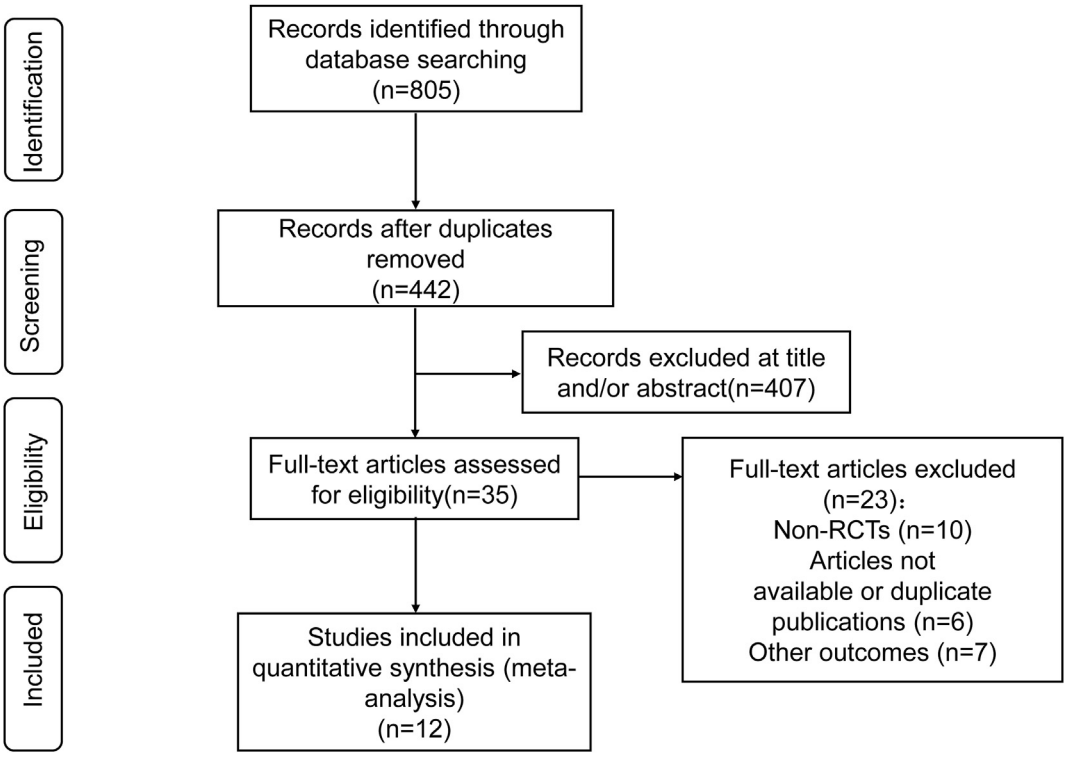

Figure 1 Flow chart of the identification of eligible studies.

in the roxadustat group, with a WMD of −52.93 (95% CI: −66.87 to −38.99, $P < 0.00001$), compared with those in the placebo group; while in the DD patients, there was no significant difference in the effect on ferritin level between the roxadustat group and the ESA group (WMD = −17.10, 95% CI: −46.71 to 12.52, $P = 0.26$; Fig. 4b).

Compared with the control group, roxadustat significantly increased the transferrin levels, with a WMD of 0.55 (95% CI: 0.41 to 0.69, $P < 0.00001$; Fig. 4c). A subgroup analysis showed that the transferrin levels were significantly different between the NDD and DD patients ($P = 0.001$; Fig. 4d). In the NDD patients, the results showed an increase in the transferrin levels, with a WMD of 0.69 (95% CI: 0.55 to 0.84, $P < 0.00001$), in the roxadustat group compared to the placebo group. Meanwhile, in the DD patients, the transferrin levels increased, with a WMD of 0.40 (95% CI: 0.30 to 0.50, $P < 0.00001$), in the roxadustat group compared to the ESA group.

