Journal of the Neurological Sciences 347 (2014) 305–309

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Diagnosis of cystathionine beta-synthase deficiency by genetic analysis

Fatemeh Suri [a], Mehrnaz Narooie-Nejad [b], Iman Safari [a], Hamidreza Moazzeni [c], Mohammad-Reza Rohani [d], Ali Khajeh [e], Brandy Klotzle [f], Jian-Bing Fan [f], Elahe Elahi [a,g,*]

[a] School of Biology, College of Science, University of Tehran, Tehran, Iran
[b] Genetics of Non-communicable Disease Research Center, Zahedan University of Medical Science, Zahedan, Iran
[c] Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[d] Division of Ophthalmology, Zahedan University of Medical Sciences, Zahedan, Iran
[e] Children and Adolescent Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[f] Illumina, San Diego, CA, USA
[g] Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 14 October 2014
Accepted 16 October 2014
Available online 22 October 2014

Keywords:
Whole genome SNP homozygosity mapping
Intellectual disability
Homocystinuria
Diagnosis
Cystathionine beta-synthase (CBS)
p.Gly116Arg

ABSTRACT

Intellectual disability like other common diseases is often complex because they are genetically heterogeneous, with many different genetic defects giving rise to clinically indistinguishable phenotypes. We present diagnosis of cystathionine beta-synthase (CBS) deficiency in a multiply affected Iranian family with obvious intellectual disability based on whole genome SNP homozygosity mapping. Diagnosis based on clinical presentations had not been made because of unavailability of appropriate medical services. Genetic analysis led to identification of homozygous c.346G>A in *CBS* that causes p.Gly116Arg in the encoded protein, cystathionine beta-synthase. *CBS* is the most common causative gene of homocystinurea. Later, the same mutation was found in three other apparently unrelated Iranian homocystinuria patients. p.Gly116Arg was reported once before in a Turkish patient, suggesting it may be a common CBS deficiency causing mutation in the Middle East. Clinical features of the patients are reported that evidence to variable presentations caused by the same mutation. Finally, observations in heterozygous carriers of the mutation suggest data that a single allele of the p.Gly116Arg causing mutation may have phenotypic consequences, including cardiac related phenotypes. Our study attests to the powers of genetic analysis for diagnosis especially for some forms of intellectual disability, with known genetic causing agents.

1. Introduction

Homocystinuria (OMIM #236200), the classical form of which is often known as cystathionine beta-synthase (CBS) (Enzyme Commission (EC) number; EC 4.2.1.22) deficiency, is an inherited disorder of methionine and sulfur metabolism whose inheritance is autosomal recessive [1,2]. Its clinical features were first described in 1962 after studying patients affected with mental retardation and analysis of their urinary amino acid profiles [3,4]. CBS is a homotetramer that catalyzes a reaction in the trans-sulfuration pathway of the methionine cycle during which serine and homocysteine are conjugated to form cystathionine. Homocysteine can also undergo remethylation to form methionine. Dysfunction of the CBS enzyme, therefore, may result in accumulation of both homocysteine and methionine.

Mutations in *CBS* on chromosome 21q22 that encodes cystathionine beta-synthase are the major cause of homocystinuria [5]. Worldwide, at least 181 mutations in *CBS* have been reported (Human Genome Mutation Database; http://www.hgmd.cf.ac.uk/ac/index.php). In addition to *CBS*, mutations in other genes involved in methionine metabolism have sometimes been reported as cause of homocystinuria (http://omim.org/entry/236200). Dietary recommendations and treatment with vitamins B6 (pyridoxal 5-phosphate), B9 (folic acid), and B12 (cobalamin) can be very advantageous in preventing clinical presentations of homocystinuria, particularly if administered early [1]. Treatment even after presentation of symptoms can be beneficial and is recommended. The vitamins are involved in the functions of the various enzymes associated with homocystinuria.

