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REVIEW ARTICLE

Association between *EPHX1* polymorphisms and carbamazepine metabolism in epilepsy: a meta-analysis

Gui-Xin Zhao[1,2] · Ming-Li Shen[1,3] · Zheng Zhang[4] · Ping Wang[1,2] · Chun-Xiang Xie[1] · Gong-Hao He[1]

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Abstract

Background EPHX1 gene polymorphisms were recently acknowledged as an important source of individual variability in carbamazepine metabolism, but the result of that association still remains controversial. *Aim of the review* To obtain a more precise estimation of the associations between *EPHX1* polymorphisms and carbamazepine metabolism and resistance. *Methods* The PubMed, EMBASE, Cochrane library, Chinese National Knowledge Infrastructure, Chinese Science and Technique Journals Database, China Biology Medicine disc and Wan fang Database were searched for appropriate studies regarding the rs1051740 and rs2234922 polymorphisms of *EPHX1* up to September 2019. The meta-analysis was carried out using the Review Manager 5.3 software. The mean difference and 95% confidence interval were applied to assess the strength of the relationship. *Results* A total of 7 studies involving 1118 related epilepsy patients were included. *EPHX1* rs1051740 polymorphism was significantly associated with adjusted concentrations of both carbamazepine (CC vs. TT: $P=0.02$; CC vs. CT + TT: $P=0.005$) and carbamazepine-10,11-epoxide (CC vs. CT + TT: $P=0.03$). Furthermore, *EPHX1* rs2234922 polymorphism was also observed to be significantly associated with decreased adjusted concentrations of carbamazepine-10,11-trans dihydrodiol (GG vs. GA + AA: $P=0.04$) and CBZD:CBZE ratio (GG vs. AA: $P=0.008$; GG vs. GA + AA: $P=0.0008$). Nevertheless, the pooled analysis showed that the *EPHX1* polymorphisms had no significant effect on CBZ resistance. *Conclusion* *EPHX1* rs1051740 and rs2234922 polymorphisms may affect the carbamazepine metabolism; but carbamazepine resistance was not related to any of the single nucleotide polymorphisms investigated. These findings provided further evidence for individualized therapy of epilepsy patients in clinics.

Keywords Carbamazepine · *EPHX1* · Epilepsy · Meta-analysis · Metabolism · Polymorphism

Impacts on Practice

- The CC genotype of rs1011740 is associated with a reduction in serum concentrations of carbamazepine when compared to the TT and CT + TT genotypes.
- The GG genotype of rs2234922 decreases serum concentrations of carbamazepine-10,11-trans dihydrodiol in the recessive model and reduces CBZD:CBZE ratio in the additive and recessive models.
- The findings of this meta analysis help to improve individualized therapy of epilepsy patients in clinics.

Gui-Xin Zhao, Ming-Li Shen and Zheng Zhang contributed equally to this work.

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✉ Gong-Hao He
gonghow@hotmail.com

[1] Department of Pharmacy, 920th Hospital of Joint Logistics Support Force, 212 Daguan Rd., Kunming 650032, China

[2] Kunming Medical University, Kunming 650500, China

[3] Dali University, Dali 671000, China

[4] Medical Engineering Section, The 306th Hospital of PLA, Beijing 100101, China

Background

Carbamazepine (CBZ), a dibenzazepines antiepileptic drug, is prescribed worldwide as a first-line treatment for focal or generalized tonic–clonic epileptic seizures [1]. It is considered as an essential medicine by the World Health Organization. However, CBZ has a relatively narrow therapeutic range (4–12 μg/mL) and a therapeutic drug monitoring of CBZ was therefore recommended to optimize its dose [2]. The causes for the inter-individual variability of daily maintenance doses of CBZ vary from environmental factors, co-medication to genetic variation [3, 4], among which genetic variation of drug-metabolizing enzymes has been widely concerned recently and was suggested to have a major impact on drug therapy for epilepsy [5, 6].

