±8.31	PCR	8
Luo	2013	China (mainland)	Asian (Chinese)	982 (0/982)	324 (0/982)	80.84 ± 5.45	80.31 ± 5.37	PCR (SNPscan [™])	8
Matyas	2018	Roma	Caucasian (Roman)	113 (56/57)	298 (180/118)	NA	42.33 ± 15,51	PCR-RFLP	7
Van Laer	2008	Belgium (Antwerp)	Caucasian (Belgian)	253	252	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	United Kingdom	Caucasian (Britisher)	98	112	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Denmark	Caucasian (Danish)	133	136	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Belgium (Ghent)	Caucasian (Belgian)	67	64	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	The Netherlands	Caucasian (Dutch)	90	94	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Finland (Oulu)	Caucasian (Finn)	164	171	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Italy	Caucasian (Italian)	114	119	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Finland (Tampere)	Caucasian (Finn)	84	87	53-67	53-67	PCR (SNaPshot [™])	7
Van Laer	2008	Germany	Caucasian (German)	130	131	53–67	53–67	PCR (SNaPshot TM)	7

F/M=female/male; NOS=Newcastle-Ottawa Scale; PCR=polymerase chain reaction; TM=trademark.

Table 0

Genotype	distribution	of	GRHL2	SNPs.

				Ca	ase			Co	ntrol		Н	WE
SNP	Author	Year	AA	AB	BB	Total	AA	AB	BB	Total	X ²	Р
rs10955255	Zhou	2015	99	125	0	224	162	63	0	225	5.963	.015
(A/G)	Lin	2011	183	107	20	310	164	122	22	308	0.011	.915
	Luo	2013	666	278	38	982	219	90	15	324	2.050	.152
	Van Laer	2008	47	118	88	253	48	122	82	252	0.048	.827
	Van Laer	2008	17	48	33	98	21	58	33	112	0.255	.613
	Van Laer	2008	16	64	53	133	17	78	41	136	4.601	.032
	Van Laer	2008	13	32	22	67	15	26	23	64	1.951	.162
	Van Laer	2008	14	41	35	90	17	45	32	94	0.029	.865
	Van Laer	2008	16	66	82	164	28	86	57	171	0.217	.641
	Van Laer	2008	12	57	45	114	31	55	33	119	0.676	.411
	Van Laer	2008	9	40	35	84	9	49	29	87	3.117	.077
	Van Laer	2008	18	51	61	130	24	61	46	131	0.228	.633
rs1981361	Zhou	2015	90	134	0	224	117	108	0	225	22.438	P <.05
(G/A)	Matyas	2018	23	73	17	113	87	162	49	298	3.299	.069
rs13263539 (G/A)	Matyas	2018	33	64	16	113	102	157	39	298	3.161	.075
rs2127034 (C/T)	Zhou	2015	116	108	0	224	171	54	0	225	4.184	.041

sources of heterogeneity. Sensitivity analysis was used to forecast the pooled results' stability. We also evaluated the HWE by the chi-square test in the control group. Studies with control groups not in HWE were still regarded for this meta-analysis, but they were omitted in the sensitivity analysis. The publication bias was measured by Egger test and Begg funnel plot. All statistical analyses were conducted using Review Manager 5.0 and Stata SE 12.0 software.