Changes in hepcidin between groups

In the included studies, hepcidin levels were reported in 2227 subjects from the roxadustat group and 1608 subjects from the control group. The random effects model was used for meta-analysis and indicated that the reduction effect on hepcidin levels was more significant in the roxadustat group than in the control group, and the difference was statistically significant (WMD = −24.04, 95% CI: −36.28 to −11.79, $P = 0.0001$; Fig. 5a). As the data ($P < 0.00001$, $I^2 = 90\%$) indicated an obvious heterogeneity, we performed a subgroup analysis. A subgroup analysis showed that the hepcidin levels were not significantly different between the NDD and DD patients ($P = 0.13$; Fig. 5b).

Changes in Hb between groups

Among 2724 patients treated with roxadustat and 2065 patients treated with placebo or ESA, a great heterogeneity was found in the Hb levels ($P < 0.00001$, $I^2 = 99\%$; Fig. 5c). The random effects model was used for meta-analysis and indicated that roxadustat significantly increases Hb levels (WMD = 0.77, 95% CI: 0.42 to 1.12, $P < 0.0001$; Fig. 5c). A subgroup analysis showed that the Hb levels were significantly different between the NDD and DD patients ($P < 0.00001$; Fig. 5d). In the NDD patients, the results showed an increase in the Hb levels, with a WMD of 1.82 (95% CI: 1.52 to 2.12, $P < 0.00001$), in the roxadustat group compared to the placebo group. Meanwhile, in the DD patients, the results also showed an increase in the Hb levels, with a WMD of 0.25 (95% CI: 0.08 to 0.42, $P = 0.005$), in the roxadustat group compared to the ESA group.

Changes in serum iron between groups

The SMD was used when iron units were inconsistent among the studies. In the included literature, 2668 patients were in the roxadustat group and 2036 patients were in the control group. Due to the low heterogeneity among included studies ($P = 0.13$, $I^2 = 32\%$), the fixed effects model was used for meta-analysis and indicated that the increased effect of the serum iron levels in the roxadustat group was more significant than that in the control group (SMD = 0.21, 95% CI: 0.15 to 0.27, $P < 0.00001$; Fig. 6a).

Table 1 Characteristics of the included studies.

Author	Location	year	No. of patients (n)	Age (year)	Usage of iron	Baseline Hb	Roxadustat dose (g/day)	Study duration (week)	Patients type	control
Chen, N.[25]	China	2017	178	50	Oral only	NDD study: Hb < 10.0 g/dL DD study: Hb:9.0—12.0 g/dL	low (1.1—1.75 mg/kg) or high (1.50—2.25 mg/kg) and low (1.1—1.8 mg/kg), medium (1.5—2.3 mg/kg) and high (1.7—2.3 mg/kg)	8 and 6	NDD-CKD, DD-CKD	Placebo and Epoetin Alfa
Chen, N.[26]	China	2019	152	54	Oral iron therapy was allowed; intravenous iron therapy was allowed during rescue therapy	Hb:7.0—10.0 g/dL	70 mg (in patients weighing 40 to <60 kg) or 100 mg (in patients weighing ≥60 kg)	8	NDD-CKD	Placebo
Chen, N.[27]	China	2019	304	49	Oral iron therapy was allowed; intravenous iron therapy was allowed during rescue therapy	Hb:9.0—12.0 g/dL	100 mg (in patients weighing 45 to <60 kg) or 120 mg (in patients weighing ≥60 kg)	26	DD-CKD	Epoetin Alfa
Akizawa, T.[28]	Japan	2019	107	63	Oral was allowed and intravenous iron was used if TSAT <5% and ferritin <30 ng/mL	Hb < 10.0 g/dL	50, 70, or 100 mg	24	NDD-CKD	Placebo
Provenzano, R.[29]	Canada	2016	144	57	Oral iron supplementation was permitted but not required	Hb:9.0—13.5 g/dL	1.0, 1.5, 1.8, or 2.0 mg/kg thrice weekly	6 and 19	DD-CKD	Epoetin Alfa
Besarab, A.[30]	USA	2015	117	66	Oral only	Hb < 11.0 g/dL	0.7, 1, 1.5 or 2.0 mg/kg,	4	NDD-CKD	Placebo
Charytan, C.[31]	USA	2021	741	58	Oral iron therapy was allowed; intravenous iron therapy was permitted if the patient did not respond adequately to oral iron, could not tolerate oral iron, and was considered iron deficient	Hb:9.0—12.0 g/dL	70, 100, 150, or 200 mg TIW based on the patient's prescribed prestudy ESA dose	52	DD-CKD	Epoetin alfa