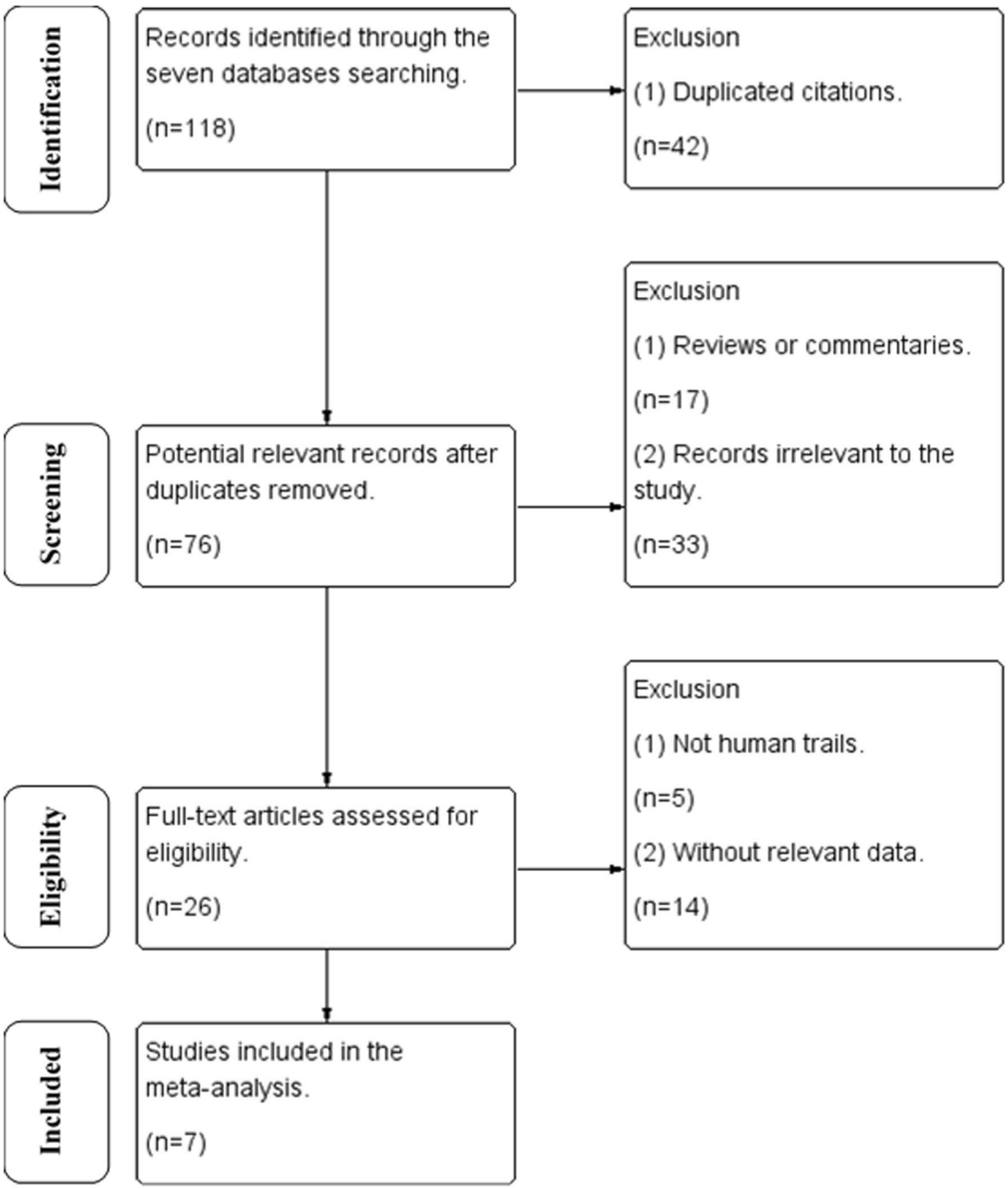


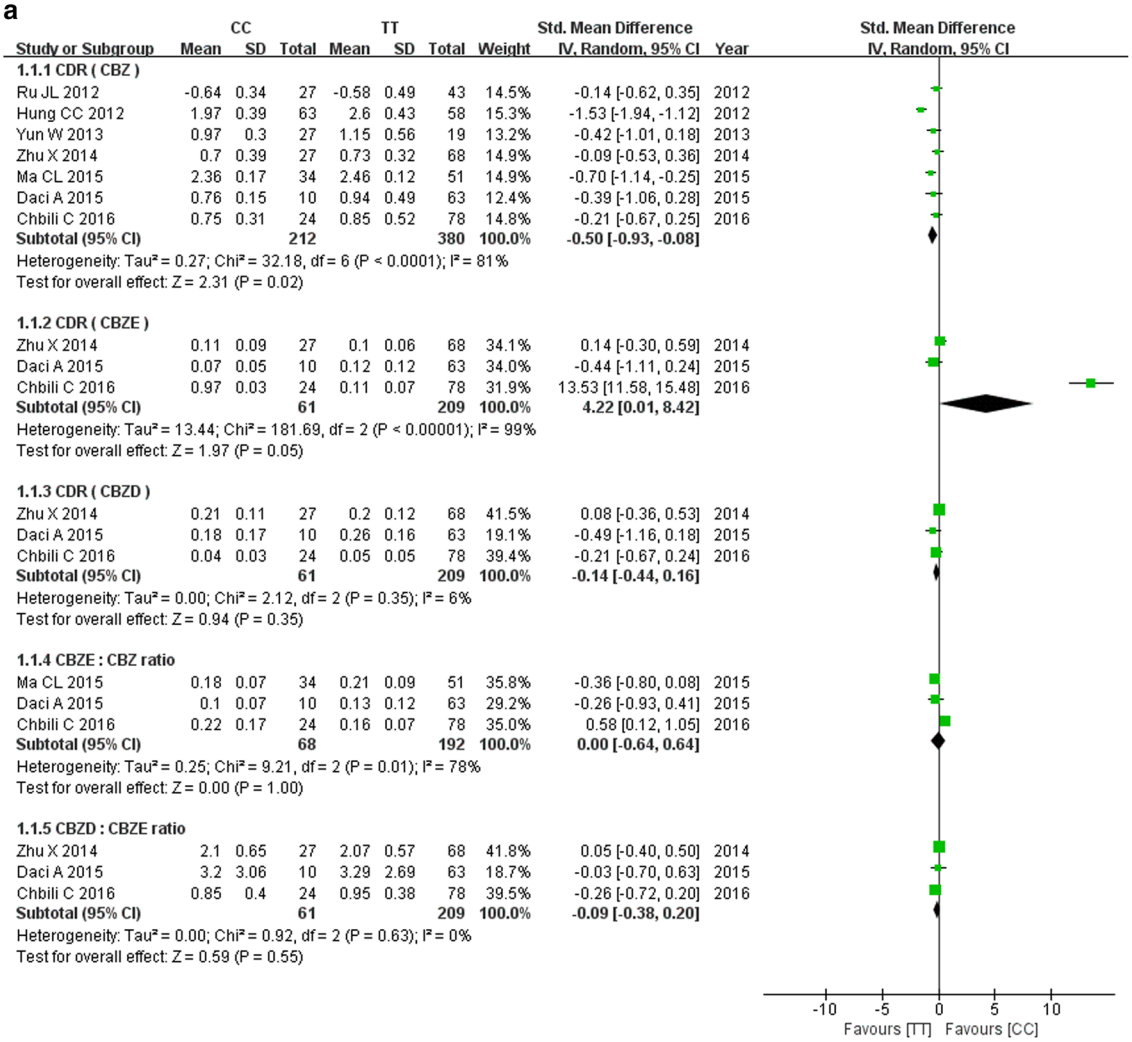
Fig. 1 Flow diagram for process of included studies selection

Table 1 Characteristics of all studies included in this meta-analysis

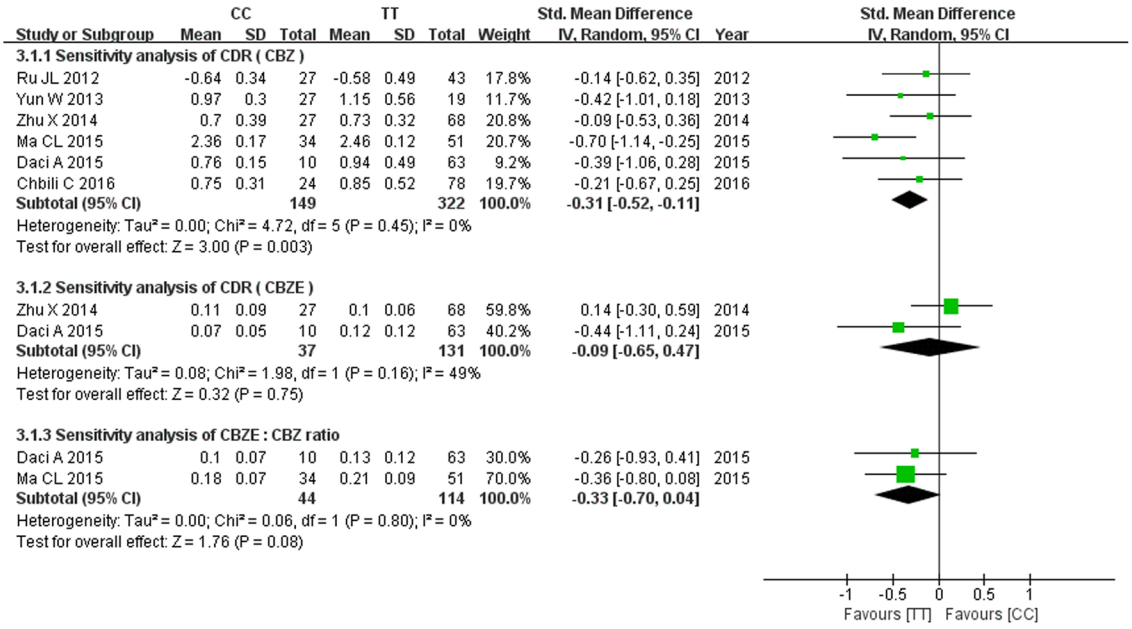
First author	Year	Population	Subjects	Drug	Dose/mg/kg/day(mean ± SD)	Treatment duration	Number (male/female)	Age/years (mean ± SD)	Weight/kg (mean ± SD)	Study design	NOS Score	Genotyping methods	DNA source	Polymorphism	Ref
Hung CC	2012	Taiwan, China	Epilepsy	CBZ	NO	Over 1 year	234(111/123)	39.23 ± 11.88	60.12 ± 10.51	Case–control	8	Taq Man and PCR–RFLP	PB	rs1051740 rs2234922	[22]
Ru JL	2012	China	Epilepsy	CBZ	NO	Over 1 year	162(104/58)	11.2 ± 3.5	43.3 ± 16.8	Case–control	5	Direct Sequencing and PCR–RFLP	PB	rs1051740 rs2234922	[25]
Yun W	2013	China	Epilepsy	CBZ	7.28 ± 2.73	Over 1 year	83(53/30)	36.2 ± 16.5	63.29 ± 7.65	Case–control	5	PCR–RFLP	PB	rs1051740 rs2234922	[30]
Zhu X	2014	China	Epilepsy	CBZ	10.93 ± 5.00	Over 1 year	210(119/91)	19 ± 15.09	49.22 ± 16.6	Case–control	5	Direct Sequencing and PCR–RFLP	PB	rs1051740 rs2234922	[24]
Daci A	2015	Kosovo	Epilepsy	CBZ	8.18 ± 3.91	Over 1 year	145(82/63)	32.9 ± 15.5	68.3 ± 16	Case–control	8	Taq Man	PB	rs1051740 rs2234922	[26]
Ma CL	2015	China	Epilepsy	CBZ	10.04 ± 3.14	Over 1 year	166(91/75)	34.75 ± 15.83	64.96 ± 11.31	Case–control	8	Direct Sequencing and Taq Man	PB	rs1051740	[21]
Chbili C	2016	Tunisia	Epilepsy	CBZ	8.51 ± 3.21	28.16 months	118(52/66)	38.26 ± 17	63.3 ± 7.6	Case–control	5	PCR–RFLP	PB	rs1051740 rs2234922	[23]

NOS Newcastle–Ottawa scale, *CBZ* Carbamazepine, *PCR–RFLP* PCR-restriction fragment length polymorphism, *PB* Peripheral blood, *SD* Standard deviation

a


CC
TT
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI Year
IV, Random, 95% CI
1.1.1 CDR (CBZ)
Ru JL 2012 -0.64 0.34 27 -0.58 0.49 43 14.5% -0.14 [-0.62, 0.35] 2012
Hung CC 2012 1.97 0.39 63 2.6 0.43 58 15.3% -1.53 [-1.94, -1.12] 2012
Yun W 2013 0.97 0.3 27 1.15 0.56 19 13.2% -0.42 [-1.01, 0.18] 2013
Zhu X 2014 0.7 0.39 27 0.73 0.32 68 14.9% -0.09 [-0.53, 0.36] 2014
Ma CL 2015 2.36 0.17 34 2.46 0.12 51 14.9% -0.70 [-1.14, -0.25] 2015
Daci A 2015 0.76 0.15 10 0.94 0.49 63 12.4% -0.39 [-1.06, 0.28] 2015
Chbili C 2016 0.75 0.31 24 0.85 0.52 78 14.8% -0.21 [-0.67, 0.25] 2016
Subtotal (95% CI) 212 380 100.0% -0.50 [-0.93, -0.08]
Heterogeneity: Tau² = 0.27; Chi² = 32.18, df = 6 (P < 0.0001); I² = 81%
Test for overall effect: Z = 2.31 (P = 0.02)
1.1.2 CDR (CBZE)
Zhu X 2014 0.11 0.09 27 0.1 0.06 68 34.1% 0.14 [-0.30, 0.59] 2014
Daci A 2015 0.07 0.05 10 0.12 0.12 63 34.0% -0.44 [-1.11, 0.24] 2015
Chbili C 2016 0.97 0.03 24 0.11 0.07 78 31.9% 13.53 [11.58, 15.48] 2016
Subtotal (95% CI) 61 209 100.0% 4.22 [0.01, 8.42]
Heterogeneity: Tau² = 13.44; Chi² = 181.69, df = 2 (P < 0.00001); I² = 99%
Test for overall effect: Z = 1.97 (P = 0.05)
1.1.3 CDR (CBZD)
Zhu X 2014 0.21 0.11 27 0.2 0.12 68 41.5% 0.08 [-0.36, 0.53] 2014
Daci A 2015 0.18 0.17 10 0.26 0.16 63 19.1% -0.49 [-1.16, 0.18] 2015
Chbili C 2016 0.04 0.03 24 0.05 0.05 78 39.4% -0.21 [-0.67, 0.24] 2016
Subtotal (95% CI) 61 209 100.0% -0.14 [-0.44, 0.16]
Heterogeneity: Tau² = 0.00; Chi² = 2.12, df = 2 (P = 0.35); I² = 6%
Test for overall effect: Z = 0.94 (P = 0.35)
1.1.4 CBZE : CBZ ratio
Ma CL 2015 0.18 0.07 34 0.21 0.09 51 35.8% -0.36 [-0.80, 0.08] 2015
Daci A 2015 0.1 0.07 10 0.13 0.12 63 29.2% -0.26 [-0.93, 0.41] 2015
Chbili C 2016 0.22 0.17 24 0.16 0.07 78 35.0% 0.58 [0.12, 1.05] 2016
Subtotal (95% CI) 68 192 100.0% 0.00 [-0.64, 0.64]
Heterogeneity: Tau² = 0.25; Chi² = 9.21, df = 2 (P = 0.01); I² = 78%
Test for overall effect: Z = 0.00 (P = 1.00)
1.1.5 CBZD : CBZE ratio
Zhu X 2014 2.1 0.65 27 2.07 0.57 68 41.8% 0.05 [-0.40, 0.50] 2014
Daci A 2015 3.2 3.06 10 3.29 2.69 63 18.7% -0.03 [-0.70, 0.63] 2015
Chbili C 2016 0.85 0.4 24 0.95 0.38 78 39.5% -0.26 [-0.72, 0.20] 2016
Subtotal (95% CI) 61 209 100.0% -0.09 [-0.38, 0.20]
Heterogeneity: Tau² = 0.00; Chi² = 0.92, df = 2 (P = 0.63); I² = 0%
Test for overall effect: Z = 0.59 (P = 0.55)
-10 -5 0 5 10
Favours [TT] Favours [CC]

