ORIGINAL RESEARCH

Use of phosphodiesterase type 5 inhibitors and risk of melanoma: a meta-analysis of observational studies

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Background: Phosphodiesterase type 5 inhibitor (PE5i) administration may stimulate the proliferation and survival of melanocytes. However, discrepancies remain regarding the association between PDE5i use and melanoma risk in observational studies in humans.
Aim: To evaluate the association between PDE5i use and melanoma in a meta-analysis.
Materials and methods: Studies were identified by searching the PubMed and Embase databases. A random-effects model was applied to synthesize the data. A stratified study was performed to evaluate the influence of study characteristics on outcomes.
Results: Four prospective cohort studies and three case–control studies with 1,534,615 male participants and 16,053 melanoma cases were incorporated. Patients who received a PDE5i had a significantly increased risk for melanoma (adjusted risk ratio [RR] =1.12, 95% CI =1.03–1.33, P=0.008) with moderate heterogeneity (I^2=54%). Cohort studies (adjusted RR =1.22, 95% CI =1.02–1.46, P=0.03) largely contributed to this result rather than case–control studies. Subsequent stratified analyses revealed that sildenafil was associated with an increased risk of melanoma (adjusted RR =1.26, 95% CI =1.07–1.50, P=0.007), but tadalafil and vardenafil were not. Also, PDE5i use was associated with a significantly increased risk of in situ melanoma (adjusted RR =1.31, 95% CI =1.01–1.69, P=0.04), but not of localized or nonlocalized melanoma.
Conclusion: PDE5i use may be associated with a significantly increased risk for melanoma in men. However, further research is needed to determine whether the association is causative.
Keywords: phosphodiesterase type 5 inhibitors, melanoma, meta-analysis, sildenafil

Introduction

Melanoma is a malignant tumor that has been correlated with endogenous factors such as skin–eye–hair color, family history, and the presence of common or atypical nevi, as well as exogenous factors such as excessive sun exposure.[1] Biologically, many dysfunctions of molecular pathways have been demonstrated to be involved in the pathogenesis of melanoma, and the activation of the RAS/RAF/mitogen-activated protein kinase and extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK signaling pathway is one of the main key signals that determine the pathogenesis and progression of melanoma.[1–3] Moreover, activation of the RAS/RAF/ERK/MEK pathway has been recognized to be a result of BRAF somatic mutation (OMIM*164757), which causes the downregulation of cyclic guanosine monophosphate (cGMP)–specific phosphodiesterase (PDE) 5A and lowers cGMP degradation, leading to an increase in intracellular Ca^{2+}.[4–6] These mechanisms have been considered to be important for the invasion and metastasis of melanocytes, and treatments targeting these signaling

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pathways have shown promising therapeutic efficacy in patients with melanoma.[7,8]

Phosphodiesterase type 5 inhibitors (PDE5is), including sildenafil, tadalafil, and vardenafil, are a category of medications approved for the treatment of erectile dysfunction.[9–11] These medications function as inhibitors of cGMP-specific PDE5A, which mimic the effects of the RAS/RAF/ERK/MEK pathway and may thereby contribute to the development and progression of melanoma. Indeed, some experimental studies in cell lines in vitro have indicated that the administration of a PDE5i was associated with enhanced proliferation and survival of melanocytes.[12–14] However, the results of subsequent observational studies regarding the relationship between PDE5i use and melanoma risk were not always consistent.[15–20] The discrepancies of these results may be due to the differences in the included participants, regimens of PDE5i administration, and limited sample sizes of some related studies, which may be underpowered to detect a significant result.[21] Therefore, in this study, we performed a meta-analysis of observational studies to elucidate the association between PDE5i use and risk of melanoma. Moreover, we also aimed to evaluate whether the relationship between PDE5i use and risk of melanoma was significantly affected by the individual application of PDE5i.

Materials and methods

The Meta-analysis of Observational Studies in Epidemiology[22] and Cochrane's Handbook guidelines[23] were followed during the performance of this meta-analysis.

Database searching

The PubMed and Embase databases were systematically searched for potential relevant records using the following searching terms: "phosphodiesterase type 5 inhibitors", "PDE5 inhibitors", "PDE5i", "avanafil", "lodenafil", "mirodenafil", "sildenafil", "tadalafil", "udenafil", or "vardenafil", combined with "melanoma". This search was limited to human studies and English language publications. The references of the original and review articles were also manually analyzed. The final literature search was performed on April 14, 2017.

Inclusion and exclusion criteria

Articles were included in this meta-analysis if the following criteria were met: 1) published as full-length articles or abstracts of scientific sessions in English; 2) reported as cohort studies or case–control studies; 3) included an adult population of ≥18 years of age; 4) identified use of PDE5i as exposure of interest; 5) included those not treated with a PDE5i as controls; 6) compared the risk of melanoma between participants who used a PDE5i vs those who did not; 7) reported the adjusted hazards ratio, relative risk (RR), or odds ratio and their corresponding 95% CIs for melanoma risk between the PDE5i users and controls. For studies that included overlapping participants, those with a larger sample size were included in the meta-analysis. Irrelevant studies, editorials, review articles, and experimental studies in cell lines or animals were excluded.

Data extraction and quality evaluation

Two authors independently performed the literature search, data extraction, and quality assessment according to the predefined inclusion criteria. Discrepancies were resolved by consensus. The extracted data included the following: 1) name of the first author, year of publication, and country where the study was conducted; 2) the general characteristics and source of the study participants; 3) the range of the study years; 4) the total number of participants, PDE5i users, and melanoma cases; 5) the follow-up durations for the included cohort studies; 6) the strategies used to confirm a diagnosis for melanoma; and 7) the adjusted variables for reporting the association between PDE5i use and melanoma risk. The quality of each study was evaluated using the Newcastle–Ottawa Scale,[24] which ranged from 1 to 9 stars, and each study was evaluated based on three aspects: the selection of the study groups, the comparability of these groups, and the ascertainment of the outcome of interest.

