Gene 531 (2013) 301–305

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Methods paper

Three most common nonsynonymous UGT1A6*2 polymorphisms (Thr181Ala, Arg184Serand Ser7Ala) and therapeutic response to deferiprone in β-thalassemia major patients

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ARTICLE INFO

Article history:
Accepted 26 August 2013
Available online 11 September 2013

Keywords:
β-Thalassemia
Deferiprone
Pharmacogenetics
UGT1A6*2 gene polymorphism
Serum ferritin

ABSTRACT

Deferiprone is used as a chelation agent in chronic iron overload in β-thalassemia patients. Patients on deferiprone therapy show variable response to this drug in terms of reduction in iron overload as well as adverse drug reactions (ADRs). The pharmacogenetic studies on deferiprone have not carried out in patients with blood disorders in India. Therefore, the present study was carried out to evaluate the three most common nonsynonymous UGT1A6 polymorphisms Thr181Ala (541 A/G), Arg184Ser (552 A/C) and Ser7Ala (19 T/G) and therapeutic response to deferiprone in β-thalassemia major patients. Two hundred and eighty six (286) β-thalassemia major patients were involved in the study. Serum ferritin levels were estimated periodically to assess the status of the iron overload and the patients were grouped into responders and non-responders depending on the ferritin levels. The UGT1A6*2 polymorphisms were detected by PCR-RFLP methods. The association between the genotypes and outcome as well as ADRs was evaluated by Open EPI software. A significant difference was observed in the genotypic distribution of UGT1A6*2 Thr181Ala polymorphism in responders and non-responders. However, there was no difference in the genotypic distribution between patients with and without ADRs. As far as the UGT1A6*2 Arg184Ser polymorphism is concerned, no significant difference was observed between responders and non-responders. Further, evaluating the association of UGT1A6*2 Ser7Ala polymorphism with drug response, there was no significant difference in the genotypic distribution between responders and non-responders. However, there was a significant difference between responders with and without ADRs and non-responders with and without ADRs. In addition to this haplotype analysis was also carried out. However, we did not find any specific haplotype to be significantly associated with the deferiprone response in β-thalassemia major patients.

1. Introduction

β-Thalassemia is the most common monogenic disorder in India, where about 10,000 children are born annually inheriting a major hemoglobin disorder (Chattopadhayay, 2006). The only treatment to sustain life is regular transfusions which inevitably lead to iron overload as humans cannot actively remove excess iron. If not treated effectively, the iron overload causes significant morbidity and mortality (Brittenham et al., 1994; Gabutti and Piga, 1996; Giardina and Grady, 1995; Olivieri and Brittenham, 1997; Olivieri et al., 1994). Therefore, iron chelation is necessary to prevent the multiple organ dysfunction and/or failure and decrease the mortality. Oral iron chelators like deferoxamine, deferiprone and deferasirox have been introduced into clinical practice. Evidence supports their use in the treatment of chronic iron overload. Although effective, there are significant challenges associated with the use of these iron chelators that can result in non-compliance. Although these iron chelators are effective, differences are observed in the pharmacokinetics of these drugs as well as adverse effect profiles. There is variability in response to these drugs. Some individuals tolerate these drugs well but may not get the desired effect. Others may have side effects and adverse drug reactions (ADRs) that make these chelators intolerable, and yet another group may get the desired effect with no side effects.

The long-term safety profile of deferiprone has been well defined. However, the factors and the mechanism involved in its adverse effects such as gastrointestinal disturbances, arthralgia, neutropenia, and agranulocytosis, are unclear (Agarwal et al., 1990; Ceci et al., 2002; Cohen et al., 2003; Franchini and Veneri, 2004; Taher et al., 2001). Therefore, there is a need to decipher the underlying factors and the mechanism involved in these ADRs. Deferiprone has been shown to

Abbreviations: ADRs, adverse drug reactions; UGTs, UDP-glucuronosyltransferases; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; WBC, white blood cell; PCM, paracetamol.