Csiky, B.[32]	Europe	2021	836	61	Intravenous iron	Hb:9.5–12.0 g/dL	according to the dose of ESA before enrollment	104	DD-CKD	ESA (epoetin alfa or darbepoetin alfa)
Hou, YP.[33]	China	2021	129	48	Oral iron therapy was allowed; intravenous iron therapy was prohibited except as rescue therapy	Hb < 12.0 g/dL	100 mg (in patients weighing 45 to <60 kg) or 120 mg (in patients weighing ≥60 kg)	24	DD-CKD	ESA
Coyne, DW.[34]	USA	2021	922	65	Oral iron therapy was encouraged; intravenous iron therapy was allowed during rescue therapy	Hb < 10.0 g/dL	70 mg (in patients weighing 45 to <70 kg) or 100 mg (in patients weighing ≥70 kg) thrice weekly	52	NDD-CKD	Placebo
Provenzano, R.[35]	USA	2021	1043	54	Oral iron therapy was encouraged; intravenous iron was allowed if the patient's Hb had not responded adequately, and the patient was considered iron deficient (ferritin <100 ng/mL and TSAT <20%)	Hb < 10.0 g/dL	70 mg (patients weighing ≤70 kg) or 100 mg (patients weighing >70 –160 kg)	52	DD-CKD	Epoetin alfa
Akizawa, T.[22]	Japan	2020	303	65	Oral was allowed and intravenous iron was used when TSAT was <20% or serum ferritin was <100 ng/mL	Hb:10.0–12.0 g/dL	70 mg or 100 mg	24	DD-CKD	Darbepoetin Alfa

Abbreviations: CKD, Chronic Kidney Disease; DD, Dialysis-Dependent; ESA, Erythropoiesis-stimulating Agent; Hb, Hemoglobin; NDD, Non-Dialysis-Dependent; TSAT, Transferrin Saturation.

