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Case Report

A novel *DDC* gene deletion mutation in two Chinese mainland siblings with aromatic L-amino acid decarboxylase deficiency

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Abstract

Background: Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM #608643) is a rare and severe disorder of biogenic amine synthesis caused by mutations in the *DDC* gene. The phenomenology of the movement disorder includes intermittent oculogyric crises and limb dystonia, generalized athetosis, and impaired voluntary movement.

Objective: To identify clinical manifestations and *DDC* gene mutations in two Chinese mainland children who are siblings with AADC deficiency.

Methods: We used targeted next-generation sequencing and quantitative polymerase chain reaction (qPCR) to reveal *DDC* mutations in these children.

Results: Two *DDC* gene mutations were found: one missense mutation, c.1040G > A (p.Arg347Gln), is a reported mutation derived from the mother; the other mutation, a whole-exon 11 and 12 deletion, is a novel mutation derived from the father. The index patient and her brother both had poor sucking power and feeding difficulty at birth and episodes of oculogyric crises, truncal hypotonia, limb hypertonia, sleep disturbances, irritability, and motor delay. The siblings both died at 1 year and 10 months due to asphyxia and pneumonia during gaze and hypertonia episodes.

Conclusion: This study identified a novel *DDC* gene deletion mutation in two siblings with AADC deficiency disease in the Chinese mainland population.

Keywords: Aromatic L-amino acid decarboxylase deficiency; AADC; *DDC*; Gene mutation; Deletion

1. Introduction

Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM #608643) is a congenital autosomal recessive metabolic disorder first identified in 1990 by Hyland et al. [1]. Clinical manifestations of AADC deficiency include hypotonia, hypokinesia, oculogyric crises, and signs of autonomic dysfunction that begin in infancy. The characteristic pattern of cerebrospinal fluid (CSF) abnormalities in patients with AADC deficiency includes the following: low homovanillic acid and 5-hydroxyindoleacetic acid levels; elevated L-dopa, 5-hydroxytryptophan, and 3-*ortho*-methyldopa levels; and normal protein levels. The diagnosis of AADC deficiency is often delayed for several years, with most

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patients receiving nonspecific diagnoses, such as cerebral palsy, before a definitive diagnosis can be obtained. CSF neurotransmitter analysis is the primary method for detecting neurotransmitter disorders [2–4]. However, CSF analysis requires lumbar puncture, which is invasive and generally not considered for patients exhibiting nonspecific presentations. Thus, AADC deficiency is likely under diagnosed by targeted next-generation sequencing to screen for probable pathogenic mutations. Here, we report a Chinese mainland family with AADC deficiency caused by two mutations in the *DDC* gene: one missense mutation, c.1040G > A (p.Arg347Gln), that is a reported mutation derived from the mother and a whole-exon 11 and 12 deletion that is a novel mutation derived from the father.

2. Materials and methods

The sibling patients' diagnoses were suspected based on clinical signs and symptoms and were supported by identified *DDC* gene mutations. This study was approved by the Beijing Children's Hospital Medical Ethics Committee (IRB approval number: 2014-10).

Genomic DNA was extracted from peripheral blood leukocytes derived from the patients and their family members. We employed targeted next-generation sequencing (NGS) to screen for probable pathogenic mutations. A custom-designed panel capturing the coding exons of 240 genes associated with developmental delay and genetic disease, including *DDC*, was synthetized using the Agilent Sure-Select Target Enrichment technique. We used Sanger sequencing to validate the probable pathogenic variation identified by targeted NGS and to determine parental origin. The mutation c.1040G > A in exon 11 in the patients is a homozygous mutation according to Sanger sequencing. The patients' mother carries a heterozygous mutation at this position, whereas the father does not. We utilized fluorescent quantitative PCR (qPCR) to evaluate the presence of a deletion around exon 11 of the *DDC* gene (NM_000790.3) and to determine parental origin [5]. Exons 10, 11, 12 and 13 were assessed using primers designed at http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi (Exon10F: AGAGGTGGTCCAGTCT AGGGTAC;R:TCCACAGAAAGCAGGCTCACAGC; Exon11F: AACACCCAGACCCAGCCTT TGG;R:CC CAGCACTCCACTAGCATTTGAG; Exon12F: CAA GAGAAATAGGCGAGCCGGTG;R:CTTGCGGAT ATAAGCCTGCAGTCC; Exon13F:TCCTCAGAGA CCTGAGCACAGTTC;R:CAGATGGCAAAGCGCA GGACAAAC). Each 20-μl qPCR reaction included 1 μl genomic DNA extracted from peripheral blood, 10 μl 2 × supermix, 0.5 μl Primer F, 0.5 μl Primer R, and 0.5 μl ddH_2O. The PCR reaction conditions were as follows: denaturation at 95 °C for 15 min and then 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s. The fluorescence intensity (SYBR Green I) of the PCR products was examined, and a melting/dissociation curve stage was generated. The Ct (ΔΔCT) method was used to evaluate the number of copies of the exons compared with the reference gene *ALB*. For ΔCT(normal) = CT (target gene)-CT (reference gene), ΔCT(test) = CT(target gene)-CT (reference gene), ΔΔCT = ΔCT(test)-ΔCT (normal), $0.7 \leq 2^{-\Delta\Delta CT} \leq 1.3$ is normal, $2^{-\Delta\Delta CT} < 0.7$ is a deletion mutation, and $2^{-\Delta\Delta CT} > 1.3$ is a duplication mutation. The novel mutations identified in the patients were assessed in 100 controls.

