Dermatology Reports
https://www.pagepress.org/journals/index.php/dr/index

eISSN 2036-7406

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Please cite this article as: Pham NTU, Nguyen TV, Nguyen1 HT. HLA-Cw6 increases the risk of psoriasis and early onset before twenty-seven years of age among the Vietnamese population. Dermatol Rep 2023 [Epub Ahead of Print] doi: 10.4081/dr.2023.9854

Submitted: 18/09/2023 – Accepted 05/10/2023

HLA-Cw6 increases the risk of psoriasis and early onset before twenty-seven years of age among the Vietnamese population

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Key words: psoriasis; polymorphism; HLA-Cw6; psoriasis susceptibility; early onset.

Acknowledgments: the authors thank the Center for Molecular Biomedicine, University of Medicine, and Pharmacy at Ho Chi Minh City for supporting the simulated gene sequencing assays for psoriasis. We also thank our colleagues from the Psoriasis Clinic of Ho Chi Minh City Hospital of Dermato-Venereology for collecting the data.

Contributions: NTUP, TVN, data acquisition; NTUP, TVN, HTN, analysis of data, and publishing edition finalization; HTN, manuscript drafting. All the authors approved the final version to be published.

Conflict of interest: the authors declare no potential conflict of interest.

Ethical approval and consent to participate: this study exhibited scrupulous adherence to the ethical guidelines in biomedical research. All laboratory examinations were meticulously conducted strictly following established protocols and testing regulations. This study was approved by the Ethics Council for Biomedical Research at Hanoi Medical University under reference number 66/GCN-HDDDNCCBH HMU, issued on March 16, 2020. The participants were clearly explained the study's objectives and protocols and written informed consent was obtained prior to study enrollment.

Availability of data and material: all data used to support the findings of this study are included within the article. Moreover, the Excel data used to support the findings of this study are available from the corresponding author upon request.

Funding: none.

Abstract

Psoriasis, a chronic inflammatory skin disorder affects people worldwide and is associated with the HLA-Cw6 allele which varies across ethnicities, and is higher in Caucasians than in Asians. We investigated the HLA-Cw6 prevalence in Vietnamese patients with psoriasis and its correlation with onset age and disease severity. We examined the association between HLA-Cw6 and the clinical features in 121 patients with psoriasis and 30 healthy controls and observed that HLA-Cw6 was significantly higher in patients with psoriasis (64.5%) than in the controls (26.7%) (p=0.0001) with an OR of 4.98 (2.04–12.15). The mean age of psoriasis onset was significantly lower in patients with positive HLA-Cw6 than those with negative HLA-Cw6. The AA genotype was more common in patients with mild psoriasis (100%), whereas the TA genotype was prevalent in patients with moderate and severe psoriasis (47.2% and 59.0%, respectively) and in those with high PASI scores (55.1% and 54.1%, respectively). Hence, HLA-Cw6 is a major genetic risk factor for psoriasis in Vietnamese patients, with higher prevalence in patients with early-onset disease. Furthermore, different HLA-Cw6 genotypes are associated with different disease severities.

Introduction

Psoriasis is a chronic inflammatory skin disorder that recurs frequently and affects more than 3% of the US adult population. [1] Besides causing skin lesions, psoriasis profoundly impacts the overall well-being of patients and their quality of life in comparison with internal medical conditions, such as cardiovascular diseases and cancer.[2] The pathogenesis of psoriasis primarily involves a complex interplay of genetics, the immune system, and environmental factors, leading to diverse clinical manifestations, prognoses, and comorbidities among patients. Among the genetic factors, the HLA-Cw6 allele has emerged as the most extensively studied, with associations ranging from disease pathogenesis, epidemiology, and clinical manifestations to comorbidities and treatment responses.

HLA-Cw6 is considered to be one of the most susceptible alleles to psoriasis. The frequency of the HLA-Cw6 allele in psoriasis varies across different ethnicities worldwide, ranging from 10.5–77.2%.[3] The prevalence of HLA-Cw6 positivity among patients with psoriasis is generally lower in Asian countries than in Caucasians. In China, the rate ranges from 32.5– 50.8%,[4,5] while in Japan, it fluctuates between 10.2–12.0%.[6,7] In contrast, in South Korea, the prevalence is relatively high at 76.1%.[8] This indicates that there is indeed variability in the prevalence of HLA-Cw6, even within the Asian population. However, these rates were consistently higher than those observed in the control groups in the aforementioned studies. This suggests a correlation between the presence of HLA-Cw6 and psoriasis, confirming its significant role in the development of this condition.

