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Gene diagnosis and pedigree analysis of two Han ethnicity families with propionic acidemia in Fujian

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Abstract

Propionic acidemia is associated with pathogenic variants in *PCCA* or *PCCB* gene. We investigated the potential pathogenic variants in *PCCA* or *PCCB* genes in Fujian Han population.

Two probands and their families of Han ethnicity containing two generations were subject to newborn screening using tandem mass spectrometry, followed by diagnosis using urine gas chromatography mass spectrometry. Sanger sequencing was used to identify potential mutations in *PCCA* and *PCCB* genes.

Compound heterozygous variants were identified in *PCCB* gene in two siblings of the first family, the youngest girl showed a novel missense variant c.1381G>C (p.Ala461Pro) in exon 13 and a heterozygous missense variant c.1301C>T (p.Ala434Val) in exon 13, which were inherited respectively from their parents. The oldest boy is a carrier with a novel missense variant c.1381G>C (p.Ala461Pro) in exon 13 which were inherited from his father. In the second family, c.1535G>A homozygous mutations were identified in the baby girl, which were inherited respectively from their parents. In silico analysis, several different types of bioinformatic software were utilized, which predicted that the novel variant c.1381G>C in *PCCB* gene was damaged. According to ACMG principle, the missense variant c.1381G>C (p.Ala461Pro) in exon 13 was a Variant of Undetermined Significance (VUS).

One novel missense variant and two missense variants in *PCCB* gene were identified in the study. The novel variant of *PCCB* gene identified VUS was identified for the first time in the Chinese population, which enriched the mutational spectrum of *PCCB* gene.

Abbreviations: GC/MS = gas chromatography mass spectrometry, LOVD = Leiden Open Variation Database, MOF = multiple organ failure, MS = mass spectrometry, NBS = newborn screening, PA = Propionic acidemia, PCC = propionyl-CoA carboxylase, VUS = Variant of Undetermined Significance.

Keywords: Newborn screening, *PCCB* gene, propionic acidemia, tandem mass spectrometry, urine gas chromatography mass spectrometry

1. Introduction

Propionic acidemia (PA; OMIM 606054) is a rare autosomal recessive disorder featured by deficiency of the mitochondrial propionyl-CoA carboxylase (PCC). PCC is considered an α6β6 multimer composed of α and β subunits, with the *PCCA* and *PCCB* genes encoding the α and β subunits.[1] These two genes encoded the subunits of the biotin-dependent propionyl-CoA carboxylase holoenzyme that converted propionyl-CoA to methylmalonyl-CoA.

Subsequently, the methylmalonyl-CoA is converted to succinyl-CoA serving as an intermediate of the citric acid cycle.[2] Most PA patients have several abnormal presentations in neonatal period including ketoacidosis, feeding refusal, lethargy, failure to thrive, as well as seizures and encephalopathy. Part of the patients exhibited a chronic late-onset form. To our best knowledge, the most common complications for PA patients are fetal anomalies, while some patients with milder symptoms present fatal neurological or cardiac symptoms without previous metabolic decompensation in the late childhood.[3–5]

To date, there are great strides and improvements in the newborn screening (NBS) ever since the addition of tandem-mass spectrometry (MS/MS) techniques. These improvements contribute to the changes in cut-offs and data analysis, which brings in elevation of sensitivity and decline of false positivity.[6] PA is characterized by elevated propionylcarnitine (C3) levels on NBS as revealed by MS/MS. Some studies also had utilized C3 ratios to

Editor: Hasanain Faisal Ghazi.

The authors have no funding and conflicts of interest to disclose.

Data availability: All the data were available upon appropriate request.

The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

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How to cite this article: Chen Y, Lin X, Lin Q, Zeng Y, Qiu X, Liu G, Zhu W. Gene diagnosis and pedigree analysis of two Han ethnicity families with propionic acidemia in Fujian. Medicine 2021;100:10(e24161).