3. Results

3.1. Clinical features of two Chinese mainland siblings with AADC deficiency

Patient 1 This index patient was a Chinese mainland girl born at 38^{+2} weeks gestation via elective cesarean section with a birth weight of 2.9 kg and good Apgar scores of 10 at 1 and 5 min. At birth, she had poor sucking power and feeding difficulty. At 2.5 months of age, it was noticed that she had frequent episodes of eye deviations, which was manifested as maintaining esotropia or gaze for a few seconds or minutes, with or without screaming. After sleeping, these symptoms were alleviated. She had frequent oculogyric crises in the evening, especially when she was fatigued. She had a history of sleep disturbances, with several night-time awakenings. She was found to have truncal hypotonia, limb hypertonia, bilateral ptosis, irritability, and increased startle response. Motor delay was observed. She could control her head for several seconds after birth but by 2 months of age, could not develop head and truncal control. She was never able to sit and crawl, nor was she able to walk with assistance or independently. Her motor skills were best in the morning, and she required frequent naps throughout the day. Cognitive delay was present. When she first presented at 1 year and 9 months of age, clinical examination showed her to be a cheerful and well-grown child (weight and height at 50th percentile). She was able to smile at people but had no speech development. She was only able to walk with assistance. She drooled intermittently and fell asleep during examination. She had poor neck and trunk control but could sit with assistance; while sitting, her head would fall forwards intermittently. There was mild bilateral ptosis, and she was able to sustain upward gaze for only 10 s. The range of extra ocular eye movements and fundoscopy were normal. She was dysarthric and was noted to speak "baba" or "mama". Mild truncal hypotonia and limb hypertonia were observed, but deep tendon reflexes were normal. Power in all limbs was grade 4, but she was unable to sustain motor effort for more than 5 s. Speech was dysarthric, but there were no other cerebellar signs. Brain magnetic resonance imaging (MRI) was normal.

Patient 2 The index patient's brother presented the same symptoms. The brother was born at 40^{+2} weeks gestation with cesarean delivery due to fetal distress and III° amniotic fluid pollution. He had poor sucking power and feeding difficulty at birth and episodes of oculogyric crises, truncal hypotonia, limb hypertonia, sleep disturbances, irritability, and motor delays, similar to his sister. The index patient and her brother both died at 1 year and 10 months due to asphyxia and pneumonia during gaze and hypertonia episodes. Their parents are healthy.

3.2. Identification of DDC mutations

Both the patients and their brother carried a heterozygous mutation, c.1040G > A (Fig. 1), which was also found in the mother. This reported missense mutation changes an amino acid: p.Arg347Gln [6]. qPCR results for *DDC* gene exons 10, 11, 12 and 13 in the sibling patients and their parents. The $2^{-\Delta\Delta Ct}$ value of exon 10 for the index patient is 1.06 and brother is 1.17, and that of the father is 1.02 and mother is 1.11, which are normal values (normal is $0.7 \leq 2^{-\Delta\Delta CT} \leq 1.3$). The $2^{-\Delta\Delta Ct}$ value of exon 11 for the index patient is 0.51 and brother is 0.51, and that of the father is 0.49 and mother is 1.14, indicating a heterozygous deletion mutation in index patient, brother and father ($2^{-\Delta\Delta CT} < 0.7$ is a deletion mutation). The $2^{-\Delta\Delta Ct}$ value for exon 12 of the index patient is 0.51 and brother is 0.55, and that of the father is 0.55 and mother is 1.01, indicating a heterozygous deletion mutation in index patient, brother and father ($2^{-\Delta\Delta CT} < 0.7$ is a deletion mutation). The $2^{-\Delta\Delta Ct}$ value for exon 13 of the index patient is 1.02 and brother is 1.07, and that of the father is 1.01 and mother is 0.98, which are normal values (normal is $0.7 \leq 2^{-\Delta\Delta CT} \leq 1.3$). Thus, the whole-exon 11 and 12 deletion in the sibling patients was derived from their father (Fig. 2). This whole-exon 11 and 12 deletion mutation is novel. Sequencing of 100 normal local controls did not reveal either mutation.