HLA-Cw6 has been extensively associated with early-onset psoriasis. Previous studies have established an association between HLA-Cw6 and the age at which psoriasis first appears, indicating a heightened susceptibility of individuals carrying the HLA-Cw6 allele to develop psoriasis at a younger age. Rubén Queiro et al. observed a significant difference in the average age of onset between individuals with a positive HLA-Cw6 (23 ± 12 years) and those with a negative HLA-Cw6 (32 ± 12 years).[9] Enerbäck C et al. revealed that individuals with an onset age between 30 and 35 had a similar prevalence of HLA-Cw6 compared with the control group. In contrast, those who developed psoriasis before the age of 21 years showed a significantly higher frequency of HLA-Cw6.[10] These findings support the association between the HLA-Cw6 allele and early-onset of psoriasis.

Furthermore, there is evidence from a few studies that mention the correlation between the HLA-Cw6 allele and the severity of psoriasis. The clinical phenotype and severity of the disease may be associated with certain genetic factors that may be predicted in advance.[11] Individuals who carry the HLA-Cw6 allele are likely to have a higher prevalence of extensive plaques on their arms, legs, and trunk.[3] However, previous studies have not elucidated the association between HLA-Cw6 and disease severity.

This novel study investigated the prevalence of the HLA-Cw6 allele in Vietnamese patients with psoriasis and its potential correlation with clinical characteristics, such as age at onset and disease severity.

Materials and Methods

Patients and controls

A total of 121 patients with psoriasis and 30 healthy controls were enrolled. The inclusion criteria were patients diagnosed with psoriasis based on the 2016 WHO criteria at Ho Chi Minh City Hospital of Dermato-Venereology, aged ≥ 18, having both parents of Vietnamese descent, and volunteering to participate. Exclusion criteria included patients with severe acute illnesses; direct family members with autoimmune, hereditary, or other immunological disorders; and pregnant or breastfeeding women. The control group comprised of healthy individuals without a personal or family history of autoimmune diseases, hereditary disorders, or psoriasis.

HLA-Cw6 sequencing

*Sample collection and DNA extraction:*2 mL of peripheral blood was collected into EDTA anticoagulant tubes, refrigerated, and processed using the ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA).

PCR primer design: The Oligo 4.1 software was used based on the standard sequence of the HLA-C gene with accession number NG_029422 in GenBank. Exon 3 of the HLA-C gene was amplified using the primer pair HLA-Cg3F (5'-AGTCGCCTTTACCCGGTTTC-3') and HLA-Cg3R (5'-AGTGCTGATCCCATTTTCCT-3'), resulting in a PCR product of 472 base pairs. Integrated DNA Technologies (Park Road, Singapore) performed primer synthesis.

PCR reaction: The 25 µl mixture included 2.5 µl of 10X PCR buffer, 2.5 µl of dNTPs (250 µM each), 2.5 µl of forward and reverse primers (0.5 µM each), 0.25 µl of TaKaRa TaqTM HotStart Polymerase (Takara Bio, Japan), and 50–100 ng of DNA. Thermal cycling conditions were 98°C for 3 minutes, followed by 35 cycles of 98°C for 10 seconds, 60°C for 30 seconds, and 72°C for 40 seconds, with a final extension at 72°C for 2 minutes. The PCR product was analyzed on a 1.5% agarose gel using the Geldoc-ItTM gel documentation system (UVP, USA) and purified with ExoSAP-IT® PCR Product Cleanup kit (Thermo Scientific) following the instructions.

Sequencing reaction: Purified PCR products were cycle-sequenced in both directions using BigDye V3.1 (Applied Biosystems, USA). DNA sequences were determined using an ABI 3500 Genetic Analyzer. Sequencher software (version 5.0) was used to analyze the results of the HLA-C gene sequence (NG_029422, GenBank).

Statistical analyses

Excel was used for data entry and R for processing. T-test, ANOVA, and Kruskal–Wallis assessed mean differences, while $\chi 2$ and Fisher's exact tests analyzed proportions. The correlation used OR with 95% CI, providing valuable insights into the research variables. Statistical significance was set at $P < 0.05$.