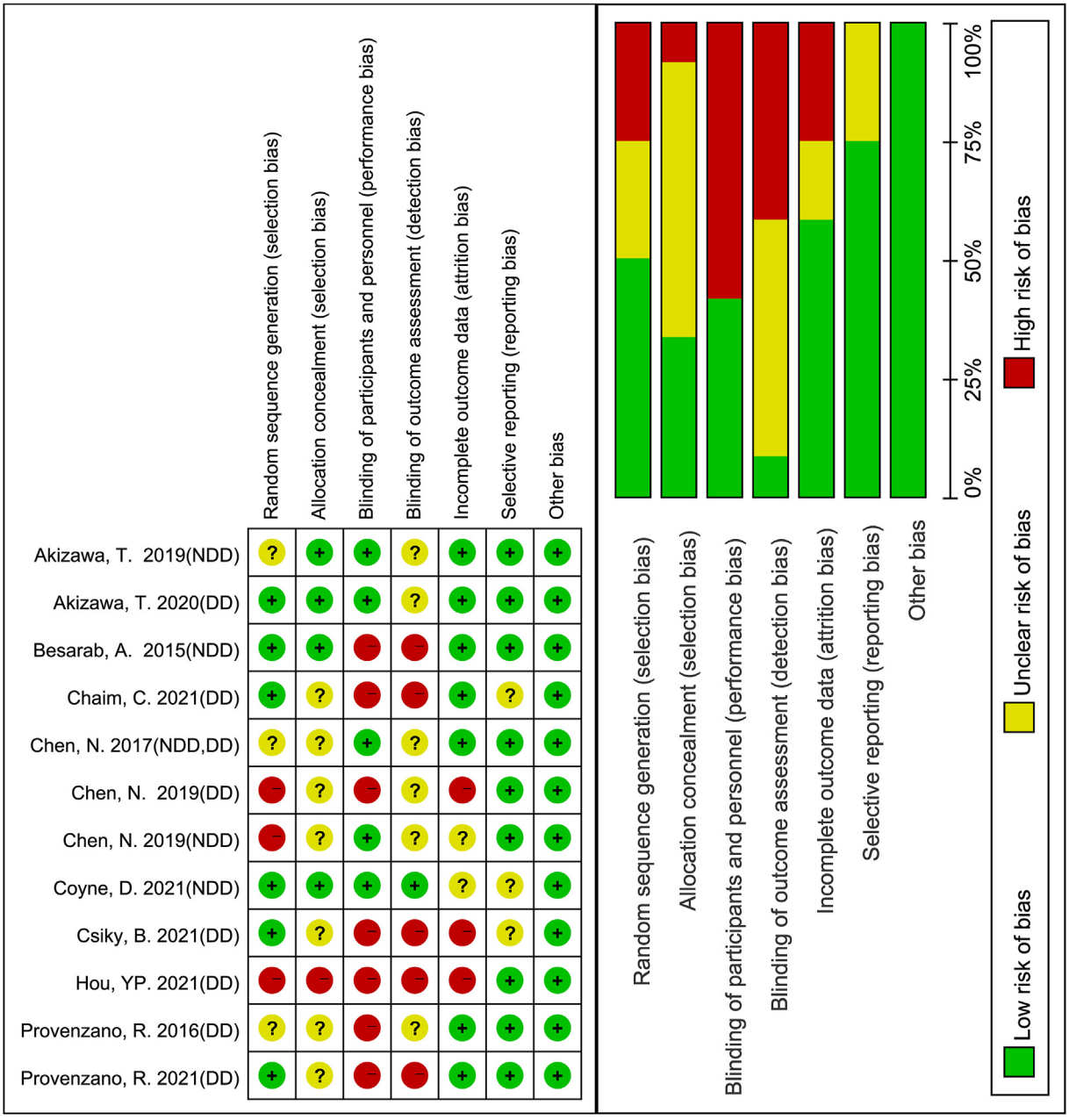

Figure 2 Cochrane risk of bias assessment.

Roxadustat safety

Next, we analyzed the extracted AEs and SAEs data to assess the safety of oral roxadustat. Among 2347 patients treated with roxadustat and 1585 patients treated with placebo or ESA, the incidence of AEs in the roxadustat group was significantly higher than that in the control group (RR = 1.03, 95% CI: 1.00 to 1.07, I^2 = 10%, P = 0.04; Fig. 6b). Among 2869 patients treated with roxadustat and 2102 patients treated with placebo or ESA, the incidence of SAEs in the roxadustat group was significantly higher than that in the control group (RR = 1.08, 95% CI: 1.00 to 1.15, I^2 = 0%, P = 0.04; Fig. 6c).

Discussion

To our knowledge, this study is a meta-analysis that specifically evaluated the efficacy of roxadustat on improving iron metabolism in NDD and DD patients with CKD. Twelve studies with 4976 patients met our inclusion criteria. Most selected studies were well-designed, but important information was missing from some reports, especially the generation of random sequences and allocation concealment, which might have led to bias.

Previous meta-analyses have shown that roxadustat can effectively improve renal anemia; however, few specific analysis of iron metabolism has been reported.[11,12] In this

