Association Between 12 Polymorphisms of VEGF/Hypoxia/Angiogenesis Pathway Genes and Risk of Urogenital Carcinomas: A Meta-Analysis Based on Case-Control Studies

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Objective: Previous studies indicated potential associations between polymorphisms in genes of VEGF/hypoxia/angiogenesis pathway and risk of urogenital carcinomas. However, the results were controversial and inconclusive. Here, we conducted an in-depth meta-analysis to investigate the precise associations between polymorphisms in VEGF/hypoxia/angiogenesis related genes and risk of urogenital carcinomas.

Methods: We searched PubMed, Web of Science, EMBASE, and Cochrane Library to identify all eligible publications. Pooled odds ratios (ORs) corresponding with the 95% confidence intervals (CIs) were calculated to evaluate their associations. Subgroup analysis was conducted to further ascertain such relationship and investigate sources of heterogeneity.

Results: In the end, a total of 96 case-control studies fulfilled the inclusion criteria were enrolled for 12 polymorphisms in 4 VEGF/hypoxia/angiogenesis related genes. The pooled results showed eNOS-rs2070744 polymorphism conferred a significantly increased overall risk of urogenital carcinomas in allele, homozygote, and recessive models, respectively. In addition, eNOS-Intron 4a/b VNTR polymorphism was identified related to an increased risk of urogenital carcinomas in recessive model. And VEGF-rs699947 polymorphism was also identified an increased risk of renal cell carcinoma (RCC) in alleleic, homozygote, dominant, homozygote, and recessive models.

Conclusion: To conclude, eNOS-rs2070744 and eNOS-Intron 4a/b VNTR polymorphisms are risk factors for urogenital carcinomas. VEGF-rs699947 polymorphism was also identified as an increased risk factor for renal carcinoma.

Keywords: VEGF/hypoxia/angiogenesis, polymorphism, urogenital carcinomas, susceptibility, meta-analysis
INTRODUCTION

Urogenital carcinomas, mainly including renal cell carcinoma (RCC), bladder carcinoma (BCa), and prostate carcinoma (PCa), are common malignancies among human neoplasms, with increasing morbidity worldwide in the last 2 decades (Dy et al., 2017). Based on the latest statistics in 2017, estimated incidence of urogenital carcinomas accounts for about 20% of all tumors in the United States. Among them, PCa ranks the highest in man with 161,360 estimated new cases in 2017 (Siegel et al., 2017). In addition to life style and occupational exposure, numerous studies indicated genetic factors such as single nucleotide polymorphisms (SNPs) may be associated with urogenital carcinomas susceptibility (Sun et al., 2008; Stadler et al., 2010).

Angiogenesis, which is the process of new blood vessels formation from original pre-existing vessels, plays a critical role in tumor initiation and development (Nicholson and Theodorescu, 2004). The vascular endothelial growth factor (VEGF) gene is highly polymorphic and several functional SNPs in the VEGF gene alter the expression of the VEGF protein, thereby affecting tumor growth and progression (Ruggiero et al., 2011). The hypoxia-inducible factor-1 alpha (HIF1α) gene is an important transcription factor in cells which regulates cellular responses, adaption, and survival under low oxygen condition in physiological and pathological processes (Li et al., 2013). Several studies demonstrated HIF1α-rs11549465 polymorphism contributed to increase the risk of prostate cancer (Orr-Urtreger et al., 2007; Foley et al., 2009). Endothelial nitric oxide synthase (eNOS) is a central mediator of several endothelium growth stimulators, such as VEGF (Duda et al., 2004; Zhao et al., 2014). Polymorphisms of the eNOS gene plays a vital role in the angiogenesis pathway and have also been found to have functional and clinical significance in malignancies (Haque et al., 2015). HRAS (Harvey rat sarcoma viral oncogene homolog) gene has been uncovered as one of the major factors in the initiation and progression of human malignancies (Zhang et al., 2008). HRAS SNP was also noted to be associated with many cancer susceptibilities.

Clarifying of the role of VEGF/hypoxia/angiogenesis gene polymorphisms in the influence of cancer may improve our knowledge of tumor angiogenesis and benefit risk stratification, disease detection, and prognosis prediction. As mentioned above, many studies have conducted investigations to clarify the associations between these polymorphisms and the risk of urogenital carcinomas, however, these results are controversial and inconsistent. In the current study, we retrieved published data and performed a comprehensive meta-analysis to systematically investigate the association between polymorphisms in VEGF/hypoxia/angiogenesis pathway genes and the risk of urogenital carcinomas.

MATERIALS AND METHODS

Acquisition of the VEGF/Hypoxia/Angiogenesis Pathway Gene Set

The gene set of VEGF/hypoxia/angiogenesis pathway was referenced from the Kyoto Encyclopedia of Genes and Genomes (KEGG) website. The VEGF/hypoxia/angiogenesis pathway gene set could be extracted via following URL link (http://software.broadinstitute.org/gsea/msigdb/geneset_page.jsp?geneSetName=BIOCARTA_VEGF_PATHWAY&keywords=angiogenesis). The gene set was originally provided via the KEGG signaling database, and encompassed the following 29 genes: ARNT, EIF1, EIF1AX, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, EIF2S1, EIF2S2, EIF2S3, ELAVL1, FLI1, FLT4, HIF1A, HRAS, KDR, eNOS, PIK3CA, PIK3CG, PIK3R1, PLCG1, PRKCA, PRKCB, PTK2, PXN, SHC1, VEGF, and VHL.

Literature Search Strategy

We performed a literature search from PubMed, Web of Science, EMBASE, and Cochrane Central Search Library according to the PRISMA guidelines (Shamseer et al., 2015) (last research update: July 20, 2017). The search terms were as follows: (gene OR synset) AND (polymorphism OR mutation OR variation OR SNP OR genotype) AND (carcinoma OR cancer OR neoplasm OR adenocarcinoma OR tumor OR malignancy) (Supplementary Table 1). The language of enrolled studies was restricted to English. Also, we identified additional articles by screening the references of enrolled articles and reviews.

Inclusion and Exclusion Criteria

Studies fitting the following inclusion criteria were enrolled: (1) articles has shown the association between polymorphisms in genes of VEGF/hypoxia/angiogenesis pathway and risk of urogenital carcinomas; (2) case-control studies; (3) publications with sufficient genotype data to assess odds ratios (ORs) and 95% confidence intervals (CIs). The exclusion criteria were as follows: (1) case-only studies without a control group, case reports, conference abstracts or reviews; (2) studies without raw data for the genotype; (3) studies with overlapping data.