b


CC
TT
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI Year
IV, Random, 95% CI
3.1.1 Sensitivity analysis of CDR (CBZ)
Ru JL 2012 -0.64 0.34 27 -0.58 0.49 43 17.8% -0.14 [-0.62, 0.35] 2012
Yun W 2013 0.97 0.3 27 1.15 0.56 19 11.7% -0.42 [-1.01, 0.18] 2013
Zhu X 2014 0.7 0.39 27 0.73 0.32 68 20.8% -0.09 [-0.53, 0.36] 2014
Ma CL 2015 2.36 0.17 34 2.46 0.12 51 20.7% -0.70 [-1.14, -0.25] 2015
Daci A 2015 0.76 0.15 10 0.94 0.49 63 9.2% -0.39 [-1.06, 0.28] 2015
Chbili C 2016 0.75 0.31 24 0.85 0.52 78 19.7% -0.21 [-0.67, 0.25] 2016
Subtotal (95% CI) 149 322 100.0% -0.31 [-0.52, -0.11]
Heterogeneity: Tau² = 0.00; Chi² = 4.72, df = 5 (P = 0.45); I² = 0%
Test for overall effect: Z = 3.00 (P = 0.003)
3.1.2 Sensitivity analysis of CDR (CBZE)
Zhu X 2014 0.11 0.09 27 0.1 0.06 68 59.8% 0.14 [-0.30, 0.59] 2014
Daci A 2015 0.07 0.05 10 0.12 0.12 63 40.2% -0.44 [-1.11, 0.24] 2015
Subtotal (95% CI) 37 131 100.0% -0.09 [-0.65, 0.47]
Heterogeneity: Tau² = 0.08; Chi² = 1.98, df = 1 (P = 0.16); I² = 49%
Test for overall effect: Z = 0.32 (P = 0.75)
3.1.3 Sensitivity analysis of CBZE : CBZ ratio
Daci A 2015 0.1 0.07 10 0.13 0.12 63 30.0% -0.26 [-0.93, 0.41] 2015
Ma CL 2015 0.18 0.07 34 0.21 0.09 51 70.0% -0.36 [-0.80, 0.08] 2015
Subtotal (95% CI) 44 114 100.0% -0.33 [-0.70, 0.04]
Heterogeneity: Tau² = 0.00; Chi² = 0.06, df = 1 (P = 0.80); I² = 0%
Test for overall effect: Z = 1.76 (P = 0.08)
-1 -0.5 0 0.5 1
Favours [TT] Favours [CC]

◂**Fig. 2** Forest plot for association between *EPHX1* rs1051740 polymorphism and CBZ metabolism in additive model (**a**). Sensitivity analysis for effect of *EPHX1* rs1051740 polymorphism on CDR_{CBZ}, CDR_{CBZE} and CBZE:CBZ ratio in additive model (**b**)

CBZ is catalyzed by cytochrome P450 3A4 (CYP3A4) to produce active metabolite carbamazepine-10,11-epoxide (CBZE), which possesses a potent anticonvulsant effect [7, 8]. Subsequently, CBZE is hydrolysed via microsomal epoxide hydrolase (mEH), which is encoded by the *EPHX1* gene, to an inactive metabolite carbamazepine-10,11-trans dihydrodiol (CBZD) [7, 9, 10]. It was previously reported that the obvious individual variability in drug pharmacokinetics and pharmacodynamics of CBZ was mainly ascribed to the genetic variation of *EPHX1* [11-14]. Therefore, the genetic polymorphisms in *EPHX1* may be an important source of interindividual variability in CBZ metabolism.

Human *EPHX1* gene spans 35.48 kb and is located on chromosome 1 (1q42.12) [15-17]. Among the validated single nucleotide polymorphisms (SNPs) in *EPHX1* gene, two SNPs (i.e., rs1051740 and rs2234922) were intensively investigated [18-20]. However, studies investigating the associations between *EPHX1* gene polymorphisms and enzyme activities exhibited inconsistent findings. For example, it was shown that patients carrying the CC genotype of rs1051740 were associated with a lower plasma concentration of CBZ as compared with the CT or TT genotype, suggesting a high metabolism in patients with the CC genotype [21, 22]. On the contrary, several other studies indicated that rs1051740 had no significant influence on plasma concentration of CBZ [23-25]. Additionally, similar inconsistent results were also observed for carriers of rs2234922 polymorphism. It was reported that the CBZD:CBZE ratio was significantly lower in rs2234922 GG carriers than in GA or AA carriers [23] although these findings were not confirmed in other studies [24, 26, 27].

Aim of the review

Based on this background, in order to achieve a more rational and individualized use of CBZ in clinical treatment, we hereby conducted a systematic meta-analysis to evaluate the associations of *EPHX1* rs1051740 and rs2234922 polymorphisms with CBZ metabolism and resistance, trying to provide necessary power to assess the effect of *EPHX1* genetic variations.

Methods

Search strategy

Relevant papers were searched through PubMed, EMBASE, Cochrane library, Chinese National Knowledge Infrastructure (CNKI), Chinese Science and Technique Journals Database (VIP), China Biology Medicine disc (CBM) and Wan fang Data. Information site using the following terms: "carbamazepine", "*EPHX1*" or "microsomal epoxide hydrolase" and "polymorphism" or "genotype". The search strategies for PubMed were provided as a sample in supplementary materials. We searched for trials without language and year of publication restrictions and references of retrieved articles were also searched and reviewed to find additional eligible studies. The last search was updated in September 2019. When multiple publications on the same study population were identified, only the most recent or complete study was used in this meta-analysis. The literature retrieval was performed by two independent authors.

