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Molecular characterization of *CPS1* deletions by array CGH

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Abstract

CPSI deficiency usually results in severe hyperammonemia presenting in the first days of life warranting prompt diagnosis. Most *CPS1* defects are non-recurrent, private mutations, including point mutation, small insertions and deletions. In this study, we report the detection of large deletions varying from 1.4 kb to >130 kb in the *CPS1* gene of 4 unrelated patients by targeted array CGH. These results underscore the importance of analysis of large deletions when only one mutation or no mutations are identified in cases where CPSI deficiency is strongly indicated.

Keywords

CPSI deficiency; Array CGH; Large deletion

1. Introduction

Urea cycle disorders are inherited defects in the disposal of nitrogenous waste from protein. Carbamoyl phosphate synthetase I (CPSI; MIM *608307; EC 6.3.4.16), the first and the rate-limiting step, synthesizes carbamoyl phosphate from ammonia, bicarbonate, and ATP. The reaction requires a cofactor, N-acetylglutamate (NAG), synthesized by N-acetylglutamate synthase. CPSI deficiency represents a continuum of symptoms ranging from neonatal onset, which is often lethal, to late onset at any age in life. The neonatal presentation of CPSI deficiency usually results in severe hyperammonemia presenting in the first days of life warranting prompt diagnosis [1–3]. *CPS1* gene consists of 38 exons and encodes a polypeptide of 1500 amino acids [4]. To date, nearly 100 mutations have been reported and documented in Human Gene Mutation Database (HGMD, <http://www.biobase-international.com>). In addition, about 200 mutations have been identified in a multicenter *CPS1* study of a large patient cohort (Häberle et al., unpublished data). Most of the *CPS1*

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Appendix A. Supplementary data

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mutations are non-recurrent, private mutations. Although small deletions and duplications are relatively common and account for about 20% of total mutations in the above-mentioned study, only a few large deletions and insertions have been reported in *CPS1* gene [5–7]. This is due to the limitation of direct DNA sequencing analysis, which does not detect large deletions. Southern blot, multiplex ligation-dependent probe amplification (MLPA), quantitative real-time PCR (qPCR), and fluorescence in situ hybridization (FISH) are used to detect large deletions. However, these methods are tedious and only useful for the detection of known deletions.

In this study, we report the detection of large deletions in *CPS1* gene by targeted oligonucleotide array CGH, MitoMet®, in 4 unrelated cases. The size of deletion varies from 1.4 kb to >130 kb.

2. Clinical descriptions

Case 1 is a 3-year old girl who initially presented with hyperammonemia, low citrulline, and respiratory alkalosis. PCR failed to amplify exons 9 and 10 of the *CPS1* gene. Deleterious point mutations were not detected in the remaining exons by sequencing analysis. These results suggested a possible homozygous deletion involving exons 9 and 10.

Case 2 was a newborn infant girl presenting in critical condition due to hyperammonemia. Sequence analysis of *CPS1* revealed a heterozygous novel variant, c.2945G>T (p.G982V). Glycine at amino acid position 982 is evolutionarily conserved from yeast to human, and is predicted to be deleterious by computer algorithms, SIFT [10] and PolyPhen [11]. Two other variants at the same amino acid position, c.2945G>A (p.G982D) and c.2944G>A (p.G982S), have been previously reported in patients with CPSI deficiency [12,13]. Collectively, these data suggested that the c.2945G>T (p.G982V) variant was likely pathogenic. However, the second mutant allele could not be identified by sequencing analysis.

Case 3 presented on the second day of life with irritability, feeding problems, and presumed sepsis. Hyperammonemia and low plasma citrulline were noted later. The child died on day of life 9. Postmortem liver analysis showed CPSI deficiency. Sequence analysis of the *CPS1* cDNA derived from cultured fibroblasts revealed an apparently homozygous mutation c.1760G>A (p.R587H) in exon 16 [14]. This mutation has been previously reported in patients with CPSI deficiency [5,12]. Sequence analysis of the parents revealed that the mother was a carrier of the c.1760G>A (p.R587H) mutation, but the father was not. cDNA analysis using RNA from phytohaemagglutinine (PHA)-stimulated white blood cells from the father revealed a deletion of exon 24 in a heterozygous state, suggesting that mRNA from the father's allele was unstable (T. Edkins, personal communication).