	Case		Contr	ol		Odds Ratio	Odds Ratio		Case		Contr	ol		Odds Ratio		Odds Ratio
itudy or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% Cl	M-H, Random, 95% Cl	Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	1	M-H, Fixed, 95% CI
in 2011	147	620	166	616	9.8%	0.84 [0.65, 1.09]		Lin 2011	20	203	22	186	14.4%	0.81 [0.43, 1.55]		
JO 2013	354	1964	120	648	10.2%	0.97 [0.77, 1.22]		Luo 2013	38	704	15	234	14.8%	0.83 [0.45, 1.54]		
an Laer1 2008	294	506	286	504	9.9%	1.06 [0.82, 1.36]		Van Laer1 2008	88	135	82	130	20.3%	1.10 [0.66, 1.81]		
an Laer2 2008	114	196	124	224	7.7%	1.12 [0.76, 1.65]		Van Laer2 2008	33	50	33	54	7.5%	1.24 [0.55, 2.75]		
an Laer3 2008	170	266	160	272	8.3%	1.24 [0.88, 1.76]		Van Laer3 2008	53	69	41	58	7.2%	1.37 [0.62, 3.04]		
an Laer4 2008	76	134	72	128	6.3%	1.02 [0.63, 1.66]		Van Laer4 2008	22	35	23	38	5.7%	1.10 [0.43, 2.84]		
in Laer5 2008	111	180	109	188	7.3%	1.17 [0.77, 1.77]		Van Laer5 2008	35	49	32	49	6.4%	1.33 [0.57, 3.12]		
an Laer6 2008	230	328	200	342	8.8%	1.67 [1.21, 2.29]		Van Laer6 2008	82	98	57	85	6.9%	2.52 [1.25, 5.08]		
in Laer7 2008	147	228	121	238	8.0%	1.75 [1.21, 2.54]		Van Laer7 2008	45	57	33	64	4.6%	3.52 [1.58, 7.87]		1
an Laero 2000	172	260	152	262	9 294	1.19 [0.76, 1.65]		Van Laer8 2008	35	44	29	38	4.4%	1.21 [0.42, 3.44]		
hou 2015	125	448	63	450	8.5%	2.38 [1.70, 3.33]		Zhou 2015	61	79 99	46	162	1.1%	1.77 [0.86, 3.64] Not estimable		
stal (95% CI)		5298		4046	100.0%	1.26 [1.05, 1.50]	+	Total (95% CD)		1622		1168	100.0%	1 33 11 07 1 651		-
tal events	2051		1681	10-120	20000000			Total (95% CI)	F 10	1022	442	1100	100.076	1.35 [1.07, 1.05]		100 m
eterogeneity; Tau ² =	0.07: Chi2	36.98	df = 11	(P=0)	0001); P	= 70%		Hotorogeneity Chille	14 67 44-	10 /0 -	413	- 228			-	
est for overall effect:	Z = 2.53 (P	= 0.01)				0.2 0.5 1 2 5 Favours [case] Favours [control]	Test for overall effect:	Z = 2.57 (F	= 0.01)	- 327			0.2	0.5 1 2 Favours [case] Favours [control]
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a start and a start	Case		Contr	ol		Odds Ratio	Odds Ratio		Case		Contr	ol		Odds Ratio		Odds Ratio
udy or Subgroup	Events	Total	Events	Total	Weight	M-H. Random, 95% Cl	M-H, Random, 95% Cl	Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	1	M-H, Fixed, 95% CI
n 2011	107	290	122	286	11.4%	0.79 [0.56, 1.10]	2	Lin 2011	20	310	22	308	7.8%	0.90 [0.48, 1.68]		
o 2013	278	944	90	309	11.8%	1.02 [0.77, 1.35]		Luo 2013	38	982	15	324	8.2%	0.83 [0.45, 1.53]		
n Laer1 2008	118	165	122	170	10.0%	0.99 [0.61, 1.59]		Van Laer1 2008	88	253	82	252	20.3%	1.11 [0.76, 1.60]		