Statistical analyses

RR was used as the general measure for the association between the use of PDE5i and risk of melanoma across studies, and the most adequately adjusted data were extracted for subsequent meta-analyses. The data of RRs and their corresponding standard errors were calculated from 95% CIs or P-values, and were logarithmically transformed to stabilize variance and normalize the distribution.[23] Cochrane's Q-test and I^2-test were applied to evaluate the heterogeneity among the included studies.[25] Significant heterogeneity was confirmed if I^2 was >50%. A random-effects model was used to synthesize the RR data, because this model is expected to retrieve a more generalized result via the incorporation of potential heterogeneity.[23] Sensitivity analyses were performed by removing one study at a time to test the robustness of the results.[26] Stratified analyses were performed to evaluate whether the associations between PDE5i use and melanoma risk were different according to different study characteristics such as the study design (cohort studies or case–control studies), the timing of PDE5i administration (recent use or early use), the number of prescriptions (the lowest or

highest prescription categories), the individual PDE5i used (sildenafil, tadalafil, or vardenafil), and the stages of melanoma evaluated (in situ, localized, or nonlocalized). Since the definitions of the recent use of PDE5i were not consistent among the included studies, the definitions included from the original articles were used. In addition, since the cut-offs for the categorization of the PDE5i prescriptions were also not consistent among these included studies, the definitions from the original articles were used, and the associations between PDE5i use and melanoma risk were summarized in the lowest and highest prescription categories, respectively. Potential publication bias was assessed by funnel plots using Egger regression asymmetry test.[27] RevMan (Version 5.1; Cochrane Collaboration, Oxford, UK) and STATA software (Version 12.0; Stata Corporation, College Station, TX, USA) were used for the meta-analysis and statistical analysis.

Results
Literature search
The literature search process is presented in Figure 1. Briefly, 726 articles were identified via the initial literature search in the PubMed and Embase databases, and 706 articles were excluded after the screening of titles and abstracts, mainly due
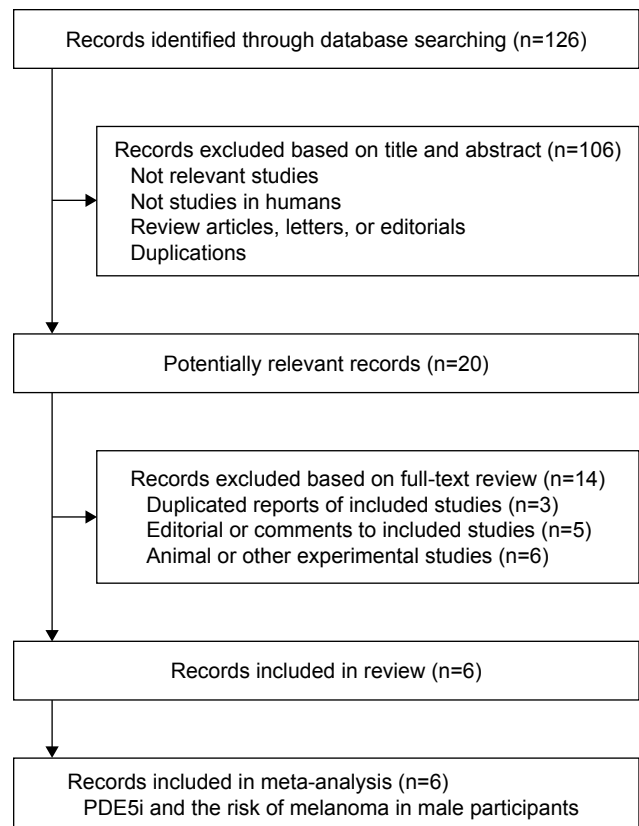


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│ Records identified through database          │
│ searching (n=126)                            │
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        │
        │      ┌──────────────────────────────────────┐
        │─────▶│ Records excluded based on title and  │
        │      │ abstract (n=106)                     │
        │      │   Not relevant studies               │
        │      │   Not studies in humans              │
        │      │   Review articles, letters, or       │
        │      │   editorials                         │
        │      │   Duplications                       │
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│ Potentially relevant records (n=20)          │
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        │─────▶│ Records excluded based on full-text  │
        │      │ review (n=14)                        │
        │      │   Duplicated reports of included     │
        │      │   studies (n=3)                      │
        │      │   Editorial or comments to included  │
        │      │   studies (n=5)                      │
        │      │   Animal or other experimental       │
        │      │   studies (n=6)                      │
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│ Records included in review (n=6)             │
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        ▼
┌─────────────────────────────────────────────┐
│ Records included in meta-analysis (n=6)      │
│ PDE5i and the risk of melanoma in male       │
│ participants                                 │
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Figure 1 Flowchart of study selection.
Abbreviation: PDE5i, phosphodiesterase type 5 inhibitor.

to the irrelevance of these articles to the purpose of this study. Subsequently, 20 potential relevant articles underwent full-text review. Among these, 14 articles were further excluded. Among these excluded articles, three articles were duplicate reports, five articles were editorial or comments, and six articles were experimental studies. Finally, six articles[15–20] were included in the meta-analysis.

Characteristics and quality of the included studies
Since one of the included articles[20] reported the data of two case–control studies (the Danish Nationwide Health Registries and the Kaiser Permanente Northern California), a total of seven studies with 1,534,615 participants and 16,053 melanoma cases were incorporated into our meta-analysis.[15–20] The characteristics and quality scores of these included studies are summarized in Table 1. All of these studies were published after 2014. Among these, four studies were designed as cohort studies,[15,17–19] one study was a nested case–control study,[16] and two studies were case–control studies.[17] Furthermore, among these included studies, three studies were conducted in the USA,[15,17,20] two studies in the UK,[18,19] and two studies in Northern Europe.[16,20] As for the general characteristics of the included participants, six studies included males from the general population[15–17,19,20] and one study included males with erectile dysfunction.[18] The mean age of the included participants ranged from 57.0 to 74.5 years, and the indexed study year ranged from 1998 to 2015. Regarding the investigation of individual PDE5i, sildenafil users were analyzed in all of the included studies, while tadalafil and vardenafil users were evaluated in five studies. Melanoma diagnosis was confirmed according to the International Classification of Diseases version 9 or 10, or with the information of national cancer registries. All studies were adjusted for multiple variables (at least for age and race) in reporting the association between the use of PDE5i and melanoma risk. The Newcastle–Ottawa scale scores ranged from 6 to 9 in the included cohort studies.