Received: 20 July 2020 / Received in final form: 8 December 2020 / Accepted: 10 December 2020

http://dx.doi.org/10.1097/MD.0000000000024161

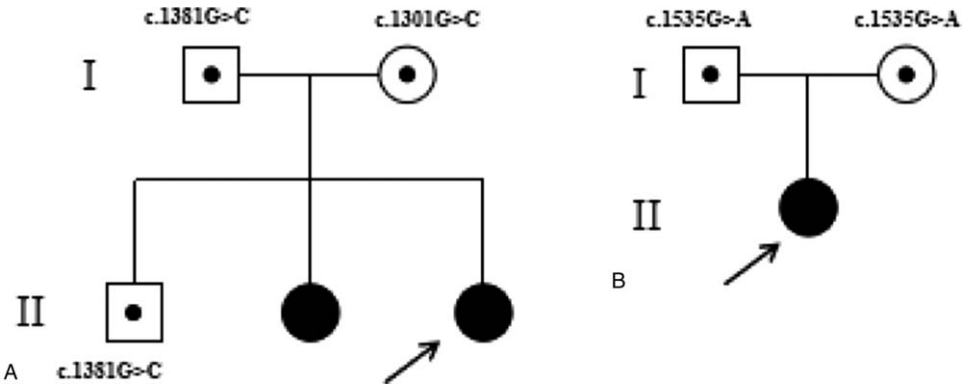

Figure 1. Pedigree of the two families (A and B). The blank with a dot symbol represented the carrier, and the filled black symbols represented the affected members. The arrow demonstrated the proband.

improve accuracy, which played important roles in the diagnosis of PA using C3 elevation by MS/MS. In total, 124 PCCA variants and 112 PCCB variants have been identified in the Human Genome Mutation Database (HGMD Professional 2018.1).[7]

Tandem mass spectrometry has been used for neonatal screening in Fujian Province since 2015. Nevertheless, some early onset PA infants may die before the release of newborn screening reports. However, little is known about the incidence of PA in Fujian Province. In this study, we reported the clinical and genetic features of PA patients through neonatal screening from unrelated families in Fujian Province, and investigate the potential pathogenic variants in *PCCA* or *PCCB* genes.

2. Methods

2.1. Subjects

This study involved 2 infants with aberrant elevation of C3 level isolated from 95,453 cases receiving neonatal screening using MS/MS between May 2015 and February 2020. Two generations from the 2 Chinese families were recruited and signed the consent. The first family included 5 members of Han ethnicity, while the second family included 3 members of Han ethnicity (Fig. 1A). A total of 100 healthy newborns with C3 in normal level by MS/MS screening from our center without any other diseases were recruited as controls after signing the informed consent. The inclusion criteria were as follows: those with an age of 72 h; those with breast-feed and received no administration of drugs, and with a MS/MS screening of C3 of >4 μmol/L. All the participants signed written informed consent. The study protocols were approved by the Ethical Committee of the Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University.

2.2. Blood acylcarnitine spectral analysis

The peripheral blood (3 droplets) collected from the neonates was dropped onto the filter paper (Whatman S&S903), followed by drying under natural conditions. The amino acid and acylcarnitine analyses were carried out using the PE commercial kit and the TQD MS/MS screening system (Waters, USA), respectively.

2.3. Sanger sequencing

Genomic DNA was extracted from dried blood spots or peripheral blood of the probands and their family members using commercial kits, according to the manufacturer's instructions. The region of interest in *PCCB* (NM_000532.4) gene was amplified using standard PCR with the specific primers listed in Table 1. The PCR cycle consisted of an initial denaturation at 95°C for 2 min, followed by 36 cycles of 95°C for 30 s, 58°C for 1 min, and 72°C for 1 min, as well as 72°C for 2 min. All PCR products were analyzed by capillary electrophoresis using an ABI Prism 3500XL Genetic Analyzer, followed by direct Sanger sequencing using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

2.4. In silico analytical tools

The disease databases such as HGMD,[8] ClinVar[9] and the Leiden Open Variation Database (LOVD)[10] were used to confirm the reported mutations. Then bioinformatic programs including PolyPhen 2, SIFT, PROVEAN and MutationTaster were employed to predict the impact of a missense change of the novel mutation on the protein structure and function[11–14] (Table 2). Additionally, multiple AA sequences were extracted from NCBI and were aligned to verify the evolutionary conservation using ClustalX (http://www.clustal.org/clustal2).[15,16]

3. Results

3.1. Patient characteristics

Two probands from different families without relationship were included from Sanming area, Fujian Province. They were diagnosed with PA based on their clinical manifestations and an abnormal metabolic profile (Fig. 1A and B) in Fujian

Table 1

Specific primers for *PCCB* variations' verification.