n Laer2 2008	48	65	58	79	7.4%	1.02 [0.49, 2.15]		Van Laer2 2008	33	98	33	112	7.7%	1.22 [0.68, 2.18]		
n Laer3 2008	64	80	78	95	7.3%	0.87 [0.41, 1.86]		Van Laer3 2008	53	133	41	136	9.2%	1.54 [0.93, 2.54]		
in Laer4 2008	32	45	26	41	6.2%	1.42 [0.57, 3.51]	and the second s	Van Laer4 2008	22	67	23	64	6.0%	0.87 [0.42, 1.79]		
n Laer5 2008	41	55	45	62	6.8%	1.11 [0.49, 2.52]		Van Laer5 2008	35	90	32	94	7.2%	1.23 [0.68, 2.25]		
in Laer6 2008	66	82	86	114	7.9%	1.34 [0.67, 2.69]		Van Laer6 2008	82	164	57	171	10.6%	2.00 [1.29, 3.11]		
in Laer7 2008	57	69	55	86	7.3%	2.68 [1.25, 5.74]		Van Laer7 2008	45	114	33	119	7.4%	1.70 [0.98, 2.94]		
In Latero 2008	40	49	49	00	7 7%	1 11 10 55 2 201		Van Laer8 2008	35	84	29	87	6.3%	1.43 [0.77, 2.66]		
hou 2015	125	224	63	225	10.8%	3.25 [2.19, 4.81]		Zhou 2015	61	130	46	131 225	9.2%	1.63 [0.99, 2.69] Not estimable		
otal (95% CI)		2137		1610	100.0%	1.24 [0.91, 1.68]	-	Total (95% Ci)		2649		2023	100.0%	1 32 [1 12 1 55]		•
otal events	1027		855			and the second se	1	Total events	512	2043	413	EVES	100.078	time [ittel trani		100 million (100 m
eterogeneity: Tau ² = est for overall effect:	0.19; Chi ² = Z = 1.35 (P	= 38.80	. df = 11	(P < 0.	0001); l ² :	= 72%	0.2 0.5 1 2 5 Favours (case) Favours (control)	Heterogeneity: Chi ² = Test for overall effect:	11.29, df = Z = 3.39 (F	10 (P =	= 0.34); F	= 11%	5		0.2	0.5 1 2 Favours (case) Favours (control)
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	Case		Contr	l		Odds Ratio	Odds Ratio									
udy or Subaroup	Events	Total	Events	Total	Weight	M-H. Random, 95% Cl	M-H, Random, 95% CI									
n 2011	127	310	144	308	11.1%	0.79 (0.57, 1.09)										
0 2013	316	982	105	324	11.5%	0.99 [0.76, 1.29]										
an Laer1 2008	206	253	204	252	9.9%	1.03 [0.66, 1.61]										
in Laer2 2008	81	98	91	112	7.5%	1.10 [0.54, 2.23]										
In Laer3 2008	117	133	119	136	7.3%	1.04 [0.50, 2.17]										
in Laer4 2008	54	67	49	64	6.5%	1.27 [0.55, 2.94]										
n Laer5 2008	76	90	77	94	7.0%	1.20 [0.55, 2.60]										
in Laer6 2008	148	164	143	171	8.0%	1.81 [0.94, 3.49]										
in Laer7 2008	102	114	88	119	7.4%	2.99 [1.45, 6.18]										
an Laer8 2008	75	84	78	87	5.5%	0.96 [0.36, 2.55]	A CONTRACT OF A									
in Laer9 2008 Iou 2015	112 125	130 224	107 63	131 225	7.9% 10.4%	1.40 [0.72, 2.72] 3.25 [2.19, 4.81]										
		2649		2023	100.0%	1.34 [0.99, 1.81]	-									
tal (95% CI)																
tal (95% CI) tal events	1539		1268													