Data Extraction

Two authors (JBC and MZ) individually extracted eligible data from each publication based on the inclusion and exclusion criteria. If there is a discrepancy, we reached agreement after discussing with a third author (XBZ). Information collected as follows: first author name, publication year, ethnicity, genotyping methods, source of controls including population-based (PB) or hospital-based (HB), Hardy-Weinberg equilibrium (HWE), cancer type, number of cases, and controls in the VEGF/hypoxia/angiogenesis genotypes. The Newcastle-Ottawa Scale (NOS) was used for assessing the quality of studies. High-quality study required score 7 to 9, and a score less than 7 defined as a low-quality study (Supplementary Table 2).

Linkage Disequilibrium (LD) Analysis Across Populations

The 1,000 genomes Project database (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) was used to extract the LD data, which comprising the polymorphisms in VEGF, HIF-1α and eNOS evaluated in the present study. Briefly, populations enrolled in the project including JPT (Japanese in Tokyo, Japan),
YRI (Yoruba in Ibadan, Nigeria), CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), and CHB (Han Chinese in Beijing, China). The LD was assessed by \( r^2 \) statistics in these populations using Haploviev software.

**Statistical Analysis**

The association between polymorphisms in four VEGF/hypoxia/angiogenesis pathway genes and the urogenital carcinomas susceptibility were evaluated using summary ORs and the corresponding 95% CIs. We used allelic (B vs. A), recessive (BB vs. BA + AA), dominant (BA + BB vs. AA), homozygous (BB vs. AA), and heterozygous (BA vs. AA) models to analyze each variable (A indicate wild allele and B indicate mutated allele). Q-statistic as well as \( I^2 \) were used to evaluate the heterogeneity within the studies (Higgins et al., 2003). Heterogeneity was considered significant when the \( P \)-value < 0.1. If there was no heterogeneity found, a fixed effect model was applied. Otherwise, the random-effect model was used to calculate pooled ORs. The significance of overall ORs was determined by Z-test. Subgroup analyses were performed based on different ethnicity, cancer type, HWE, and the source of control. Sensitivity analysis was conducted to assess the results stability by omitting one study each time. Publication bias was analyzed by Begg’s funnel plot and Egger’s test (Begg and Mazumdar, 1994; Egger et al., 1997). All analyses were performed using STATA 12.0 statistical software (Stata Corporation, College Station, TX, USA).

**RESULTS**

**Main Characteristics of the Eligible Studies**

According to the inclusion and exclusion criteria, only 12 polymorphisms in four genes of VEGF/hypoxia/angiogenesis pathway were identified (VEGF-rs10434, VEGF-rs1570360, VEGF-rs2010963, VEGF-rs2052039, VEGF-rs699947, VEGF-rs833061, HIF1α-rs11549465, HIF1α-rs11549467, eNOS-rs1799983, eNOS-rs2070744, eNOS-Intron 4a/b VNTR, and HRAS-rs12628). Details of the enrolled studies were listed in Table 1 (Clifford et al., 2001; Abe et al., 2002; McCarron et al., 2002; Medeiros et al., 2002; Johne et al., 2003; Lin et al., 2003; Sanyal et al., 2004; Chau et al., 2005; Kim et al., 2005; Marangoni et al., 2006; Sfar et al., 2006; Fukuda et al., 2007; Garcia-Closas et al., 2007; Li et al., 2007, 2012; Orr-Urtreger et al., 2007; Jacobs et al., 2008; Nadaoka et al., 2008; Onen et al., 2008; Foley et al., 2009; Lee et al., 2009; Morris et al., 2009; Ricketts et al., 2009; Bruyère et al., 2010; VanCleave et al., 2010; Ajaz et al., 2011; Ryk et al., 2011; Sanli et al., 2011; Asamaly et al., 2012; Henríquez-Hernández et al., 2012; Qin et al., 2012, 2014; Traczyk et al., 2012; Brankovic et al., 2013; Ianni et al., 2013; Jaiswal et al., 2013; Martinez-Fierro et al., 2013; Pandith et al., 2013; Sáenz-López et al., 2013; Safarinejad et al., 2013; Verim et al., 2013; Wang et al., 2013; Ziaei et al., 2013; Fraga et al., 2014; Yang et al., 2014; Lu et al., 2015; Polat et al., 2015; Shen et al., 2015; Xian et al., 2015; Ceylan et al., 2016; Diler and Oden, 2016). The study selection processes were presented in Figure 1.

For polymorphisms in VEGF gene (VEGF-rs10434, VEGF-rs1570360, VEGF-rs2010963, VEGF-rs3025039, VEGF-rs699947, VEGF-rs833061), a total of 49 case-control studies with 8,070 cases and 10,024 controls have met the inclusion criteria. Specifically, there are 918 cases and 1,330 controls in VEGF-rs10434, 3,522 cases and 3,167 controls in VEGF-rs1570360, 3,106 cases and 4,152 controls in VEGF-rs2010963, 3,582 cases and 4,890 controls in VEGF-rs3025039, 3,252 cases and 4,432 controls in VEGF-rs699947, 2,533 cases and 3,550 controls in VEGF-rs833061, respectively. Twenty-two studies of them were performed in Caucasians, 25 studies in Asians, and others were in mixed ethnic groups (including at least one race). Controls of 35 studies were hospital-based (H-B) and 13 studies were population-based (P-B), only one study didn’t show whether it was H-B or P-B. Additionally, the distributions of polymorphisms in VEGF for control groups were consistent with HWE, except for these studies listed here (Lin et al., 2003; Garcia-Closas et al., 2007; Onen et al., 2008; Ianni et al., 2013; Lu et al., 2015; Shen et al., 2015; Xian et al., 2015).

For HIF1α-rs11549465 and rs11549467 polymorphisms, 19 eligible studies comprising 6,064 cases and 6,784 controls were enrolled. There are 5,789 cases and 6,360 controls in HIF1α-rs11549465, 3,336 cases and 4,013 controls in HIF1α-rs11549467, respectively. Of them, eight studies were performed on subjects in Caucasians, six in Asians and the other five were in mixed ethnic groups. Moreover, nine studies were H-B, eight were P-B and the other two did not show H-B or P-B. There were several studies not consistent with HWE (Clifford et al., 2001; Chau et al., 2005; Jacobs et al., 2008).

