Hemoglobin
international journal for hemoglobin research

ISSN: 0363-0269 (Print) 1532-432X (Online) Journal homepage: https://www.tandfonline.com/loi/ihem20

First Description of Hb San Diego (*HBB*: c.328G>A) in a Chinese Family with Congenital Erythrocytosis

Huixia Xiong & Suning Chen

To cite this article: Huixia Xiong & Suning Chen (2019) First Description of Hb San Diego (*HBB*: c.328G>A) in a Chinese Family with Congenital Erythrocytosis , Hemoglobin, 43:2, 126-128, DOI: 10.1080/03630269.2019.1615940

To link to this article: https://doi.org/10.1080/03630269.2019.1615940

HEMOGLOBIN
2019, VOL. 43, NO. 2, 126–128
https://doi.org/10.1080/03630269.2019.1615940

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

First Description of Hb San Diego (*HBB*: c.328G>A) in a Chinese Family with Congenital Erythrocytosis

Huixia Xiong and Suning Chen

Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, People's Republic of China

ABSTRACT

Congenital erythrocytosis is a rare and hereditary disorder of red blood cell (RBC) production that can be caused by high oxygen affinity hemoglobin (Hb) variants. We applied a genetic approach including whole exome sequencing and Sanger sequencing. We identified a heterozygous β-globin gene (Hb San Diego or *HBB*: c.328G>A) in exon 3 as a causative germline mutation in a Chinese family with congenital erythrocytosis. We concluded that in erythrocytosis with a dominant inheritance and normal or inappropriately high erythropoietin (EPO) levels, the high oxygen affinity Hb variants should be considered. In addition, as a tool for identification of mutations in congenital erythrocytosis, whole exome sequencing improves diagnostic accuracy and provides the opportunity for discovery of novel variants.

ARTICLE HISTORY
Received 17 February 2019
Revised 25 March 2019
Accepted 25 March 2019

KEYWORDS
Erythrocytosis; Hb San Diego; hemoglobin (Hb) variants; high oxygen affinity

Congenital erythrocytosis represents an uncommon and hereditary disease where patients present with elevated hemoglobin (Hb) and packed cell volume (PCV) levels with no apparent reason. Contrary to polycythemia vera (PV), it is characterized by isolated erythrocytosis and lack of clonal markers. It can be primary or secondary. Primary congenital erythrocytosis results from mutations of the erythropoietin (EPO) receptor gene, whereas secondary congenital erythrocytosis may be associated with high oxygen affinity Hb variants, 2,3-biphosphoglycerate (2,3-BPG) mutase deficiency and dysregulation of oxygen-sensing pathway.

At the present time, eight genes are known to have been involved in congenital erythrocytosis but in about 70.0% of the patients, the underlying molecular defects remain unknown [1]. High oxygen affinity Hb variants are the most common causes of congenital erythrocytosis [2], particularly the *HBB* genes, are the most frequent mutated Hb subunit [3]. The molecular classification of high oxygen affinity Hb variants recognizes three different structural modifications relating to the transition from 'T' (tense, deoxygenated) to 'R' (relaxed, oxygenated) states, the 2,3-BPG binding sites and the heme pocket [4].

Hb San Diego (*HBB*: c.328G>A), characterized as an electrophoretically silent high oxygen affinity Hb variant, was first described in 1974 in a Filipino family [5] and subsequently reported in subjects of different origins [2]. The amino acid substitution [β109(G11)Val→Met; codon 109 (G>A)] affects the α1β1 interaction region and reduces release of oxygen [6] that leads to tissue hypoxia and stimulates erythropoietic drive. Here, we present the first report of a Chinese family with congenital erythrocytosis caused by Hb San Diego.

Case report

The proband is a 43-year-old Chinese male, who was referred to our outpatient department with an 8-year history of erythrocytosis and complaints of intermittent headaches and dizziness. He had no history of thrombosis or pulmonary hypertension. He did not smoke or drink alcohol and his body mass index was normal. The laboratory data showed that he had an isolated erythrocytosis (Hb 22.5 g/dL and PCV 0.64 L/L) with normal leukocyte and platelet counts. Additionally, the red blood cell (RBC) morphology and serum EPO level were normal. His bone marrow showed an erythroid hyperplasia with no blasts or fibrosis and chromosomal abnormalities were not detected. There was no splenic enlargement at the ultrasound examination. No mutations were found in *JAK2* (exons 14 or 12), *CALR* (exon 9), *MPL* (exon 10) and *BCR/ABL* gene rearrangements were negative. He was initially diagnosed with idiopathic erythrocytosis and treated with occasional phlebotomies for the high value of PCV.

Erythrocytosis was also present in other family members subsequently, but the only findings were facial and mucosal erythrosis. We collected the laboratory data provided by the regular health examinations of his 22 family members. The family history showed an autosomal dominant pattern of inheritance affecting at least six family members spanning three generations (Figure 1). The clinical and laboratory

CONTACT Professor Suning Chen chensuning@sina.com Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, Jiangsu Province, People's Republic of China.

Supplemental data for this article can be accessed here.