4. Discussion

AADC deficiency is an autosomal recessive inherited disease. As the AADC enzyme decarboxylates L-dopa and 5-hydroxytryptophan (5-HT), deficiency results in impaired synthesis of dopamine and serotonin. The gene encoding AADC (*DDC* gene) maps to chromosome 7p12.2-p12.3 and is composed of 15 exons spanning 85 kb. Symptoms of deficiency include poor sucking power, feeding difficulty, ptosis, lethargy, hypothermia, and hypotension during the neonatal period. Neurologic dysfunction and movement abnormalities are common [7,8]. Both the index patient and her brother in our report exhibited oculogyric crises, truncal hypotonia, limb hypertonia, sleep disturbances, irritability, and

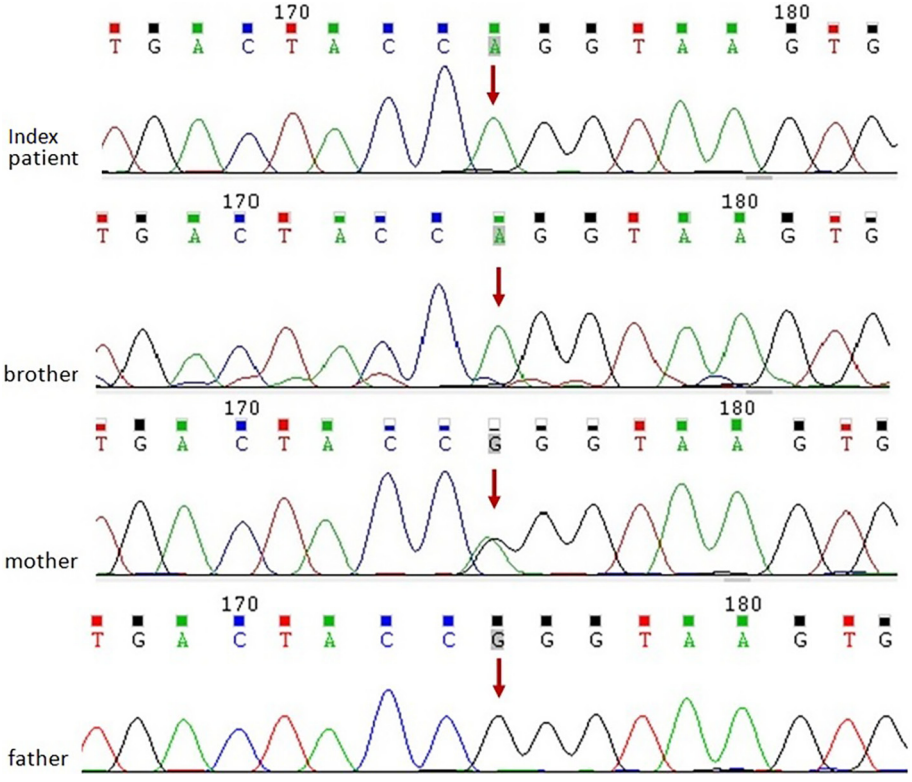

Fig. 1. A heterozygous mutation (c.1040G > A) in the index patient and her brother, which has been reported, that was derived from their mother.