For polymorphisms in eNOS gene (eNOS-rs1799983, eNOS-rs2070744, and eNOS-Intron 4a/b VNTR), 24 case-control studies with 4,286 cases and 5,009 controls were included in the current work. Specifically, there are 3,777 cases and 4,429 controls in eNOS-rs1799983, 813 cases, and 854 controls in eNOS-rs2070744, 709 cases and 1,111 controls in eNOS-Intron 4a/b VNTR, respectively. Of them, 23 studies were performed in Caucasian, 1 was in mixed ethnic groups. In addition, among these studies, 13 were H-B, and 11 were P-B. All the studies were consistent with HWE. While for HRAS-rs12628 polymorphism, finally, 4 eligible case-control studies comprising 886 cases and 747 controls were included. Of them, 3 studies were performed in Caucasian, 1 in Asian. Of these studies, 2 were H-B, and 2 were P-B. Two studies were not consistent with HWE (Johne et al., 2003; Sanyal et al., 2004).

**Quantitative Synthesis**

Table 2 and Supplementary Table 3 listed the main results of current meta-analysis work of polymorphisms in VEGF/hypoxia/angiogenesis pathway genes and risk of urogenital carcinomas.

**eNOS-rs2070744**

The pooled results of five included studies had shown eNOS-rs2070744 polymorphism conferred a significantly higher overall risk to urogenital carcinomas in allele, homozygote and recessive models (B vs. A: OR = 1.379, 95%CI = 1.187–1.602,
TABLE 1 | Detail characteristics of enrolled studies.

| SNP       | References | Ethnicity | Source of control | Cancer type | Case  | Control | Y(HWE) |
|-----------|------------|-----------|-------------------|-------------|-------|---------|--------|
|           |            |           |                   | AA          | AB    | BB      |        |
|           |            |           |                   | AA          | AB    | BB      |        |
|           |            |           |                   | AA          | AB    | BB      |        |
|           |            |           |                   | AA          | AB    | BB      |        |
| VEGF-rs10434 | Abe et al., 2002 | Asian | HB | RCC | 113 | 31 | 1 | 109 | 33 | 3 | Y |
|           | Shen et al., 2015 | Asian | HB | RCC | 152 | 170 | 39 | 166 | 164 | 30 | Y |
|           | Lu et al., 2015 | Asian | HB | RCC | 172 | 191 | 49 | 365 | 375 | 85 | Y |
| VEGF-rs1570360 | McCarron et al., 2002 | Caucasian | PB | PCa | 114 | 109 | 15 | 120 | 109 | 34 | Y |
|           | Stär et al., 2006 | Caucasian | HB | PCa | 58 | 37 | 6 | 36 | 50 | 14 | Y |
|           | Garcia-Closas et al., 2007 | Caucasian | HB | BCa | 431 | 383 | 78 | 389 | 407 | 82 | Y |
|           | Jacobs et al., 2008 | Caucasian | PB | PCa | 557 | 489 | 126 | 210 | 194 | 54 | Y |
|           | Ricketts et al., 2009 | Caucasian | PB | RCC | 134 | 143 | 47 | 146 | 130 | 38 | Y |
|           | Bruyère et al., 2010 | Caucasian | PB | RCC | 27 | 17 | 5 | 94 | 83 | 25 | Y |
|           | Yang et al., 2014 | Asian | HB | BCa | 224 | 187 | 69 | 213 | 162 | 45 | Y |
|           | Xian et al., 2015 | Asian | HB | RCC | 115 | 112 | 39 | 232 | 220 | 80 | N |
| VEGF-rs2010963 | Stär et al., 2006 | Caucasian | HB | PCa | 29 | 57 | 15 | 44 | 46 | 10 | Y |
|           | Garcia-Closas et al., 2007 | Caucasian | HB | BCa | 388 | 396 | 96 | 387 | 396 | 93 | Y |
|           | Bruyère et al., 2010 | Caucasian | PB | RCC | 15 | 25 | 8 | 86 | 92 | 20 | Y |
|           | Sáenz-López et al., 2013 | Caucasian | PB | RCC | 101 | 93 | 20 | 129 | 118 | 32 | Y |
|           | Qin et al., 2014 | Asian | HB | RCC | 287 | 391 | 146 | 410 | 429 | 144 | Y |
|           | Shen et al., 2015 | Asian | HB | RCC | 121 | 170 | 69 | 134 | 163 | 63 | Y |
|           | Lu et al., 2015 | Asian | HB | RCC | 139 | 194 | 79 | 299 | 377 | 148 | Y |
|           | Xian et al., 2015 | Asian | HB | RCC | 30 | 132 | 104 | 49 | 256 | 227 | Y |
| VEGF-rs3025039 | Abe et al., 2002 | Asian | HB | RCC | 97 | 41 | 7 | 90 | 52 | 3 | Y |
|           | Stär et al., 2006 | Caucasian | HB | PCa | 79 | 20 | 2 | 72 | 27 | 1 | Y |
|           | Garcia-Closas et al., 2007 | Caucasian | HB | BCa | 852 | 217 | 17 | 787 | 385 | 11 | N |
|           | Bruyère et al., 2010 | Caucasian | PB | RCC | 29 | 17 | 1 | 141 | 53 | 2 | Y |
|           | Sáenz-López et al., 2013 | Caucasian | PB | RCC | 156 | 57 | 2 | 200 | 73 | 7 | Y |
|           | Wang et al., 2013 | Asian | HB | BCa | 293 | 153 | 24 | 539 | 275 | 36 | Y |
|           | Yang et al., 2014 | Asian | HB | BCa | 307 | 149 | 24 | 284 | 121 | 15 | Y |
|           | Shen et al., 2015 | Asian | HB | RCC | 139 | 81 | 55 | 240 | 148 | 46 | Y |
|           | Lu et al., 2015 | Asian | HB | RCC | 262 | 91 | 59 | 554 | 166 | 105 | N |
|           | Xian et al., 2015 | Asian | HB | RCC | 70 | 127 | 69 | 196 | 236 | 100 | Y |
| VEGF-rs699947 | Kim et al., 2005 | Asian | HB | BCa | 13 | 69 | 71 | 11 | 69 | 73 | Y |
|           | Garcia-Closas et al., 2007 | Caucasian | HB | BCa | 261 | 471 | 220 | 268 | 447 | 214 | Y |
|           | VanCeave et al., 2010 | Mixed | PB | PCa | 125 | 53 | 12 | 402 | 198 | 35 | Y |
|           | Ajaz et al., 2011 | Asian | NA | RCC | 30 | 81 | 32 | 44 | 41 | 21 | Y |
|           | Henríquez-Hernández et al., 2012 | Caucasian | HB | BCa | 11 | 25 | 23 | 14 | 16 | 13 | Y |
|           | Sáenz-López et al., 2013 | Caucasian | PB | RCC | 54 | 114 | 48 | 77 | 142 | 53 | Y |
|           | Ianni et al., 2013 | Caucasian | PB | PCa | 115 | 54 | 55 | 75 | 57 | 24 | N |
|           | Jaiswal et al., 2013 | Asian | HB | BCa | 67 | 116 | 17 | 106 | 112 | 32 | Y |
|           | Martinez-Fierro et al., 2013 | Caucasian | HB | PCa | 37 | 38 | 2 | 70 | 78 | 24 | Y |
|           | Shen et al., 2015 | Asian | HB | RCC | 171 | 174 | 67 | 397 | 332 | 95 | N |
|           | Lu et al., 2015 | Asian | HB | RCC | 99 | 119 | 48 | 243 | 225 | 64 | Y |
| VEGF-rs833061 | Lin et al., 2003 | Asian | HB | PCa | 60 | 32 | 4 | 43 | 72 | 4 | N |
|           | Fukuda et al., 2007 | Asian | HB | PCa | 143 | 103 | 24 | 132 | 97 | 23 | Y |
|           | Garcia-Closas et al., 2007 | Caucasian | HB | BCa | 237 | 434 | 216 | 243 | 432 | 198 | Y |
|           | Onen et al., 2008 | Mixed | PB | PCa | 33 | 89 | 11 | 50 | 94 | 13 | N |
|           | Bruyère et al., 2010 | Caucasian | PB | RCC | 19 | 29 | 1 | 47 | 109 | 46 | Y |
|           | Sáenz-López et al., 2013 | Caucasian | PB | RCC | 56 | 111 | 49 | 77 | 138 | 58 | Y |
|           | Wang et al., 2013 | Asian | HB | BCa | 255 | 178 | 37 | 475 | 307 | 88 | Y |
|           | Lu et al., 2015 | Asian | HB | RCC | 228 | 93 | 91 | 513 | 168 | 143 | N |