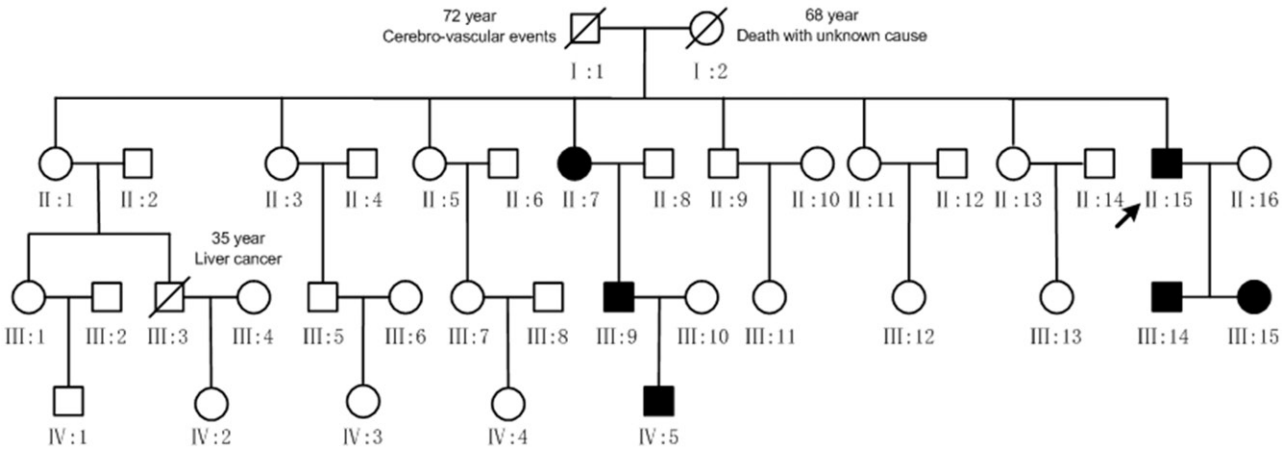

Figure 1. Pedigree of known family members. The arrow indicates the proband. Solid symbols represent family members with erythrocytosis.

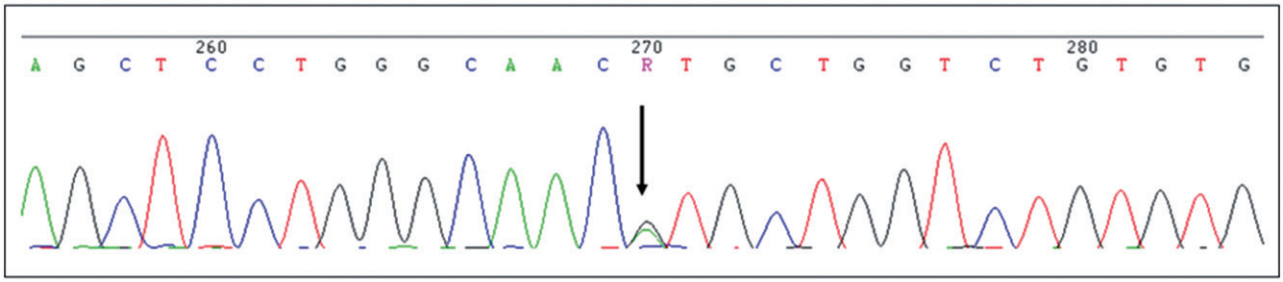

Figure 2. Identification of the *HBB*: c.328G>A mutation by Sanger sequencing.

findings are summarized in the Supplementary Table S1 (available online). These patients have no history of thrombosis but his father died from cerebro-vascular events, while information of erythrocytosis was not available.

To screen for the potential mutation causing disease, two affected members and one unaffected member were subjected to whole exome sequencing using the Hiseq platform (Illumina Inc., San Diego, CA, USA). Genomic DNA was extracted from peripheral blood leukocytes, while the germline or somatic origin of the mutation was confirmed by their identification on DNA from buccal cells. Whole exome sequencing revealed a germline heterozygous missense mutation on the β-globin gene at *HBB*: c.328G>A, p.V110M in exon 3, which consistent with Hb San Diego. We then confirmed the same mutation (Figure 2) using Sanger sequencing (3730 DNA analyzer; Applied Biosystems, Foster City, CA, USA) in all patients and another three asymptomatic subjects (Figure 1; subjects II-3, II-5 and II-11).

This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, PRC. All studied subjects gave their informed consent.

According to published data, 224 high oxygen affinity Hb variants have been described in the HbVar database [7]. Phenotypic assessment by laboratory techniques remains a main step in their diagnosis [8]. Although the finding of reduced P_{50} may suggest the presence of a high oxygen affinity Hb variant, a confirmatory test is necessary. Combination of routine biochemical techniques, such as capillary electrophoresis or isoelectric focusing, cation exchange liquid chromatography and reverse phase liquid chromatography of globin chains, can improve the detection of Hb variants [9–11]. It is important to note that many of the variants are not identified by conventional laboratory approaches alone and required DNA sequencing performed for definitive identification. Recently, next generation sequencing (NGS) approaches have been used to identify new genes and new pathogenic variants segregating within families with congenital erythrocytosis [1]. Our experience shows that NGS methodologies can provide rapid and accurate mutation analyses in congenital erythrocytosis. As targeted NGS is cost-efficient, Camps *et al.* [12] recently developed a custom-made erythrocytosis gene panel to identify new genes associated with RBC diseases. We hope this high-throughput technology could be applied directly to erythrocytosis cases where a genetic cause is suspected.