The most common clinical manifestations of CBS deficiency include anomalies in nervous, skeletal, ocular, and vascular systems [1]. The earliest manifestations usually occur in the first or second decade of life. Ectopia lentis is the most consistent presentation of the disorder. Other ocular manifestations include high myopia, glaucoma, cataract, and retinal detachment [6]. Skeletal abnormalities include dolichostenomelia which results in a Marfan syndrome-like appearance [7]. Ectopia lentis and skeletal anomalies may in part be due to disruptions of proteins with high concentrations of cysteine such as fibrillin-1 which is involved in the etiology of both conditions [8]. The disruptions

* Corresponding author at: College of Science, University of Tehran, Enghelab Ave., Tehran 1417614411, Iran. Tel.: +98 9122181251; fax: +98 2166405141.
E-mail addresses: elahe.elahi@gmail.com, elaheelahi@ut.ac.ir (E. Elahi).

http://dx.doi.org/10.1016/j.jns.2014.10.031
0022-510X/

are expected to be caused by malfunction of sulfur metabolism. Intellectual disability of varying severity is the most frequent abnormality of the nervous system and usually the earliest manifestation of CBS deficiency [9]. Vascular occlusions can result in thromboembolism and are the most frequent cause of death [10]. Additionally, homocystinuria may affect the liver and skin; hypopigmentation of the skin is common [9]. Although the total clinical presentation among various patients is heterogeneous, a "full-blown" presentation is likely to progressively develop if the condition is left untreated [11].

Biochemical testing of homocystinuria is performed by urinary and preferably blood amino acid profiling [1]. The hallmark of CBS deficiency is increased levels of homocysteine and methionine. Based on biochemical screening of more than 200,000 newborns in various countries, a detection rate of 1 in 344,000 was reported [9]. The actual prevalence is thought to be higher, particular in certain populations such as those of Ireland (1 in 65,000), Norway (1 in 6400), and Qatar (1 in 1800) [12-14]. Its incidence in Iran is unknown. A controversial issue with regard to CBS deficiency is potential pathological consequences of being a heterozygous carrier of a *CBS* mutation [15-17]. This is important because an association between increased plasma homocysteine and cardiovascular disease has been reported [18-21].

To the best of our knowledge, we here report for the first time clinical findings and genetic analysis of Iranian patients affected with CBS deficiency. Correct diagnosis was based on results of genetic analysis.

2. Subjects and methods

2.1. Subjects

The CBS deficiency family (HCU-220) studied here included three affected siblings (HCU-220-4, HCU-220-5, and HCU-220-6) (Fig. 1). Blood samples of the patients and family members were sent to us for genetic analysis. The patients were originally reported to be affected with intellectual disability (ID). With the objective of finding the causative gene of the condition, we proceeded to perform whole genome linkage analysis. Recently, genetic analysis on a large cohort of Iranian ID patients had resulted in identification of 73 causative genes [22]. Autosomal recessive inheritance of ID in the family was inferred on the basis of multiple affected children being born to consanguineous unaffected parents. The family was of Sistani ethnicity, and lived in a remote village in the province of Sistan and Baluchestan. The results, as described below, along with biochemical testings and clinical examinations, led to correct diagnosis. After completion of studies on family HCU-220, three other CBS deficiency families were identified and genetic analyses on these were done as well.

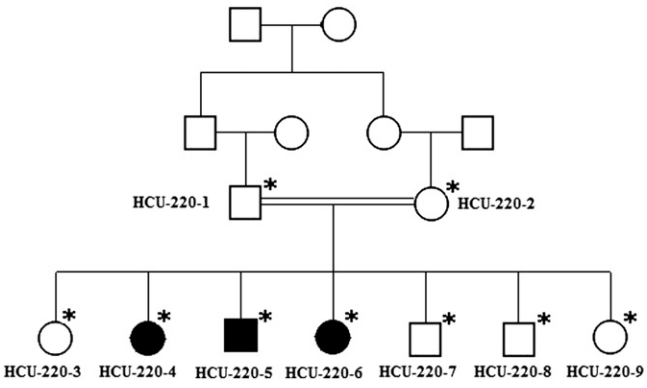

Fig. 1. Pedigree of cystathionine beta-synthase deficiency pedigree HCU-220. ■, ●: affected; □, ○: not affected. *, Individuals on whom genome-wide SNP genotyping was performed.