Overall results for the association between PDE5i use and melanoma risk
The pooled results of the six studies were analyzed with the random-effects model to evaluate the overall association between PDE5i use and risk of melanoma. The results revealed that PDE5i treatment was associated with a significantly increased risk for melanoma (adjusted RR =1.12, 95% CI =1.03–1.33, P=0.008; Figure 2A). Furthermore, considerable heterogeneity was detected among the included studies (P for Cochrane's Q-test =0.05, I^2=54%). Sensitivity

Table 1 Characteristics of the included studies

Study	Design	Country	Population	Study year	No of participants	Mean age Years	Users of PDE5i Meds (n)	Follow-up Person-years	Confirmation of melanoma diagnosis	No of melanoma cases	Adjusted factors	NOS
Li et al[15] 2014	PC	USA	US male health professionals	2000–2010	25,848	64.8	Sildenafil (1,378)	204,870	Pathologically confirmed invasive cases in the medical records	142	Age, BMI, smoking, physical activity, childhood reaction to sun, number of severe sunburns, mole count, hair color, family history of melanoma, sun exposure, UV index, and other treatments for ED	8
Loeb et al[16] 2015	NCC	Sweden	Swedish men with no prostate cancer	2006–2012	24,390	74.5	Sildenafil, vardenafil, or tadalafil (2,148)	NR	The Swedish Prescribed Drug Register	4,065	CCI, marital status, educational level, and disposable income	8
Pottegard et al[20] 2016 (DNHR)	CC	Denmark	Danish men	2000–2012	77,495	61.0	Sildenafil, or tadalafil (448)	NA	The Danish Cancer Registry	7,045	Age, calendar time, use of medications, diagnoses of NMSC, DM, COPD, alcohol-related disease, renal disease, and education status	7
Pottegard et al[20] 2016 (KPNC)	CC	USA	Northern California men	2000–2014	32,279	64.0	Sildenafil or vardenafil (568)	NA	KPNC Cancer Registry	2,972	Age, calendar time, use of medications, diagnoses of NMSC, DM, COPD, alcohol-related disease, renal disease, and socioeconomic status	7
Lian et al[18] 2016	PC	UK	ED men	1998–2015	142,983	59.0	Sildenafil, vardenafil, or tadalafil (58,732)	698,479	UK Clinical Practice Research Datalink	440	Age, year of cohort entry, alcohol-related disorders, smoking status, BMI, CCI, precancerous skin lesions, use of antiparkinsonian drugs, immunosuppression, influenza vaccination, referral to colonoscopy, and PSA	9
Boor[17] 2016	RC	USA	USA men	2010–2014	525,523	NR	Sildenafil, vardenafil, or tadalafil (12,357)	NR	ICD9	74	Age and race	6
Matthews et al[19] 2016	PC	UK	UK men	1999–2014	706,097	57.0	Sildenafil, vardenafil, or tadalafil (145,104)	3,459,875	Data by National Health Service and ICD10	1,315	Age, index year, BMI, alcohol use, current drinker	9

Abbreviations: BMI, body mass index; CC, case–control; CCI, Charlson comorbidity index; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; DNHR, Danish Nationwide Health Registries; ED, erectile dysfunction; ICD, International Classification of Diseases; KPNC, Kaiser Permanente Northern California; NA, not applicable; NCC, nested case–control; NMSC, nonmelanoma skin cancer; NOS, Newcastle–Ottawa Scale; NR, not reported; PC, prospective cohort; PDE5i, phosphodiesterase type 5 inhibitor; PSA, prostate-specific antigen; RC, retrospective case–control; UV, ultraviolet.
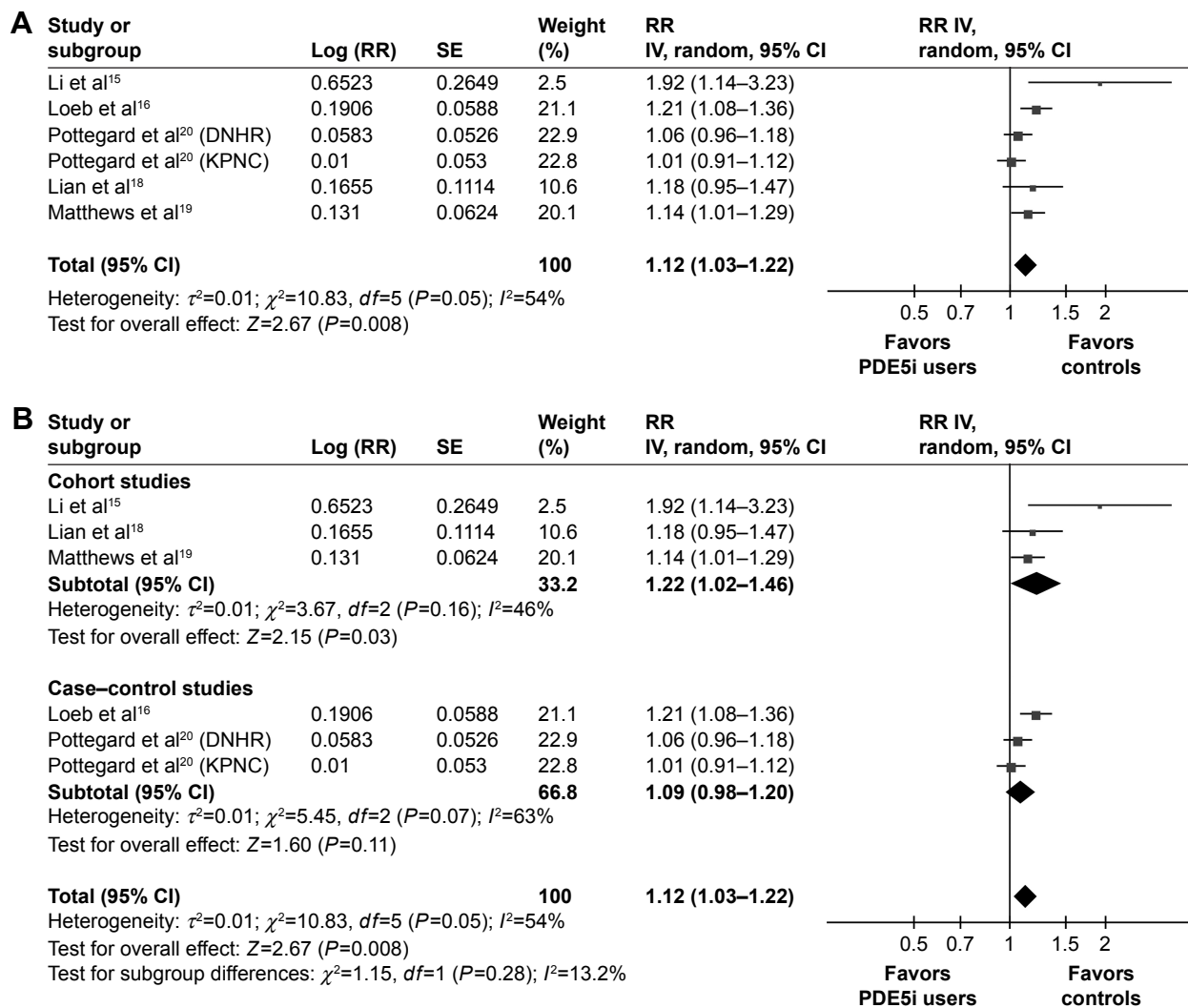
A