Prognostic evaluations of all high oxygen affinity Hb variants, are not associated with the same clinical picture. Erythrocytosis due to Hb San Diego is usually well tolerated by young patients and generally believed to have a favorable prognosis, although thrombotic events have been reported in elderly patients or when other vascular risk factors are associated [13]. Additionally, symptoms of hypoxia worsen with age, due to an alteration of the microcirculation, lowering tissue oxygen extraction capacity.

Management of congenital erythrocytosis caused by a high oxygen affinity Hb variant should be personalized and the benefit-risk ratio of each therapeutic strategy should be evaluated. It should depend primarily on cessation of smoking, and greater physical activity. Phlebotomy and

prescriptions of antiplatelet agents can be discussed. When the hyperviscosity symptoms such as a headache are present, phlebotomy therapy may be effective. In case of thrombosis or association with a major hazard factor of thrombosis, it is reasonable to give an antiplatelet aggregation therapy. There is no contraindication to flights, high-altitude conditions. Nevertheless, blood donation must be proscribed [14]. An increase in fetal losses during pregnancy has not been reported in affected families. The use of low-dose aspirin or low molecular weight heparin are sufficient to manage these pregnancies [15]. Genetic risk assessment and genetic counseling should be offered to family members of a diseased person [14].

In the family presented here, the proband was given antiplatelet drugs and underwent occasional phlebotomies because of his hyperviscosity syndromes. One of his affected sisters was treated with low-dose aspirin considering that the risk of cardiovascular events were increased because of her age and menopause. The other four patients and three healthy mutation carriers required a follow-up once every 6-12 months. It would be helpful to accumulate more information on long-term outcomes of such patients.

Acknowledgments

We are grateful to our patients and their families for participating in this study.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Bento C. Genetic basis of congenital erythrocytosis. Int J Lab Hematol. 2018;40(Suppl 1):62–67.

[2] Bento C, Almeida H, Maia TM, *et al.* Molecular study of congenital erythrocytosis in 70 unrelated patients revealed a potential causal mutation in less than half of the cases (Where is/are the missing gene(s)?). Eur J Haematol. 2013;91(4):361–368.

[3] Hussein K, Percy M, McMullin MF. Clinical utility gene card for: familial erythrocytosis. Eur J Hum Genet. 2012;20(5). doi: 10.1038/ejhg.2011.252

[4] Thom CS, Dickson CF, Gell DA, *et al.* Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med. 2013;3(3):a011858.

[5] Nute PE, Stamatoyannopoulos G, Hermodson MA, *et al.* Hemoglobinopathic erythrocytosis due to a new electrophoretically silent variant, Hemoglobin San Diego (β109 (G11)Val→Met). J Clin Invest. 1974;53(1):320–328.

[6] Anderson NL. Hemoglobin San Diego (β 109 (G11) val→met). Crystal structure of the deoxy form. J Clin Invest. 1974;53(1):329–333.

[7] Giardine B, Borg J, Viennas E, *et al.* Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res. 2014;42 (Database issue): D1063–D1069 (http://globin. cse.psu.edu).

[8] Orvain C, Joly P, Pissard S, *et al.* Diagnostic approach to hemoglobins with high oxygen affinity: experience from France and Belgium and review of the literature. Ann Biol Clin (Paris). 2017;75(1):39–51.

[9] Basset P, Beuzard Y, Garel MC, *et al.* Isoelectric focusing of human hemoglobin: its application to screening, to the characterization of 70 variants, and to the study of modified fractions of normal hemoglobins. Blood. 1978;51(5):97–982.

[10] Lin C, Cotton F, Fontaine B, *et al.* Capillary zone electrophoresis: an additional technique for the identification of hemoglobin variants. Hemoglobin. 1999;23(2):97–109.

[11] Hoyer JD, Scheidt RM. Identification of hemoglobin variants by HPLC. Clin Chem. 2005;51(7):1303–1304.

[12] Camps C, Petousi N, Bento C, *et al.* Gene panel sequencing improves the diagnostic work-up of patients with idiopathic erythrocytosis and identifies new mutations. Haematologica. 2016;101(11):1306–1318.

[13] Gonzalez Fernandez FA, Villegas A, Ropero P, *et al.* Haemoglobinopathies with high oxygen affinity. Experience of Erythropathology Cooperative Spanish Group. Ann Hematol. 2009;88(3):235–238.

[14] Mangin O. High oxygen affinity hemoglobins. Rev Med Interne. 2017;38(2):106–112.

[15] McMullin MF, Bento C, Rossi C, *et al.* Outcomes of pregnancy in patients with congenital erythrocytosis. Br J Haematol. 2015;170(4):586–588.