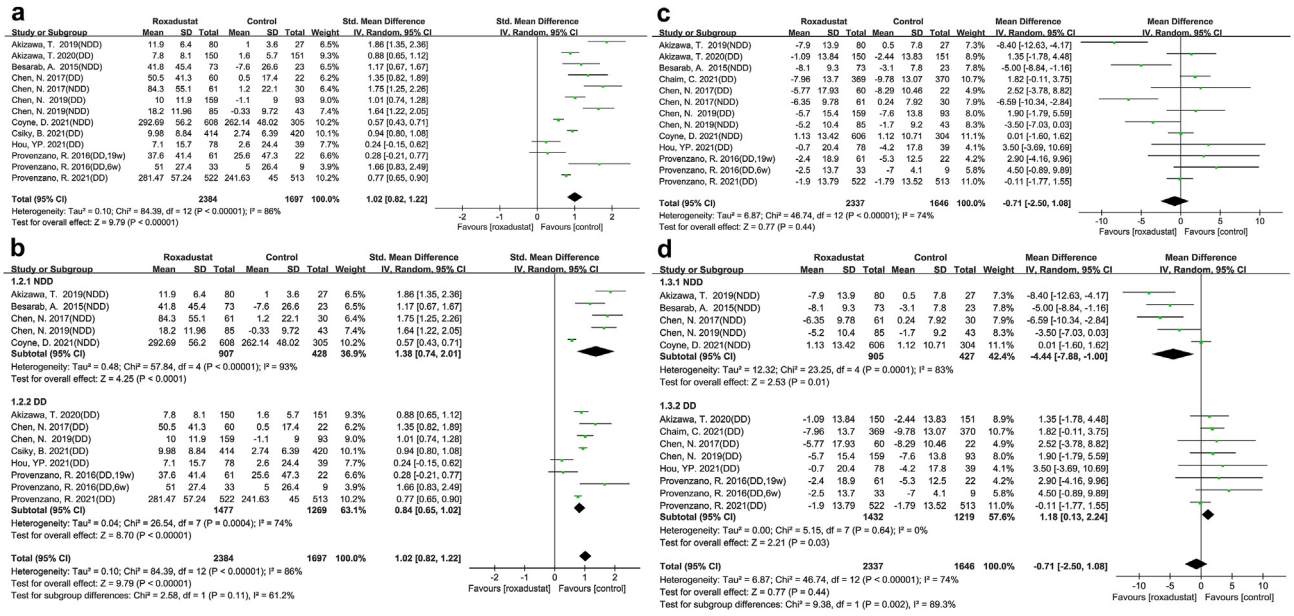

Figure 3 Forest plots for comparisons of total iron binding capacity (a) and transferrin saturation (c). Subgroup analysis of effect of roxadustat on outcome based on non-dialysis-dependent and dialysis-dependent patients: total iron binding capacity (b); transferrin saturation (d).

