Novel Mutation in the ATP-Binding Cassette Transporter A3 (ABCA3) Encoding Gene Causes Respiratory Distress Syndrome in A Term Newborn in Southwest Iran

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1. Introduction

ABCA3 glycoprotein belongs to the ATP-binding cassette (ABC) superfamily of transporters which can be found in all organisms from smallest prokaryotes to human. These proteins utilize the energy derived from hydrolysis of ATP for the translocation of a wide variety of substrates across the plasma membrane. Mutations in the ABCA3 gene are knowingly causative for fatal surfactant deficiency, particularly respiratory distress syndrome (RDS) in term babies.

Case Presentation: In this study, Sanger sequencing of the whole ABCA3 gene (NCBI NM_001089) was performed in a neonatal boy with severe RDS. A homozygous mutation has been identified in the patient. Parents were heterozygous for the same missense mutation GGA > AGA at position 202 in exon 6 of the ABCA3 gene (c.604G > A; p.G202R). Furthermore, 70 normal individuals have been analyzed for the mentioned change with negative results.

Conclusions: Regarding Human Genome Mutation Database (HGMD) and other literature recherche, the detected change is a novel mutation and has not been reported before. Bioinformatics mutation predicting tools prefer it as pathogenic.

Keywords: Surfactant, ABCA3 Gene Mutation, Respiratory Distress Syndrome (RDS), Southwest Iran

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2. Case Presentation

The index patient was a 2.8 kg full-term male infant born after an uncomplicated pregnancy at 38 weeks gestational age, via a vaginal delivery, to healthy and consanguineous parents (first cousin couple). His symptoms demonstrated atelectasis, abnormal lung findings and strenuous respiration. There was no family history of lung disease, abortion, mental retardation. He developed severe respiratory distress syndrome.

The patient was urgently hospitalized, ventilation and other routine cares were applied, but unfortunately he was resistant to treatments and died in early days of life.

2.1. Genetic Analysis

Genomic DNA was prepared from blood leukocytes (10 mL) of the proband, his parents and 70 matched unrelated controls (from related ethnicity) without any respiratory distress syndrome by routine salting out protocol.

The 30 coding regions (exons 3 - 32) of the ABCA3 gene (NCBI NM_001089) and the intron-exon boundaries were amplified by PCR with designed primer pairs using Primer3 software (Table 1) (http://primer3.ut.ee/).

Polymerase chain reaction contained: 100 ng genomic DNA, 12.5 µL Master Mix (Amplicon Co.), 25 pmol of each primer and the total of PCR volume was 25 µL. The PCR was done in 35 cycles: 93°C for 1 minute, 63°C for 30 s and 72°C for 30 s and finally the productions of PCR put in the temperature 72°C for 5 minutes to complete extension. PCR products were subsequently sequenced using same primers as mentioned in Table 1 and Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems 3500 DNA Analyzer). Finally, chromatograms were read and analyzed with by Chromas and DNA Baser v4 software.

| Primer | Sequence |
|--------|----------|
| abca3-E3-F | CTCTGGGCTTTGCCATATGCTG |
| abca3-E3-R | AGACAGCCCTCTCTTCAGAG |
| abca3-E4-F | CTTGGGGAAGGGGCTTCCTG |
| abca3-E4-R | CGTGGCCACACAGATCTAGG |
| abca3-E5-F | CCTGAGCAAGCAGATCTAGG |
| abca3-E5-R | CGATGGCCTCTGACTGCT |
| abca3-E6-F | CCTGCTGTCGATCTCTCAGG |
| abca3-E6-R | TGTGACACTAGTCACACTAGC |
| abca3-E7-F | AGGGTTTCTGAGATGTTGGG |
| abca3-E7-R | GGAAGCTCGGACTCAAGGCC |
| abca3-E8-F | CTCTCCGGCTCTCACTCCA |
| abca3-E8-R | AGTCTCCTGCTCTCACCCTCCT |
| abca3-E9-F | CAGCCTCTGCTGTATTTCACA |
| abca3-E9-R | CCCTTCTGAGGCGCAGTAC |
| abca3-E10-F | TGATTCCGGAAAAAGAACAGGCT |
| abca3-E10-R | AGACATGCTGCACTGGAGG |
| abca3-E11-F | ACCCTTCGACACTGAGGAGCC |
| abca3-E12-F | GTTGGCTTGGCTGCTGCAAA |
| abca3-E12-R | ACTGCCCCGCTGTAGATCT |
| abca3-E13-F | GAGCCAGGAGGGCTTCG |
| abca3-E13-R | TGAAGTGGTCTGATTAAAGGGG |
| abca3-E14-F | GAGATCGACTGTAAGG |
| abca3-E14-R | TCTAGACATGATCTGAG |
| abca3-E15-F | TTCCCTCTCATGGAGACCTC |
| abca3-E15-R | GTGAGCAAGGAGGGAAAC |
| abca3-E16-F | GTGGAGGCTCTACAGCAGTC |
| abca3-E16-R | AAGTGAGCCATATCCCTTC |
| abca3-E17-F | GGATCCAGCAAGAGAACCTC |
| abca3-E17-R | GGTGATCCATCTTTGAGATG |
| abca3-E18-F | GGGGTTGGTCTTGTAGG |
| abca3-E18-R | GAGCCAGGGCTCTCAGG |
| abca3-E19-F | ACCATATGCTCTCCTCCAC |
| abca3-E19-R | GGCTTTGACTGAGGGGCT |
| abca3-E20-F | GCACACAGGACAGACCT |
| abca3-E20-R | ATACCCGAGAAGACCCACCTC |
| abca3-E21-F | CCTGGCTTCCTCTCAGG |
| abca3-E21-R | GTAGCTGACTGTAAGG |
| abca3-E22-F | ATACCCGAGAAGACCCACCTC |
| abca3-E22-R | GTAGCTGACTGTAAGG |
| abca3-E23-F | GTAGCTGACTGTAAGG |
| abca3-E23-R | CTGAGGCTCTCCTCTG |
| abca3-E24-F | GTTGGAGGCTCTAGG |
| abca3-E24-R | GCAGCTGACAGGCGTACAG |
| abca3-E25-F | GAACTTGGAAGGGGAGG |
| abca3-E25-R | AGAGACGTGGGGAGCATC |
| abca3-E26-F | CCAAGCTCCTCACCACCTC |
| abca3-E26-R | GTAGCTGACTGTAAGG |
| abca3-E27-F | GGAGCTGACTGTAAGG |
| abca3-E27-R | CTGAGGCTCTCCTCTG |
| abca3-E28-F | GTAGCTGACTGTAAGG |
| abca3-E28-R | TGTATGGGAAAGCTTGT |
| abca3-E29-F | AACTCATGCTGACCTTCCCC |
| abca3-E29-R | ACCAAGATGCTGATGGCTTC |
| abca3-E30-F | GTAGCTGACTGTAAGG |
| abca3-E30-R | GTAGCTGACTGTAAGG |
| abca3-E31-F | GAAGCTGGAAGGGGAGC |
| abca3-E31-R | CAATGCTGACTGTAAGG |
| abca3-E32-F | CTATGCTGGAAGGAGC |
| abca3-E32-R | GATCTGCGATGCTGACTT |