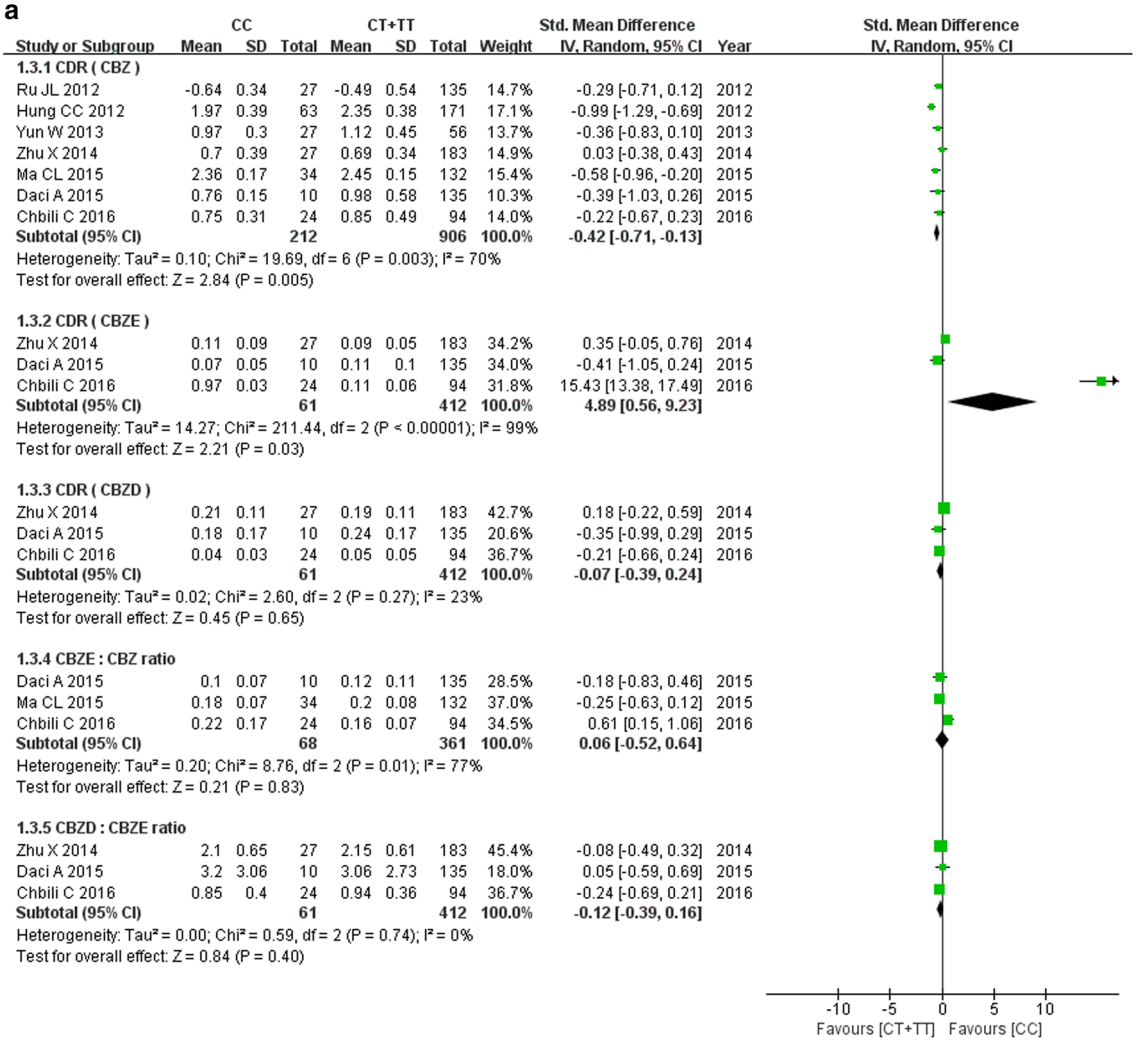
Inclusion and exclusion criteria

Studies fulfilling the following criteria were considered as eligible for inclusion in this meta-analysis: (a) case–control studies (b) evaluation of the associations between the genotypes of *EPHX1* rs1051740 and rs2234922 polymorphisms and plasma concentration or resistance of CBZ in epilepsy (c) each patient was administrated with CBZ monotherapy and not receiving pharmacological treatment for other pathologies (d) no liver or renal diseases were reported (e) including at least three studies for each polymorphism to allow calculation of publication bias between studies, and (f) studies based on any ethnicity population were eligible. Exclusion criteria were the following: (a) insufficient information for data extraction (b) reviews and case reports (c) not human research, and (d) outcomes not in our focus of interest.

Data extraction

Information was carefully extracted from all eligible publications by two independent authors, including author's name, publication date, country, sample size, plasma concentration and adjusted serum concentration of CBZ (CDR_{CBZ}), CBZE (CDR_{CBZE}) and CBZD (CDR_{CBZD}), CBZE:CBZ and CBZD:CBZE ratios, genotypes distribution, drug resistant patients (defined as no change or less than 50% reduction in seizure frequency for at least 1 year during treatment with CBZ) and drug responsive patients (defined as seizure-free or a 50% or greater reduction in seizure frequency for at least 1 year during treatment with CBZ) [26, 28]. If diverse results were generated, the disagreement was resolved by discussion between the two authors or by asking a third investigator. The authors of the studies were contacted for additional data when necessary and applicable.

a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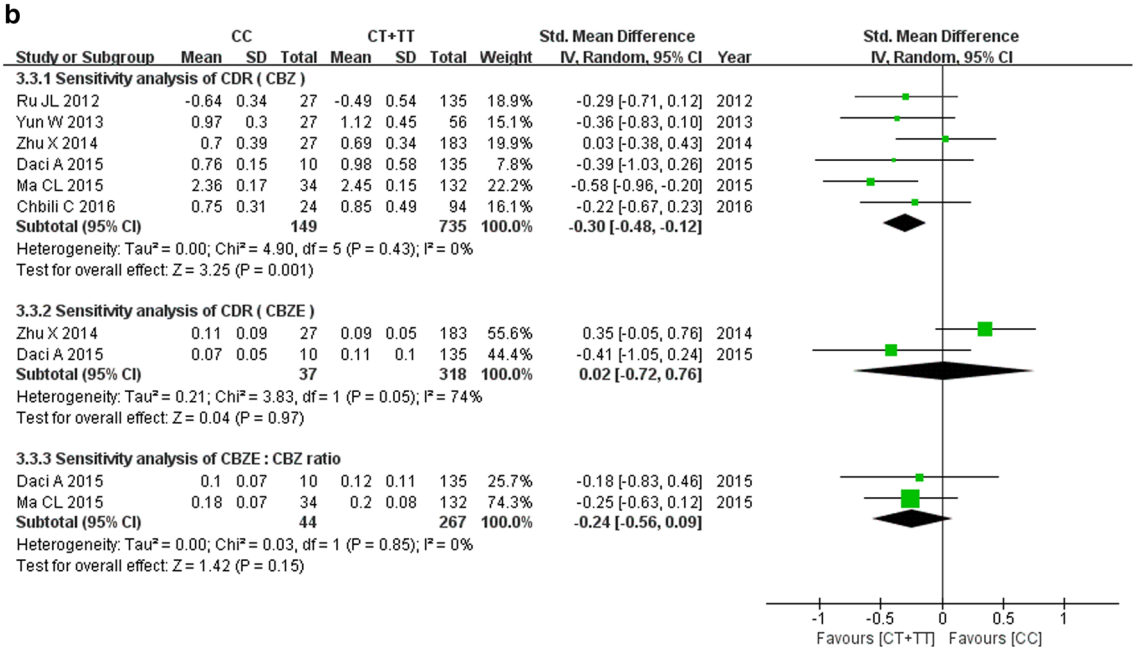
CT+TT
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI Year
IV, Random, 95% CI
1.3.1 CDR (CBZ)
Ru JL 2012 -0.64 0.34 27 -0.49 0.54 135 14.7% -0.29 [-0.71, 0.12] 2012
Hung CC 2012 1.97 0.39 63 2.35 0.38 171 17.1% -0.99 [-1.29, -0.69] 2012
Yun W 2013 0.97 0.3 27 1.12 0.45 56 13.7% -0.36 [-0.83, 0.10] 2013
Zhu X 2014 0.7 0.39 27 0.69 0.34 183 14.9% 0.03 [-0.38, 0.43] 2014
Ma CL 2015 2.36 0.17 34 2.45 0.15 132 15.4% -0.58 [-0.96, -0.20] 2015
Daci A 2015 0.76 0.15 10 0.98 0.58 135 10.3% -0.39 [-1.03, 0.26] 2015
Chbili C 2016 0.75 0.31 24 0.85 0.49 94 14.0% -0.22 [-0.67, 0.23] 2016
Subtotal (95% CI) 212 906 100.0% -0.42 [-0.71, -0.13]
Heterogeneity: Tau² = 0.10; Chi² = 19.69, df = 6 (P = 0.003); I² = 70%
Test for overall effect: Z = 2.84 (P = 0.005)
1.3.2 CDR (CBZE)
Zhu X 2014 0.11 0.09 27 0.09 0.05 183 34.2% 0.35 [-0.05, 0.76] 2014
Daci A 2015 0.07 0.05 10 0.11 0.1 135 34.0% -0.41 [-1.05, 0.24] 2015
Chbili C 2016 0.97 0.03 24 0.11 0.06 94 31.8% 15.43 [13.38, 17.49] 2016
Subtotal (95% CI) 61 412 100.0% 4.89 [0.56, 9.23]
Heterogeneity: Tau² = 14.27; Chi² = 211.44, df = 2 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.21 (P = 0.03)
1.3.3 CDR (CBZD)
Zhu X 2014 0.21 0.11 27 0.19 0.11 183 42.7% 0.18 [-0.22, 0.59] 2014
Daci A 2015 0.18 0.17 10 0.24 0.17 135 20.6% -0.35 [-0.99, 0.29] 2015
Chbili C 2016 0.04 0.03 24 0.05 0.05 94 36.7% -0.21 [-0.66, 0.24] 2016
Subtotal (95% CI) 61 412 100.0% -0.07 [-0.39, 0.24]
Heterogeneity: Tau² = 0.02; Chi² = 2.60, df = 2 (P = 0.27); I² = 23%
Test for overall effect: Z = 0.45 (P = 0.65)
1.3.4 CBZE : CBZ ratio
Daci A 2015 0.1 0.07 10 0.12 0.11 135 28.5% -0.18 [-0.83, 0.46] 2015
Ma CL 2015 0.18 0.07 34 0.2 0.08 132 37.0% -0.25 [-0.63, 0.12] 2015
Chbili C 2016 0.22 0.17 24 0.16 0.07 94 34.5% 0.61 [0.15, 1.06] 2016
Subtotal (95% CI) 68 361 100.0% 0.06 [-0.52, 0.64]
Heterogeneity: Tau² = 0.20; Chi² = 8.76, df = 2 (P = 0.01); I² = 77%
Test for overall effect: Z = 0.21 (P = 0.83)
1.3.5 CBZD : CBZE ratio
Zhu X 2014 2.1 0.65 27 2.15 0.61 183 45.4% -0.08 [-0.49, 0.32] 2014
Daci A 2015 3.2 3.06 10 3.06 2.73 135 18.0% 0.05 [-0.59, 0.69] 2015
Chbili C 2016 0.85 0.4 24 0.94 0.36 94 36.7% -0.24 [-0.69, 0.21] 2016
Subtotal (95% CI) 61 412 100.0% -0.12 [-0.39, 0.16]
Heterogeneity: Tau² = 0.00; Chi² = 0.59, df = 2 (P = 0.74); I² = 0%
Test for overall effect: Z = 0.84 (P = 0.40)
-10 -5 0 5 10
Favours [CT+TT] Favours [CC]