Figure 2. The association between polymorphism rs10955255 and age-related hearing impairment (AHL) in different genetic models. The results indicated that there is a close association in the allele, homozygous and recessive model between the SNP rs10955255 in the GRHL2 gene and AHL susceptibility.

3. Results

3.1. Search results and characteristics of the eligible studies

Fig. 1 shows the flow chart of the study selection in the metaanalysis. After primary literature search, 31 possibly related articles were found. Twenty articles retained for screening the title and abstract after erasing duplicate records. Eight articles were excluded as the studies were not about the relationship between GRHL2 and AHL. The remaining 12 articles were evaluated through full-text screening for qualification. Seven articles were further omitted for the reason that they are reviews (n = 3) or not about the SNPs in GRHL2 genes (n = 4). Finally, 5 articles on 13 independent studies (2762 cases and 2321 controls) satisfied the inclusion criteria and were covered in our metaanalysis. The elementary information, consisting of the NOS scores of the 13 qualified studies were summarized and showed in Table 1. Table 2 shows the genotype distribution of SNP sites in GRHL2 gene in the 13 qualified studies.

3.2. Meta-analysis of the SNPs in GRHL2 gene and AHL

Two SNPs (rs10955255 and rs1981361) in GRHL2 gene were selected for the meta-analysis. We chose 5 genetic models for the genotype comparison. There were 2649 cases and 2023 controls

included in 9 studies on Caucasian populations and 3 studies on Asian populations for SNP rs10955255. The pooled ORs (95% CI) were 1.26 (1.05–1.50, P = .01), 1.33 (1.07–1.65, P = .01), and 1.32 (1.12–1.55, P = .0007) in the allele, homozygote, and recessive model separately. (Fig. 2 and Table 3). The evidence suggested that there may be a close relationship between the SNP site rs10955255 in the GRHL2 gene and AHL susceptibility. Regarding SNP site rs1981361, the pooled ORs (95% CI) in mixed population were 1.26 (1.02–1.56, P = .04), 1.64 (1.21–2.23, P = .001), and 1.61 (1.19–2.19, P = .02) in the allele, heterozygote, and dominant models separately (Fig. 3 and Table 3). For rs2127034 authors did not provide the original dates, so we could not include it in our study, but in the Oulu region of Finland, it is associated with AHL (P = .0009, OR = 0.58). Allele homozygote GG appears to be more common in people with hearing impairment. Homozygote AA may be a protective factor in AHL in older patients. The detailed information of the overall analysis of the association between the 2 SNP sites in GRHL2 gene and AHL susceptibility are listed in Table 3. (Figs. 4 and 5)

3.3. Publication bias and sensitivity analysis

As shown in Fig. 7 there is no publication bias was found in all 5 genetic models of SNP site rs10955255 via Egger test. Due to lack

Table 3

SNP	Ethnicity	Study number	Case/Control	Genetic medel	l² (%)	Model	OR (95% CI)	Z score	P (Z)
rs10955255	Mixed population	12	2649/2023	G vs A	70	R	1.26 (1.05,1.50)	2.53	.01
(A >G)				GG vs AA	32	F	1.33 (1.07,1.65)	2.57	.01
Dverall analys SNP \$10955255 A > G) \$1981361 G > A)				AG vs AA	72	R	1.24 (0.91,1.68)	1.35	.18
				GG+AG vs AA	74	R	1.34 (0.99,1.81)	1.86	Fore $P(Z)$ 53 .01 57 .01 35 .18 36 .06 39 .0007 73 .46 35 .39 73 .46 35 .39 78 .43 77 .44 36 .51 99 $P < .0001$ 1 .0007 31 .19 43 .02 39 $P < .0001$ 21 .03 37 .01 27 .79 38 .0007 1 .04 24 .46 39 .002 34 .73 39 .05 51 .01 51 .01 54 .73
				GG vs AG+AA	11	F	1.32 (1.12,1.55)	3.39	
	Asian subgroup	3	1516/857	G vs A	92	R	1.23 (0.71,2.15)	0.73	.46
				GG vs AA	0	F	0.82 (0.53,1.29)	0.85	.39
				AG vs AA	94	R	1.36 (0.63,2.95)	0.78	.43
				GG+AG vs AA	94	R	1.35 (0.63,2.89)	0.77	.44
				GG vs AG+AA	0	F	0.86 (0.56,1.34)	0.66	.51
	Caucasian subgroup	9	1133/1166	G vs A	16	F	1.27 (1.13,1.43)	3.99	P <.0001
				GG vs AA	11	F	1.54 (1.20,1.97)	3.41	.0007
				AG vs AA	0	F	1.17 (0.92,1.49)	1.31	.19
				GG+AG vs AA	2	F	1.32 (1.06,1.65)	2.43	.02
				GG vs AG+AA	0	F	1.40 (1.18,1.67)	3.89	P <.0001
	High quality	11	2425/1798	G vs A	50	R	1.17 (1.02,1.36)	2.21	.03
	subgroup			GG vs AA	32	F	1.33 (1.07,1.65)	2.57	.01
				AG vs AA	1	F	1.02 (0.87,1.20)	0.27	.79
				GG+AG vs AA	33	F	1.07 (0.92,1.25)	0.92	.36
				GG vs AG+AA	11	F	1.32 (1.12,1.55)	3.39	.0007
rs1981361	Mixed population	2	337/523	A vs G	0	F	1.26 (1.02,1.56)	2.1	.04
rs10955255 М (A >G) // Сал (G >A) // Сал				AA vs GG	NA	F	1.31 (0.64,2.69)	0.74	.46
				GA vs GG	0	F	1.64 (1.21,2.23)	3.18	.001
				AA+GA vs GG	0	F	1.61 (1.19,2.19)	3.08	.002
SNP rs10955255 (A >G) rs1981361 (G >A)				AA vs GA+GG	NA	F	0.90 (0.49,1.64)	0.34	.73
	Asian subgroup	1	224/225	A vs G			1.35 (1.00,1.82)	1.99	.05
				AA vs GG			NA		
				GA vs GG			1.61 (1.11,2.34)	2.51	.01
				AA+GA vs GG			1.61 (1.11,2.34)	2.51	.01
				AA vs GA+GG			NA		
	Caucasian subgroup	1	113/298	A vs G			1.16 (0.85,1.58)	0.96	.34
				AA vs GG			1.31 (0.64,2.69)	0.74	.46
				GA vs GG			1.70 (1.00,2.91)	1.95	.05
				AA+GA vs GG			1.61 (0.96,2.72)	1.8	.07
rs1981361 (G >A)				AA vs GA+GG			0.90 (0.49,1.64)	0.34	.73