Gene	Nucleotide change	Primer sequence (5′-3′)	Size
PCCB	c.1301C>T c.1381G>C	Forward, TGGTTTCCTGGGGTCTTT Reverse, GCCTCTCTCCAACATTTCC	266 bp
	c.1535G>A	Forward, GGTTGGGCACTGCTTAT Reverse, AATAATTTTACAGTAAGGCACAA	409 bp

Table 2

Pathogenicity prediction analysis of *PCCB* c.1381G>C alteration.

Bioinformatic program	URL	Score	Prediction effect
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2/	1	Damaging
SIFT	http://sift.jcvi.org/	0.000	Damaging
PROVEAN	http://provean.jcvi.org/index.php	−4.41	Deleterious effect
MutationTaster	http://www.mutationtaster.org/	1.000	Disease causing

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3.2. Clinical data and auxiliary examination

In the first family, the non-consanguineous parents were all healthy individuals. The proband was their youngest daughter who was screened by MS/MS and diagnosed by urine gas chromatography mass spectrometry (GC/MS). Then the inherited metabolic diseases panel was utilized to analyze the mutation was from *PCCA* gene or *PCCB* gene, in which a novel mutation was identified in *PCCB* gene. According to blood propionyl carnitine concentration, the child was recommended to adjust the mixing ratio of milk powder, and oral administration of levocarnitine 100 to 200 mg/kg every day. Then the blood propionyl carnitine concentration was tested a month later.

The eldest son was a carrier without clinic symptoms, while the second daughter died of a serious multiple organ failure (MOF) during the neonatal period. In the second family, despite the fact that the parents were non-consanguineous healthy individuals, they were from the same village. The proband was their only daughter who was admitted to our hospital with the clinical symptoms as follow: feeding difficulties, lethargy, coma, hypomyotonia, as well as severe metabolic acidosis and hyperammonemia. Unfortunately, the baby died of MOF after discharge requested by the parents. Details of auxiliary examination and the genotypes of the siblings were shown in Table 3.

3.3. Lineage analysis of PCCB gene mutation

In the first proband, compound heterozygous variants were identified in *PCCB*, in which a novel heterozygous variant c.1381G > C (p.Ala461Pro) was identified in exon 3 inherited from her father. Meanwhile, there was a heterozygous variant c.1301C>T (p.Ala434Val) in exon 3, which was inherited from her mother (Fig. 2). Her sister showed the same genotype, and her brother was a carrier with a heterozygous variant c.1381G>C (p.Ala461Pro). The second proband showed the homozygous variant c.1535G>A (p.Arg512His) in *PCCB* (Figs. 2 and 3).

4. Discussion

To date, PA is still a challenge with a higher prevalence among the neonates, which severely affects the life quality of the neonates. Several aspects have been reported to be associated with the onset of the disease, especially gene mutation. Some efforts have been made to illustrate the roles of gene mutation in the pathogenesis of PA. For example, Desviat et al[17] identified 34 novel mutations in PA patients, and analyzed the functional characterization of missense variants and phenotype associations. In this study, we focused on the roles of potential pathogenic variants in *PCCA* or *PCCB* genes in the pathogenesis of PA. We identified a novel variant of *PCCB* gene in the Chinese population.