Table 1. Primers Used for the Amplification of the ABCA3 Gene
Figure 1. Sequence Analysis of Entire Coding Exons and the Flanking Intronic Regions of the ABCA3 Gene

(A)

(B)

(C)

Sequence analysis of entire coding exons and the flanking intronic regions of the ABCA3 gene from affected infant revealed a homozygous missense mutation GGA→AGA substitution at exon 6, which causes an amino acid exchange of glycine to arginine at codon 202 (p.Gly202Arg/G202R). In this way, his healthy parents were heterozygous for the same missense mutation, while sequence analysis of exon 6 of healthy control individuals demonstrated no change at this position (Figure 1).

3. Discussion

ABCA3 is a member of ABCA subfamily that has a role in transport of lipids and it is most likely that ABCA3 has same work. The ABCA3 gene is larger than other proteins genes involved in surfactant. Nevertheless, it is reasonable that more mutations event in ABCA3 gene, also because the maximum amount of pulmonary surfactant consists of lipids which in turn are transported by ABCA3 proteins. In this view, ABCA3 gene mutations appear to be the most essential cause of surfactant metabolism and respiratory diseases in newborns.

RDS is a multi-factorial disease with a variety range of clinical symptoms, often occurs in preterm infants and ABCA3 gene mutations are considered as an important factor causing heredity RDS. Otherwise, neonatal RDS is major cause of morbidity for prematurely born infants. Some of ABCA3 gene mutations cause fatal respiratory distress syndrome of newborns while others of moderate mutations result in chronic interstitial lung disease. The most reported types of mutations with clinical significance are missense, deletions, splicing and insertion mutations (Table 2).

Interestingly, the risk of affected RDS is much more in male than in female neonates. To date, there is no rapid and true test for detecting ABCA3 deficiency; indeed some of children with ABCA3 mutations have undergone lung transplantation for their respiratory failure. Other treatment options for mild cases are ventilation, oxygenation and surfactant replacement.
Table 2. Types of Currently Reported ABCA3 Gene Mutations (http://www.hgmd.cf.ac.uk/)

| Mutation Type              | Number of Mutations |
|----------------------------|---------------------|
| Missense/nonsense          | 56                  |
| Splicing                   | 8                   |
| Regulatory                 | 0                   |
| Small deletions            | 12                  |
| Small insertions           | 5                   |
| Small indels               | 1                   |
| Gross deletions            | 0                   |
| Gross insertions/duplica-  | 0                   |
| tions                      |                     |
| Complex rearrangements     | 0                   |
| Repeat variations          | 0                   |
| Total                      | 82                  |

ABCA3 gene analysis revealed one unreported homozygous mutation in the patient, but both parents were heterozygous (carrier) with normal lung function.

In this case, it is likely the missense mutation G202R (Gly202Arg substitution) in exon 6 caused dysfunction of the ABCA3 protein, changing the polarity of amino acid chain, which surely must affect the functionality. This mutation has never been reported in either public data base of single nucleotide polymorphisms (http://www.ncbi.nlm.nih.gov/SNP/), and is located in the first extracellular loop, which intervenes in extracellular interactions that probably have severe effect on protein function.

To confirm the pathogenicity of the detected missense mutation, 70 unrelated healthy (without any clinical signs of lung disease) were searched for the mentioned change.

We used the sorting intolerant from tolerant (SIFT), polymorphism phenotyping 2 (Polyphen 2), predict SNP and mutation taster (www.mutationtaster.org) software to predict pathogenicity of detected mutation and its impact on ABCA3 function. All the mentioned programs predicted G202R as damaging. The clinical phenotype, genetic findings and computational analysis support its deleterious nature. However, our finding would expand the mutation database of the ABCA3 gene and might be useful for further individual screening, at least in Iran.

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