(Continued)
| SNP             | References             | Ethnicity | Source of control | Cancer type | Case Control | Y(HWE) |
|-----------------|------------------------|-----------|-------------------|-------------|--------------|--------|
|                 |                        |           |                   |             | AA AB BB     | AA AB BB |
| HIF1α-rs11549465| Clifford et al., 2001 | Caucasian | HB                | RCC         | 42 6 0.1    | 110 27 6  N |
|                 | Chau et al., 2005      | Mixed     | NA                | PCa         | 161 29 6    | 179 14 3  N |
|                 | Orr-Urtreger et al., 2007 | Caucasian | PB                | PCa         | 287 99 16   | 217 80 3  Y |
|                 | Li et al., 2007        | Mixed     | PB                | PCa         | 818 209 14  | 995 221 18 Y |
|                 | Jacobs et al., 2008    | Mixed     | PB                | PCa         | 1156 252 12 | 1138 284 28 N |
|                 | Nadaoka et al., 2008   | Asian     | HB                | BCa         | 197 21 1    | 419 42 0.1 Y |
|                 | Foley et al., 2009     | Caucasian | PB                | PCa         | 65 30 0.1   | 175 13 0.1 Y |
|                 | Morris et al., 2009    | Caucasian | PB                | RCC         | 290 39 3    | 262 46 5  Y |
|                 | Li et al., 2012        | Asian     | HB                | PCa         | 612 48 2    | 659 57 0.1 Y |
|                 | Qin et al., 2012       | Asian     | HB                | RCC         | 572 46 2    | 578 43 2  Y |
|                 | Fraga et al., 2014     | Caucasian | HB                | PCa         | 579 164 11  | 566 156 14 Y |
| HIF1α-rs11549467| Clifford et al., 2001 | Caucasian | HB                | RCC         | 47 1 0.1    | 140 4 0.1  Y |
|                 | Chau et al., 2005      | Mixed     | NA                | PCa         | 195 1 0.1   | 196 0.1 0.1 N |
|                 | Orr-Urtreger et al., 2007 | Caucasian | PB                | PCa         | 198 2 0.1   | 298 2 0.1  Y |
|                 | Li et al., 2007        | Mixed     | PB                | PCa         | 1053 13 0.1 | 1247 17 0.1 Y |
|                 | Nadaoka et al., 2008   | Asian     | HB                | BCa         | 204 13 2    | 421 40 0.1 Y |
|                 | Morris et al., 2009    | Caucasian | PB                | RCC         | 313 10 2    | 294 15 0.1 Y |
|                 | Li et al., 2012        | Asian     | HB                | PCa         | 614 47 1    | 685 31 0.1 Y |
|                 | Qin et al., 2012       | Asian     | HB                | RCC         | 575 45 0.1  | 584 39 0.1 Y |
| eNOS-rs1799983  | Medeiros et al., 2002  | Caucasian | HB                | PCa         | 49 61 15    | 70 65 18 Y |
|                 | Marangoni et al., 2006 | Caucasian | HB                | PCa         | 30 50 4     | 30 29 6  Y |
|                 | Jacobs et al., 2008    | Caucasian | PB                | PCa         | 659 632 129 | 682 600 164 Y |
|                 | Lee et al., 2009       | Caucasian | PB                | PCa         | 517 468 103 | 607 557 129 Y |
|                 | Lee et al., 2009       | Mixed     | PB                | PCa         | 77 20 0.1   | 280 88 5  Y |
|                 | Ryk et al., 2011       | Caucasian | PB                | BCa         | 128 106 28  | 75 62 13 Y |
|                 | Zaei et al., 2013      | Caucasian | HB                | PCa         | 44 23 11    | 48 33 6  Y |
|                 | Safarinejad et al., 2013 | Caucasian | HB                | PCa         | 120 48 2    | 248 89 3  Y |
|                 | Verim et al., 2013     | Caucasian | HB                | BCa         | 7 49 10     | 31 44 13 Y |
|                 | Brankovic et al., 2013 | Caucasian | HB                | PCa         | 76 65 9     | 54 40 6  Y |
|                 | Polat et al., 2015     | Caucasian | PB                | BCa         | 7 59 9      | 48 75 20 Y |
|                 | Ceylan et al., 2016    | Caucasian | HB                | PCa         | 46 23 9     | 47 23 5  Y |
|                 | Diler et al., 2016     | Caucasian | PB                | PCa         | 6 55 23     | 65 41 10 Y |
| eNOS-rs2070744  | Ryk et al., 2011       | Caucasian | PB                | BCa         | 152 142 40  | 84 63 8  Y |
|                 | Safarinejad et al., 2013 | Caucasian | HB                | PCa         | 52 93 25    | 150 159 31 Y |
|                 | Brankovic et al., 2013 | Caucasian | HB                | PCa         | 54 68 28    | 34 51 15 Y |
|                 | Polat et al., 2015     | Caucasian | PB                | BCa         | 24 40 11    | 56 72 15 Y |
|                 | Diler et al., 2016     | Caucasian | PB                | PCa         | 30 30 24    | 47 56 13 Y |
| eNOS-Intron 4a/b VNTR | Medeiros et al., 2002  | Caucasian | HB                | PCa         | 87 32 6     | 121 29 3  Y |
|                 | Sanli et al., 2011     | Caucasian | HB                | PCa         | 87 40 5     | 104 48 6  Y |
|                 | Amasyali et al., 2012  | Caucasian | HB                | BCa         | 52 63 8     | 137 59 5  Y |
|                 | Safarinejad et al., 2013 | Caucasian | HB                | PCa         | 101 54 15   | 249 88 3  Y |
|                 | Polat et al., 2015     | Caucasian | PB                | BCa         | 50 24 1     | 97 43 3  Y |
|                 | Diler et al., 2016     | Caucasian | PB                | PCa         | 65 16 3     | 83 31 2  Y |
| HRAS-rs12628    | Johne et al., 2003     | Caucasian | HB                | BCa         | 151 119 42  | 164 170 26 N |
|                 | Sanyal et al., 2004    | Caucasian | PB                | BCa         | 153 147 2   | 54 61 6  N |
|                 | Traczyk et al., 2012   | Caucasian | PB                | PCa         | 45 64 23    | 49 48 9  Y |
|                 | Pandith et al., 2013   | Asian     | HB                | BCa         | 90 42 8     | 135 25 0.1 Y |