b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Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI Year
IV, Random, 95% CI
3.3.1 Sensitivity analysis of CDR (CBZ)
Ru JL 2012 -0.64 0.34 27 -0.49 0.54 135 18.9% -0.29 [-0.71, 0.12] 2012
Yun W 2013 0.97 0.3 27 1.12 0.45 56 15.1% -0.36 [-0.83, 0.10] 2013
Zhu X 2014 0.7 0.39 27 0.69 0.34 183 19.9% 0.03 [-0.38, 0.43] 2014
Daci A 2015 0.76 0.15 10 0.98 0.58 135 7.8% -0.39 [-1.03, 0.26] 2015
Ma CL 2015 2.36 0.17 34 2.45 0.15 132 22.2% -0.58 [-0.96, -0.20] 2015
Chbili C 2016 0.75 0.31 24 0.85 0.49 94 16.1% -0.22 [-0.67, 0.23] 2016
Subtotal (95% CI) 149 735 100.0% -0.30 [-0.48, -0.12]
Heterogeneity: Tau² = 0.00; Chi² = 4.90, df = 5 (P = 0.43); I² = 0%
Test for overall effect: Z = 3.25 (P = 0.001)
3.3.2 Sensitivity analysis of CDR (CBZE)
Zhu X 2014 0.11 0.09 27 0.09 0.05 183 55.6% 0.35 [-0.05, 0.76] 2014
Daci A 2015 0.07 0.05 10 0.11 0.1 135 44.4% -0.41 [-1.05, 0.24] 2015
Subtotal (95% CI) 37 318 100.0% 0.02 [-0.72, 0.76]
Heterogeneity: Tau² = 0.21; Chi² = 3.83, df = 1 (P = 0.05); I² = 74%
Test for overall effect: Z = 0.04 (P = 0.97)
3.3.3 Sensitivity analysis of CBZE : CBZ ratio
Daci A 2015 0.1 0.07 10 0.12 0.11 135 25.7% -0.18 [-0.83, 0.46] 2015
Ma CL 2015 0.18 0.07 34 0.2 0.08 132 74.3% -0.25 [-0.63, 0.12] 2015
Subtotal (95% CI) 44 267 100.0% -0.24 [-0.56, 0.09]
Heterogeneity: Tau² = 0.00; Chi² = 0.03, df = 1 (P = 0.85); I² = 0%
Test for overall effect: Z = 1.42 (P = 0.15)
-1 -0.5 0 0.5 1
Favours [CT+TT] Favours [CC]

◂**Fig. 3** Forest plot for association between *EPHX1* rs1051740 polymorphism and CBZ metabolism in recessive model (**a**). Sensitivity analysis for effect of *EPHX1* rs1051740 polymorphism on CDR_{CBZ}, CDR_{CBZE} and CBZE:CBZ ratio in recessive model (**b**)

Assessment of methodological quality

We used the Newcastle–Ottawa scale (NOS) for assessing the quality of the included studies in this meta-analysis [29]. Using this scale system, each included study was judged on three broad perspectives: the selection of the study groups, the comparability of the groups, and the ascertainment of outcome of interest. High-quality studies were identified with a NOS score of five or more, whereas those with less than a score of five were considered as low-quality studies.

Statistical analysis

We conducted the review using the Review Manager 5.3 software. Comparisons of the dichotomous variables were performed using odds ratio (OR) with 95% confidence interval (CI). For continuous variables, the standard mean differences (Std. MD) with 95% CI were used. Z-test was performed to determine the statistical significance of results with statistical significance defined as $P < 0.05$. Heterogeneity between articles was assessed by Cochrane's Q-test. Data with low heterogeneity ($P \geq 0.10$ and $I^2 \leq 50\%$) were analyzed by a fixed-effects model while a random-effect model was used for data with high heterogeneity. To explore sources of heterogeneity, the leave-one-out sensitivity analysis was also conducted. Furthermore, a funnel plot was used to evaluate the publication bias when applicable.

Results

Study selection and qualitative assessment

As shown in Fig. 1, the initial search strategy identified 118 studies in the electronic databases, from which 42 were excluded for duplication. Then, we excluded 69 studies after a careful full-text review of the remaining 76 studies, which included 33 irrelevant studies, 17 review articles or commentaries, 5 in vitro or animal studies, 14 studies with insufficient data. Eventually, 7 studies [21-26, 30] including 1118 participants were included according to the present inclusion and exclusion criteria.

The characteristics of included studies are presented in Table 1. These studies were published between 2012 and 2016 from different countries. All participants in the studies were epilepsy patients and were treated with CBZ monotherapy at a stable maintenance dose. The classification of epilepsies and epileptic syndromes were conducted according to the guidelines of the International League Against Epilepsy [31]. All the patients were treated with CBZ in a dose range of 400–1200 mg/day. The source of DNA was peripheral venous blood, and the genetic polymorphisms were assayed either by PCR–RFLP, Tap Man Assay or direct DNA sequencing. The NOS scores of the included studies ranged from 5 to 8 based on the NOS evaluation system, indicating a relatively high quality of included studies. Furthermore, a test of funnel plot was not conducted due to the insufficient number of studies in the present study.

Associations between *EPHX1* rs1051740 polymorphism and CBZ metabolism

Additive model (CC vs. TT)

Seven studies evaluated the association between rs1051740 polymorphism and CBZ metabolism. Outcomes, such as the adjusted concentrations of CBZ, CBZE and CBZD, were reported in 7, 3 and 3 articles, respectively; while the metabolic ratios (i.e., CBZE:CBZ and CBZD:CBZE) data were evaluated in 3 articles. In the additive model, the CC genotype significantly decreased CDR_{CBZ} compared with TT genotype ($P = 0.02$, $I^2_{Het} = 81\%$). But no significant associations were observed in the CDR_{CBZE} ($P = 0.05$, $I^2_{Het} = 99\%$), CDR_{CBZD} ($P = 0.35$, $I^2_{Het} = 6\%$), CBZE:CBZ ratio ($P = 1.00$, $I^2_{Het} = 78\%$) or CBZD:CBZE ratio ($P = 0.55$, $I^2_{Het} = 0\%$) (Fig. 2a). The sensitivity analysis was performed by excluding each study successively, which effectively abrogated the heterogeneity and did not influence the present results (Fig. 2b).

Recessive model (CC vs. CT + TT)

Similar to the CC versus TT data, the CC genotype also decreased CDR_{CBZ} as compared with CT + TT genotype ($P = 0.005$, $I^2_{Het} = 70\%$), but did not significantly affect the CDR_{CBZD} ($P = 0.65$, $I^2_{Het} = 23\%$), CBZE:CBZ ratio ($P = 0.83$, $I^2_{Het} = 77\%$) or CBZD:CBZE ratio ($P = 0.40$, $I^2_{Het} = 0\%$) (Fig. 3a). The leave-one out sensitivity analysis decreased heterogeneity and did not affect the overall results (Fig. 3b). Moreover, the CC genotype was initially found to increase CDR_{CBZE} but with substantial between-study heterogeneity ($P = 0.03$, $I^2_{Het} = 99\%$) (Fig. 3a). However, when the study by Chbili et al. [23] was removed in sensitivity analysis, the association became insignificant with the heterogeneity relatively reduced ($P = 0.97$, $I^2_{Het} = 74\%$), indicating instability of this outcome (Fig. 3b).