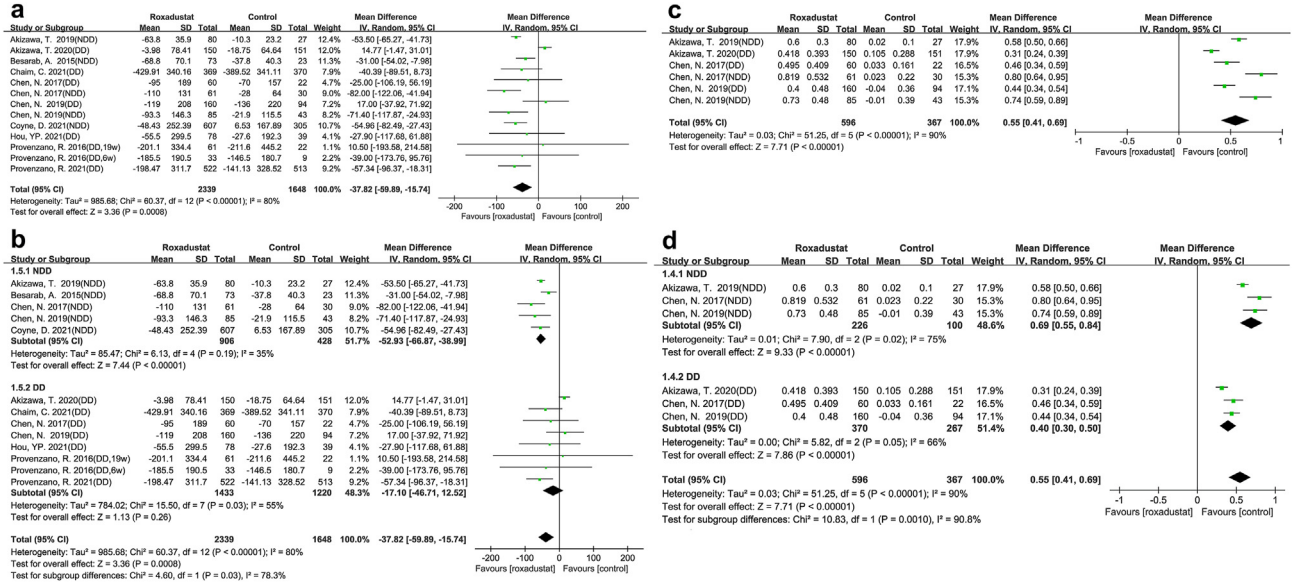

Figure 4 Forest plots for comparisons of ferritin (a) and transferrin (c). Subgroup analysis of effect of roxadustat on outcome based on non-dialysis-dependent and dialysis-dependent patients: ferritin (b); transferrin (d).

study, apart from Csiky B's study, other trials limited intravenous iron but allowed oral iron supplementation. Under these conditions, our meta-analysis showed that roxadustat increased the serum iron, increased TIBC and transferrin, and decreased ferritin and hepcidin. These findings showed that roxadustat could effectively increase the utilization of iron. In particular, in patients with functional iron deficiency, roxadustat can improve the absorption and utilization of iron and prevent the harm caused by iron overload.[13] Roxadustat is an orally bioavailable HIF–PHI that was the first to enter a phase III clinical trial.[14] In this review, research of DD and NDD patients in different disease states may have been a source of bias. To reduce this risk, we performed a subgroup analysis of patients in both DD and NDD groups, which showed that TSAT was significantly reduced in the roxadustat group for NDD patients ($P = 0.01$) and TSAT was significantly reduced in the ESA group for DD patients ($P = 0.03$). Similarly, roxadustat significantly reduced ferritin levels compared with placebo in the NDD group ($P < 0.00001$) and there no significant difference between roxadustat and ESA in the DD group ($P = 0.26$), indicating