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http://dx.doi.org/10.1016/j.gene.2013.08.078

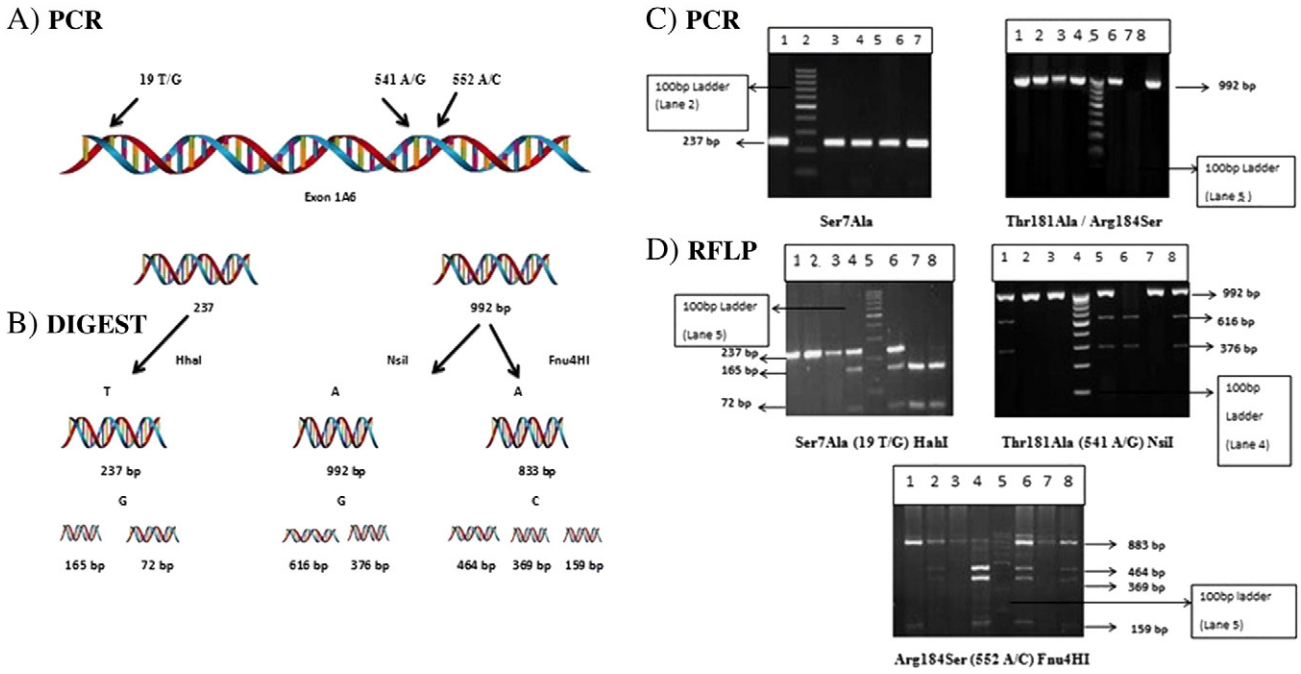

Fig. 1. UGT1A6*2 PCR-RFLP genotype assay. A) Separate amplification of segments of UGT1A6*2 with PCR. B) Resulting amplicons digested by HhaI, NsiI, Fnu4HI to detect the Ser7Ala, Thr181Ala and Arg184Ser polymorphisms respectively. C) PCR pictures. D) RFLP pictures.

have good bioavailability, but its clearance is accelerated by rapid biotransformation, approximately 85% of the drug is metabolized to a non-chelating 3-O-glucuronide conjugate by UDP-glucuronosyltransferases (UGTs) (Huang et al., 2006). Of all the known human UGTs, 12 have been evaluated previously for deferiprone glucuronidation by Haverfield et al (2005). This study revealed that only UGT1A6 was capable of glucuronidation of deferiprone. The impact of genetic variations of UGTs on drug response and ADR's has not been carried out in India. Therefore, the present study was carried out with an aim to evaluate the association of three most common UGT1A6*2 polymorphismsThr181Ala (541 A/G), Arg184Ser (552 A/C) and Ser7Ala (19 T/G) with drug response and ADRs in β-thalassemia major patients on deferiprone therapy.