Case 4 presented with early-onset hyperammonemia and was referred for the analysis of the *CPS1* gene based on the low plasma citrulline and urine orotic acid. The patient died from metabolic crisis at age 7 months. Genetic analysis revealed an apparently homozygous mutation, c.3484delC in both cDNA and genomic DNA. However, the mutation was only found in the mother, therefore paternal DNA was analyzed by array CGH for the detection of intragenic deletions in this region.

3. Materials and methods

3.1. MitoMet® array CGH

A custom designed clinical oligonucleotide array CGH (MitoMet®) was manufactured using the Agilent microarray platform (Agilent Technologies, Santa Clara, CA). This is a clinically validated 44 K oligonucleotide array with a complete coverage of the mitochondrial genome (6400 probes for the 16.6-kb genome) and 192 nuclear genes related to mitochondrial structure/function and metabolic diseases, including *CPS1*, *NAGS*, *OTC*, *ASS1*, *ASL*, and *ARG1* genes in the urea cycle, with an average spacing of approximately 250 bp/probe [8,9].

4. Results

4.1. Case 1

PCR failed to amplify exons 9 and 10 of the *CPS1* gene. MitoMet® array CGH analysis detected a homozygous deletion measuring at least 1.3 kb (nucleotide position 211,163,672–211,164,925, NCBI36/hg18) in the long arm of chromosome 2, encompassing exons 9 and 10 of the *CPS1* gene (Fig. 1A). PCR across the putative breakpoints and sequence analysis identified junction points to be positions c.841–194 and c.1086+77, corresponding to a 1440 bp deletion including exons 9–10 with a direct repeat of 3 nucleotides (TTG) at the breakpoints (Fig. 1A). The deletion of exons 9–10 is predicted to result in an inframe deletion of the CPSI protein (p.I281_E362del).

4.2. Case 2

Sequencing analysis of the *CPS1* gene revealed a heterozygous mutation, c.2945G>T (p.G982V). Array CGH analysis demonstrated an intragenic deletion encompassing exons 9 to 11 of the *CPS1* gene. Sequence analysis of the PCR products of the deletion junction revealed a deletion of 2395 bp (c.841–212_c.1164+26) with a direct repeat of 5 nucleotides (TATTA) flanking the breakpoints (Fig. 1B) and resulting in an in-frame deletion, p.I281_E388del, of 108 amino acids of the CPSI protein. These results are consistent with the diagnosis of CPSI deficiency.

4.3. Case 3

The deletion of *CPS1* in this case was first detected by cDNA analysis from PHA-stimulated white blood cells and later defined by standard sequencing of the flanking regions of the deletion breakpoint. MitoMet® array CGH confirmed the deletion (Fig. 1C). The 5' end of the breakpoint involved a poly-A tract of greater than 20 bp in size. The deletion size of 2559 bp, including exon 24, is predicted to result in an out-of-frame deletion (p.E966AfsX27).

4.4. Case 4

DNA samples from the deceased child were not available. MitoMet® array CGH analysis of *CPS1* of the proband's father revealed a heterozygous deletion of exons 3–38. The size of deletion was estimated to range from 134 kb to 767 kb based on the backbone probe positions including the extended *CPS1* 3'-UTR region (Fig. 1D). No other known gene is located in the 3' side of the deleted region.

5. Discussion

Oligonucleotide array-based CGH is a powerful tool to detect large intragenic rearrangements [8,9]. The high-density probe coverage of the target coding regions allowed us to identify the approximate break-points (Figs. 1A–D). In this study, the size of deletions ranged from 1.4 kb to >134 kb. Each targeted coding exon is covered on average by 5 probes. The immediate flanking introns are also densely covered. To score a deletion a cut-off of a minimum of 5 consecutively absent probes was set. In our experience, an intragenic deletion as small as 240 bp has been detected (Wong L-J, unpublished observation). When the deletion breakpoints fall outside of the densely probed coding region, it may be difficult to estimate the breakpoint location due to the sparse coverage of backbone region by probes (Fig. 1D).