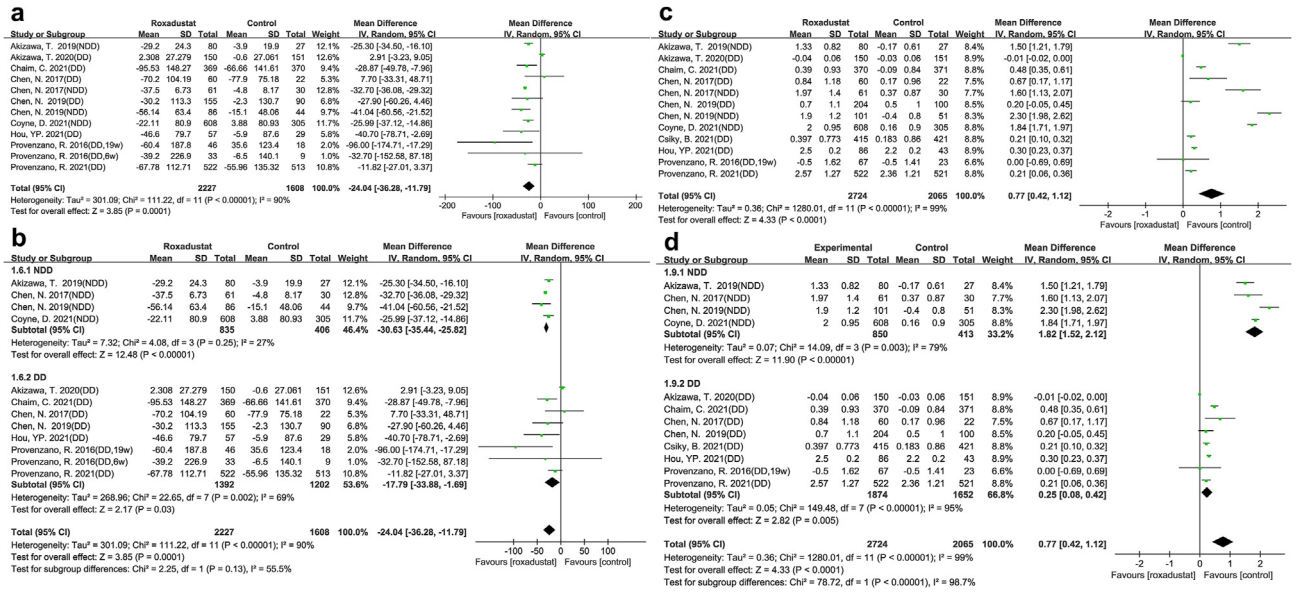

Figure 5 Forest plots for comparisons of hepcidin (a) and hemoglobin (c). Subgroup analysis of effect of roxadustat on outcome based on non-dialysis-dependent and dialysis-dependent patients: hepcidin (b); hemoglobin (d).

that the source of the heterogeneity of the data between the two groups was dialysis or non-dialysis. Hepcidin is a key factor in iron metabolism, which is affected by iron status, inflammation, and the oxygen concentration and can predict disease progression and mortality in patients with CKD.[15] Our meta-analysis showed a decrease in the hepcidin level, which indicated that roxadustat enhanced iron uptake and mobilization by reducing hepcidin levels. Consistently, a study has shown that reducing hepcidin concentrations can improve iron homeostasis and anemia in renal animal models.[16]

The HIF oxygen-sensing pathway is considered an important pathway for iron homeostasis.[17] HIF binds directly to hypoxia response elements in DNA[18] and regulates the expression of iron-related proteins. HIF-1 induces the expression of transferrin, transferrin receptor 1, and ceruloplasmin to improve iron utilization, while HIF-2 regulates divalent metal-regulatory protein 1 and duodenal cytochrome *b* and reduces the trivalent iron ion to the ferrous ion for absorption by intestinal cells.[19] A recent study on HIF-2α knockout mice also showed that the HIF played a critical role in iron absorption and utilization.[20] Compared with the DD patients, the NDD patients with CKD showed a greater reduction in hepcidin levels and a greater increase in transferrin, suggesting a better iron metabolism in the latter patients, which may be related to their residual renal function.

Long-term use of HIF-PHIs may have AEs due to persistent activation of HIF-regulated genes. In particular, the activation of vascular endothelial growth factor is involved in various biological processes, such as cell differentiation, mitochondrial metabolism, and tumor growth.[21] However, in a phase III study in Japan,[22] the incidences of new or worsening retinal hemorrhages were similar between the darbepoetin alfa and roxadustat groups, and no clinically significant changes were found in the retinal thickness. AEs and SAEs are considered major challenges in clinical application of roxadustat. A recent study of pooled RCTs in NDD patients, with a noninferior study design, reported roxadustat is noninferior to placebo with respect to MACE.[23] And in DD patients, roxadustat was non-inferior to ESA for risks for MACE and MACE+.[24] However, FDA Roxadustat Briefing Document of Roxadustat (NDA 213805) released on July 15, 2021 reported that roxadustat may increase the risk of thrombosis and even mortality. In our included studies, the incidences of AEs and SAEs were significantly increased in roxadustat group, which indicates that the roxadustat has higher risks than ESA. Therefore, larger and longer follow-up studies are needed to discover and confirm its safety in clinical use.

To ensure the quality of the study, we only included RCTs. Nevertheless, our research has some limitations. First, the limited number of studies may affect the strength of meta-analysis. Second, due to the relatively short duration of selected studies, the long-term efficacy of roxadustat cannot be predicted, and the long-term incidence of AEs and SAEs cannot be obtained. Third, although we did a subgroup analysis, several results had high heterogeneity. This may be related to the baseline patient characteristics, the differences in roxadustat and iron dosages, the differences of baseline iron parameters and treatment durations in the included studies.

In this study, we investigated the therapeutic effectiveness of roxadustat in the treatment of iron metabolism disorders in NDD and DD patients with CKD using a meta-analysis. In general, roxadustat could increase iron bioavailability via enhanced intestinal iron absorption and release of iron from functional storages by improving TIBC and transferrin, and reducing hepcidin levels. From a clinical perspective, roxadustat is especially useful for patients with renal anemia with functional iron deficiency.

