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Review Article
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Severe Phenotype of De Barsy Syndrome in Two Siblings with Novel Mutations in the *ALDH18A1* Gene

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Abstract

De Barsy syndrome is a rare autosomal recessive genetic disorder characterized by growth retardation, intellectual disability, a prematurely-aged appearance (progeroid features) and loose skin (cutis laxa) as well as eye abnormalities and others. Some cases of de Barsy syndrome have been linked with mutations *PYCR1* or *ALDH18A1*. We describe a family with two siblings with clinically severe de Barsy syndrome in whom two novel mutations in *ALDH18A1* (p.Glu100* and p.Arg724His) were found by clinical exome sequencing using TruSight One panel. The p.Glu100* is a novel mutation predicted to cause absence of the protein. The p.Arg724His has been found with low frequency (0.000016) but not in association with human disease; it has been scored as pathogenic by CADD, MetaSVM, Polyphen2, MutationAssessor, SIFT and MutationTaster. The level of ammonia in serum was determined in second sibling and was in normal range. Amino acid profile in serum revealed decreased concentration of arginine, cytruline, homocysteine, PHE and ornithine. The patients suffer from severe symptoms of GERD such as vomiting, feeding problems instead of multistage therapy including Nissen fundoplication procedure as well as from epilepsy requires complex multidrug therapy. L-Arginine (200 mg/kg) and citrulline (100 mg/kg) were supplemented in the second sibling. The disease leads to premature apoptosis, so antioxidants (coenzyme Q, vitamin A and E) as well as carnitine were supplemented but without spectacular clinical results.

We provide clinical description of severe phenotype of de Barsy syndrome. Our molecular report broadens the spectrum of *ALDH18A1* mutations causing de Barsy syndrome.

Keywords: De Barsy syndrome; ALDH18A1 gene; Recessive mutations; Amino acids profile; Severe developmental delay

Introduction

De Barsy syndrome known as De Barsy-Moens-Dierckx syndrome is an ultrarare, autosomal recessive disease characterized by distinctive, dysmorphic facial features suggesting in the neonatal period progeria-like appearance, cutis laxa, ocular defects and orthopedic abnormalities as well as athetoid movements, developmental delay and intellectual disability [1-4]. Whereas the genetic defect in de Barsy syndrome cannot be always established, the known causes are recessive mutations in *PYCR1* gene (OMIM179035), encoding pyrroline-5-carboxylate reductase 1 as well as *ALDH18A1* gene (OMIM138250), encoding D1-pyrroline-5-carboxylate synthase (P5CS) [5-7]. Defects of the *ALDH18A1* and *PYCR1* genes also cause separate entities such as autosomal recessive cutis laxa type I (OMIM219100), autosomal recessive cutis laxa type II (ARCL2; OMIM219200), wrinkly skin syndrome (WSS; OMIM278250), gerodermia osteodysplastica (OMIM231070) or hyperammonemia, hypoornithinemia, hypocitrullinemia, hypoargininemia and hypoprolinemia [8,9].

We have ascertained a family with two siblings with clinical diagnosis of severe de Barsy syndrome molecularly confirmed by identification of two novel mutations in the *ALDH18A1* gene.

Clinical Report

First proband was the third male child of healthy non-consanguineous couple and was born at 38 weeks of gestation by natural delivery after complicated pregnancy (hypotrophy) with following birth parameters: weight 2460 g (-2,1 SD), length 50 cm, OFC 31 cm, Apgar score 9 points. After birth hypotonia, feeding and sucking problems as well as facial dysmorphism suggesting progeria-like appearance, irregular lack of subcutaneous tissue, prominent vessels on the skin and wrinkling skin were observed. Moreover, severe GERD was diagnosed requiring Nissen fundoplication procedure and gastrostomy. Developmental milestones were severely delayed. The neurological examination showed spasticity and severe athetoid movements involving limbs and head. With time the abnormal movements were exaggerated together with hypersensitivity to the touch and anxiety.

Brain NMR study showed hypoplasia of corpus callosum. Ophthalmological examination revealed cloudy corneas; during re-consultation bilateral cataract were diagnosed needed surgical procedure. Hearing test showed bilateral deafness (70dB). EEG results performed several times were abnormal, but revealed no specific pattern that could suggests a particular the type of epilepsy.

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Facial dysmorphism was present including microcephaly, sharpened facial features, large fontanelles, shallow eyeballs and prominent eyes, wrinkling skin in periorbital region, infra-orbital creases, small, short nose, long philtrum and prominent ears. Moreover, atrophy of subcutaneous tissue, dry and thin skin, wrinkling skin, especially on feet and hands as well as diffuse and protuberant abdomen. The child died at the age of 2 years with respiratory and circulatory insufficiency.