SNP, single nucleotide polymorphism; HB, hospital-based; PB: population-based; RCC, renal cell carcinoma; PCa, prostate cancer; BCa, bladder cancer; HWE, Hardy Weinberg equilibrium; Y, controls conformed to HWE; N, controls were not conformed to HWE; Mixed, more than two ethnicities.
**TABLE 2** | Representative results of meta-analysis for polymorphisms in VEGF/ Hypoxia/Angiogenesis genes and risk of Urogenital Carcinomas.

| SNP                | Comparison | Subgroup | N | $P_H$ | $P_A$ | Random | Fixed |
|--------------------|------------|----------|---|-------|-------|--------|-------|
| eNOS-rs2070744     | B vs. A    | Overall  | 5 | 0.471 | 2.566E-05** | 1.379 (1.188–1.602) | 1.379 (1.187–1.602) |
|                    | B vs. A    | PB       | 3 | 0.746 | 6.260E-04  | 1.436 (1.167–1.768) | 1.437 (1.167–1.768) |
|                    | B vs. A    | BCa      | 2 | 0.599 | 7.782E-03* | 1.387 (1.089–1.767) | 1.388 (1.090–1.768) |
|                    | B vs. A    | PCa      | 3 | 0.196 | 1.113E-03* | 1.362 (1.060–1.751) | 1.373 (1.135–1.662) |
|                    | BA+BB vs. AA | BCa   | 2 | 0.922 | 3.913E-02* | 1.402 (1.017–1.932) | 1.402 (1.017–1.932) |
|                    | BA+BB vs. AA | PCa   | 3 | 0.125 | 2.278E-02* | 1.307 (0.865–1.974) | 1.375 (1.045–1.808) |
|                    | BB vs. AA  | Overall  | 5 | 0.466 | 1.814E-05** | 2.082 (1.481–2.926) | 2.097 (1.495–2.942) |
|                    | BB vs. AA  | PB       | 3 | 0.657 | 2.220E-04** | 2.453 (1.510–3.985) | 2.481 (1.532–4.019) |
|                    | BB vs. AA  | BCa      | 2 | 0.438 | 6.251E-03* | 1.436 (1.167–1.768) | 1.437 (1.167–1.768) |
|                    | BB vs. AA  | PCa      | 3 | 0.235 | 9.759E-04* | 2.001 (1.211–3.306) | 2.000 (1.325–3.018) |
|                    | BB vs. BA  | Overall  | 5 | 0.414 | 5.468E-05** | 1.876 (1.370–2.567) | 1.898 (1.390–2.581) |
|                    | BB vs. BA  | PB       | 3 | 0.393 | 1.915E-02* | 2.241 (1.225–4.099) | 2.298 (1.271–4.019) |
|                    | BB vs. BA  | BCa      | 2 | 0.358 | 1.503E-02* | 1.946 (1.099–3.448) | 2.006 (1.145–3.516) |
|                    | BB vs. BA  | PCa      | 3 | 0.214 | 1.300E-03* | 1.869 (1.166–2.997) | 1.848 (1.271–2.687) |
| eNOS-Intron 4a/b VNTR | BB vs. BA  | Overall  | 6 | 0.109 | 1.970E-04** | 2.436 (1.109–5.351) | 2.725 (1.608–4.619) |
| eNOS-rs1799983     | B vs. A    | BCa      | 3 | 0.190 | 1.078E-02* | 1.361 (1.024–1.809) | 1.324 (1.067–1.642) |
| HIF1α-rs11549467   | B vs. A    | PCa      | 4 | 0.490 | 4.413E-02* | 1.461 (1.003–2.128) | 1.465 (1.010–2.124) |
| HRAS-rs12628       | BA+BB vs. AA | Y     | 2 | 0.127 | 3.647E-05** | 2.220 (1.245–3.960) | 2.211 (1.517–3.222) |
| VEGF-rs2010963     | BA vs. AA  | RCC      | 6 | 0.530 | 1.762E-04** | 1.168 (1.027–1.327) | 1.168 (1.027–1.328) |
| VEGF-rs3025039     | B vs. A    | Asian    | 6 | 0.337 | 4.545E-04** | 1.179 (1.067–1.303) | 1.180 (1.076–1.294) |
| VEGF-rs699947      | B vs. A    | Asian    | 6 | 0.286 | 9.201E-07** | 1.273 (1.139–1.424) | 1.277 (1.158–1.408) |