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
Li et al[15]	0.6523	0.2649	2.5	1.92 (1.14–3.23)	
Loeb et al[16]	0.1906	0.0588	21.1	1.21 (1.08–1.36)	
Pottegard et al[20] (DNHR)	0.0583	0.0526	22.9	1.06 (0.96–1.18)	
Pottegard et al[20] (KPNC)	0.01	0.053	22.8	1.01 (0.91–1.12)	
Lian et al[18]	0.1655	0.1114	10.6	1.18 (0.95–1.47)	
Matthews et al[19]	0.131	0.0624	20.1	1.14 (1.01–1.29)	
Total (95% CI)			**100**	**1.12 (1.03–1.22)**	

Heterogeneity: τ^2=0.01; χ^2=10.83, df=5 (P=0.05); I^2=54%
Test for overall effect: Z=2.67 (P=0.008)

0.5 0.7 1 1.5 2
Favors PDE5i users — Favors controls

B

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
Cohort studies					
Li et al[15]	0.6523	0.2649	2.5	1.92 (1.14–3.23)	
Lian et al[18]	0.1655	0.1114	10.6	1.18 (0.95–1.47)	
Matthews et al[19]	0.131	0.0624	20.1	1.14 (1.01–1.29)	
Subtotal (95% CI)			**33.2**	**1.22 (1.02–1.46)**	

Heterogeneity: τ^2=0.01; χ^2=3.67, df=2 (P=0.16); I^2=46%
Test for overall effect: Z=2.15 (P=0.03)

Case–control studies					
Loeb et al[16]	0.1906	0.0588	21.1	1.21 (1.08–1.36)	
Pottegard et al[20] (DNHR)	0.0583	0.0526	22.9	1.06 (0.96–1.18)	
Pottegard et al[20] (KPNC)	0.01	0.053	22.8	1.01 (0.91–1.12)	
Subtotal (95% CI)			**66.8**	**1.09 (0.98–1.20)**	

Heterogeneity: τ^2=0.01; χ^2=5.45, df=2 (P=0.07); I^2=63%
Test for overall effect: Z=1.60 (P=0.11)

| **Total (95% CI)** | | | **100** | **1.12 (1.03–1.22)** | |

Heterogeneity: τ^2=0.01; χ^2=10.83, df=5 (P=0.05); I^2=54%
Test for overall effect: Z=2.67 (P=0.008)
Test for subgroup differences: χ^2=1.15, df=1 (P=0.28); I^2=13.2%

0.5 0.7 1 1.5 2
Favors PDE5i users — Favors controls

Figure 2 Forest plots for the meta-analysis of the association between PDE5i use and risk of melanoma presented as adjusted RR.
Notes: (**A**) Forest plots for all available observational studies; (**B**) forest plots stratified by study design (cohort studies or case–control studies). The effect size of each study is proportional to the statistical weight. The diamond indicates the overall summary estimate for the analysis; the width of the diamond represents the 95% CI.
Abbreviations: df, degrees of freedom; DNHR, Danish Nationwide Health Registries; IV, inverse variance; KPNC, Kaiser Permanente Northern California; PDE5i, phosphodiesterase type 5 inhibitor; RR, risk ratio; SE, standard error.

analyses conducted by omitting one study at a time did not significantly change these results, suggesting the robustness of the results. Subsequent subgroup analyses by study design revealed that PDE5i use was associated with a significantly increased risk for melanoma in these cohort studies (three studies,[15,18,19] adjusted RR =1.22, 95% CI =1.02–1.46, P=0.03, I^2=46%; Figure 2B), but the association was not significant in the pooled results for three case–control studies[16,20] (adjusted RR =1.12, 95% CI =1.03–1.22, P=0.11, I^2=63%; Figure 2B).