Other models

As for the rest genetic models, our meta-analysis did not observe any associations between rs1051740 polymorphism

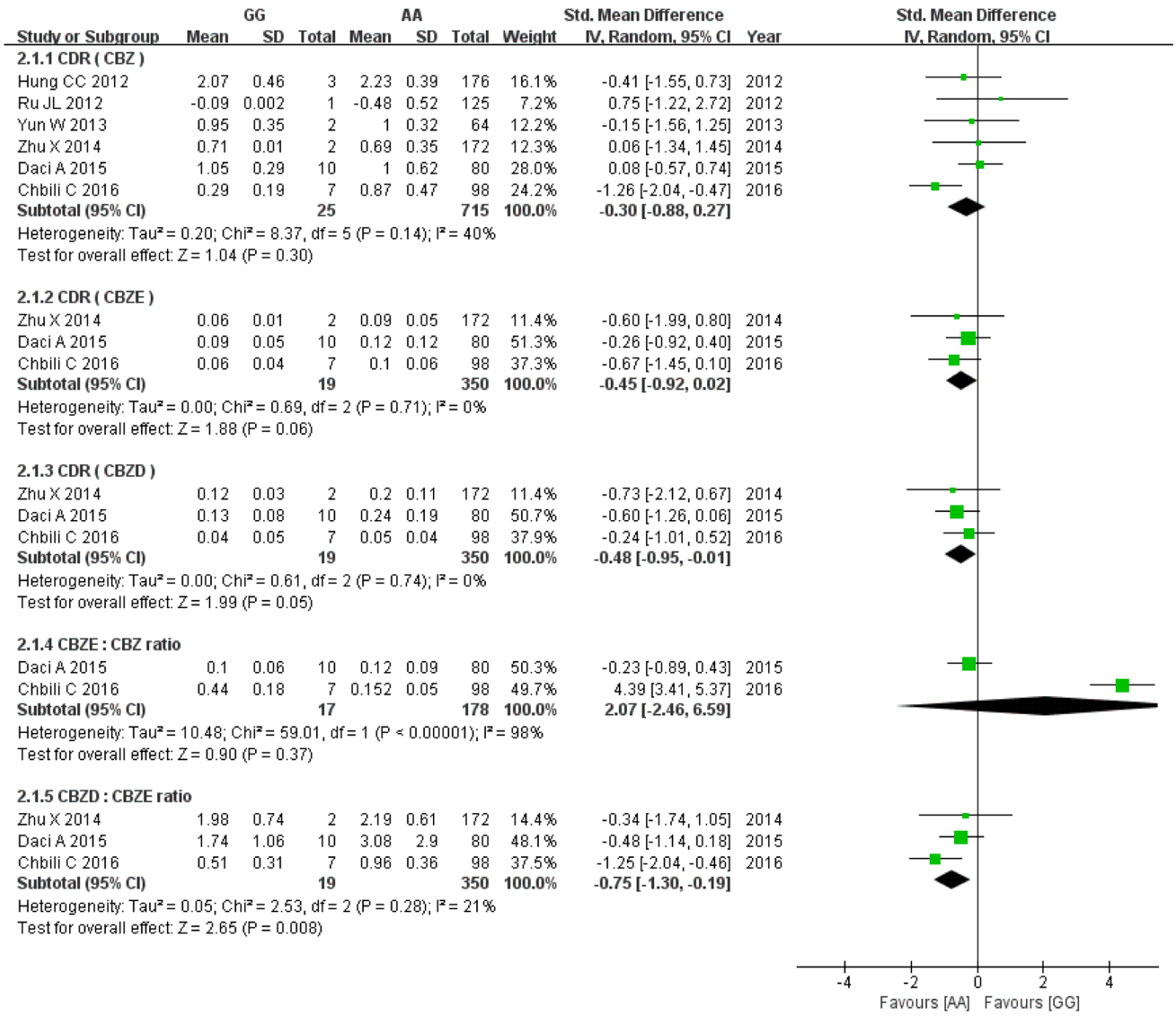

Fig. 4 Forest plot for association between *EPHX1* rs2234922 polymorphism and CBZ metabolism in additive model

and the metabolism of CBZ in heterozygous model (CT vs. TT), dominant model (CC + CT vs. TT) and co-dominant model (CT vs. CC + TT) (supplemental Fig. S1a, Fig. S2a and Fig. S3). The sensitivity analysis was also conducted by excluding each study successively, which abrogated the heterogeneity but did not influence the present results (supplemental Fig. S1b and Fig S2b).

Associations between *EPHX1* rs2234922 polymorphism and CBZ metabolism

Additive model (GG vs. AA)

Six studies assessed the association between *EPHX1* rs2234922 polymorphism and CBZ metabolism. The adjusted concentrations of CBZ, CBZE and CBZD were reported in 6, 3 and 3 articles, respectively. There were 2 articles that measured CBZE:CBZ ratio, while CBZD:CBZE ratio was evaluated in 3 articles. For additive model, the results showed that the GG genotype reduced CBZD:CBZE ratio compared with the wild-type homozygote AA genotype ($P = 0.008$, $I^2_{Het} = 21\%$), and no statistically significant

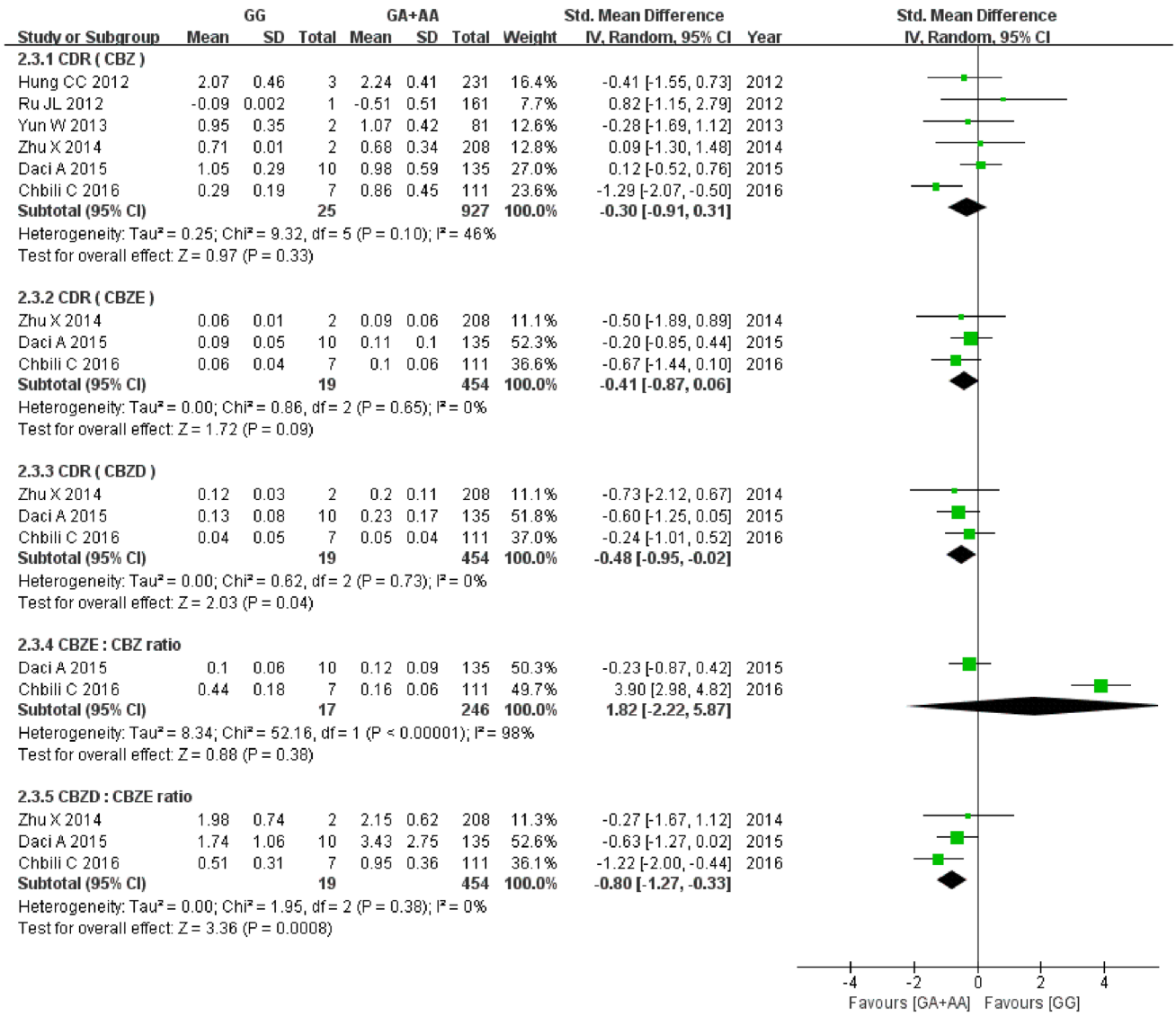

Fig. 5 Forest plot for association between *EPHX1* rs2234922 polymorphism and CBZ metabolism in recessive model

heterogeneity was observed (Fig. 4). As for other outcomes, we did not find any association in this model (Fig. 4). Significant heterogeneity was found regarding the CBZE:CBZ ratio. However, the sensitivity analysis was not performed because of the limited number of included studies regarding this outcome.

Recessive model (GG vs. GA + AA)

When compared with the GA + AA genotypes, the GG genotype decreased CDR_{CBZD} ($P = 0.04$, $I^2_{Het} = 0\%$) and CBZD:CBZE ratio ($P = 0.0008$, $I^2_{Het} = 0\%$) (Fig. 5). No associations were observed in the CDR_{CBZ} ($P = 0.33$, $I^2_{Het} = 46\%$), CDR_{CBZE} ($P = 0.09$, $I^2_{Het} = 0\%$), or CBZE:CBZ ratio ($P = 0.38$, $I^2_{Het} = 98\%$) (Fig. 5). The sensitivity analysis was not conducted because this outcome was only involved in 2 studies.