PA was firstly reported by Childs et al[18] in 1961. Nowadays, the overall incidence was in a range of 1/100,000 to 1/50,000.[19,20] Additionally, the detection rates in US, southwest Germany and Kuwait were 0.41, 0.35 and 1.68 per 100,000 newborns, respectively.[21] In a study performed in Saudi Arabia, the incidence of PA was relatively high, which was associated with the frequent consanguineous marriages in the Saudi society.[22] Indeed, there are significant differences in the gene mutation types of PA. For example, the *PCCA* mutation in Japanese population was predominantly featured by 923–924insT, IVSl8–6C>G and R399Q, and that for the *PCCB* gene was featured by R410W, T428I and A153P.[23] In Taiwan, the mutation spectrum for PA patients were the two frequent demographic mutations (c.-4156-+183+3713del andc.1301C>T) in the *PCCB* gene identified in their study were linked to low enzyme activity and the classic phenotypic form of propionic acidemia.[24]

In this study, we reported two patients with PA screened by MS/MS and diagnosed by urine GC/MS. We identified a novel mutation in *PCCB* gene in the first family. We identified compound heterozygous variants in *PCCB* in the first proband.

Table 3

Clinical, biochemical and genetic characteristics of the two families.

Symbol	Age of onset	Gender	Clinical features	pH*	C3† (μmol/L)	C3/C2‡	Ammo§ (μmol/L)	3-hydroxy-propionic acid‖	Methylcitric acid#	Genotype	Evolution
1-proband	1.5m	F	Vomiting	7.33	22.01	3.0	140.3	179.6	32.2	c.1301C>T c.1381G>C	Alived at 1.5y
1-brother	N	M	N	ND	3.21	0.11	ND	ND	ND	c.1381G>C	Alived at 5y
1-sister	12d	F	Feeding difficulties	7.30	21.18	1.06	337	230.2	32.7	c.1301C>T c.1381G>C	Died at 17d
2-proband	21d	F	Feeding difficulties, low response	7.40	11.92	1.77	528.28	171.7	26.01	c.1535G>A	Died at 24d

d = day, F = female, M = male, m = month, ND = not detected, y = year.

* Normal range 7.35-7.45 in blood.

† Normal range 0.3–4 μmol/L in blood.

‡ Normal range 0.03–0.2 in blood.

§ Normal range 9.00–30.00 μmol/L in blood.

‖ Normal range 0–4 μmol/L in urine.

Normal range 0–0.7 μmol/L in urine.