Stratified analysis of the association between PDE5i use and risk of melanoma

A stratified analysis was performed according to the timing of PDE5i use, the number of prescriptions, the individual PDE5I used, and the stage of melanoma. Two studies[15,16] provided stratified data according to the timing of PDE5i use, and these pooled results revealed that recent use of PDE5i was associated with a significantly increased risk for melanoma (adjusted RR =1.38, 95% CI =1.02–1.86, P=0.04, I^2=35%; Figure 3A), whereas early use of these medications was not (adjusted RR =1.46, 95% CI =0.76–2.80, P=0.26, I^2=77%; Figure 3A). Moreover, the stratified analysis of five studies[16,18–20] indicated that PDE5i use was associated with an increased risk of melanoma in both the lowest (adjusted RR =1.10, 95% CI =1.00–1.20, P=0.04, I^2=40%; Figure 3B) and highest (adjusted RR =1.14, 95% CI =1.01–1.29, P=0.04, I^2=32%; Figure 3B) prescription categories. Further analyses regarding the individual use of PDE5i revealed that the use of sildenafil was associated with
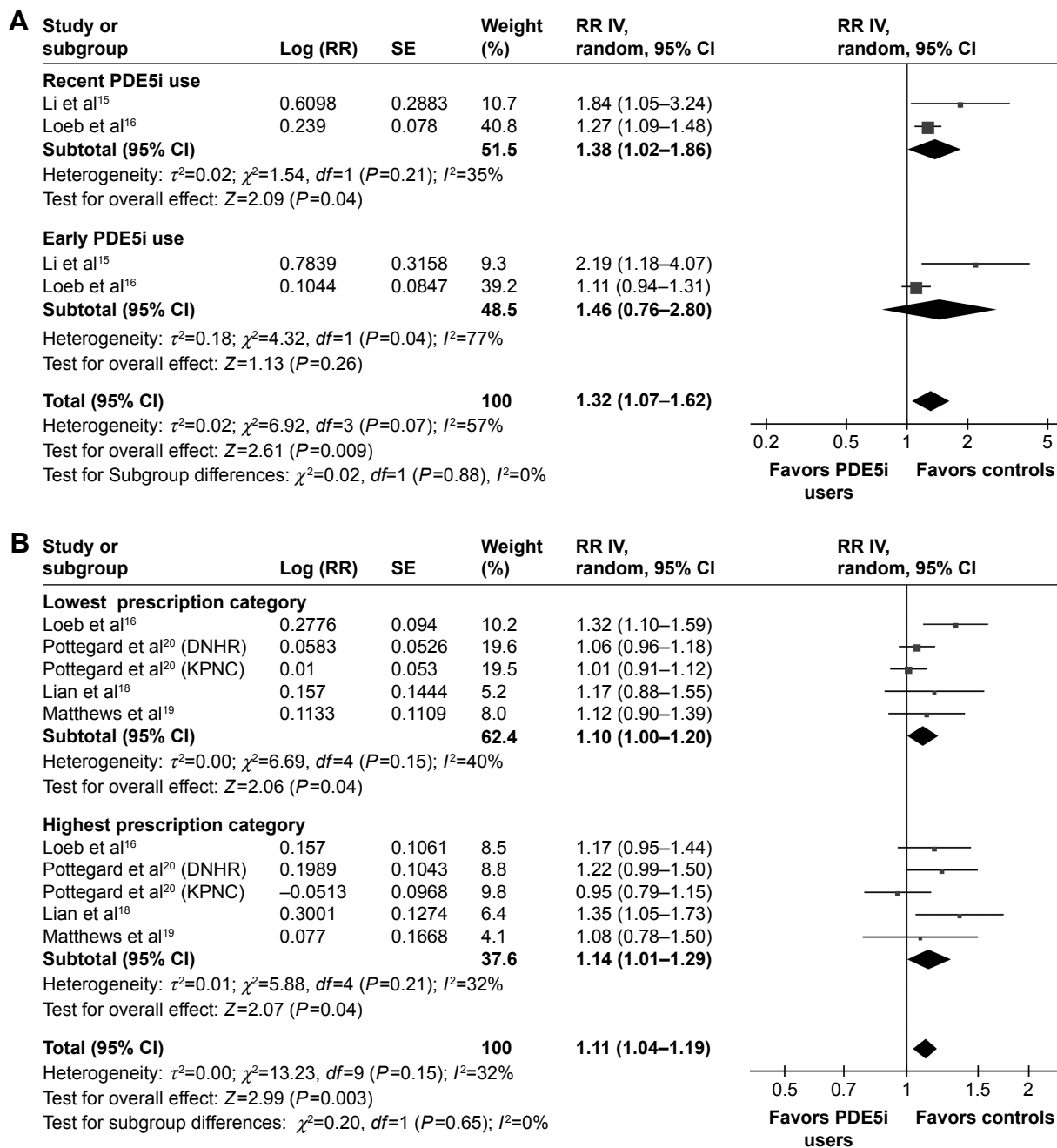
A

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
Recent PDE5i use					
Li et al[15]	0.6098	0.2883	10.7	1.84 (1.05–3.24)	
Loeb et al[16]	0.239	0.078	40.8	1.27 (1.09–1.48)	
Subtotal (95% CI)			**51.5**	**1.38 (1.02–1.86)**	

Heterogeneity: τ^2=0.02; χ^2=1.54, df=1 (P=0.21); I^2=35%
Test for overall effect: Z=2.09 (P=0.04)

Early PDE5i use					
Li et al[15]	0.7839	0.3158	9.3	2.19 (1.18–4.07)	
Loeb et al[16]	0.1044	0.0847	39.2	1.11 (0.94–1.31)	
Subtotal (95% CI)			**48.5**	**1.46 (0.76–2.80)**	

Heterogeneity: τ^2=0.18; χ^2=4.32, df=1 (P=0.04); I^2=77%
Test for overall effect: Z=1.13 (P=0.26)

Total (95% CI)			**100**	**1.32 (1.07–1.62)**	

Heterogeneity: τ^2=0.02; χ^2=6.92, df=3 (P=0.07); I^2=57%
Test for overall effect: Z=2.61 (P=0.009)
Test for Subgroup differences: χ^2=0.02, df=1 (P=0.88), I^2=0%

0.2 0.5 1 2 5
Favors PDE5i users   Favors controls

B

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
Lowest prescription category					
Loeb et al[16]	0.2776	0.094	10.2	1.32 (1.10–1.59)	
Pottegard et al[20] (DNHR)	0.0583	0.0526	19.6	1.06 (0.96–1.18)	
Pottegard et al[20] (KPNC)	0.01	0.053	19.5	1.01 (0.91–1.12)	
Lian et al[18]	0.157	0.1444	5.2	1.17 (0.88–1.55)	
Matthews et al[19]	0.1133	0.1109	8.0	1.12 (0.90–1.39)	
Subtotal (95% CI)			**62.4**	**1.10 (1.00–1.20)**	

Heterogeneity: τ^2=0.00; χ^2=6.69, df=4 (P=0.15); I^2=40%
Test for overall effect: Z=2.06 (P=0.04)

Highest prescription category					
Loeb et al[16]	0.157	0.1061	8.5	1.17 (0.95–1.44)	
Pottegard et al[20] (DNHR)	0.1989	0.1043	8.8	1.22 (0.99–1.50)	
Pottegard et al[20] (KPNC)	−0.0513	0.0968	9.8	0.95 (0.79–1.15)	
Lian et al[18]	0.3001	0.1274	6.4	1.35 (1.05–1.73)	
Matthews et al[19]	0.077	0.1668	4.1	1.08 (0.78–1.50)	
Subtotal (95% CI)			**37.6**	**1.14 (1.01–1.29)**	

Heterogeneity: τ^2=0.01; χ^2=5.88, df=4 (P=0.21); I^2=32%
Test for overall effect: Z=2.07 (P=0.04)

Total (95% CI)			**100**	**1.11 (1.04–1.19)**	

Heterogeneity: τ^2=0.00; χ^2=13.23, df=9 (P=0.15); I^2=32%
Test for overall effect: Z=2.99 (P=0.003)
Test for subgroup differences: χ^2=0.20, df=1 (P=0.65); I^2=0%

