LARGE DELETION OF CYP1B1 IN A CASE OF PRIMARY CONGENITAL GLAUCOMA IDENTIFIED WITH MLPA

Tran Thu Ha[1], Dao Thi Mai Anh[1], Nguyen Van Huy[1] and Tran Van Khanh[2,✉]

[1]Vietnam National Eye Hospital
[2]Hanoi Medical University

Primary congenital glaucoma (PCG) is an inherited ocular abnormality that is caused by mutation in CYP1B1 gene. Report of large deletion or gene copy number variants of CYP1B1 in PCG is uncommon. In this case report we describe a rare case of PCG with whole CYP1B1 deletion with implication for changes in detecting mutation of CYP1B1 gene. Clinical, ophthalmological, genetic, and pedigree information of the proband is described and discussed in detail in this case report. The deletion was identified using the Multiplex ligation-dependent probe amplification (MLPA) method. Whole deletion of CYP1B1 was identified using MLPA. Intra ocular pressure (IOP) of the right eye was 38mmHg and that of the left eye was 35mmHg. Corneal diameters of both eyes were 13mm horizontal and 12mm vertical. The patient underwent a total of five operations with the first operation performed when he was 2.5 months old. Further investigation revealed that the patient's grandfather was exposed to a large quantity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. CYP1B1 deletion albeit rare can cause Primary Congenital Glaucoma with poor prognosis and MLPA should be used to detect large deletion and gene copy number variation when sequencing method fails to detect mutations.

Keywords: Primary congenital glaucoma, CYP1B1, MLPA.

I. INTRODUCTION

Primary congenital glaucoma (PCG; OMIM #231300) is an autosomal recessive disorder caused by mutations in the *CYP1B1* gene. Characteristic of the patients with PCG include early onset (before 3 years of age), buphthalmos (enlarged ocular globe) as the consequence of increased intraocular pressure (IOP), and breaks in Decsemet's membrane (Haab's striae).[1,2] Delayed of diagnosis and treatment can lead to irreversible optic nerve damage and loss of vision.[2] While homozygous mutation of CYP1B1 is associated with the congenital form, heterozygous or compound heterozygous mutation are also responsible for primary open angle glaucoma (POAG) in juvenile and adult.[3]

Corresponding author: Tran Van Khanh
Hanoi Medical University
Email: tranvankhanh@hmu.edu.vn
Received: 14/11/2022
Accepted: 19/11/2022

CYP1B1 has been reported to play an important role in other ocular structure abnormalities such as Anterior Segment Dysgenesis.[8] Della Paolera et al. reported that the identification of CYP1B1 in Congenital Primary Glaucoma correlated to higher need for surgical intervention although no difference in outcome was observed. The identification of CYP1B1 is important both in clinical management of the patients and in counseling for the affected families. To date, over 400 disease-causing variants have been reported and archived in CYP1B1 mutation databases such as the Leiden Open Variant Database (LOVD, lovd.nl) and the Human Gene Mutation Data base.[9] The majority of studies on CYP1B1 mutation only reported point mutation or small deletions and suggested CYP1B1 deletion or gene copy number variation was not the main cause of PCG.[9,11,12] In Vietnam, one previous

study has analyzed thirty families with PCG in which 5 variants were identified.[10] In this study, we report a case of a PCG patient with complete deletion of CYP1B1 detected through MLPA, which warrant further discussion and consideration in regard to future diagnostic protocol.

II. MATERIALS AND METHODS

Clinical and ophthalmic evaluation was carried out for the proband and family members including his unaffected sister and parents. Intra Ocular Pressure was measured using Icare ic100 tonometer instrument (Icare, Finland).

Multiplex Ligation-dependent Probe Amplification (MLPA)

Genomic DNA was purified from peripheral blood using the QiaAmp DNA blood mini kit per the manufacturer's instructions (Qiagen, Germany).

To identify large exonic deletions that were not detected by direct sequencing and to measure the copy number, MLPA method was employed using the MLPA Kit P128 (MRC- Holland). The kit contains two probes for *CYP1B1* (CYP1B1-1-136nt and CYP1B1-3-176nt). MLPA is a high-throughput and straightforward technique for quantification of gene copy number. Products from amplification were analyzed on a 3100-Avant Genetic Analyzer ABI-PRISM (ThermoFisher, US). The results were analysed with MRC-Coffalyser software (MRC-Holland) and the copy number was described using the dosage quotient (DQ). A range of $0.8 < DQ < 1.2$ was considered normal, and a range of $0.4 < DQ < 0.7$ was considered to indicate a heterozygous deletion.