Results

We collected and analyzed the genotype of HLA-Cw6 in 121 patients with psoriasis and 30 controls at the Ho Chi Minh City Hospital of Dermato-Venereology.

Characteristics of the study group

All basic characteristics of the study group are presented in Table 1.

The mean age of the patients was 42.5 ± 12.4 years (ranging from 21–73 years). Male patients were predominantly in the psoriasis group, accounting for 63.3% of the total patients.

The duration of psoriasis in patients was 12.22 ± 10.22 years, with the shortest duration being < one year and the longest duration reaching up to 69 years. The mean age of onset for the psoriasis patient group was 27.79 ± 10.94, ranging from as young as four years old to as old as 60. Among

patients with psoriasis, 25.6% had a family history. The average body mass index (BMI) was recorded as 23.39 ± 5.33, while the mean Psoriasis Area and Severity Index (PASI) score was reported as 20.31 ± 12.70.

The association between HLA-Cw6 and clinical features of psoriasis

Our study involved sequencing the HLA-Cw6 gene in wells 49: AA, 50: TT, and 51: TA (Figure 1). Sequencing of 151 cases, including the disease and control groups, revealed that HLA-Cw6 was positive in 57% of the total samples and negative in 43%. Specifically, patients with psoriasis showed a significantly higher proportion of HLA-Cw6 positivity (64.5 %), whereas in the control group, the positivity rate for HLA-Cw6 was only 26.7%. The difference between the two groups was significant (p=0.0001). Those with a positive HLA-Cw6 had 4.98 times (95% CI:2.04–12.15, $p < 0.01$) higher odds of having psoriasis than those with a negative HLA-Cw6. On analysis of the allele genotypes of HLA-Cw6, the results reveal that patients with psoriasis had HLA-Cw6 genotypes in a heterogeneous (TA) form. Homogeneous (TT) genotypes comprised 14.1% of cases, whereas genotype AA represented 35.5 %. The proportion of genotype AA in the control group was higher in the control group than that in the psoriasis group (73.3% vs. 35.5%). This difference was significant (P =0.001). The details of this analysis are presented in Table 2.

Our study observed that the group of psoriasis patients with positive HLA-Cw6 had a mean onset age of 26.1 ± 10.9 years, which was significantly different from the group of patients with psoriasis with negative HLA-Cw6 (31.9 ± 10.3 years) (p=0.002). There was a significant difference between the HLA-Cw6 gene and the age of onset of the patients, with the TT genotype showing the earliest onset, followed by the TA and AA genotypes (p =0.01) (Table 3).

Our results revealed that the AA phenotype was commonly observed in mild cases according to the Body Surface Area (BSA) classification (100%). The TA phenotype was more prevalent in the moderate and severe cases (47.2% and 59.0%, respectively). This difference was significant (p=0.01). Similarly, based on the BSA classification, the AA phenotype was commonly found in mild cases according to the Psoriasis Area and Severity Index (PASI) classifications (73.3%). The TA phenotype was more prevalent in the moderate and severe cases (55.1% and 54.1%, respectively). This difference was significant (P =0.03). However, there was no significant difference between the HLA-Cw6 genotypes and the dermatology life quality index (DLQI) classification (p=0.28). (Table 4)

Discussion

The HLA-Cw6 gene is a major risk factor for psoriasis and is present in people of all races and ethnicities.[12] The global prevalence of the HLA-Cw6 allele in patients with psoriasis ranges from