In cases 1–3, the sequences at the breakpoints were identified by PCR across the deletion junctions, followed by sequencing. Short two to five nucleotide microhomologies at the breakpoints were identified in case 1 (TTG), case 2 (TATTA) and case 3 (AA). Common or recurrent breakpoints were not detected in this study. Analysis of the junction sequences using RepeatMasker (<http://www.repeatmasker.org/>) web tool did not reveal any interspersed or simple repeat sequence homology. ClustalW (<http://www.clustal.org/>) analysis revealed no significant increase of sequence homology in the breakpoint regions compared to homology in a randomly selected *CPSI* gene region. The lack of recurrent deletion breakpoints, a paucity of repeat elements in the *CPSI* genomic environment indicates that nonallelic homologous recombination (NHAR) unlikely to be the mechanism involved in *CPSI* large deletion. On the other hand, the presence of microhomology regions (TTG, TATTA and AA) suggests the possible involvement of microhomology-mediated break-induced replication (MMBIR) or a single event of fork stalling and template switching (FoSTeS X1) in our cases [15–17]. However, the alternative mechanism microhomology-mediated end joining (MMEJ, also known as Ku-independent NHEJ) could not be excluded [18].

Although *CPSI* is larger than *OTC*, large *OTC* deletions account for 5–10% of all *OTC* deficiency cases [9], compared to about 1–2% of *CPSI* deficiency cases (Häberle et al., unpublished data). This may be explained by the unique genomic environment of chromosome X surrounding the *OTC* gene and extend to include the *DMD* and *GK* (glycerol kinase) gene regions [9,19]. All four patients in this study presented with hyperammonemia in early infancy, suggesting that *CPSI* deletions confer risk of the early-onset *CPSI* deficiency. The possibility of large intragenic deletions in the *CPSI* gene should be considered if the clinical presentation, biochemical and/or enzymatic studies strongly support *CPSI* deficiency, but only one or no deleterious mutations were identified. This report demonstrates the great utility of a targeted oligonucleotide array CGH for this purpose [8].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Suzuki Y, Matsushima A, Ohtake A, Mori M, Tatibana M, Orii T. Carbamyl phosphate synthetase I deficiency with no detectable mRNA activity. *Eur. J. Pediatr.* 1986; 145:406–408. [PubMed: 3792387]
2. Finckh U, Kohlschutter A, Schafer H, Sperhake K, Colombo JP, Gal A. Prenatal diagnosis of carbamoyl phosphate synthetase I deficiency by identification of a missense mutation in CPS1. *Hum. Mutat.* 1998; 12:206–211. [PubMed: 9711878]
3. Haberle J, Schmidt E, Pauli S, Rapp B, Christensen E, Wermuth B, Koch HG. Gene structure of human carbamylphosphate synthetase 1 and novel mutations in patients with neonatal onset. *Hum. Mutat.* 2003; 21:444. [PubMed: 12655559]
4. Summar ML, Hall LD, Eeds AM, Hutcheson HB, Kuo AN, Willis AS, Rubio V, Arvin MK, Schofield JP, Dawson EP. Characterization of genomic structure and polymorphisms in the human carbamyl phosphate synthetase I gene. *Gene.* 2003; 311:51–57. [PubMed: 12853138]
5. Summar ML. Molecular genetic research into carbamoyl-phosphate synthase I: molecular defects and linkage markers. *J. Inherit. Metab. Dis.* 1998; 21(Suppl 1):30–39. [PubMed: 9686343]
6. Loscalzo ML, Galczynski RL, Hamosh A, Summar M, Chinsky JM, Thomas GH. Interstitial deletion of chromosome 2q32–34 associated with multiple congenital anomalies and a urea cycle defect (CPS I deficiency). *Am. J. Med. Genet. A.* 2004; 128A:311–315. [PubMed: 15216554]
7. Haberle J, Koch HG. Genetic approach to prenatal diagnosis in urea cycle defects. *Prenat. Diagn.* 2004; 24:378–383. [PubMed: 15164414]
8. Wong LJ, Dimmock D, Geraghty MT, Quan R, Lichter-Konecki U, Wang J, Brundage EK, Scaglia F, Chinault AC. Utility of oligonucleotide array-based comparative genomic hybridization for detection of target gene deletions. *Clin. Chem.* 2008; 54:1141–1148. [PubMed: 18487280]
9. Shchelochkov OA, Li FY, Geraghty MT, Gallagher RC, Van Hove JL, Lichter-Konecki U, Fernhoff PM, Copeland S, Reimschisel T, Cederbaum S, Lee B, Chinault AC, Wong LJ. High-frequency detection of deletions and variable rearrangements at the ornithine transcarbamylase (OTC) locus by oligonucleotide array CGH. *Mol. Genet. Metab.* 2009; 96:97–105. [PubMed: 19138872]
10. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res.* 2001; 11:863–874. [PubMed: 11337480]
11. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res.* 2002; 30:3894–3900. [PubMed: 12202775]
12. Kurokawa K, Yorifuji T, Kawai M, Momoi T, Nagasaka H, Takayanagi M, Kobayashi K, Yoshino M, Kosho T, Adachi M, Otsuka H, Yamamoto S, Murata T, Suenaga A, Ishii T, Terada K, Shimura N, Kiwaki K, Shintaku H, Yamakawa M, Nakabayashi H, Wakutani Y, Nakahata T. Molecular and clinical analyses of Japanese patients with carbamoylphosphate synthetase 1 (CPS1) deficiency. *J. Hum. Genet.* 2007; 52:349–354. [PubMed: 17310273]
13. Eeds AM, Hall LD, Yadav M, Willis A, Summar S, Putnam A, Barr F, Summar ML. The frequent observation of evidence for nonsense-mediated decay in RNA from patients with carbamyl phosphate synthetase I deficiency. *Mol. Genet. Metab.* 2006; 89:80–86. [PubMed: 16737834]
14. Rapp B, Haberle J, Linnebank M, Wermuth B, Marquardt T, Harms E, Koch HG. Genetic analysis of carbamoylphosphate synthetase I and ornithine transcarbamylase deficiency using fibroblasts. *Eur. J. Pediatr.* 2001; 160:283–287. [PubMed: 11388595]
15. Hastings PJ, Ira G, Lupski JR. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* 2009; 5:e1000327. [PubMed: 19180184]
16. Zhang F, Carvalho CM, Lupski JR. Complex human chromosomal and genomic rearrangements. *Trends Genet.* 2009; 25:298–307. [PubMed: 19560228]
17. Zhang F, Gu W, Hurles ME, Lupski JR. Copy number variation in human health, disease, and evolution. *Annu. Rev. Genomics Hum. Genet.* 2009; 10:451–481. [PubMed: 19715442]