Figure 3. The association between polymorphism rs1981361 and AHL in different genetic models. The results indicated that there may be an association in the allele, heterozygote and dominant genetic models between the SNP rs1981361 in the GRHL2 gene and AHL susceptibility.

of sufficient data, we did not check the publication bias for rs1981361. The stability of the pooled ORs of the SNP site rs10955255 was calculated by removing each of the covered studies by turns. The results show no matter which of the covered studies was excluded, no significant change of the pooled ORs was discovered (data not shown). The consequences suggest that the relevant ORs were comparatively sturdy. For rs1981361, we did not execute the sensitivity analysis due to insufficient number of included studies.

4. Discussion

The general aim of this research was to discover the positive correlation between AHL susceptibility and 2 SNP sites (rs10955255, rs1981361) of GRHL2 gene. In our results, a significant relationship was observed between the SNP sites (rs10955255, rs1981361) and AHL, which indicated that the A allele is a potential risk factor to AHL. We first analyzed the mixed population to assess the relationship between rs10955255 and AHL. The pooled ORs showed there were significant

correlations in the allele, homozygous, and recessive model, respectively. But with a high heterogeneity, to explore where it came from, we conducted a subgroup analysis according to 9 case-control studies in Caucasians and 3 case-control studies in Asians. The outcomes revealed the heterogeneity between-study was decreased to accredited level after analysis by ethnicity, suggesting that the source of heterogeneity may mainly come from ethnicity. Subgroup analysis revealed that a significant association was observed between rs10955255 and AHL in the allele model, homozygote model, dominant model, and recessive model in the Caucasian population, but no statistical significance in each gene model of Asian population. In groups of Caucasians, a significant association was observed between rs10955255 and AHL in the allele model, homozygote model, dominant model, and recessive model, while no statistical significance in each gene model of Asians. In addition, Caucasians with the G allele were especially vulnerable to AHL (allele model, G versus A: OR (95%) CI = 1.27 (1.13–1.43)). Also, the NOS scale was employed to validate the quality of these studies (Fig. 6 and Table 3) and eventually confirmed it maybe one of the sources of heterogene-



Figure 4. The association between polymorphism rs10955255 and AHL in 5 genetic models in Asian subgroup analysis. The results indicated that there is no statistical significance in each gene model of Asian population.