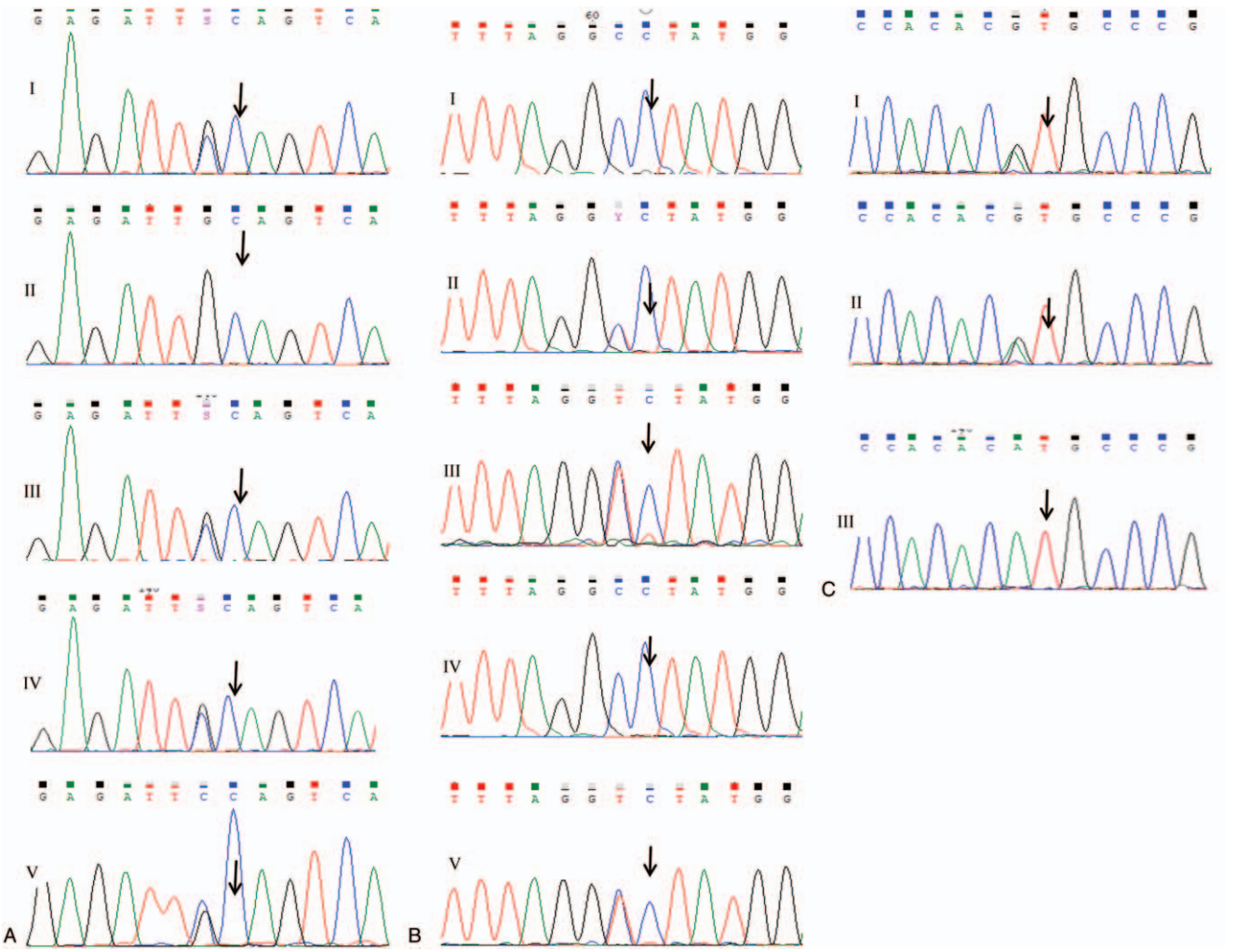

Figure 2. Gene mutation analysis. (A) Sequence analysis of *PCCB* gene in the first family separately identified the heterozygous c.1381G > C variant in the proband (III), her father (I), her brother (IV) and her sister (V). (B) Sequence analysis of *PCCB* gene in the first family separately identified the heterozygous c.1301C > T variant in the proband (III), her mother (II) and her sister (V). (C) Sequence analysis of PCCB gene in the second family identified the homozygous c.1535G > A variant in the proband (III), the heterozygous c.1535G > A variant in her father (I) and mother (II).

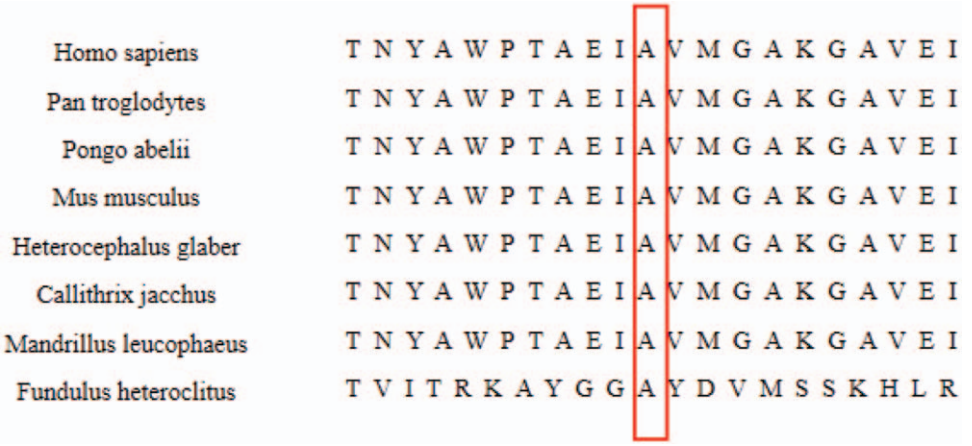

Figure 3. Amino acid alignment of the P-protein from several organisms. The position of Ala461 residue (highlighted by a red box) was highly conserved among different species.