The second proband was a male born to the same parents at 38 weeks with hypotrophy (weigh birth 2040 g, -2,3 SD). The same clinical symptoms were observed as in the older brother: hypotrophy, hypotony, sucking and feeding problems, facial and skin dysmorhism. The boy was operated on because of bilateral inguinal hernias and GERD (Nissen fundoplication and gastrostomy procedures were performed). Severe feeding problems, failure of thrive, permanent vomiting, psychomotor and developmental delay with hypotony, facial and skin dysmorphism, severe athetoid movements and lack of social contact were observed. Additional examinations revealed: abnormal EEG (variably localized changes alternately), vision problem in VEP study, hypoplasia of corpus callosum in MRI of brain, cloudy corneas (bilateral cataract) in ophthalmological examinations. The child requires complex multidrug therapy. Treatment with valproic acid and levetiracetam led to no clinical improvement. Replacement of valproic acid with lamotrigine improved neither the seizures (reduction in seizure frequency) nor the EEG (electroencephalography) pattern reaching a plateau in terms of severity of the clinical condition and then remaining stable.

Then clinical suspicion of de Barsy syndrome was considered.

Genetic Studies

Written informed consent was obtained prior to genetic testing from all subjects or their legal guardians.

The chromosomal analysis of first proband revealed a normal male karyotype (46,XY). *PLP1* mutation screening (MLPA: Multiplex Ligation-Dependent Probe Amplification, direct sequencing) didn't revealed any abnormality. Moreover, no subtelomeric aberrations or interstitial microdeletion syndromes were found on MLPA testing (P070-A2 human telomere-5, P245-A2 microdeletion syndromes-1). Next, array-comparative genomic hybridization (array-CGH) was performed and showed two microduplications (Agilent 180 K, hg 18): 5q23.3 (0,9 kbp) including the *FBN2* gene and 13q13.23 (0,5 kbp) including *NBEA* gene. The identified microduplications were not considered as a pathogenic according to literature; however parents' studies did not disclose any of these alterations. Since this could suggest *de novo* events array CGH study was performed in the second proband but it did not disclose any abnormalities.

NGS analysis was performed using TruSight One kit according to manufacturer instructions (Illumina). The sample was run on 1/4 of lane on HiSeq 1500 using 2x100 bp paired-end reads. Bioinformatics analysis was performed as previously described [10]. Briefly, after initial processing with CASAVA, the sequencing reads were aligned to the hg19 reference genome with Burrows-Wheeler Alignment Tool and further processed by Genome Analysis Toolkit [11]. Base quality score recalibration, indel realignment, duplicate removal and the SNP/INDEL calling were done as described [12]. The detected variants were annotated using Annovar and converted to MS Access format for final manual analyses. Alignments were viewed with Integrative Genomics Viewer [13,14]. The min. 20x and 10x coverage of the target was 98.4% and 99.5%, respectively.

Sanger sequencing was performed using BigDye Terminators kit v 3.1 (Life Technologies) with the following primers: forward 5' GGC ATG CAT TTC TGC ATA GTT 3', Reverse: 5' GCA ATT GCT GCT CTT GAG TG 3').

NGS analysis generated 97,099,776 reads. We filtered the results to retain high quality variants changing protein coding sequence or affecting splice sites with population frequency <0.01 (according to the EXAC database and an in house database of ~500 Polish exomes). After filtering there were 247 variants left. These variants were searched for biallelic mutations consistent with autosomal recessive inheritance as well as for hemizygous variants potentially causative of recessive sex-linked condition or heterozygous variants consistent with autosomal dominant de novo mutation (here we considered variants not found in available databases and predicted to cause loss of protein function, i.e., introduce premature stop codon or affect a splice site). The variants left after filtering are shown in Table 1.