2.2. Genome-wide genotyping and homozygosity mapping

DNA was isolated according to standard phenol-chloroform methods. Genome-wide single-nucleotide polymorphism (SNP) genotyping was carried out using HumanCytoSNP-12v1-0_D BeadChips and the iScan reader (Illumin; www.illumin.com). Nine individuals, including seven siblings and their parents were genotyped (Fig. 1). SNPs that had not been genotyped in one or more individual and SNPs that exhibited Mendelian error were removed from the analysis. MERLIN was used for linkage analysis under an autosomal recessive model [23]. Additionally, homozygous regions common to the three affected siblings with a minimum length of 1 Mb and absent in non-affected individuals were sought using the Homozygosity Detector Tool within the GenomeStudio V2010.3 program (Illumina). The SNP chip data output was subsequently exported to Microsoft Excel software and homozygous regions were confirmed within the EXCLUDEAR spreadsheet. Genomic regions are reported with reference to Human Genome Build 37.3.

2.3. Mutation screening

The 15 coding exons and flanking intronic sequences of *CBS* in all three affected children were amplified by polymerase chain reaction (PCR) and then sequenced using the dye terminator chemistry (Big Dye kit and the Prism 3700 sequencer; Applied Biosystems, Foster City, CA, USA). Sequences were analyzed with the Sequencher 4.8 software (Gene Codes, Ann Arbor, MI, USA). Sequence variations were identified by comparison with reference sequences available at the National Center for Biotechnology Information: NC_000021.9, NM_000071.2, and NP_000062.1. Having identified the putative disease associated sequence variation in *CBS*, the mutation status was assessed in the remaining members of the family by direct sequencing. The mutation was also screened in CBS deficiency patients of three additional families from the same province who report to be unrelated. Sequences of all primers used are available upon request.

3. Results

3.1. Genetic analysis

Linkage analysis using MERLIN showed that the highest logarithm of odds (LOD) score (2.8) was associated with a region on chromosome 21q22 (Fig. 2A). A score higher than 1.6 was not obtained anywhere else on the genome. The GenomeStudio Homozygosity Detector tool also only identified the same region on 21q22 to be homozygous in the three affected siblings and not homozygous in the unaffected parents and siblings (Fig. 2B). The homozygous region common to the three affected siblings expanded ~14 Mb and was bound by proximal and distal markers, respectively, rs2018308 (21:33898917 bp) and rs7278087 (21:48098824 bp). The locus includes 296 annotated protein coding genes, one of which is *CBS*. *CBS* was considered a candidate causative gene because homocystinuria is accompanied with ID. Sequencing of the gene revealed c.346G>A that causes p.Gly116Arg in the homozygous state in the DNAs of the three affected siblings (Fig. 2C). It was the only variation observed in *CBS*, and this variation was either absent or present in the heterozygous state in the remaining members of the family (Table 1). C.346G>A was previously reported as a *CBS* causative mutation in a Turkish patient who harbored it in the compound heterozygous state [16]. P.Gly116 is positioned close to p.Lys119, which is the putative binding site of phosphopyridoxal phosphate [24]. The PolyPhen (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) bioinformatics tools both predict that p.Gly116Arg is damaging. The results of the genetic analysis suggested that the sequence variation that causes p.Gly116Arg in *CBS* is the cause of disease in family HCU-220, and that the disease of the affected individuals is homocystinuria due to cystathionine beta-synthase deficiency. To obtain confirmatory evidence for this diagnosis and for gaining a more