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Research article

The association between the C282Y and H63D polymorphisms of HFE gene and the risk of Parkinson's disease: A meta-analysis

Jianjian Xia, Huamin Xu, Hong Jiang, Junxia Xie*

Department of Physiology, Shandong Provincial Key Laboratory of Pathogenesis and Prevention of Neurological Disorders and State Key Disciplines: Physiology, Medical College of Qingdao University, Qingdao 266071, China

HIGHLIGHTS

- We performed a meta-analysis to assess the C282Y and H63D polymorphisms of HFE in PD.
- The C282Y polymorphism in HFE could be a potential protective factor for PD.
- No significant associations were found for any genetic model for the H63D mutation.

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ABSTRACT

Impaired brain iron homeostasis has been considered as an important mechanism in Parkinson's diseases (PD). There are indications that C282Y and H63D polymorphisms of HFE genes involved in iron metabolism might contribute to the pathogenesis of PD in some cases. However, the investigation of the relationship between PD and the two polymorphisms had produced contradictory results. We performed a meta-analysis to assess the C282Y and H63D polymorphisms of HFE in PD susceptibility. PubMed, EMBASE and Web of Science were systematically searched to identify relevant researches. The strict selection criteria and exclusion standard were applied. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of associations. A fixed-effect or random-effect model was selected, depending on the results of the heterogeneity test. Fifteen studies were included in the meta-analysis (eight studies with 1631 cases and 4548 controls for C282Y; seven studies with 1192 cases and 4065 controls for H63D). For the C282Y polymorphism, significant associations were observed in the Recessive model (YY vs CY + CC: OR = 0.22, 95% CI = 0.09–0.57, $P = 0.002$). This indicated that the C282Y polymorphism in HFE might be a potential protective factor for PD. However, no significant associations were found for any genetic model for the H63D polymorphism, suggesting that the H63D polymorphism might not be associated with PD.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease, characterized by the loss of pigmented dopaminergic neurons in the substantia nigra pars compacta (SNc). Although the exact mechanisms underlying the etiology of PD are not clear, increasing evidence has shown that nigral iron accumulation contributed to the neurodegeneration of dopamine neurons in PD [1–5]. Iron-induced oxidative stress via Fenton reaction is believed to be the main putative mechanism underlying iron-induced neurodegeneration of dopamine neurons.

Due to the important role of iron accumulation in PD, elevation of total body iron stores or iron overload might be one of the risks of PD. Our previous studies have shown that misregulation of iron transporters including divalent metal transporter 1 and ferroportin 1 were involved in the nigral iron accumulation [6–8]. Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism, leading to increased iron absorption and excessive iron accumulation [9]. It is reported that HH is most often caused by mutations of HFE gene on chromosome 6p21.3. Two mutations of this gene (C282Y and H63D) have been described to contribute to iron overload. Evidence has showed that these two polymorphisms in the HFE gene could trigger serum iron overload. In addition, HFE protein has been reported to be involved in the regulation

* Corresponding author at: Department of Physiology, Shandong Provincial Key Laboratory of Pathogenesis and Prevention of Neurological Disorders and State Key Disciplines: Physiology, Medical College of Qingdao University, No. 308 Ningxia Road, Qingdao 266071, China. Tel.: +86 532 83780191; fax: +86 532 83780136.
E-mail address: jxiaxie@public.qd.sd.cn (J. Xie).

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of iron homeostasis by binding to the transferrin receptor (TfR) and decreasing the transport of iron [10]. Studies have also demonstrated that an interaction between HFE and TfR was lost when HFE is mutated [10], suggesting that HFE might play a key role in regulating iron transportation to cells. This leads to the hypothesis that HFE gene mutations might be a possible mechanism contributing to iron overload and the pathogenesis of PD. However, studies on correlation between the HFE mutations and risk of PD have produced conflicting results [11–16]. Some reports indicated that there was a positive association between HFE mutations and risk of PD, and others showed no association was observed. Thus, in the present study, we performed a meta-analysis to clarify the association between HFE gene polymorphisms and risk of PD.