III. CASE PRESENTATION

1. The proband

The patient is a 14-year-old boy who was diagnosed with PCG in the first month after birth. No other complication or trauma was noted at birth. At diagnosis, for both eyes, the corneal diameters were 13mm in the horizontal and 12mm in the vertical. Surgery was planned and performed at 2.5 months of age; however the patient condition did not improve, the second surgery was done at 4.5 months and subsequent operations was done at age 2, 3 and 4. At the time of data collection for this study, the patient had lost vision in both eyes with intra ocular pressure (IOP) of 38mmHg in the right eye and 35mmHg in the left eye. Further investigation revealed that the patient's grandfather was a military veteran and was exposed directly to a large quantity of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Figure 1 shows the MLPA result of the patient and family members. Two probes CYP1B1-1 and CYP1B1-3 were used to detect deletion in exon 1 and exon 3, respectively. Reference samples and control probe were also used to rule out the possibility of a loss of heterozygosity (LOH). The result revealed the patient had a homozygous deletion of the CYP1B1 gene. MLPA also showed the parents were both heterozygous for CYP1B1 deletion.

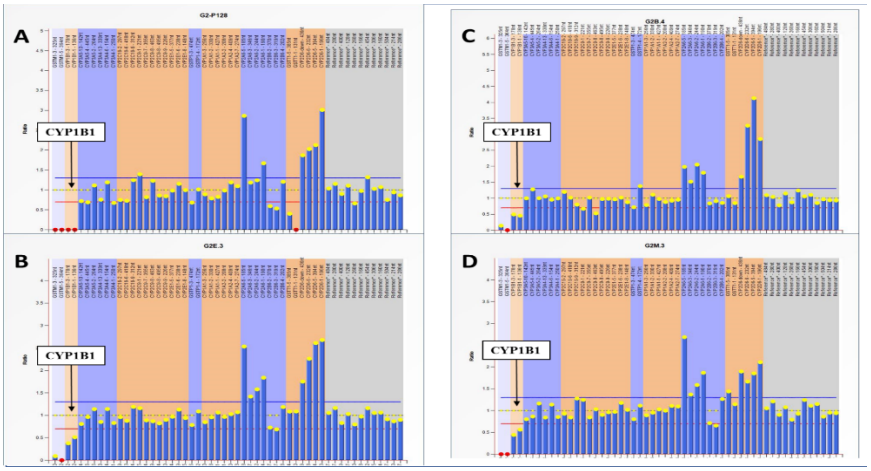

Figure 1. MLPA result of the proband

(A), his sibling (B), father (C) and mother (D). The red arrow indicates the probe to flank for CYP1B1 exon 1 and exon 3. Absence of the 2 probe means the whole CYP1B1 gene is deleted. MLPA result shows the proband is homozygous while the parents are heterozygous for CYP1B1 deletion.

IV. DISCUSSION

In many heritable and congenital diseases, the genetic mutation type is frequently associated with the severity of the symptoms and clinical outcomes. For example, in Hemophilia A, deletional or nonsense mutation are often associated with higher treatment failure. In PCG, such association have not been mentioned which may be confounded by other factor such as age of diagnosis and timely intervention. For our patient, even with timely diagnosis and early intervention, he still required a number of operations and still had poor clinical outcomes. This can be partly attributed to the homozygous deletional mutation, which leads to no CYP1B1 enzymatic activity, further exacerbated his problems.

CYP1B1 belongs to the super family cytochrome P450, a group of enzyme which have important role in metabolism and development.[4] Compounds metabolized by CYP1B1 include 17-beta-estradiol, retinols, arachidonic acid and melatonin, all of which have important function in ocular development.[5] CYP1B1 is also activated in the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin), a compound which heavily polluted the environment in Vietnam. Dioxin was described as a mutagenic by a recent study of a Vietnamese family, resulting in point mutations and deletional mutations confirmed by whole genome sequencing.[13] Other evidence shown that dioxin can induce gene mutations including point mutation, large deletion and rearrangement in offspring of exposed individual.[7] There are many hypotheses about the mechanism that leads to mutation. Minakkam et al. described how dioxin could induce epigenetic transgenerational mutation and sperm epimutations.[14] However, the interaction between CYP1B1 and dioxin in the pathogenesis of CPG is still unknown. Further investigation is needed to assess how the presence of dioxin change the dynamic of PCG. Animal models study might

be the appropriate next step to explore the mechanism of CYP1B1 in the presence of high dioxin pollution and whether dioxin could directly cause mutation in CYP1B1.

Large deletion mutation in CYP1B1 can have implication for mutation screening and future diagnostic methods for PCG in Vietnam. Large deletion or rearrangement could be detected using MLPA for patient where traditional sequencing methods fail to identify mutation. As such, potential carrier can be identified, and this information can be valuable in genetic counseling. To our knowledge, this is the first study to identify CYP1B1 deletion using MLPA method. Because of the robustness of the method, it can be easily incorporated into the current diagnostic protocol. Instead of sequencing alone, we propose a diagnostic protocol of sequencing followed by MLPA to identify gene copy number variants in patients without point mutation. Because Sanger's sequencing alone cannot distinguish a deletion carrier from a deletion non-carrier, we suggest the use of this protocol to help diagnose PCG and avoid overlooking potential deletional mutation and carrier. Our study's patient and family shall be provided with counseling and further testing of family members to identify other carriers, as well as prenatal diagnosis for future pregnancy.