10.5– 77.2%, with significant variation across different ethnic groups.[3] Our study discovered that the overall frequency of HLA-Cw6 was 57%. A review by Chen et al. showed that the prevalence of HLA-Cw6 in patients with psoriasis is higher in Caucasians than in Asians.[3] However, the prevalence varies among countries and ethnicities. The prevalence in our study of Vietnamese patients was 64.5%, which is higher than that reported in certain European studies, such as Croatia (59.3%),[13] Spain (39.0–49.4%),[14,15] and Iceland (64.2%).[16] A few Asian countries have a high prevalence of HLA-Cw6, such as South Korea (76.1%)[8] and India (67.6%-71.1%),[17–19] which is higher than that in our study. Meanwhile, our study is more prevalent than in other Asian countries, such as China (32.5–50.8%),[4,5] Japan (10.2–12.0%),[6,7] and Taiwan (12.1–32.8%).[20–22] These findings suggest that genetic characteristics may vary among different populations, and not only due to geographical location. Studies on the genetic characteristics of Vietnamese people have contributed to global gene mapping. Our study revealed that the proportion of patients with psoriasis was significantly higher (64.5%) than that of the controls (26.7%) (p=0.0001). This result correlates with those of studies conducted in other countries around the world. Although the prevalence of HLA-Cw6 positivity varies among different races and nationalities, [3]it is clear that the prevalence in patients with psoriasis is always significantly higher than that in controls. This finding indicated that individuals with psoriasis were more likely to carry the HLA-Cw6 allele than those without psoriasis in Vietnamese people. The odds ratio of having psoriasis for people with HLA-Cw6 was 4.98. This indicates that people with HLA-Cw6 were 4.98 times more likely to develop psoriasis than those without HLA-Cw6. Our study confirms that HLA-Cw6 is a genetic factor associated with psoriasis in the Vietnamese population. Individuals with HLA-Cw6 have a significantly higher risk of developing psoriasis than those without HLA-Cw6. Therefore, HLA-Cw6 may play a role in the development of psoriasis. Our study also revealed that the most common genotype in patients with psoriasis was TA (50.4%), followed by TT (14.1%), and AA (35.5%). This finding shows that the TA genotype is the most common in patients with psoriasis. In contrast, the AA genotype was the most common in the control group (73.3%). Genotypes TA and TT were associated with a higher risk of the disease, whereas genotype AA was associated with a lower risk. The clear difference between the two groups suggests that HLA-Cw6 polymorphisms are associated with the risk of psoriasis.

Our study revealed that individuals with HLA-Cw6 had a significantly earlier onset of psoriasis than those without the gene. The results demonstrated a significant lower mean age of onset of psoriasis in patients with positive HLA-Cw6 (26.1 ± 10.9 years) than those with negative HLA-Cw6 (31.9 ± 10.3 years). Furthermore, our study is consistent with the findings of Rubén Queiro et al., who noted a significant difference in the average age of onset of psoriasis between people with the HLA-Cw6 gene (23 ± 12 years) and those without the gene (32 ± 12 years). [9] Previous studies

have shown that HLA-Cw6 is associated with early-onset psoriasis. Additionally a study by T Henseler et al. suggested that the prevalence of HLA-Cw6 positivity was 85.3% in patients with early-onset psoriasis. [23] Enerbäck et al. observed that the frequency of HLA-Cw6 was significantly higher in patients with psoriasis that developed before age 21 than in patients with psoriasis that developed at age ≥21.[10] Our findings suggestthat individuals with the HLA-Cw6 allele are more likely to develop psoriasis at an earlier age. The difference in the age at onset was significant (p=0.002). The TT genotype showed the earliest onset, at 24 ± 12.1 years, while the TA and AA genotypes had a later onset, at 27 ± 10.3 years and 30 ± 9.5 years, respectively. This difference in the age at onset was significant (p = 0.01). Additionally, our study indicates that HLA-Cw6 may affect the age of psoriasis onset. We observed that patients with psoriasis in Vietnam with HLA-Cw6 positivity had a higher risk of early onset psoriasis, with the onset age possibly < 27 years of age. This is an important finding because it may help doctors identify individuals who are at an increased risk of developing psoriasis at an early age.

Recent studies have suggested a possible association between the HLA-Cw6 allele and the severity of psoriasis. This suggests that people who carry the HLA-Cw6 allele may be more likely to experience severe psoriasis, with more extensive plaques on their arms, legs, and trunk. [3] The clinical phenotype and severity of the disease may be associated with certain genetic factors, which may be predicted in advance. [11] The study results indicate that the AA phenotype was more common in mild cases of psoriasis, while the TA phenotype was more common in moderate and severe cases. This difference was significant for both BSA (p=0.01) and PASI classifications (p=0.03). The AA phenotype is the most common genotype in the general population (73.3%), however, it is less common in people with psoriasis (35.5%). This suggests that the AA genotype is associated with milder cases of psoriasis. In contrast, the TA phenotype was less common in the general population (19.3%) but more common in patients with psoriasis (50.4%). This suggests that the TA genotype may be associated with severe psoriasis. Our findings insinuate that the HLA-Cw6 genotype may contribute to the severity of psoriasis. However, further studies are required to confirm these findings and understand the exact mechanism of this association.