0.5 0.7 1 1.5 2
Favors PDE5i users   Favors controls

Figure 3 Forest plots for the meta-analysis of the association between PDE5i use and risk of melanoma presented as adjusted RR.
Notes: (**A**) Forest plots stratified by the timing of PDE5i administration (recent or early use of PDE5i); (**B**) forest plots stratified by the categories of PDE5i prescription (lowest or highest prescription category). The effect size of each study is proportional to the statistical weight. The diamond indicates the overall summary estimate for the analysis; the width of the diamond represents the 95% CI.
Abbreviations: df, degrees of freedom; DNHR, Danish Nationwide Health Registries; IV, inverse variance; KPNC, Kaiser Permanente Northern California; PDE5i, phosphodiesterase type 5 inhibitor; RR, risk ratio; SE, standard error.

a significantly increased risk for melanoma (six studies,[15–18,20] adjusted RR =1.26, 95% CI =1.07–1.50, P=0.007, I^2=79%; Figure 4A), whereas use of tadalafil (three studies,[17,18,20] adjusted RR =1.17, 95% CI =0.91–1.50, P=0.22, I^2=53%; Figure 4A) or vardenafil (two studies,[18,20] adjusted RR =1.02, 95% CI =0.90–1.16, P=0.73, I^2=0%; Figure 4A) was not. Finally, the stratified analysis according to the stage of

melanoma revealed that PDE5i use was associated with a significantly increased risk for in situ melanoma (two studies,[16,20] adjusted RR =1.31, 95% CI =1.01–1.69, P=0.04, I^2=69%; Figure 4B), showed a trend toward increased risk for localized melanoma (three studies,[16,20] adjusted RR =1.13, 95% CI =0.99–1.29, P=0.06, I^2=23%; Figure 4B), and showed a trend toward reduced risk for nonlocalized melanoma
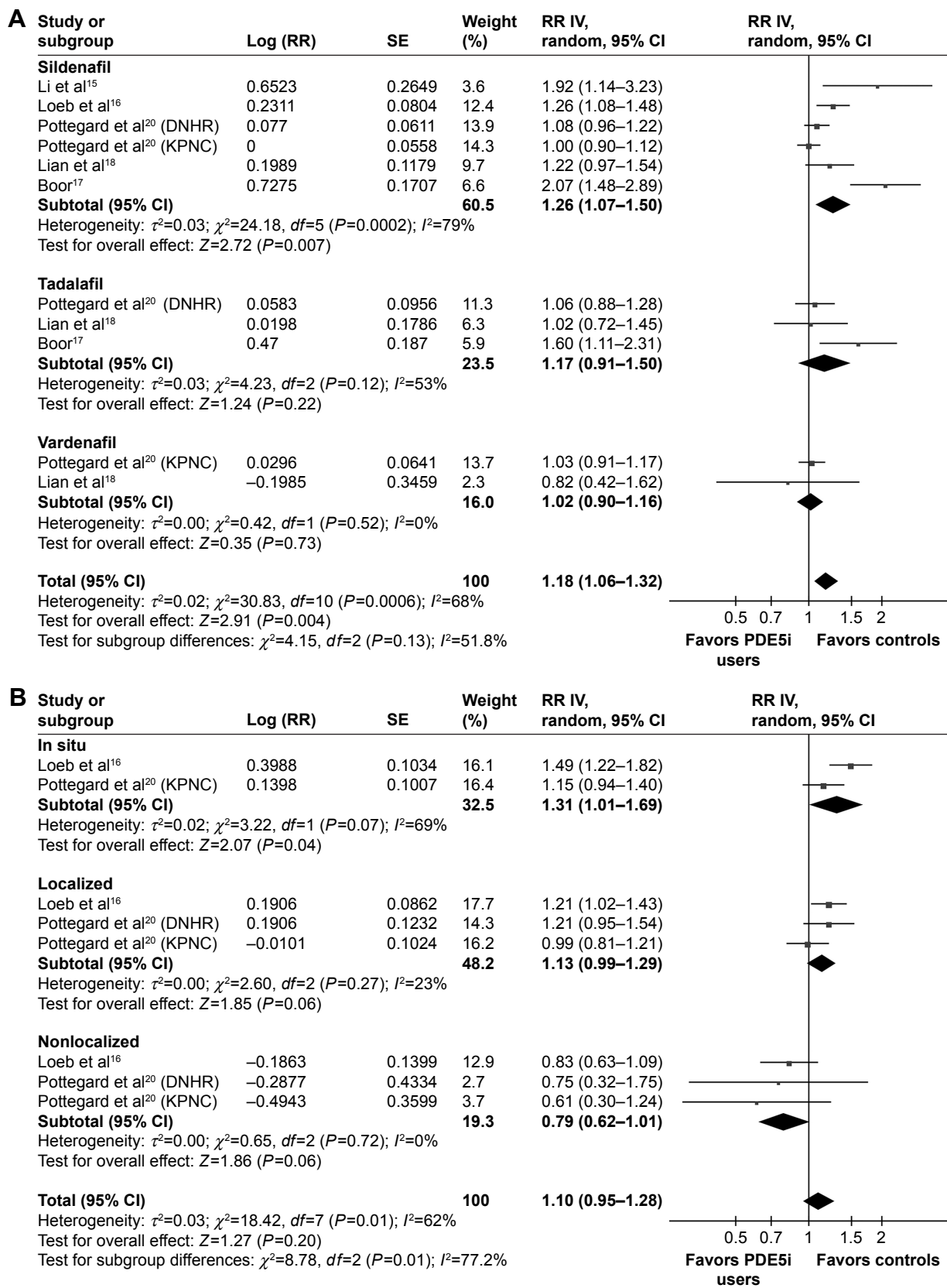
A

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
Sildenafil					
Li et al[15]	0.6523	0.2649	3.6	1.92 (1.14–3.23)	
Loeb et al[16]	0.2311	0.0804	12.4	1.26 (1.08–1.48)	
Pottegard et al[20] (DNHR)	0.077	0.0611	13.9	1.08 (0.96–1.22)	
Pottegard et al[20] (KPNC)	0	0.0558	14.3	1.00 (0.90–1.12)	
Lian et al[18]	0.1989	0.1179	9.7	1.22 (0.97–1.54)	
Boor[17]	0.7275	0.1707	6.6	2.07 (1.48–2.89)	
Subtotal (95% CI)			**60.5**	**1.26 (1.07–1.50)**	