Allele mode	el								Homozygot	e mo	del							
	Experime	Intal	Contr	lo		Odds Ratio	Odds Ratio			Experim	ental	Contre	l		Odds Ratio	Odds	Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fixed, 95% Cl		Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	M-H, Fix	ed, 95% Cl	
Van Laer1 2008	294	506	286	504	24.7%	1.06 [0.82, 1.36]			Van Laer1 2008	88	135	82	130	28.6%	1.10 (0.66, 1.81)			
Van Laer2 2008	114	196	124	224	10.0%	1.12 [0.76, 1.65]			Van Laer2 2008	33	50	33	54	10.6%	1.24 [0.55, 2.75]		A	
Van Laer3 2008	170	266	160	272	11.7%	1.24 [0.88, 1.76]			Van Laer3 2008	53	69	41	58	10.2%	1.37 [0.62, 3.04]			
Van Laer4 2008	76	134	72	128	6.6%	1.02 (0.63, 1.66)			Van Laer4 2008	22	35	23	38	8.1%	1.10 [0.43, 2.84]			
Van Laer5 2008	111	180	109	188	8.4%	1.17 (0.77, 1.77)			Van Laer5 2008	35	49	32	49	9.0%	1.33 (0.57, 3.12)	1		
Van Laer6 2008	230	328	200	342	12.0%	1.67 [1.21, 2.29]			Van Laer6 2008	82	98	57	85	9.8%	2.52 [1.25, 5.08]			-
Van Laer7 2008	147	228	121	238	8.7%	1.75 [1.21, 2.54]			Van Laer7 2008	45	57	33	64	6.4%	3.52 [1.58, 7.87]			-
Van Laer8 2008	110	168	107	174	7.5%	1,19 (0.76, 1.85)			Van Laer8 2008	35	44	29	38	6.3%	1.21 [0.42, 3,44]			-
Van Laer9 2008	173	260	153	262	10.5%	1.42 [0.99, 2.02]			Van Laer9 2008	61	79	46	70	10.9%	1.77 [0.86, 3.64]	-		-
Total (95% CI)		2266		2332	100.0%	1.27 [1.13, 1.43]	•		Total (95% CI)		616		586	100.0%	1.54 [1.20, 1.97]		-	
Total events	1425		1332				1. The second se		Total events	454		376					in the second second	
Heterogeneity: Chi ² = ! Test for overall effect:	9.57, df = 8 Z = 3.99 (P	(P = 0.: < 0.000	30); f² = 1 01)	6%			0.2 0.5 1 2 Favours (experimental) Favours (control)	5	Heterogeneity: Chi ² = Test for overall effect:	9.03, df = 8 Z = 3.41 (F	B (P = 0.3 P = 0.000	4); l ² = 11 7)	%			0.2 0.5 Eavours lexperimental	1 2 Favours (control)	5
Heterozygo	te mo	del					, around for house in a second former all		Recessive	mode	el					, areas leitermentel	, mount footmost	
	Experime	Intal	Contr	ol		Odds Ratio	Odds Ratio			Experim	ental	Contro	I		Odds Ratio	Odds	Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	M-H, Fixed, 95% Cl		Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	M-H, Fix	ed, 95% Cl	
Van Laert 2008	118	165	122	170	27.2%	0 99 (0 61 1 59)			Van Laer1 2008	88	253	82	252	24 2%	1 11 10 76 1 601			
Van Laer2 2008	48	65	58	79	10.9%	1.02 [0.49, 2.15]			Van Laer2 2008	33	98	33	112	9.2%	1.22 [0.68, 2.18]			
Van Laer3 2008	64	80	78	95	11.3%	0.87 [0.41 1.86]			Van Laer3 2008	53	133	41	136	11.0%	1 54 10.93 2 541			
Van Laer4 2008	32	45	26	41	6.2%	1.42 (0.57, 3.51)			Van Laer4 2008	22	67	23	64	7.1%	0.87 [0.42, 1.79]			
Van Laer5 2008	41	55	45	62	8.6%	1 11 10 49 2 521			Van Laer5 2008	35	90	32	94	8.6%	1.23 [0.68, 2.25]		-	
Van Laer6 2008	66	82	86	114	11.2%	1.34 [0.67, 2.69]			Van Laer6 2008	82	164	57	171	12.6%	2.00 [1.29, 3.11]			
Van Laer7 2008	57	69	55	86	6.8%	2.68 [1.25, 5.74]			Van Laer7 2008	45	114	33	119	8.8%	1.70 (0.98, 2.94)			
Van Laer8 2008	40	49	49	58	6.5%	0.82 (0.30, 2.25)			Van Laer8 2008	35	84	29	87	7.5%	1.43 [0.77, 2.66]			
Van Laer9 2008	51	69	61	85	11.3%	1.11 [0.55, 2.28]			Van Laer9 2008	61	130	46	131	11.0%	1.63 [0.99, 2.69]			
Total (95% CI)		679		790	100.0%	1.17 [0.92, 1.49]	-		Total (95% CI)		1133		1166	100.0%	1.40 [1.18, 1.67]		•	
Total events	517		580				(Total events	454		376						
Heterogeneity: Chi ² = Test for overall effect:	6.57, df = 8 Z = 1.31 (P	(P = 0.1 = 0.19)	58); I ² = 0	1%			0.2 0.5 1 2 Favours [experimental] Favours [control]	5	Heterogeneity: Chi ² = Test for overall effect:	7.11, df = 8 Z = 3.89 (F	8 (P = 0.5 P < 0.000	2); I ² = 09 1)	6			0.2 0.5 Favours [experimental]	1 2 Favours [control]	5
Dominant n	nodel																	
	Experime	Intal	Contr	lo		Odds Ratio	Odds Ratio											
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Fixed, 95% C	M-H, Fixed, 95% CI											
Van Laer1 2008	206	253	204	252	28.3%	1.03 (0.66, 1.61)												
Van Laer2 2008	81	98	91	112	11.0%	1.10 (0.54, 2.23)												
Van Laer3 2008	117	133	119	136	10.6%	1.04 [0.50, 2.17]												
Van Laer4 2008	54	67	49	64	7.3%	1.27 [0.55, 2.94]												
Van Laer5 2008	76	90	77	94	8.7%	1.20 [0.55, 2.60]												
Van Laer6 2008	148	164	143	171	10.2%	1.81 [0.94, 3.49]		•										
Van Laer7 2008	102	114	88	119	6.8%	2.99 [1.45, 6.18]												
Van Laer8 2008	75	84	78	87	6.1%	0.96 [0.36, 2.55]												
Van Laer9 2008	112	130	107	131	11.0%	1.40 [0.72, 2.72]												
Total (95% CI)		1133		1166	100.0%	1.32 [1.06, 1.65]	•											
Total events	971		956				2 2 2 X											
Heterogeneity: Chi ² = 1	8.13, df = 8	(P = 0.4	42); I ² = 2	1%			02 05 1 2	5										
Test for overall effect:	Z = 2.43 (P	= 0.02)					Favours [experimental] Favours [control]	3										