Other models

Our meta-analysis found no associations in heterozygous model (GA vs. AA), dominant model (GG + GA vs. AA) and co-dominant model (GA vs. GG + AA) (supplemental Fig. S4a, Fig. S5a and Fig. S6a). Sensitivity analysis abrogated the heterogeneity but did not alter the initial results of CDR_{CBZ}, CDR_{CBZE} and CDR_{CBZD} (supplemental Fig. S4b, Fig. S5b and Fig. S6b). However, for CBZD:CBZE ratio, sensitivity analysis changed the association result. After excluding one study [26], significant main effects (for CBZD:CBZE ratio) were observed in heterozygous model

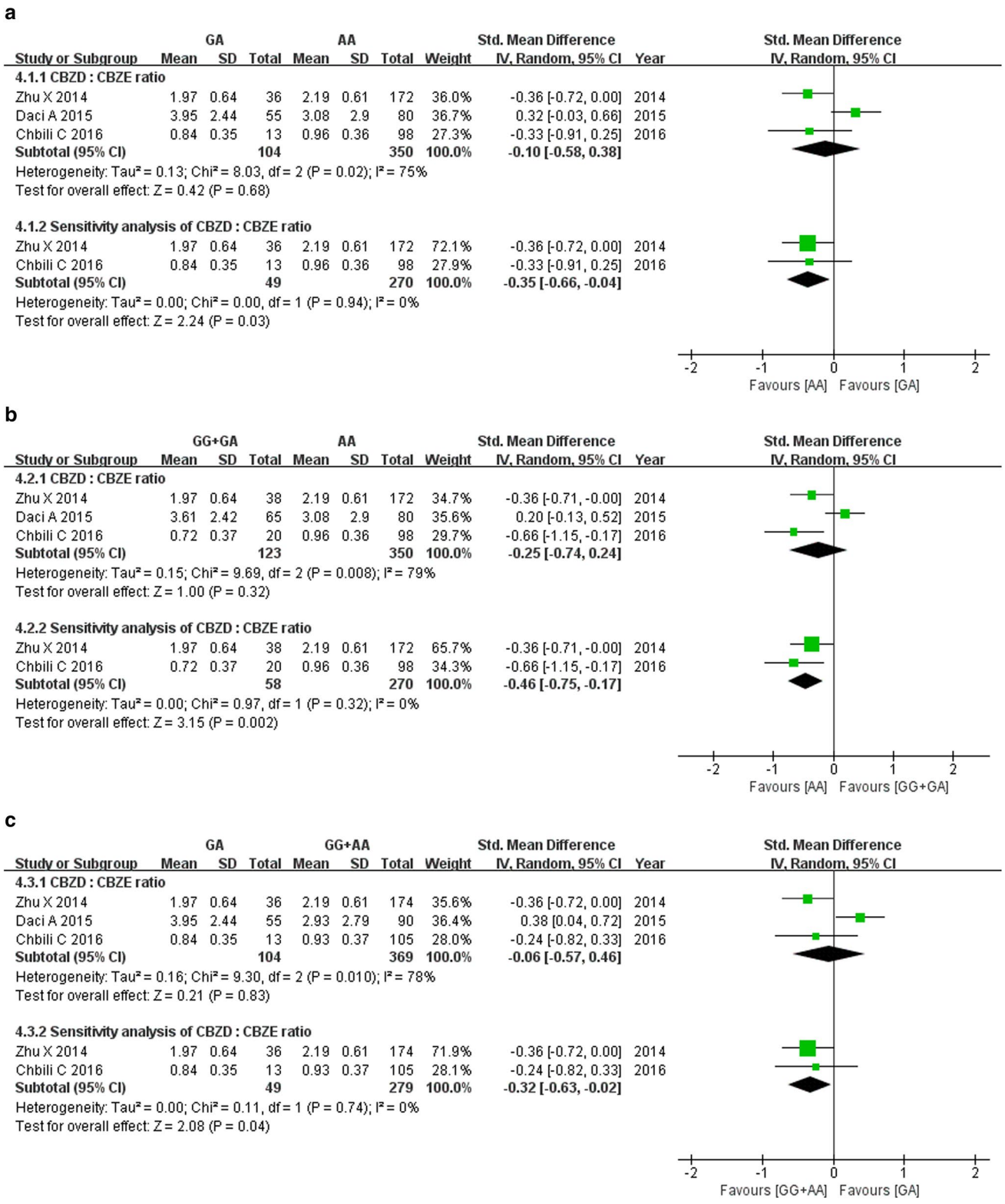

Fig. 6 Sensitivity analysis for effect of *EPHX1* rs2234922 polymorphism on CBZD:CBZE ratio in heterozygous model (**a**), dominant model (**b**) and co-dominant mode (**c**)

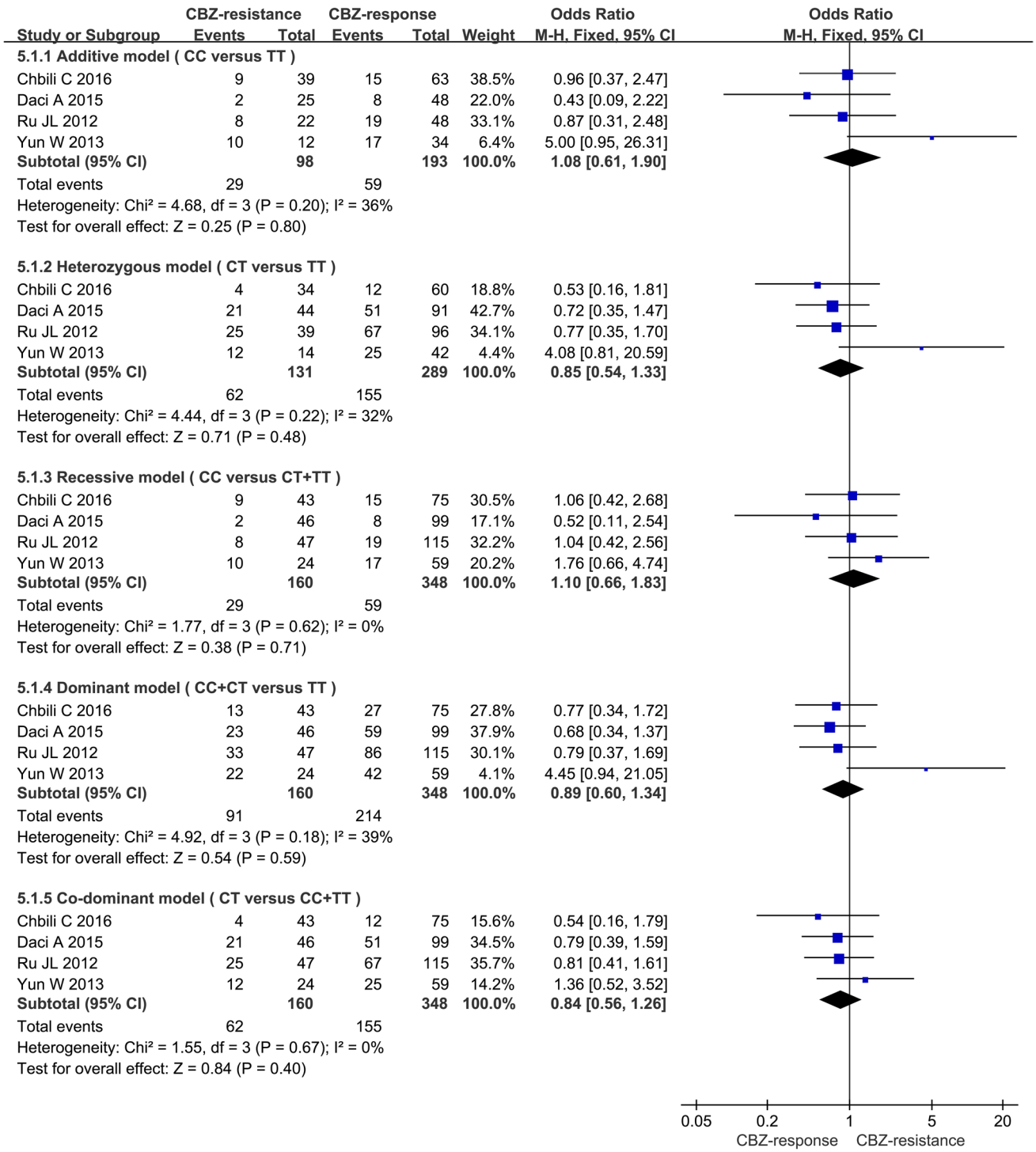

Fig. 7 Forest plot for association between *EPHX1* rs1051740 polymorphism and CBZ resistance in additive model, heterozygous model, recessive model, dominant model and co-dominant model