Heterogeneity: τ^2=0.03; χ^2=24.18, df=5 (P=0.0002); I^2=79%
Test for overall effect: Z=2.72 (P=0.007)

Tadalafil					
Pottegard et al[20] (DNHR)	0.0583	0.0956	11.3	1.06 (0.88–1.28)	
Lian et al[18]	0.0198	0.1786	6.3	1.02 (0.72–1.45)	
Boor[17]	0.47	0.187	5.9	1.60 (1.11–2.31)	
Subtotal (95% CI)			**23.5**	**1.17 (0.91–1.50)**	

Heterogeneity: τ^2=0.03; χ^2=4.23, df=2 (P=0.12); I^2=53%
Test for overall effect: Z=1.24 (P=0.22)

Vardenafil					
Pottegard et al[20] (KPNC)	0.0296	0.0641	13.7	1.03 (0.91–1.17)	
Lian et al[18]	−0.1985	0.3459	2.3	0.82 (0.42–1.62)	
Subtotal (95% CI)			**16.0**	**1.02 (0.90–1.16)**	

Heterogeneity: τ^2=0.00; χ^2=0.42, df=1 (P=0.52); I^2=0%
Test for overall effect: Z=0.35 (P=0.73)

Total (95% CI)			**100**	**1.18 (1.06–1.32)**	

Heterogeneity: τ^2=0.02; χ^2=30.83, df=10 (P=0.0006); I^2=68%
Test for overall effect: Z=2.91 (P=0.004)
Test for subgroup differences: χ^2=4.15, df=2 (P=0.13); I^2=51.8%

0.5 0.7 1 1.5 2
Favors PDE5i users   **Favors controls**

B

Study or subgroup	Log (RR)	SE	Weight (%)	RR IV, random, 95% CI	RR IV, random, 95% CI
In situ					
Loeb et al[16]	0.3988	0.1034	16.1	1.49 (1.22–1.82)	
Pottegard et al[20] (KPNC)	0.1398	0.1007	16.4	1.15 (0.94–1.40)	
Subtotal (95% CI)			**32.5**	**1.31 (1.01–1.69)**	

Heterogeneity: τ^2=0.02; χ^2=3.22, df=1 (P=0.07); I^2=69%
Test for overall effect: Z=2.07 (P=0.04)

Localized					
Loeb et al[16]	0.1906	0.0862	17.7	1.21 (1.02–1.43)	
Pottegard et al[20] (DNHR)	0.1906	0.1232	14.3	1.21 (0.95–1.54)	
Pottegard et al[20] (KPNC)	−0.0101	0.1024	16.2	0.99 (0.81–1.21)	
Subtotal (95% CI)			**48.2**	**1.13 (0.99–1.29)**	

Heterogeneity: τ^2=0.00; χ^2=2.60, df=2 (P=0.27); I^2=23%
Test for overall effect: Z=1.85 (P=0.06)

Nonlocalized					
Loeb et al[16]	−0.1863	0.1399	12.9	0.83 (0.63–1.09)	
Pottegard et al[20] (DNHR)	−0.2877	0.4334	2.7	0.75 (0.32–1.75)	
Pottegard et al[20] (KPNC)	−0.4943	0.3599	3.7	0.61 (0.30–1.24)	
Subtotal (95% CI)			**19.3**	**0.79 (0.62–1.01)**	

Heterogeneity: τ^2=0.00; χ^2=0.65, df=2 (P=0.72); I^2=0%
Test for overall effect: Z=1.86 (P=0.06)

Total (95% CI)			**100**	**1.10 (0.95–1.28)**	

Heterogeneity: τ^2=0.03; χ^2=18.42, df=7 (P=0.01); I^2=62%
Test for overall effect: Z=1.27 (P=0.20)
Test for subgroup differences: χ^2=8.78, df=2 (P=0.01); I^2=77.2%

0.5 0.7 1 1.5 2
Favors PDE5i users   **Favors controls**

Figure 4 Forest plots for the meta-analysis of the association between PDE5i use and risk of melanoma presented as adjusted RR.
Notes: (**A**) Forest plots stratified by the individual PDE5i used (sildenafil, tadalafil, or vardenafil); (**B**) forest plots stratified by the stages of melanoma (in situ, localized, or nonlocalized melanoma). The diamond indicates the overall summary estimate for the analysis; the width of the diamond represents the 95% CI.
Abbreviations: *df*, degrees of freedom; DNHR, Danish Nationwide Health Registries; IV, inverse variance; KPNC, Kaiser Permanente Northern California; PDE5i, phosphodiesterase type 5 inhibitor; RR, risk ratio; SE, standard error.
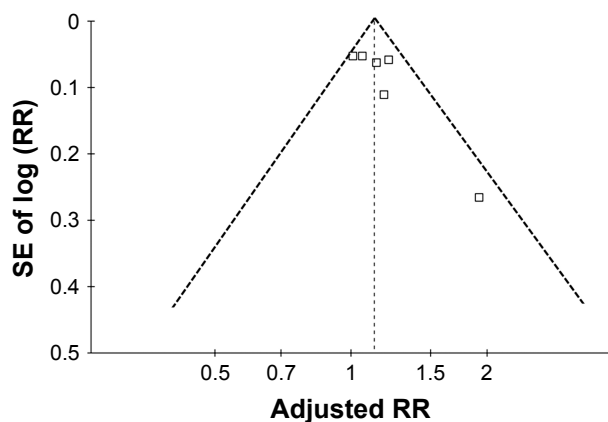
Figure 5 Funnel plot for the association between PDE5i use and risk of melanoma.
Notes: Each square indicates an included study. The x-axis represents the adjusted RR of the association between PDE5i use and risk of melanoma reported in each study, and the y-axis represents the SE of log (RR).
Abbreviations: PDE5i, phosphodiesterase type 5 inhibitor; RR, risk ratio; SE, standard error.

(three studies,[16,20] adjusted RR =0.79, 95% CI =0.62–1.01, P=0.06, I^2=0%; Figure 4B).

Publication bias

The funnel plot regarding the association between PDE5i use and risk of melanoma in the overall study is shown in Figure 5. The funnel plot was symmetric on visual inspection. Based on the Egger regression test, no significant publication bias was detected for the association between PDE5i use and risk of melanoma (P=0.623).