**SNP**, single nucleotide polymorphism; $P_H$, $P$ value of $Q$ test for heterogeneity test; $P_A$, $P < 0.05$ was considered as statistically significant (bold font mark) for cancer type subgroup analysis. And multiple testing $P$ value according to Bonferroni correction ($P < 0.05 / (12$ polymorphisms $\times 5$ models)) was considered as statistically significant (bold font mark); PCa, prostate cancer; RCC, renal cell carcinoma; BCa, bladder cancer; HB, hospital based; PB, population based; HWE, Hardy Weinberg equilibrium. Heterogeneity was considered significant when the $P$-value $< 0.1$. A fixed effects model (Der-Simonian Laird) was used if there was no significant heterogeneity; otherwise, a random effects model (Der-Simonian Laird) was used.
\( P_A = 2.566E-05; \) BB vs. AA: OR = 2.097, 95%CI = 1.495–2.942, \( P_A = 1.814E-05 \) and BB vs. BA + AA: OR = 1.898, 95%CI = 1.590–2.591, \( P_A = 5.468E-05 \), respectively. In the stratification analysis by source of control, an increased risk of urogenital neoplasms was also identified for P-B groups in allele, homozygote, and recessive models (B vs. A: OR = 1.437, 95%CI = 1.167–1.768, \( P_A = 6.260E-04 \); BB vs. AA: OR = 2.481, 95%CI = 1.532–4.019, \( P_A = 2.220E-04 \) and BB vs. BA + AA: OR = 2.352, 95%CI = 1.501–3.687, \( P_A = 1.915E-04 \)). Moreover, when the stratification analysis conducted by cancer type \( (P_A < 0.05, \) without Bonferroni correction), we also identified an increased risk of BCa in allelic, dominant, homozygote and recessive models (B vs. A: OR = 1.388, 95%CI = 1.090–1.768, \( P_A = 7.822E-03 \); BA + BB vs. AA: OR = 1.402, 95%CI = 1.017–1.932, \( P_A = 3.913E-02 \); BB vs. AA: OR = 2.298, 95%CI = 1.266–4.172, \( P_A = 6.251E-03 \) and BB vs. BA + AA: OR = 2.006, 95%CI = 1.145–3.516, \( P_A = 1.503E-02 \)). An increased risk of PCa in allelic, dominant, homozygote, and recessive models (B vs. A: OR = 1.373, 95%CI = 1.135–1.662, \( P_A = 1.113E-03 \); BA + BB vs. AA: OR = 1.375, 95%CI = 1.045–1.808, \( P_A = 2.278E-02 \); BB vs. AA: OR = 2.000, 95%CI = 1.325–3.018, \( P_A = 9.759E-04 \) and BB vs. BA + AA: OR = 1.848, 95%CI = 1.271–2.687, \( P_A = 1.300E-03 \)).

**eNOS-Intron 4a/b VNTR**

For eNOS-Intron 4a/b VNTR polymorphism, a total of six eligible case-control studies were included. The final analysis has shown that eNOS-Intron 4a/b VNTR polymorphism was related to an increased risk of urogenital neoplasms in recessive model (BB vs. BA + AA: OR = 2.725, 95%CI = 1.608–4.619, \( P_A = 1.970E-04 \)). Subgroups analysis \( (P_A \text{ value without Bonferroni correction}) \) identified an increased risk of BCa in homozygote models (BB vs. AA: OR = 2.661, 95%CI = 1.044–7.050, \( P_A = 4.899E-02 \)).

**eNOS-rs1799983**

Overall, there was no significant association between eNOS-rs1799983 polymorphism and the risk of urogenital neoplasms. However, subgroups analysis by cancer type revealed an increased risk of BCa in allelic and heterozygote models (B vs. A:...
FIGURE 2 | Linkage disequilibrium analyses for VEGF, eNOS, and HIF1α polymorphisms in populations from 1,000 genomes. The number of each cell represents $r^2$ and white color cells shows no LD between polymorphisms. Population descriptors: JPT, Japanese in Tokyo, Japan; YRI, Yoruba in Ibadan, Nigeria; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China.

**HIF1α-rs11549467**
Overall, there was no significant association between HIF1α-rs11549467 polymorphism and the risk of urogenital neoplasms. Subgroups analysis by cancer type revealed an increased risk of PCa in allelic and heterozygote models (B vs. A: $OR = 1.324, 95\% CI = 1.067–1.642, P_A = 1.078E-02$; BB vs. AA: $OR = 1.887, 95\% CI = 1.103–3.230, P_A = 2.056E-02$) ($P_A$ value without Bonferroni correction).

**HRAS-rs12628**
No significant association was uncovered for the association between HRAS-rs12628 polymorphism and urogenital carcinomas risk. However, when conducting the stratification analysis by HWE status, we identified an increased risk of urogenital carcinomas (all are BCa studies) in dominant and
homozygote model for HWE (Y) groups (BB + BA vs. AA: OR = 2.211, 95%CI = 1.517–3.222, \( P_A = 3.647E-05 \); BB vs. AA: OR = 4.174, 95%CI = 1.851–9.412, \( P_A = 5.725E-04 \)). Moreover, in the stratification analysis by source of control, an increased risk of urogenital carcinomas for H-B groups was also found (BB vs. BA + AA: OR = 2.396, 95%CI = 1.458–3.938, \( P_A = 5.642E-04 \)).