Limitations and recommendations

This study has a few limitations. However, these limitations do not detract from the significance of our findings. The study was conducted at a single center, however the sample size was large enough to be considered significant. This study did not assess environmental factors, which is a common limitation in genetic studies. Overall, this study provides valuable insights into the genetic basis of psoriasis in the Vietnamese population. Future studies are needed to confirm these findings

and identify other genetic and environmental factors that may contribute to the development of psoriasis.

Conclusions

In conclusion, the findings of this study suggest that HLA-Cw6 is a major genetic risk factor for psoriasis in the Vietnamese population. This is supported by the higher prevalence of HLA-Cw6 in patients with early-onset disease, and the association between different HLA-Cw6 genotypes and disease severity.

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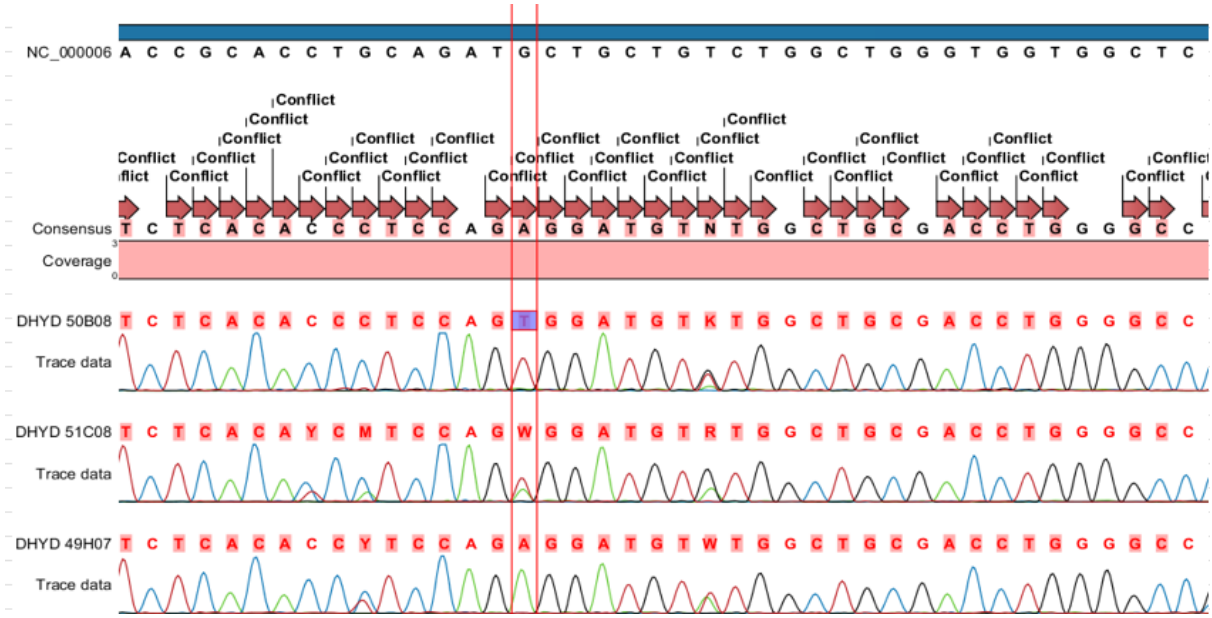

Figure 1. HLA-Cw6 sequencing.

Table 1. Basic characteristics.