Discussion

In this meta-analysis, upon pooling the results of all available studies, we found that the use of a PDE5i was associated with an increased risk of melanoma among male participants. Moreover, subgroup analyses indicated that the association between PDE5i use and risk of melanoma remained significant in the prospective cohort studies, in participants with recent PDE5i administration, and in participants with both the lowest and highest categories of PDE5i prescriptions. These results suggest that the association between PDE5i use and risk of melanoma appears to be sequential and probably is not dose dependent. Interestingly, we also found that the use of sildenafil was associated with a significantly increased risk for melanoma, whereas use of tadalafil or vardenafil was not. Moreover, PDE5i use was associated with a significantly increased risk for in situ melanoma, but not for localized or nonlocalized melanoma. Taken together, these results demonstrate that the use of a PDE5i, particularly sildenafil, may be associated with an increased risk of melanoma in men.

Notably, although the results of our meta-analysis support the potential association between PDE5i use and risk of melanoma, at present, we could not determine whether the association between PDE5i use and melanoma is causative. The subgroup analysis in this study, which was conducted by including prospective cohort studies, only indicated that the use of PDE5i was independently associated with an increased risk of the subsequent incidence of melanoma in men, which supports a sequential relationship between PDE5i administration and melanoma incidence. This was further enhanced by analysis of adequately adjusted results. However, since the cut-offs for categorization of the number of PDE5i prescriptions varied among the included studies, we compared the association between PDE5i use and risk of melanoma in participants based on the lowest and highest prescription categories. Although these results revealed that PDE5i use was associated with an increased risk of melanoma in men with both the lowest and highest prescription categories, the differences in the results among subgroups were not statistically significant (P for subgroup interaction =0.65). This finding also does not support a dose–response dependency in the association between PDE5i use and melanoma risk. To the best of our knowledge, no randomized controlled trial (RCT) has evaluated whether patients who received PDE5i have a significantly increased melanoma incidence compared to controls. Since RCTs with an adequate sample size and a sufficient follow-up duration appear to be the most reliable strategy to determine whether PDE5i is causative of melanoma incidence, these studies are warranted to confirm our results. In addition, the stratified analysis in this study regarding the use of individual PDE5i indicates that sildenafil was associated with an increased melanoma incidence, but tadalafil or vardenafil were not. However, few studies have reported the relationship between the use of tadalafil or vardenafil and risk of melanoma (only two or three studies). Therefore, the associations between other PDE5is, besides sildenafil, and risk of melanoma deserve further evaluation.

The mechanisms underlying the potential association between PDE5i use and risk of melanoma could be explained through the pharmacologic effects of PDE5i, which mimics the inhibition of cGMP-specific PDE5A induced by the activation of the RAS/RAF/ERK/MEK pathway in melanocytes.[4] Direct evidence was reported in a recently published in vitro study, which discovered a cGMP-dependent growth-promoting pathway in murine and human melanoma cells and revealed that sildenafil treatment further increases intracellular cGMP concentrations, potentiating the activation

of cell proliferation.[14] However, it remains to be determined whether other PDE5is, besides sildenafil, have a similar influence on the proliferation and survival of melanocytes. Moreover, results from recent studies have suggested that the association between PDE5i use and risk of melanoma may be complicated, and the role of myeloid-derived suppressor cells (MDSCs) may be important.[28] Activation of MDSCs has been associated with blocked antitumor immunity,[28] while PDE5is such as sildenafil[29] and tadalafil[30,31] could suppress the activated MDSCs, thereby inhibiting the growth of tumor cells. The suppressive efficacy of PDE5i for MDSCs has been confirmed in melanoma-bearing animals[30,32] and human patients with melanoma,[30,33,34] and a recent study showed that the PDE5i tadalafil can improve the clinical outcomes for advanced melanoma patients by enhancing antitumor immunity.[35] Interestingly, considering the stage of melanoma, we found that PDE5i use was associated with a significantly increased risk for in situ melanoma and also exhibited a trend toward increased risk for localized melanoma and reduced risk for nonlocalized melanoma. These findings may reflect that, for patients with advanced melanoma, PDE5i may exert an adjunctive antitumor effect by suppressing activated MDSCs. Moreover, previous studies have indicated that a BRAF somatic mutation is a major cause to the activation of the RAS/RAF/ERK/MEK pathway and the subsequent cGMP-specific PDE5A inhibition in melanocytes, which underlies the pathogenesis of melanoma related to BRAF somatic mutations. Considering that the potential stimulatory effect of PDE5i for melanoma is by mimicking the inhibitory effect of the RAS/RAF/ERK/MEK pathway for PDE5A, the carcinogenic effect of PDE5i may be insignificant in patients for whom the cGMP-specific PDE5A has already been inhibited via BRAF somatic mutation and activation of RAS/RAF/ERK/MEK signaling. Interestingly, melanoma patients with BRAF somatic mutations are more likely to have advanced melanoma with poor prognosis, as indicated by previous clinical studies[36–38] and a meta-analysis.[39] Therefore, the association between PDE5i use and risk of melanoma may become insignificant in patients with BRAF somatic mutations, who usually have a higher risk for advanced melanoma.

Our study has limitations that should be considered when interpreting these results. First, our meta-analysis was based on a limited number of observational studies, and we did not have access to the individual patient-based data of the original studies. Although we conducted a stratified analysis to explore the potential influence of study characteristics on the association between PDE5i use and risk of melanoma,

these results should be interpreted with caution. Second, as mentioned above, the results of our study could only suggest a possible association between PDE5i use and risk of melanoma. However, future studies, preferably RCTs, are needed to determine whether this relationship is causative. Moreover, as a meta-analysis of observational studies, although we combined the most adequately adjusted results, we could exclude the possibility of residual confounding factors, such as the frequencies of BRAF somatic mutations, which may influence the results of our study. In addition, when analyzing the potential influence of PDE5i prescriptions on the association between PDE5i use and risk of melanoma, we used the amount of prescriptions, which was not as accurate as the exact dose the participants actually took. This may further confound the results. Finally, our study participants were mostly Western men. Studies should also be performed to determine the association between PDE5i use and risk of melanoma in Asians.

Conclusion

PDE5i use may be associated with a significantly increased risk of melanoma in men. However, whether the association between PDE5i and melanoma is causative requires further investigation.

Author contributions

All authors contributed to the data analysis and drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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