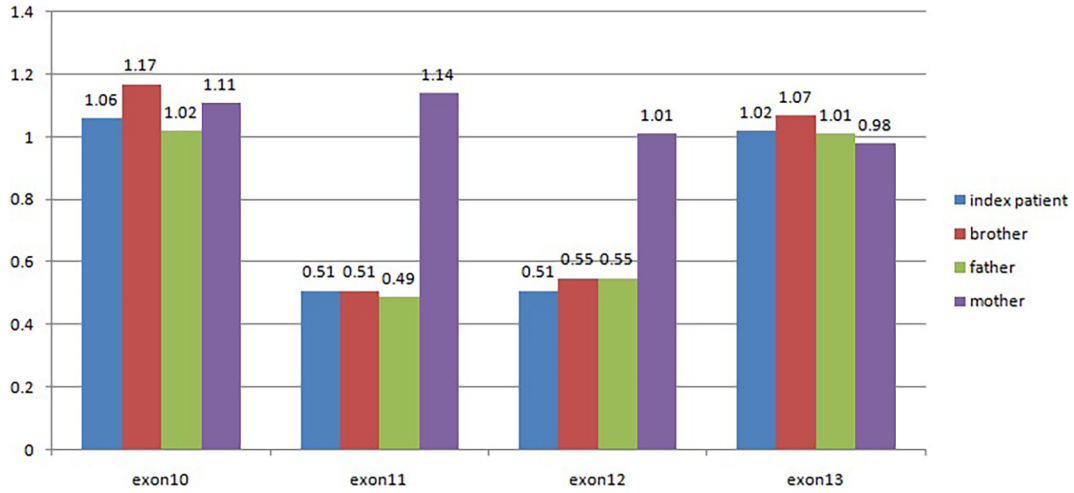

Fig. 2. qPCR results for *DDC* gene exons 10, 11, 12 and 13 in the sibling patients and their parents. The $2^{-\Delta\Delta Ct}$ value of exon 10 for the index patient is 1.06 and brother is 1.17, and that of the father is 1.02 and mother is 1.11, which are normal values (normal is $0.7 \leq 2^{-\Delta\Delta CT} \leq 1.3$). The $2^{-\Delta\Delta Ct}$ value of exon 11 for the index patient is 0.51 and brother is 0.51, and that of the father is 0.49 and mother is 1.14, indicating a heterozygous deletion mutation in index patient, brother and father ($2^{-\Delta\Delta CT} < 0.7$ is a deletion mutation). The $2^{-\Delta\Delta Ct}$ value for exon 12 of the index patient is 0.51 and brother is 0.55, and that of the father is 0.55 and mother is 1.01, indicating a heterozygous deletion mutation in index patient, brother and father ($2^{-\Delta\Delta CT} < 0.7$ is a deletion mutation). The $2^{-\Delta\Delta Ct}$ value for exon 13 of the index patient is 1.02 and brother is 1.07, and that of the father is 1.01 and mother is 0.98, which are normal values (normal is $0.7 \leq 2^{-\Delta\Delta CT} \leq 1.3$). Thus, the whole-exon 11 and 12 deletion in the sibling patients was derived from their father.

motor delay. Molecular analysis showed both the index patient and her brother to be compound heterozygotes with the missense mutation c.1040G > A, which has been reported [6], and a novel whole-exon 11 and 12 deletion. Due to the heterogeneity of known mutations, sequencing of the entire *DDC* gene is necessary for genetic diagnosis of AADC deficiency. Since the initial description of the index family in 1990, approximately 100 patients have been described in case reports or case series to date. Although the global incidence of AADC deficiency is unknown, it is higher in specific Asian (especially Taiwanese and Japanese) populations, most likely due to a founder effect. Various mutations in AADC-deficient patients have been identified, most of which are missense mutations and large deletions [9]. The intervening sequence (IVS) 6 + 4 A > T mutation, which might be common among Taiwanese AADC-deficient patients, can be homozygous or heterozygous with another heterozygous missense or insertion mutation [10]. For example, Jie Zhu et al reported one Chinese mainland patient carrying c.1063dupA (p.I355fs) and c.250A > C (p.S84R) [11].

Chien YH et al demonstrated that an elevated concentration of L-dopa metabolite 3-O-methyldopa (3-OMD) in dried blood spots can be integrated into newborn screening programs to precisely predict AADC deficiency [3]. Currently, no effective drug treatment exists for AADC deficiency, and patients typically die at approximately 5–6 years of age [9]. The mutation c.1040G > A our siblings taking was reported as a small influence on the phenotype of AADC deficiency [12], but the siblings were died earlier at 1 year and 10 months of age, most likely as a result of their whole-exon 11 and 12 deletion mutation which may induce flame shift and early termination of AADC protein and more functional analyses are required. The mainstay of treatment for AADC-deficient patients is dopamine (DA), monoamineoxidase (MAO) inhibitors and vitamin B6, a cofactor for AADC. Response to treatment varies. Males appear to respond favorably to therapy, which had been reported previously [13]. Lee HF et al reported cases of two Taiwanese AADC-deficient siblings with an unusually mild phenotype and slow clinical progression. Both carried the compound heterozygous mutations IVS 6 + 4 A > T and 853C > T and showed excellent response to vitamin B6, MAO inhibitor and DA. However, whether a homozygous or heterozygous IVS 6 + 4 A > T mutation, all the cases reported exhibit poor response to treatment [14].

In conclusion, AADC deficiency should be considered in the differential diagnosis of patients who have small hands and feet and symptoms of oculogyric crises, floppiness, and dysautonomia. Clinical outcomes of AADC deficiency remain poor. More patients need to be collected and more research should be performed to clarify these findings. In addition, a systemic study needs to be carried out to assess the incidence of AADC deficiency and the mutation spectrum in Chinese mainland populations. Further functional studies are needed to delineate the correlation between *DDC* gene defects and the pathogenesis of AADC deficiency.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.braindev.2018.08.003.

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