Figure 5. The association between polymorphism rs10955255 and AHL in 5 genetic models in Caucasian subgroup analysis. The results indicated that there is a close association in the allele model, homozygote model, dominant model and recessive model between the SNP rs10955255 in the GRHL2 gene and AHL susceptibility.



Figure 6. The association between polymorphism rs10955255 and AHL in 5 genetic models in high quality subgroup analysis. The results indicated that there is a close association in the allele model, homozygote model and recessive model between the SNP rs10955255 in the GRHL2 gene and AHL susceptibility.



Figure 7. Publication bias in different genetic models of SNP rs10955255. The results indicated that no publication bias was found in all 5 genetic models of SNP rs10955255 via Bigg' test and Egger's test.

ity. For rs1981361 polymorphism, an increased risks of AHL was found under allele model (A vs G), heterozygote model (GA vs GG) and dominant model (AA+GA vs GG), but not homozygote model (AA vs GG) and recessive model (AA vs GA+GG), suggesting that the risk of AHL is increased in general population with A allele. However, there was only 1 study in Asian and 1 study in Caucasian population separately, so we were unable to do subgroup analysis.

The present study has several restrictions that should be acknowledged. The sample size included in the meta-analysis is relatively small. In our meta-analysis, there were only 2 studies included for SNP site rs1981361, its funnel plots were highly asymmetric, which suggested there is a significant publication bias (data not shown). Limited studies also increase the heterogeneity. For rs2127034 and 13263539, there is only 1 related research respectively, which restricted our next analysis. In addition, Zhou's study showed that the distribution of rs10955255, rs2127034, rs1981361 did not comply with the HWE, the same to Van Laer, L' study of rs10955255 conducted in Denmark. We need a larger sample of reports to further update our conclusion, though 2321 controls and 2762 cases are included in this meta-analysis. The majority of the included studies were about Caucasians, and only a few about Asians, which could make our findings less relevant for the Asian population. We only detected the studies that published in Chinese and English. Maybe there are studies in other languages not covered in this meta-analysis. Therefore, the results could increase random mistake, publication bias, and so on.

As far as we know, fairly complex natural environmental factors and multiple genetic factors, as well as other unknown factors, together led to the occurrence and development of AHL. At present, AHL has become a severe health and social problem. Accumulating testimony showed the allele G in rs10955255 and the allele A rs1981361 (simply in Caucasians) may be the genetic predisposition factor of AHL. However, given the above

limitations, larger studies and further meta-analyses based on populations and other factors should be performed to clarify the association of GRHL2 gene polymorphisms and the risks of AHL.

Author contributions

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