**VEGF-rs2010963**

Overall, there was no significant association between eNOS-rs1799983 polymorphism and the risk of urogenital neoplasms. Nevertheless, subgroups analysis by cancer type revealed an increased risk of RCC in heterozygote, dominant, and homozygote models (BA vs. AA: OR = 1.168, 95%CI = 1.027–1.328, \( P_A = 1.762E-02 \); BA + BB vs. AA: OR = 1.181, 95%CI = 1.047–1.333, \( P_A = 6.790E-03 \); BB vs. AA: OR = 1.196, 95%CI = 1.010–1.416, \( P_A = 3.784E-02 \) (\( P_A \) value without Bonferroni correction).

**VEGF-rs3025039**

No significant association was found between VEGF-rs3025039 polymorphism and the risk of urogenital carcinomas. Nevertheless, subgroup analysis by ethnicity showed an increased risk of urogenital carcinomas in allelic and homozygote model for Asian population (B vs. A: OR = 1.180, 95%CI = 1.076–1.294, \( P_A = 4.545E-04 \); BB vs. AA: OR = 1.401, 95%CI = 1.152–1.705, \( P_A = 7.532E-04 \)). Moreover, in the stratification analysis by source of control, an increased risk of urogenital carcinomas for H-B groups was also found (BB vs. AA: OR = 1.458–3.938, \( P_A = 5.642E-04 \)).

**VEGF-rs699947**

Overall, there was no significant association between VEGF-rs699947 polymorphism and the risk of urogenital neoplasms. Nonetheless, an increased risk of urogenital carcinomas for Asian populations in allelic, heterozygote, dominant, and homozygote models were found in the stratification analysis by ethnicity (B vs. A: OR = 2.277, 95%CI = 1.158–1.408, \( P_A = 9.201E-07 \); BB vs. AA: OR = 1.352, 95%CI = 1.166–1.568, \( P_A = 6.730E-05 \); BB + BA vs. AA: OR = 1.410, 95%CI = 1.226–1.620, \( P_A = 1.339E-06 \) and BB vs. AA: OR = 1.572, 95%CI = 1.279–1.931, \( P_A = 1.706E-05 \)). Moreover, when the stratification analysis conducted by cancer type, we also identified an increased risk of RCC in allelic, heterozygote, dominant, homozygote, and recessive models (B vs. A: OR = 2.277, 95%CI = 1.158–1.408, \( P_A = 9.201E-07 \); BB vs. AA: OR = 1.352, 95%CI = 1.166–1.568, \( P_A = 6.730E-05 \); BB + BA vs. AA: OR = 1.410, 95%CI = 1.226–1.620, \( P_A = 1.339E-06 \) and BB vs. AA: OR = 1.572, 95%CI = 1.279–1.931, \( P_A = 1.706E-05 \)).

**VEGF-rs10434, VEGF-rs1570360, VEGF-rs833061, and HIF1α-rs11549465**

There was no significant association between VEGF-rs10434, VEGF-rs1570360, VEGF-rs2010963, VEGF-rs833061, and HIF1α-rs11549465 polymorphisms and the risk of urogenital carcinomas. Furthermore, in the subgroup analysis by ethnicity, source of controls, cancer type or HWE status, similar results were also obtained.

**Sensitivity Analysis and Publication Bias**

In order to assess the stability of current meta-analysis result, sensitivity analysis was performed. The influence of the individual dataset on the pooled ORs was investigated after sequentially excluding each single case-control study. The study material alteration did not change the corresponding pooled ORs for all 12 polymorphisms (Supplementary Figure 1 and Supplementary Table 4). Furthermore, Begg’s funnel plot and Egger’s regression test were performed to evaluate the publication bias (Begg and Mazumdar, 1994; Egger et al., 1997). As for these 12 polymorphisms, no evidence of publication bias was identified by viewing the shape of Begg’s funnel plot, which was further validated by Egger’s regression test (Supplementary Figure 2 and Supplementary Table 5).

**LD Analyses Across Populations**

To better understand the quantitative synthesis, we performed LD analysis to test for the existence of bins in the region comprising these polymorphisms of each angiogenesis related genes (VEGF-rs10434, VEGF-rs1570360, VEGF-rs2010963, VEGF-rs3025039, VEGF-rs699947, VEGF-rs833061, HIF1α-rs11549465, HIF1α-rs11549467, eNOS-rs1799983, and eNOS-rs2070744), respectively. LD plots for polymorphisms in each gene were presented in Figure 2 and Supplementary Table 6. Although we have uncovered significant LD for several polymorphisms in separate genes, such as VEGF, they were not statistically associated with urological neoplasms’ risk in current work. As for the two significant polymorphisms (eNOS-Intron 4a/b VNTR and eNOS-rs2070744), LD analysis cannot be performed because eNOS-Intron 4a/b VNTR polymorphism was mismatched.

**DISCUSSION**

Angiogenesis is well-recognized as a key element for sustained tumor growth and a critical factor for tumor metastasis (McConkey et al., 2016; De Palma et al., 2017). Our previous studies also demonstrated upregulation of angiogenesis genes, e.g., VEGF played vital roles in bladder cancer progression (Zu et al., 2006; Lei et al., 2015; Zhou et al., 2015; Chen et al., 2016; Long et al., 2016). Other researchers also suggested that polymorphisms in angiogenesis pathway genes might be an important risk factors for the initiation and progression of urogenital neoplasms (Jacobs et al., 2008; Jaiswal et al., 2013; Orlandi et al., 2013; Dornbusch et al., 2016; Gong et al., 2017).

Some investigators have conducted case-control studies to evaluate the association between polymorphisms in angiogenesis related genes and the risk of urological tumors (Foley et al., 2013; Orlandi et al., 2013; Dornbusch et al., 2016; Gong et al., 2017).
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However, most of prior studies addressed on limited polymorphisms in single angiogenesis related gene while neglected potential multiple gene's influence on urological carcinogenesis. Xian et al. found VEGF-rs3025039 genetic variant was associated with increased risk of RCC (Xian et al., 2015), but another study revealed no association (Abel et al., 2002). Li P et al. reported HIF1α-rs11549467 rather than HIF1α-rs11549465 polymorphism increased prostate cancer susceptibility (Li et al., 2012). Other studies failed to detect any association between these two polymorphisms and the risk of urinary cancers (Clifford et al., 2001; Morris et al., 2009). One study showed the eNOS-rs1799983 was associated with increased prostate cancer risk (Medeiros et al., 2002), while another study data suggested no association (Brankovic et al., 2013). HRAS-rs12628 polymorphism could mediate urinary bladder cancer development and predict tumors advancing (Amasyali et al., 2012), but another study showed no significant correlation (Sanyal et al., 2004). In the current study, we presented a comprehensive meta-analysis for 12 polymorphisms in four VEGF/hypoxia/angiogenesis pathway genes (VEGF, HIF1α, eNOS, and HRAS) including 96 case-control studies to examine the precise associations between these polymorphisms and risk of urogenital neoplasms.