V. CONCLUSION

Ethic approval and consent to participate

The study design was reviewed and approved by the Ethical board of Hanoi Medical University. The study conformed with the Declaration of Helsinki regarding the use of human samples and identifiable information. Informed consent was obtained from the parents of the patients regarding the use of the samples for research purpose.

Consent to publish

The study contains no identifiable data of the patients. Consent to publish not applicable in this context.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. We however cannot provide personal information or data contain identification of the patients in any form.

Competing interests

The authors declare no conflict of interest

Author Contributions

Van Khanh Tran, Van Huy Nguyen conceived and design the study and analysis. Thu Ha Tran and Thi Mai Anh Dao contributed in data collection and carry out the experiments., performed analysis and finalizing the results. Thu Ha Tran, Thi Mai Anh Dao and Van Khanh Tran contributed in drafting of the manuscript. All authors have read and approved of the final version for publication.

ACKNOWLEDGMENTS

We sincerely thank to the patients and their parents for giving us consents and allowing us to publish the data. We thank our colleagues at Vietnam National Eye Hospital who helped us in patient recruitment and clinical data collection. This work was supported by funding from the Ministry of Health of Vietnam.

REFERENCES

1. DeLuise VP, Anderson DR. Primary infantile glaucoma (congenital glaucoma). *Surv Ophthalmol*. 1983 Jul 1; 28(1): 1-19.

2. Ho CL, Walton DS. Primary Congenital Glaucoma: 2004 Update. *J Pediatr Ophthalmol Strabismus*. 2004 Sep 1; 41(5): 271-88.

3. Sarfarazi M, Stoilov I. Molecular genetics of primary congenital glaucoma. *Eye*. 2000 May; 14(3b): 422–8.

4. Li F, Zhu W, Gonzalez FJ. Potential role of CYP1B1 in the development and treatment of metabolic diseases. *Pharmacol Ther*. 2017 Oct; 178: 18-30.

5. Banerjee A, Chakraborty S, Chakraborty A, Chakrabarti S, Ray K. Functional and Structural Analyses of CYP1B1 Variants Linked to Congenital and Adult-Onset Glaucoma to Investigate the Molecular Basis of These Diseases. Anderson MG, editor. *PLOS ONE*. 2016 May 31; 11(5): e0156252.

6. Das DN, Panda PK, Sinha N, Mukhopadhyay S, Naik PP, Bhutia SK. DNA damage by 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced p53-mediated apoptosis through activation of cytochrome P450/aryl hydrocarbon receptor. *Environ Toxicol Pharmacol*. 2017 Oct; 55: 175-85.

7. Ton ND, Nakagawa H, Ha NH, Duong NT, Nhung VP, Hien LTT, et al. Whole genome sequencing and mutation rate analysis of trios with paternal dioxin exposure. *Hum Mutat*. 2018 Oct; 39(10): 1384-92.

8. Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders: *Curr Opin Ophthalmol*. 2011 Sep; 22(5): 314–24.

9. Li N, Zhou Y, Du L, Wei M, Chen X. Overview of Cytochrome P450 1B1 gene mutations in patients with primary congenital glaucoma. *Exp Eye Res*. 2011 Nov; 93(5): 572-9.

10. Do T, Shei W, Chau PTM, Trang DL, Yong VHK, Ng XY, et al. CYP1B1 and MYOC Mutations in Vietnamese Primary Congenital Glaucoma Patients. *J Glaucoma*. 2016; 25(5): e491-498.

11. Souzeau E, Hayes M, Ruddle JB, Elder JE, Staffieri SE, Kearns LS, et al. CYP1B1 copy number variation is not a major contributor to primary congenital glaucoma. *Mol Vis*. 2015 Feb 11; 21: 160-4.

12. Liu Y, Garrett ME, Yaspan BL, Bailey JC, Loomis SJ, Brilliant M, et al. DNA copy number variants of known glaucoma genes in relation to primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2014 Nov 20; 55(12): 8251–8.

13. Ton ND, Nakagawa H, Ha NH, Duong NT, Nhung VP, Hien LTT, et al. Whole genome sequencing and mutation rate analysis of trios with paternal dioxin exposure. *Hum Mutat*. 2018 Oct; 39(10): 1384-92.

14. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Dioxin (TCDD) Induces Epigenetic Transgenerational Inheritance of Adult Onset Disease and Sperm Epimutations. PLoS ONE [Internet]. 2012 Sep 26 [cited 2018 Oct 8]; 7(9). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3458876/.