2. Methods and materials

2.1. Data collection

Two investigators independently reported studies on the associations between HFE polymorphisms and PD. The PubMed, EMBASE and Web of Science, from their inception to November 25, 2014, were searched to identify potentially relevant researches. The following search strategy was used, which combined both the medical subject heading (MeSH) and keywords (("HFE" or "C282Y" or "H63D" or "Cys282Tyr" or "His63Asp" or "rs1800562" or "rs1799945") and ("Parkinson disease" or "Parkinsonism" or "Parkinson's disease" or "Parkinson" or "PD") and ("SNP" or "SNPs" or "single nucleotide polymorphism" or "polymorphism" or "genetic polymorphism" or "mutation" or "variant" or "variation")). When necessary, the authors of articles were contacted to obtain missing data. The search was done without restriction on language, but only published articles in English were included in the last.

2.2. Selection criteria

Two investigators independently identified potentially relevant studies and evaluated each trial according to predefined eligibility criteria. Studies were included if they matched the following criteria: (1) association study, using a cohort-design or case-control; (2) available data for C282Y or H63D mutations with risk of PD; and (3) the genotypes distribution in the control population were in Hardy–Weinberg equilibrium (HWE). The exclusion criteria were (1) reviews and animal studies; (2) lack of data on genotype number or frequency; and (3) genotype distribution in the control groups was not consistent with HWE. If the same author published more than one study, only the most recent or complete report was included in the meta-analysis.

2.3. Data extraction

After removing duplicate studies and adding any additional studies, two investigators extracted data independently in a standard time and entered the information into a common database. When discrepancies arose, all investigators assessed the data. The following information was collected: first author, year of publication, country, ethnicity, characteristics, study design, sample sizes of patients and controls, genotype numbers, and *P* value for HWE. Therefore, we combined the data and analyzed the pooled controls from those series as one group and compared with another one from the cases.

2.4. Statistical analysis

We performed our meta-analysis based on the PRISMA checklists and followed the guideline [17]. Hardy–Weinberg equilibrium (HWE) was evaluated for each study by chi-square test in control groups, and $P<0.05$ was considered a significant departure from HWE. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the strength of the association between C282Y/H63D SNPs and susceptibility to PD. Pooled ORs were per-

