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Validation and evaluation of two porphobilinogen deaminase activity assays for diagnosis of acute intermittent porphyria

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ABSTRACT

Background: Acute intermittent porphyria (AIP) is caused by diminished activity of porphobilinogen deaminase (PBGD). The purpose of this study was to validate and compare two assays for PBGD activity. The clinical sensitivity of the PBGD activity assays in AIP diagnosis was also evaluated.
Methods: This study included 74 subjects from 18 Taiwanese families including symptomatic patients with AIP, asymptomatic carriers, and healthy family members. The specific mutations in AIP patients were identified by DNA sequencing. PBGD activity was measured in erythrocytes by quantifying formation of coproporphyrin or uroporphyrin by the enzyme using porphobilinogen (PBG) as a substrate and fluorimetry for detection.
Results: The calibration curves obtained with pure coproporphyrin or uroporphyrin were linear with correlation coefficients > 0.99 in the range of 0–200 nM for coproporphyrin and 0–150 nM for uroporphyrin. The coefficients of variation for within-run and between-day imprecision were < 9.8% for both assays. The three groups of subjects were used to establish the best cut-off of PBGD activity for identifying symptomatic AIP patients by using area under receiver operating characteristic curve analysis. The symptomatic AIP patients and asymptomatic carriers had significantly lower PBGD activity compared with the healthy family members (all $p < .001$).
Conclusion: Two different PBGD activity assays were validated. The best cut-off for coproporphyrin was derived as 46.4 nmol/h/mL RBC with corresponding sensitivity of 100% and specificity of 100% and the best cut-off for uroporphyrin was derived as 43.7 nkat/L RBC with corresponding sensitivity of 100% and specificity of 97.4%.

1. Introduction

Acute intermittent porphyria (AIP) is an autosomal dominant metabolic disease that is caused by a partial deficiency of the enzyme porphobilinogen deaminase (PBGD; EC 4.3.1.8) [1,2]. This enzyme (also called hydroxymethylbilane synthase, HMBS) is the third enzyme in the pathway for the synthesis of heme [1]. There are two isoforms of PBGD, one, which is referred to as the "housekeeping" form, is found in all types of cells, the other is only found in erythrocytes [2]. Partial deficiency of PBGD results from mutations of the *PBGD* gene [1]. Clinical features of AIP are characterized by neurological dysfunction manifested as various symptoms including abdominal pain, seizures, neuropsychiatric symptoms, and polyneuropathy [3,4]. Tachycardia and hypertension accompany the symptoms [1]. Delayed diagnosis and treatment may be fatal. Besides using standard clinical criteria for diagnosis, the traditional biochemical diagnosis of AIP is made by finding increased concentrations of the porphyrin precursors porphobilinogen (PBG) and δ-aminolevulinic acid (ALA) in the urine after exclusion of hereditary coproporphyria and variegate porphyria [3,5]. The diagnosis can be confirmed by decreased PBGD activity [4] and *PBGD* gene mutation analysis [6].

The *PBGD* gene is located on chromosome 11 at the 11q24.1-11q24.2 region [3,7]. The gene has 15 exons [1]. The isoform that can be found in any type of cell is encoded by exon 1 and exons 3–15; the isoform found only in erythroid cells is encoded by exons 2–15 [1,3,7]. A large number of mutations of the *PBGD* gene have been identified, although the clinical manifestations of these mutations in patients with AIP are similar [1].

There are several methods for measuring PBGD activity in laboratories and no international standardization has been established for PBGD activity. Assays for PBGD activity have been frequently UV-based using methods such as spectrophotometry or spectrofluorimetry [5,8,9]. The determination of erythrocyte PBGD activity using PBG as a substrate has been found to be a superior method compared with using