Characteristics Mean ± SD (Range)	Total	Study Group		*P*
		Psoriasis (n = 121)	Control (n = 30)	
	n (%)/ Mean ± SD(Range)	n (%)/ Mean ± SD(Range)	n (%)/ Mean ± SD(Range)	
Age	42.5 ± 12.3 (21–73)	42.5 ± 12.4 (21–73)	42.4 ± 12.2 (24–67)	1
Ethnicity Kinh (Vietnamese) Ethnic minorities of Vietnamese	 148 (98.0%) 3 (2.0%)	 119 (98.4%) 2 (1.6%)	 29 (96.7%) 1 (3.3%)	 0.48
Sex Male Female	 95 (62.9%) 56 (37.1%)	 77 (63.6%) 44 (36.4%)	 18 (60.0%) 12 (40.0%)	 0.71
Address Ho Chi Minh City Outside Ho Chi Minh City	 64 (52.9%) 57 (47.1%)	 64 (52.9%) 57 (47.1%)		
Education Primary school Secondary school High school University and postgraduate	 3 (2.5%) 10 (8.3%) 21 (17.4%) 44 (36.3%)	 3 (2.5%) 10 (8.3%) 21 (17.4%) 44 (36.3%)	- 	
Career Office worker Unskilled laborer Self-employed Student Laborer Homemaker Others	 33 (27.3%) 23 (29.7%) 11 (9.1%) 10 (8.3%) 4 (3.3%) 4 (3.3%) 36 (29.7%)	 33 (27.3%) 23 (29.7%) 11 (9.1%) 10 (8.3%) 4 (3.3%) 4 (3.3%) 36 (29.7%)	- 	
Disease duration (years)	12.22 ± 10.22 (0-69)	12.22 ± 10.22 (0-69)	-	

Age of onset (years)	27.79 ± 10.94 (4-60)	27.79 ± 10.94 (4-60)	-	
Family history of psoriasis Yes No	 31 (25.6%) 90 (74.4%)	 31 (25.6%) 90 (74.4%)	-	
Body Mass Index (BMI)	23.39 ± 5.33 (15.43-37.04)	23.39 ± 5.33 (15.43-37.04)	-	
Psoriasis Area and Severity Index (PASI)	20.31 ± 12.70 (1.8 – 61.6)	20.31 ± 12.70 (1.8 – 61.6)	-	

Table 2. HLA-Cw6 allele genotypes (n=151).

HLA-Cw6	Total n (%)	Study Group		*P*	OR (95%CI)
		Psoriasis n (%)	Control n (%)		
HLA-Cw6	(N=151)	(N=121)	(N=30)		
Positive	86 (57.0)	78(64.5)	8 (26.7)	0.0001	4.98 (2.04-12.15)
Negative	65 (43.0)	43 (35.5)	22 (73.3)		
HLA-Cw6 genotypes	(N=151)	(N=121)	(N=30)		
Heterogeneous (TA)	66 (43.7)	61 (50.4)	5 (16.7)	0.001	-
Homogeneous (TT)	20 (13.3)	17 (14.1)	3 (10.0)		
Negative (AA)	65 (43.0)	43 (35.5)	22 (73.3)		

Table 3. HLA-Cw6 and age of onset (n=121).

	n	Age of onset TB ± ĐLC	*p*
HLA-Cw6			

	n	Age of onset TB ± ĐLC	*p*
Positive	78	26.1 ± 10.9	0.002
Negative	43	31.9 ± 10.3	
HLA-Cw6 Genotypes			
Heterogeneous (TA)	61	26.4 ± 10.6	0.01
Homogeneous (TT)	17	24 ± 12.1	
Negative (AA)	43	31.9 ± 10.3	

Table 4. HLA-Cw6 genotypes and clinical characteristics of the psoriasis group (n=121)

Gen HLA-Cw6	AA n (%)	TA n (%)	TT n (%)	*p*
Body surface area (BSA)	N= 43	N=61	N=17	
Mild (BSA < 10)	7 (100.0)	0 (0.0)	0 (0.0)	0.01
Moderate (10 ≤ BSA ≤ 30)	18 (33.9)	25 (47.2)	10 (18.9)	
Severe (BSA > 30)	18 (29.5)	36 (59.0)	7 (11.5)	
Psoriasis area and severity index (PASI)				
Mild (PASI < 10)	11 (73.3)	3 (20.0)	1 (6.7)	0.03
Moderate (10 ≤ PASI < 20)	21 (30.4)	38 (55.1)	10 (14.5)	
Severe (PASI ≥ 20)	11 (29.7)	20 (54.1)	6 (16.2)	
Dermatology life quality index (DLQI) (effect on patient's life)				
No effect	0 (0.0)	1 (100.0)	0 (0.0)	0.28
Small effect	10 (55.6)	5 (27.8)	3 (16.6)	
Moderate effect	11 (39.3)	12 (42.9)	5 (17.8)	
Very large effect	15 (33.3)	26 (57.8)	4 (8.9)	
Extremely large effect	7 (24.1)	17 (58.6)	5 (17.3)	