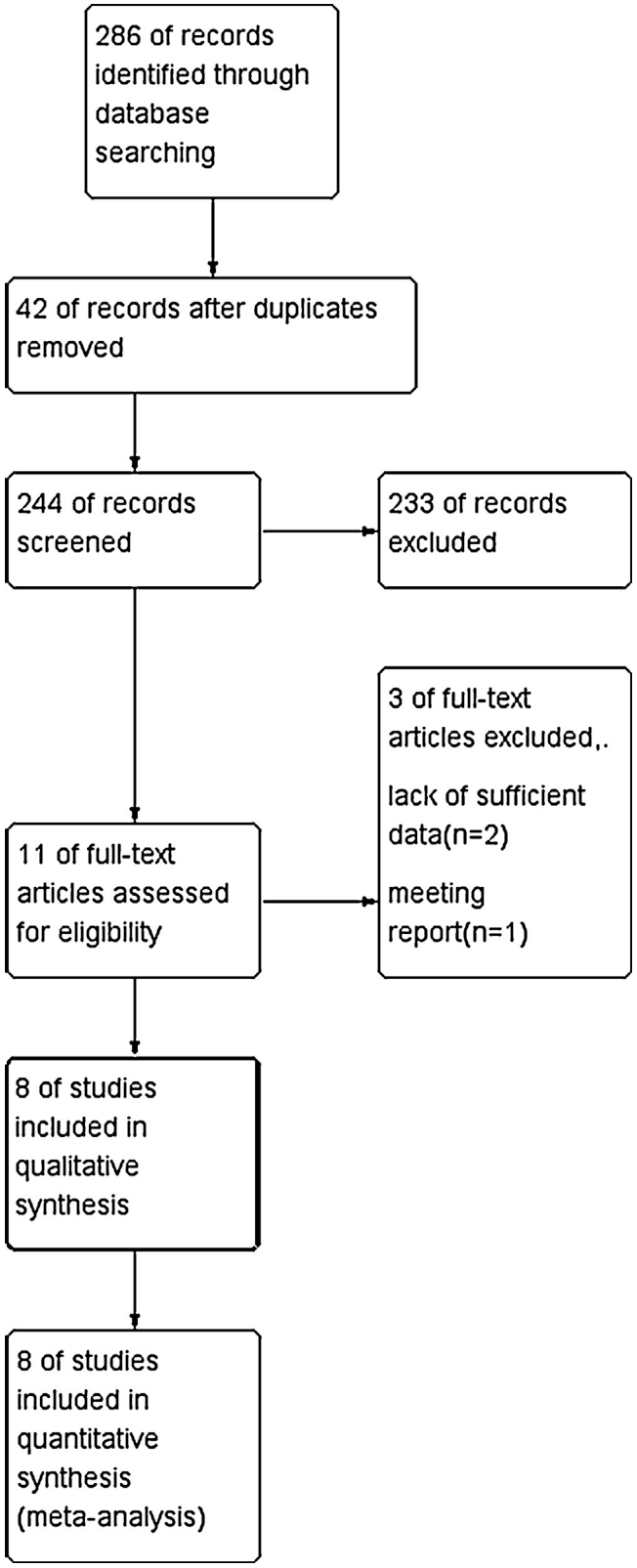

Fig. 1. Flow chart of study selection.

formed for allelic comparison (C282Y: Y versus C, H63D: D versus H), heterozygote model (C282Y: CY versus YY, H63D: HD versus DD), homozygote model (C282Y: YY versus CC, H63D: DD versus HH), dominant model (C282Y: YY + CY versus CC, H63D: DD + HD versus HH), recessive model (C282Y: YY versus CY + YY, H63D: DD versus HD + HH), respectively. The statistical significance was determined by *Z*-test with *P*-value < 0.05. Heterogeneity was evaluated by *Q* statistic (significance level of $P < 0.1$) and I^2 statistic (greater than 50% as evidence of significant inconsistency) [18]. Either fixed-effect or random effect model was used to pool the effect sizes according to the heterogeneity [19]. The studies without deviation from HWE among controls were used to do a supplementary meta-analysis. Potential publication bias was checked by Begg's funnel plots. An asymmetric plot and the *P*-value of Begg's test less than 0.05 was considered a significant publication bias [20]. All statistical analyses were performed with Stata 12.0 software (StataCorp, USA).

3. Results

3.1. Eligible studies and meta-analysis databases

Fig. 1 described the flow chart of articles' selection and specific reasons for exclusion from the meta-analysis. Firstly, 286 records were identified through database searching and hand searching of the references available by November 2014; after excluding the overlapping studies in different databases, 244 potentially relevant articles were identified for further review; through screening of titles, abstracts or full text if essential, we identified 11 potentially relevant articles; finally, after excluding 2 articles [15,21] for insufficient and one meeting report [22], 8 articles (including 8 independent studies [11–14,16,23–25] about C282Y and 7 independent studies [11,13,14,16,23–25] about H63D) were identified to analyze the association between HFE polymorphisms and susceptibility to PD. The subjects were all Caucasians. The distribution of the HFE genotype frequencies among PD cases and controls are listed in Tables 1 and 2.

3.2. Association between C282Y polymorphism and PD susceptibility

The main results of this meta-analysis and the heterogeneity test were included in Table 1. For C282Y mutation, on the basis of 1631 cases and 4548 controls included in 8 studies, a significant depress risk of PD was observed in Recessive model (YY vs CY + CC: OR = 0.22, 95% CI = 0.09–0.57, $P = 0.002$). There are no significant association in other genetic model (Y vs C: OR = 0.99, 95% CI = 0.82–1.20, $P = 0.953$; CY vs YY: OR = 0.99, 95% CI = 0.81–1.20, $P = 0.905$; YY vs CC: OR = 1.08, 95% CI = 0.43–2.71, $P = 0.862$; YY + CY vs CC: OR = 0.99, 95% CI = 0.82–1.20, $P = 0.929$) Fig. 2.