2. Materials and methods

2.1. Study population

A total of 286 β-thalassemia major patients evaluated in Thalassemia and Sickle Cell Society, Hyderabad, were included in the study. The study was approved by the Institutional Ethical Committee and informed consent was obtained from all the subjects included in the study. Patients were confirmed to have β-thalassemia by HPLC technique (β-thalassemia Short Program of Variant Bio-Rad) and reverse dot blot (RDB) hybridization as reported previously (Munshi et al., 2009). After 15 transfusions all the patients were put on iron chelation therapy using deferiprone (Kelfer). Initially the dosage was 50 mg/kg/day and increased up to 75 mg/kg/day depending upon the serum ferritin levels of the patient. The information on clinical characteristics was collected by using a structured questionnaire.

2.2. Follow up

All the patients on deferiprone therapy were monitored every month for iron overload and adverse drug reactions. The patients were grouped into responders and non-responders depending upon serum ferritin levels (<2500 mg/mL for responders and >2500 mg/mL for non-responders) as well as the frequency of transfusions. The responders and non-responders were further grouped into patients with and without adverse drug reactions.

2.3. Molecular studies

Genomic DNA was extracted from the whole blood samples using the phenol–chloroform method. UGT1AThr181Ala, Arg184Ser and Ser7Ala polymorphisms were detected by PCR-RFLP technique. The primers used for amplifying Thr181Ala and Arg184Ser are: forward 5′-GGAAATACCTAGGAGCCCTGTGA-3′ and reverse 5′-AGGAGCCAAATG AGTGAGGGAG-3′. The 992 bp PCR product was digested with NsiI restriction enzyme for the Thr181Ala substitution and Fnu4HI restriction enzyme for the Arg184Ser substitution (Fermentas Fast Digest) by incubating at 37 °C for 5 minutes followed by separation of fragments on 3%

Table 1
Clinical characteristics of responders and non-responders.

Parameters	Responders	Non-responders	*p*-Value
Age (years)	6.68 ± 3.82	7.56 ± 4.41	0.22
Male:female	30:56	120:80	
Weight (kg)	17.38 ± 7.34	18.32 ± 7.47	0.46
Baseline Hb level (g/dL)	7.85 ± 1.26	7.77 ± 1.43	0.71
Hematocrit (%)	26.03 ± 3.78	24.25 ± 4.05	<0.001
WBC (C/cu)	8108.6 ± 180	10119.0 ± 175.6	0.0001
Platelet (L/cu)	3734.3 ± 120	2490.4 ± 88.5	0.0001
Serum ferritin (ng/dL)	1760.4 ± 640.0	5536.7 ± 237.03	.0001

Table 2
Clinical characteristics of responders with and without ADRs.

Parameters	Responders with ADR	Responders without ADR	*p*-Value
Age (years)	9.42 ± 3.52	5.5 ± 3.36	0.0002
Male:female	11:13	32:30	
Weight (kg)	22.5 ± 7.87	15.0 ± 5.79	0.0002
Baseline Hb level (g/dL)	7.65 ± 1.60	7.95 ± 1.08	0.40
Hematocrit (%)	27.16 ± 4.01	25.13 ± 5.01	0.0001
WBC (C/cu)	6805.2 ± 125.6	7877.5 ± 131.3	0.0001
Platelet (L/cu)	2118 ± 119	2081 ± 108.6	0.24
Serum Ferritin (ng/dL)	1734.3 ± 575.0	1771.0 ± 675.0	0.86