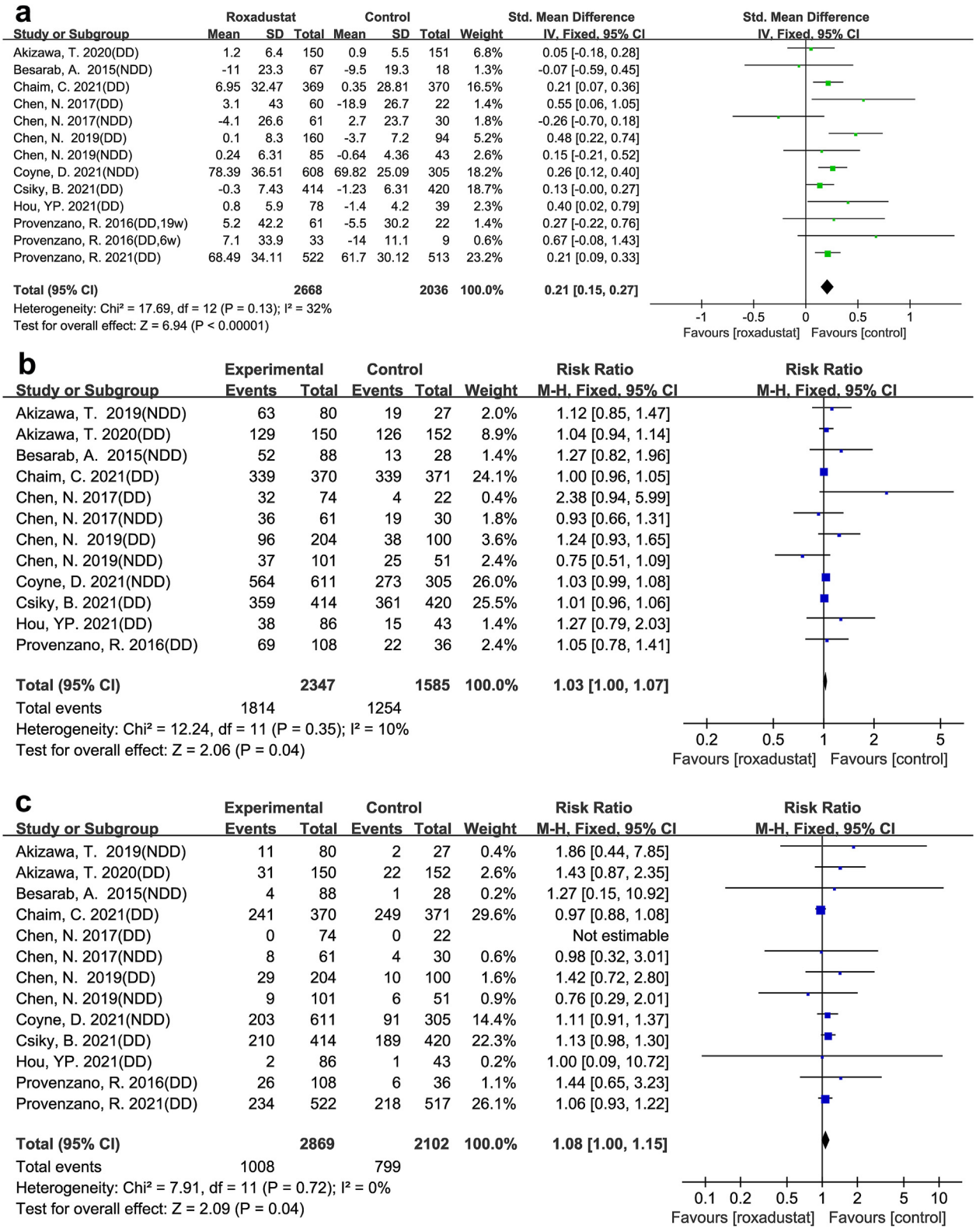

Figure 6 Forest plots for comparisons of iron (a). AEs (b) and SAEs (c) in a treatment group compared to control group. Abbreviations: AE: Adverse Event; SAE: Severe Adverse Event.