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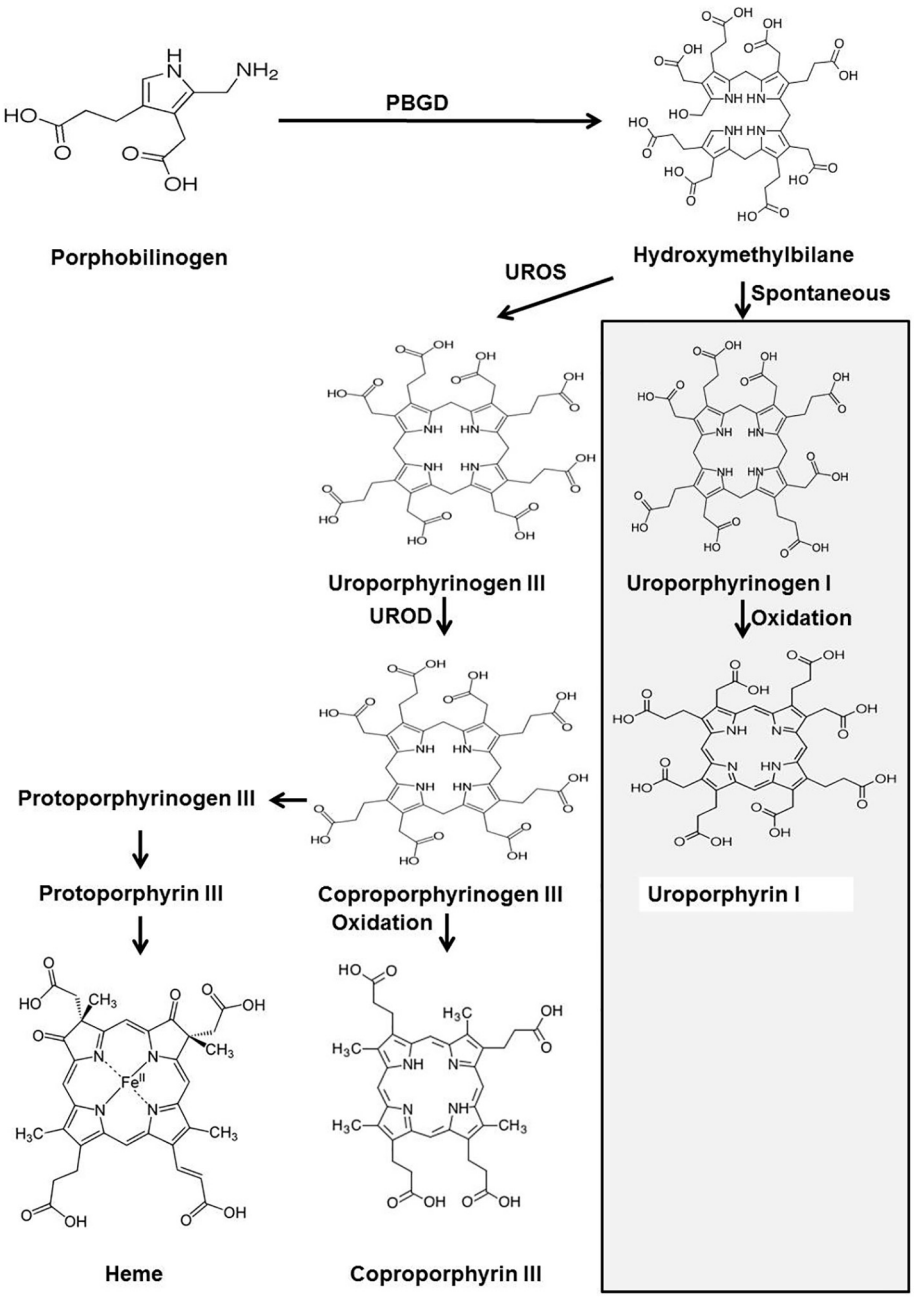

Fig. 1. The heme biosynthesis pathway. PBG is catalyzed by PBGD, Uroporphyrinogen III synthase (UROS) and Uroporphyrinogen III decarboxylase (UROD) to form heme. Hydroxymethylbilane can cyclize non-enzymatically to from uroporphyrinogen I (shaded reactions). Our experiment determined coproporphyrin and uroporphyrin as end products to calculate PBGD activity.

ALA as a substrate [10]. Also, tandem mass spectrometry has been used to measure PBGD activity [8]. In addition to quantitation of coproporphyrin to determine PBGD activity, it has been found that when the only end product of the chemical pathway is uroporphyrin I in human erythrocytes, the quantification of uroporphyrin I can be used to quantify PBGD activity. This can be accomplished by inactivation of two enzymes in the heme biosynthesis pathway, uroporphyrinogen III synthase (UROS) and uroporphyrinogen III decarboxylase (UROD) [11].

We established two PBGD assays in our laboratory and evaluated the efficacy of these two methods by studying Taiwanese families with AIP patients confirmed with *PBGD* gene mutation analysis. The connections between specific *PBGD* gene mutations and PBGD activity were investigated. We hypothesized that using smaller volumes would make the procedure easier to perform and that creating a standard curve for calculation and calibration would improve the methods.

2. Materials and methods

2.1. Subjects

There were 74 subjects from 18 Taiwanese families included in this study. Patients with AIP had histories of clinical features and elevated urinary ALA and PBG concentrations. Blood samples in EDTA were collected for measurement of hematocrit, hemoglobin and DNA analysis. All of the patients, asymptomatic carriers and healthy family members were confirmed by *PBGD* gene mutation analysis and urinary PBG and ALA measurement. Heparinized blood samples were collected from the subjects for the PBGD activity assay. The PBGD activity in erythrocytes during remission was determined in all subjects. The protocol was approved by the institutional review board of Chang Gung Memorial Hospital (101-5008A3, 102-2871C and 103-3839C).