Previous studies revealed that eNOS could modulate cancer-related events, such as VEGF-induced angiogenesis, invasion, and metastasis (Jadessi et al., 2000; Duda et al., 2004). The eNOS-rs2070744 polymorphism is a point mutation of thymine to cytosine (T>C) at −786 nucleotide in the 5′-flanking region of the eNOS gene (Nakayama et al., 1999). This mutation position in eNOS promoter region can alter gene activity and serum NO level, indicating the CC homozygote carriers may have an increased risk of cancer (Zhang et al., 2014). Present meta-analysis indicated that eNOS-rs2070744 polymorphism conferred a significantly increased overall risk to urogenital neoplasms. This is consistent with several previously published studies (Ryk et al., 2011; Brankovic et al., 2013; Safarinejad et al., 2013; Polat et al., 2015; Diler and Oden, 2016). The eNOS-Intron 4a/b VNTR polymorphism is a variable number of tandem repeats (27 nt) in intron 4 participating in basal plasma NO generation (Wang et al., 1997). Our final analysis results suggested that this polymorphism was related to an increased risk of urogenital neoplasms in recessive model. However, 6 case-control studies for eNOS-Intron 4a/b VNTR polymorphism enrolled in our study, there were two studies showed no significant relationship between this polymorphism and urogenital neoplasms (Sanli et al., 2011; Diler and Oden, 2016).

Although no significant association was uncovered between the association between VEGF-rs699947 polymorphism and the risk of urogenital neoplasms. In the subgroup analysis by ethnicity, we found an increased risk of urogenital neoplasms in Asian populations. Moreover, when the stratification analysis conducted by cancer type, we also identified an increased risk for RCC subtype. Similar results were found between VEGF-rs3025039 polymorphism and risk of urogenital neoplasms. Nevertheless, in the subgroup analysis of ethnicity, we observed an increased risk of urogenital neoplasms in allelic model for Asian populations. Moreover, in stratification analysis by source of control, an increased risk of urogenital neoplasms for HB groups was also uncovered. Deviations in HWE status were influenced by methodological problems, such as the genotyping errors, the population stratification or selection bias, et al. Thus, we have conducted subgroup analyses by HWE status. For the HRAS-rs12628 polymorphism, we identified an increased risk of urogenital neoplasms in the dominant model for HWE (Y) groups.

While for VEGF (rs3025039, rs10434, rs1570360, rs2010963, and rs833061), HIF1α (rs11549465 and rs11549467), HRAS-rs12628 and eNOS-rs1799983 polymorphisms, our meta-analysis indicated there were no significant associations between them and urogenital neoplasms susceptibility even in subgroup analysis by ethnicity, HWE status, source of controls and cancer type (multiple testing P-value according to Bonferroni correction \( P < 0.05/(12 \text{ polymorphisms} \times 5 \text{ models}) \) was considered as statistically significant). However, we also prefer to identify markers for specific tumors (eg. RCC, BCa, and PCa). For these SNPs, \( P_\text{A} < 0.05 \) was considered as statistically significant for cancer type subgroup analysis. Results suggested VEGF-rs699947, VEGF-rs3025039, and VEGF-rs2010963 polymorphisms may be a potential risk factor for RCC. And eNOS-rs2070744, eNOS-Intron 4a/b VNTR, eNOS-rs1799983, and HRAS-rs12628 polymorphisms may be a risk factor for BCa. In addition, eNOS-rs2070744 and HIF1α-rs11549467 polymorphisms may be a risk factor for PCa. Accordingly, these subgroups results suggested these SNPs might be used as potential diagnostic markers for RCC, BCa, and PCa, respectively. There are several important advantages for the current meta-analysis. First and foremost, we implemented a comprehensive database search to identify all eligible studies in the VEGF/hypoxia/angiogenesis pathway, making our meta-analysis more substantial and persuasive. Second, all included studies were assessed by Newcastle Ottawa Scale, aiming to exclude low quality studies and elevate the overall quality. Third, various subgroup analyses based on ethnicity, HWE status, source of controls and cancer type were performed, trying to further stratify the sources of heterogeneity. Fourth, in order to making our analysis more accurate, the recognized formula was performed to adjust the results. In addition, sensitivity analysis was applied to confirm the stability of included studies, and Begg's funnel plot and Egger's test was utilized to analyze publication bias.

However, several limitations should also be noted in our present meta-analysis. First, for several polymorphisms, particularly when the case number in the studies was small, it may result in an insufficient power for identifying weak association between these polymorphisms and urogenital neoplasms susceptibility. Second, we currently didn't validate these polymorphisms with related clinical consequences. In the follow-up study, we will focus on the functional aspects of how these polymorphisms affect genes expression and ultimately associate with tumorigenesis. Third, all of the included studies were restricted in English, exclusion of other languages studies may increase the publication bias. Fourth, we didn't consider...
several potential confounding factors in this study, such as the age, gender, smoking habit, drinking status, and environmental factors, etc. This limited us further study the genetic and environmental interaction model. Therefore, the current result should be cautious interpreted.

In summary, in conjunction with other studies, current meta-analysis results suggest that eNOS-rs2070744 and eNOS-Intron 4a/b VNTR polymorphisms are associated with elevated risk of urogenital carcinomas. In addition, VEGF-rs699947 polymorphism was also identified as an risk factor for renal carcinoma. Further well-designed case-control studies with large population size are warranted to strengthen our findings.

AUTHOR CONTRIBUTIONS
J-BC, MZ, and X-BZ conceived and designed the study, performed the literature search and data extraction. J-BC, MZ, YC, P-HL, Y-WQ, CL, XC, W-BR, and Q-QL performed the meta-analysis and drafted the manuscript. L-FL, M-FC, H-QC, and X-BZ revised the manuscript. H-QC and X-BZ final approval of the version to be submitted.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.00715/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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