Besides, a novel heterozygous variant c.1381G > C (p.Ala461Pro) was identified in exon 3 inherited from her father, and a heterozygous variant c.1301C > T(p.Ala434Val) was identified in exon 3 inherited from her mother. Her sister had the same genotype, and her brother was a carrier with a heterozygous variant c.1381G > C (p.Ala461Pro). The second proband had the homozygous variant c.1535G>A (p.Arg512His) in *PCCB*. Three mutations (c.1381G > C, c.1301C > T and c.1535G>A) in the PCCB gene identified in this study were linked to low enzyme activity and the classic phenotypic form of PA. All these mutations may affect αff heteromeric and β –β homomeric assembly.[25]

Computational analysis including PolyPhen 2, SIFT, PROVEAN and MutationTaster predicted that the novel variant c.1381G > C (p.Ala461Pro) was likely to present pathogenic significance. Meanwhile, a conservative analysis in different species showed that this AA was highly conserved across a broad range of species. This strongly suggested that the variant at this site might be deleterious. According to ACMG,[26] the novel mutation pathogenicity analysis was a VUS (PM1+PP1). The first proband's sister died of sever MOF, and the clinical symptoms and laboratory test results were consistent with PA. Clinical features demonstrated that the novel mutations were associated with a classic PA phenotype. Based on NBS findings, the probands were recommended to take the special milk to limit the protein intake. However, the compliance of the patient was poor as the parents didn't follow the doctor's advice, which led to severe conditions after discharge.

Genotyping analysis using standard or massive parallel sequencing approaches promotes the identification of novel candidate variants in the *PCCA* or *PCCB* genes in PA patients confirmed using biochemical and/or enzymatic diagnosis. A significant number of PA patients would develop cardiac complications.[27] In our study, we presented two Chinese families that were representatives of neonatal PA patients without obvious cardiac symptoms. The probands exhibited severe and progressive manifestation such as lethargy, coma, hypomyotonia, as well as great elevation in the blood ammonia and plasma C3. Molecular genetic analysis by inherited metabolic diseases panel and Sanger sequencing were conducted to confirm the variation of such site.

There are some limitations in this study. Firstly, we only included two families and therefore the sample size is not large. Secondly, we did not investigate the potential mechanisms of the *PCCA* or *PCCB* in the pathogenesis of PA. In our subsequent study, we will focus on the functional roles of the identified genes.

5. Conclusions

In this study, we describe the clinical and genetic features of two Han Chinese families in Fujian Province affected with PA. A novel variant c.1381G > C (p.Ala461Pro) and reported mutation c.1301C>T (p.Ala434Val) in PCCB gene were identified in the three siblings in the first family. In the second family, a reported mutation c.1535G>A (p.Arg512His) which is likely to be associated with the pathogenesis of PA[17] was identified. Our findings suggested that the novel variant in *PCCB* gene probably underlie the pathogenesis of PA in this family. NBS is beneficial to the early detection of inherited metabolic diseases in newborns. In future, large sample sized studies are required to further investigate the exact roles of these mutations in the pathogenesis of PA.

Acknowledgments

We thank the families for participating in the study.

Research involving Human Participants and/or Animals.

The study protocols were approved by the Ethical Committee of the Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University.

Informed consent: All the participants signed written informed consent.

Authorship: CY wrote the manuscript; ZWB, LGH revised the manuscript; ZYL, LQY did the data analysis; LXH, QXL did the data collection.

Author contributions

Conceptualization: Xuehua Lin.
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Formal analysis: Xuehua Lin.
Investigation: Qingying Lin, Guanghua Liu.
Methodology: Qingying Lin.
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Supervision: Yinglin Zeng, Xiaolong Qiu.
Validation: Yinglin Zeng, Xiaolong Qiu.
Visualization: Yinglin Zeng, Guanghua Liu.
Writing – original draft: Yao Chen.
Writing – review & editing: Wenbin Zhu.

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