3.3. Association between H63D polymorphism and PD susceptibility

The main results of this meta-analysis and the heterogeneity test were included in Table 2. For H63D mutation, on the basis of 1192 cases and 4065 controls included in 7 studies. Overall, significant association was not identified in any genetic model (D vs H: OR = 1.00, 95% CI = 0.86–1.15, $P = 0.949$; HD vs HH: OR = 0.99, 95% CI = 0.84–1.17, $P = 0.911$; DD vs HH: OR = 1.03, 95% CI = 0.61–1.74, $P = 0.908$; DD + HD vs HH: OR = 0.99, 95% CI = 0.84–1.17, $P = 0.925$; DD vs HD + HH: OR = 1.03, 95% CI = 0.61–1.73, $P = 0.916$).

3.4. Publication bias

No publication bias for the association between C282Y/H63D and PD susceptibility was identified by Begg's funnel plot (Fig. 3 in Recessive model of C282Y, YY vs CY + CC, $P = 0.308$). Symmetrical funnel plots were obtained in all the genetic models.

4. Discussion

HFE gene mutations have been reported to be involved in some neurodegenerative diseases, including PD, Alzheimer's disease and amyotrophic lateral sclerosis [26,27]. Many studies have explored the relationship between the HFE gene mutation and the development of PD across populations worldwide. But the results are

Table 1
Distribution of C282Y genotypes among PD cases and controls, and *P* values of HWE in controls.

First author	Total number		Cases/frequency			Controls/frequency			HWE(P)
	Cases	Controls	CC	CY	YY	CC	CY	YY	Controls
Borie [11]	71	57	66	5	0	52	5	0	0.729
Buchanan [12]	438	485	391	46	1	405	76	4	0.835
Dekker [13]	269	2914	228	38	3	2567	339	8	0.362
Guerreiro [14]	132	115	114	18	0	110	5	0	0.812
Aamodt [16]	388	505	330	56	2	428	75	2	0.501
Halling [23]	79	153	67	12	0	125	26	2	0.628
Greco [24]	181	180	178	3	0	177	3	0	0.910
Mariani [25]	73	139	70	3	0	137	2	0	0.932

HWE: Hardy–Weinberg equilibrium.

Table 2
Distribution of H63D genotypes among PD cases and controls, and *P* values of HWE in controls.

First author	Total number		Cases/frequency			Controls/frequency			HWE(*P*)
	Cases	Controls	HH	HD	DD	HH	HD	DD	Controls
Borie [11]	66	59	42	23	1	39	20	0	0.117
Dekker [13]	269	2914	203	64	2	2136	710	68	0.325
Guerreiro [14]	132	115	89	38	5	74	39	2	0.215
Aamodt [16]	388	505	315	66	7	396	102	7	0.882
Halling [23]	79	153	54	22	3	110	38	5	0.450
Greco [24]	181	180	125	49	7	141	36	3	0.691
Mariani [25]	77	139	53	24	0	99	38	2	0.438

HWE: Hardy–Weinberg equilibrium.