18. Lieber MR. The mechanism of human nonhomologous DNA end joining. *J. Biol. Chem.* 2008; 283:1–5. [PubMed: 17999957]
19. Stanczak CM, Chen Z, Zhang YH, Nelson SF, McCabe ER. Deletion mapping in Xp21 for patients with complex glycerol kinase deficiency using SNP mapping arrays. *Hum. Mutat.* 2007; 28:235–242. [PubMed: 17089405]

Fig. 1. MitoMet® array CGH results. A. A homozygous deletion encompassing exons 9–10 of the *CPS1* gene was detected. PCR across the deletion junction and sequencing confirmed a 1440 bp deletion from c.841–194 to c.1086+77, which is predicted to be an in-frame deletion (p.I281_E362del). B. A heterozygous deletion encompassing exons 9–11 of the *CPS1* gene was detected. A 2395 bp deletion was confirmed by PCR and sequencing, which is predicted to be an in-frame deletion (p.I281_E388del). C. A heterozygous deletion of exon 24 of *CPS1* gene was detected by MitoMet® array CGH and confirmed by PCR and sequencing. The 5' end of the deletion junction included a poly-A tract, the size of the deletion is 2559 bp. The deletion of exon 24 is predicted to be a frameshift deletion (p.E966AfsX27). D. A large heterozygous deletion encompassing intron 2 to 3' UTR was detected by MitoMet® array CGH. The size of deletion ranges from 134 kb to 767 kb based on backbone probe positions. The deletion does not include any known genes at the 3' end of *CPS1* gene.