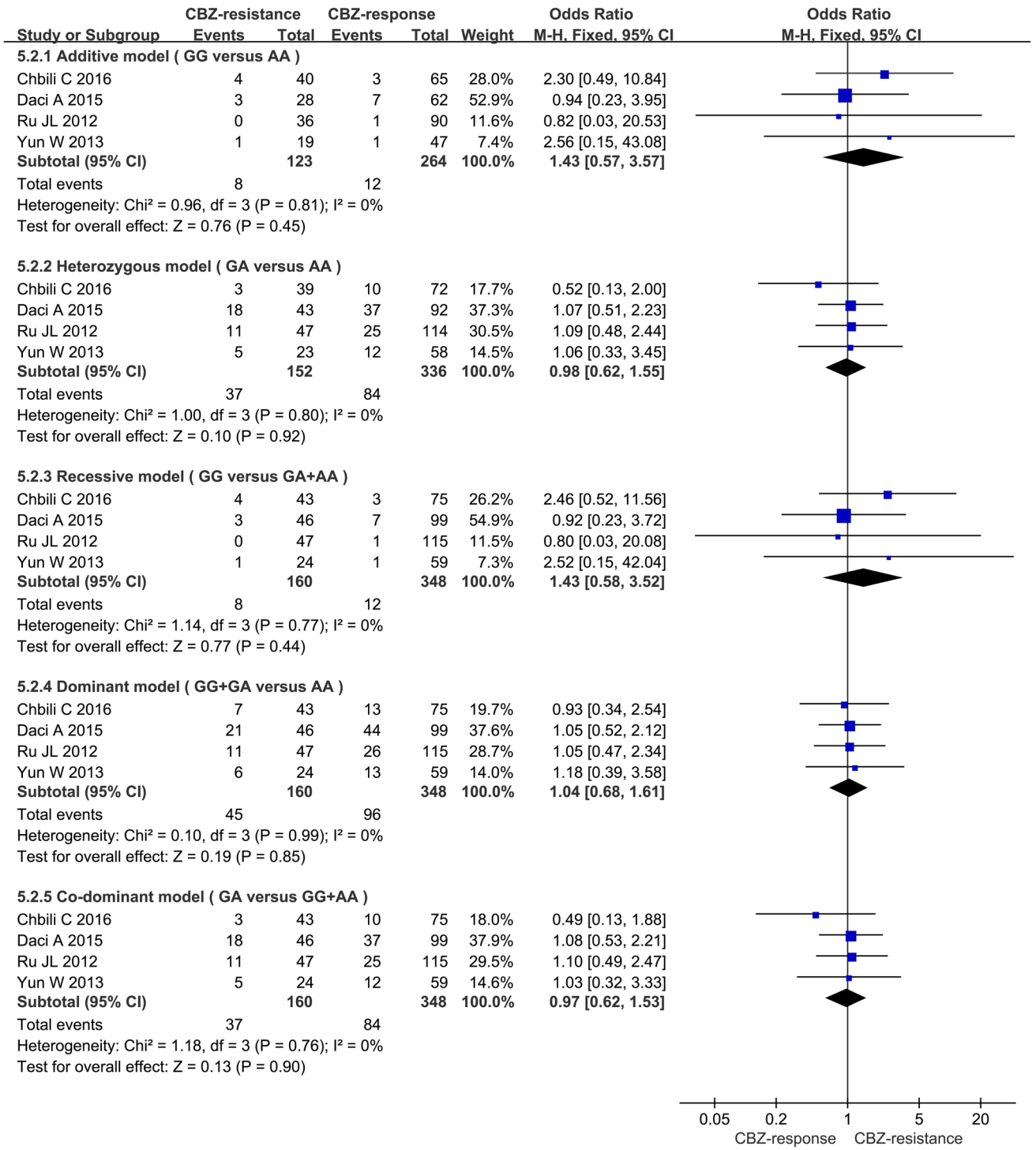

Fig. 8 Forest plot for association between *EPHX1* rs2234922 polymorphism and CBZ resistance in additive model, heterozygous model, recessive model, dominant model and co-dominant model

($P = 0.03$, $I^2_{Het} = 0\%$) (Fig. 6a), dominant mode ($P = 0.002$, $I^2_{Het} = 0\%$) (Fig. 6b) and co-dominant model ($P = 0.04$, $I^2_{Het} = 0\%$) (Fig. 6c).

Associations between *EPHX1* rs1051740 and rs2234922 polymorphisms and CBZ resistance

There were 4 studies that evaluated the roles of *EPHX1* rs1051740 and rs2234922 polymorphisms in CBZ resistance, respectively. For rs1051740, the overall OR with its 95% CI did not show any associations between this polymorphism and CBZ resistance in all models, and no statistically significant heterogeneity was observed (Fig. 7). With regard to the rs2234922, we also did not find any associations in all genetic models (Fig. 8).

Discussion

Epilepsy is the most common chronic neurological disease characterized by recurrent seizures [32]. The therapeutic goal is to maximize seizure control while minimizing adverse drug effects, thus improving quality of life [33]. CBZ is one of the most commonly used anticonvulsants in the treatment of epilepsy [3]. However, its slow absorption and wide inter-individual variability in oral administration may lead to variation in its therapeutic efficacy [34, 35]. Genetic variants are known to influence the individual daily maintenance doses for epilepsy in addition to influencing a patient's response to therapy [10, 36]. Therefore, elucidating the role of genetic polymorphisms on pharmacokinetics and pharmacodynamics of CBZ would contribute to effective individualized therapy of epilepsy patients in clinics.

To our knowledge, this study for the first time reviewed the existing literatures and explored the associations between *EPHX1* polymorphisms and CBZ metabolisms by meta-analysis. Our results provided comprehensive evidence to suggest that the rs1051740 and rs2234922 polymorphisms were associated with metabolisms of CBZ, which reflected the potential effect of *EPHX1* gene on clinical treatment of epilepsy. Furthermore, the outcomes of included studies were reported completely and the quality score represented a reliable quality of evidence, indicating relatively fine completeness of proof in our present meta-analysis.

The rs1051740 within exon 3 of *EPHX1* is characterized by substitution of histidine for tyrosine at the amino acid position 113 (site T337C, amino acid change Tyr113His) [17]. In this meta-analysis, the rs1051740 polymorphism was found to be associated with decreased CDR_{CBZ}, indicating that this SNP had certain effect on the serum CBZ concentration in epilepsy patients. Furthermore, we found that patients with the rs1051740 CC genotype exhibited higher CDR_{CBZE} compared with CT + TT genotype. These might be owing to the possibility that the CC genotype impedes mEH enzymatic activity according to previous reports [37, 38]. Overall, our results suggested that the rs1051740 polymorphism may affect CBZ plasma levels and therapeutic efficacy of CBZ although further original studies based on high-quality are still needed.

With regard to rs2234922, it is located in the exon 4 of *EPHX1* and characterized by substitution of arginine for histamine at the position 139 (A416G, His139Arg) [17]. It was previously indicated that this kind of amino acid substitution affected the affinity of mEH to its substrate and resulted in enzyme activity change [18]. Our present results observed that this SNP decreased CDR_{CBZD} in the recessive model and reduced CBZD:CBZE ratio in additive and recessive models, which further confirmed that rs2234922 decreased enzymatic activity of mEH and were consistent with previous fundamental research findings [13]. However, it should be noted that this SNP was also reported to increase mEH activity according to earlier in vitro studies [18, 20]. This differential effect of rs2234922 on mEH activity may be ascribed to the facts either that the protein stabilities of Arg substitution in the transfected cells were different from those in human tissues [13, 39] or that this SNP influenced enzyme activity in a substrate-specific way [15]. In this regard, additional studies are warranted to clarify these issues and validate the present meta-analysis results.

As for the data instability of CDR_{CBZE} (for rs1051740) and CBZD:CBZE ratio (for rs2234922) according to the leave-one-out sensitivity analysis, the reason might be that the total sample sizes of the 2 outcomes were relatively limited, which led to downgrading the quality of the evidence. Therefore, these results should be interpreted cautiously and well-designed studies are necessary.

It was noteworthy that, on one hand, one previous study demonstrated that rs1051740 CC and CT genotypes were significantly associated with CBZ resistance in Thai patients with epilepsy [40]. Regretfully, this article could not be included in the present work because of data insufficiency. On the other hand, no associations were found based on the present 4 included studies conducted in Chinese, Kosovar, and Tunisian epilepsy patients, respectively [23, 25, 26, 30], which was also inconsistent with the results of CBZ metabolism. This might be due to the different geographical distributions of subjects, age or environmental factors [4, 41]. However, the included studies did not provide any information regarding these confounding factors for further evaluation. In addition, the number of studies that evaluated the roles of EPHX1 rs1051740 and rs2234922 polymorphisms in CBZ resistance is also limited, which might mask their actual connection as a result. Consequently, further investigations based on more well-designed experiments

and larger sample size should be performed to verify the effects of *EPHX1* gene on CBZ resistance.

Some limitations of our meta-analysis should be addressed. Firstly, the relatively small sample size may cause false positives and reduce the statistical power of our results. Secondly, because only hospitalized patients were focused on in the present study, selection bias might influence the result reliability. Thirdly, lack of certain significant original data led to restricting further evaluation of other indicators such as concentration–time curve, metabolic clearance rate, therapeutic effect, and adverse events. Furthermore, CBZ optimal dose might be influenced by several confounding factors such as age, sex, ethnicity and the interaction between the SNPs. But original data deficiency limited our further assessment of these factors. Fourthly, a substantial degree of heterogeneity was found in results of CBZ and CBZE, which limited the generalizability of these results. Finally, the included studies used the flexible dosing designs and diverse duration of therapy, which may influence observable effects of pharmacokinetic genes. These limitations should be considered and overcome in future clinical studies.

Conclusion

In conclusion, our results showed that *EPHX1* rs1051740 was associated with altered CBZ and CBZE concentration and that the rs2234922 polymorphism was related to decrease CBZD concentration, which confirmed important effects of *EPHX1* gene on pharmacokinetics and pharmacodynamics of CBZ and would help improve individualized therapy of epilepsy patients in clinics.

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Conflicts of interest The authors declare that they have no conflict of interest.

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