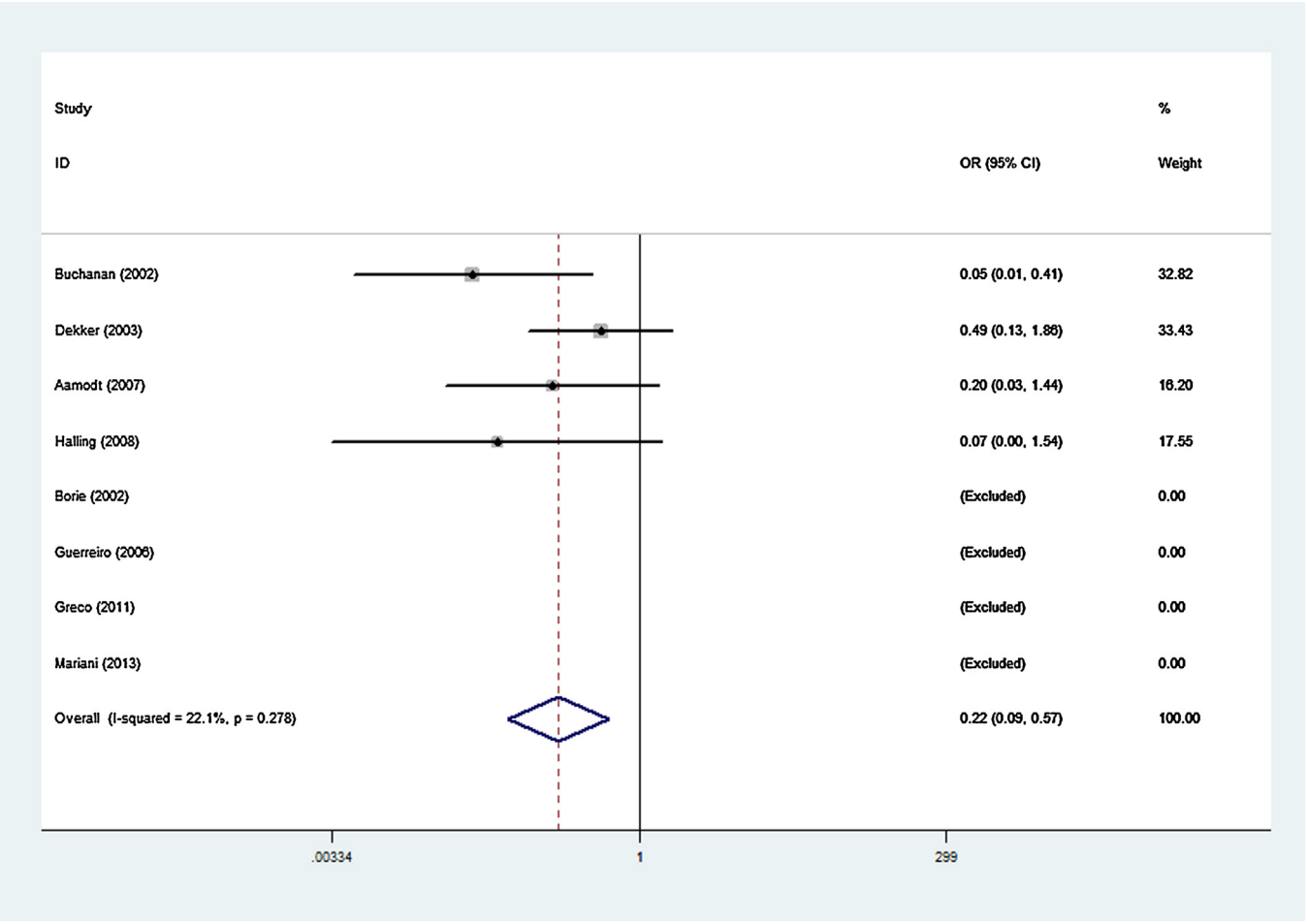

Fig. 2. Forest plot about the Recessive model of C282Y for overall comparison (YY vs CY + CC). OR: Odds ratios.

conflicting. Some results suggested that there were no significant association between PD and HFE common mutations including C282Y and H63D [11,16,23–25]. Some reports demonstrated that the presence of the C282Y variant allele in HFE gene might confer higher risk for developing PD [13,14]. However, other results showed that possession 282Tyr might provide protection against the development of PD [12,28]. In addition, most of these studies have had small sizes sample and inconclusive findings. To overcome these shortcomings, meta-analysis can be used.

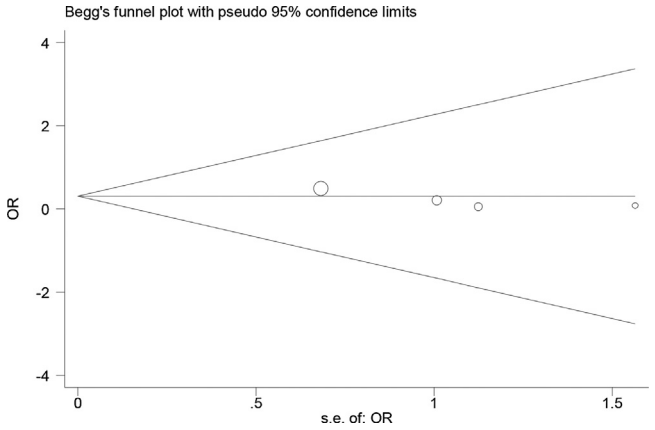

Fig. 3. Begg's funnel plot for publication bias analysis for C282Y polymorphisms (YY vs CY + CC).
OR: Odds ratios.

In the present meta-analysis, we used data from 8 observational studies. We found evidence for a significant association for the C282Y polymorphism in the Recessive model, but no evidence for a relationship of the H63D polymorphism with PD risk. The observed protective effect of the 282Tyr allele in PD is somewhat paradoxical, as both homozygosis and heterozygosis for the 282Tyr allele are associated with significantly higher serum iron [29]. However, somewhat paradoxical results might be explained. Although increased iron levels were found in the brains of patients with PD, epidemiological evidence on the possible effect of serum iron levels on PD risk is inconclusive. Recent results showed that increased serum iron levels are causally associated with a decreased risk of developing PD [29]. This is consistent with our results and provides evidence to further confirm the protective effect of the 282Tyr allele in PD in this study.

We did not observe heterogeneity among the studies, which led us to apply a fix-effects model. We performed a sensitivity analysis, removing each study one by one and rerunning the model to determine the effect on the overall estimate. The estimates changed very little when a study was removed. This finding suggests that the results were stable.

Compared with traditional research, our meta-analysis has some specific features. First and foremost, a larger sample size was used to estimate its effect in the meta-analysis. So our results are more reliable. Besides, to the best of our knowledge, no previous meta-analysis has explored the role of the C282Y/H63D polymorphism in the development and progression of PD. To achieve a more reliable and comprehensive conclusion on the roles of both

variants, we performed a meta-analysis to assess the association between C282Y/H63D polymorphisms and PD risk. Last but not least, we performed a sensitivity analysis to estimate the influence of each study on the overall estimate, the results of which suggested that the result was stable. In view of these findings, we are convinced that the results of our meta-analysis should be more reliable than the results of previous researches.

In our meta-analysis, all of the available results from studies were pooled, which significantly increased the statistical effect of the study. Nevertheless, to some extent, the results should be interpreted with caution, in light of some limitations. To begin with, PD is a multifactorial disease that involves complex interactions between environmental and genetic factors. We did not consider certain factors, such as living habits and employment, which could affect the significance of the independent role of HFE polymorphisms in PD development. Then, the number of studies of the HFE was relatively small. Investigations involving large study samples of different ethnicities are necessary for a more reliable evaluation of its associations. Furthermore, although we made every effort to find all appropriate publications, we may have missed some studies or retrieved some studies erroneously.

We believe that the positive loci identified in our meta-analysis represent particularly promising HFE mutations, in spite of these limitations, for PD candidate genes. In conclusion, our data support the hypothesis that a change in iron metabolism may confer susceptibility to PD. The results of this meta-analysis suggest that the C282Y polymorphism in HFE could be a potentially protective factor for PD. However, the H63D polymorphism does not appear to be associated with PD risk. To obtain a better understanding of the potential mechanism for PD in humans, large well-designed epidemiological studies on the susceptibility to PD are needed to confirm this association. It will also be necessary to consider genetic factors, ethnicity, gender, and environmental risk factors.

Competing interests

The authors declare no conflict of interest.

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