Based on the known association between *ALDH18A1* and de Barsy syndrome we strongly prioritized two heterozygous mutations in this gene: chr10:097402754-C>A, NM_001017423.1: p.Glu100*/c.298G>T and chr10:097369983-C>T, NM_001017423.1: p.Arg724His. The: p.Glu100* variant has not been reported before and the SNPeff software predicted that it leads to nonsense mediated decay (NMD) of mRNA and thus causes loss of function. The p.Arg724His variant (rs773714478) has not been previously associated with human disease has but it is present in ExAC database with frequency of 0.000016. The p.Arg724His mutation has been scored as pathogenic by CADD (score=34), MetaSVM (D), Polyphen2 (D), MutationAssessor (H), SIFT (D) and MutationTaster (D). The identified *ALDH18A1* mutations were confirmed by standard Sanger sequencing. Both parents were tested and single mutation was observed in mother (p.Glu100*) as well as in father (p.Arg724His) indicating inherited origin of the variants. Testing of the affected brother revealed the same genotype as in the proband, i.e., presence of both mutations. Testing of healthy siblings was refused by the parents.

We also considered the p.Leu71* variant in *PDK3* as possibly contributing to disease (Table 1) given that a mutation in this gene (p.R158H) has been described by Kennerson et al. (PubMed: 23297365) as causing X-linked dominant Charcot-Marie-Tooth disease-6 (CMTX6; OMIM: 300905). Sanger sequencing confirmed the hemizygous *PDK3* p.Leu71* variant in the proband and showed that it was inherited from the heterozygous mother. The affected brother of the proband did not have the *PDK3* p.Leu71* variant. We provide evidence suggesting non-pathogenicity of loss of function variants in *PDK3*.

Metabolic and Biochemical Results

Metabolic test such as GC/MS (gas chromatography-mass spectrometry) and tandem mass spectrometry (tandem MS), VLCFA level, CDG screening were normal in both patients. During the diagnostic process amino acids profile and ammonia level in serum were not assayed. Afterwards, when mutations in *ALDH18A1* gene were identified, the level of ammonia in serum was determined in second sibling and was in normal range (69,22 umol/l and 50,6 umol/l). Amino acid profile in serum revealed decreased concentration of arginine (22,8 umol/l, normal range: 46-128 umol/l), cytruline (11 umol/l, normal range: 16-46 umol/l), homocysteine (1,2 umol/l, normal range: 3,3-8,3 umol/l), PHE (33 umol/l, normal range: 39-74 umol/l) and ornitine (7,6 umol/l, normal range: 27-98 umol/l). The remaining amino acids were in normal range including glicyne. Vitamin B12 level (825 pg/ml), folic acid level (18 pg/ml) were decreased.

Gene	Position	ID	Effect	Disease (inheritance, comments)
Potentially AR (potentially biallelic variants, frequency<0.01 in available databases)				
ALDH18A1	chr10:97402754	-	NM_001017423.1:p.Glu100*/c.298G>T	De Barsy syndrome or autosomal recessive cutis laxa type
	chr10:97369983	rs773714478	NM_001017423.1:p.Arg724His/c.2171G>A, scored as pathogenic by CADD (score=34), MetaSVM (D), Polyphen2 (D), Mutation Assessor (H), SIFT (D) and MutationTaster (D)	III (http://omim.org/entry/219150)
NLRC5	chr16:57059318	rs148647729	NM_032206.4:p.Ala155Thr/c.463G>A, apparently homozygous (5 reads), scored as benign by CADD, MetaSVM, Polyphen2, MutationAssessor, SIFT and MutationTaster	Hematopoietic and immune system's phenotypes in homozygous knock-out mice (http://www.informatics.jax.org)
FAM186A	chr12:50747016	rs200241170	NM_001145475.1:p.Ala1200Asp/c.3599C>A	High (>100) frequency of loss of function alleles and homozygotes in ExAC database
	chr12:50744108	rs113065589	NM_001145475.1:c.6504G>C,NM_001145475.1:c.6503+4G>C	(http://exac.broadinstitute.org/gene/ENSG00000185958)
Potentially AD (freq. 0 in available databases, predicted loss of function)				
ATP5G1	chr17:46970814	-	NM_001002027.1:p.Ala12_Leu13fs/c.36_37insTCTG	None (pLI=0.33)
CRLS1	chr20:6012011	-	NM_001127458.1:p.Thr120fs/c.359delC	None (pLI=0.03)
KIF12	chr9:116854866	-	NM_138424.1:p.Trp413*/c.1239G>A	None (pLI=1.78E-05)
Potentially X liked (freq. 0 in available databases, predicted loss of function)				
PDK3	chrX:24512964	-	NM_001142386.2:p.Leu71*/c.212T>A	Charcot-Marie-Tooth disease (X-linked dominant)

pLI: Probability that a gene is intolerant of loss-of-function variation (http://exac.broadinstitute.org)

Table 1: Variants found by NGS in the proband that were considered as potentially disease-causing.

Discussion

We have ascertained a family with two male siblings with clinical diagnosis of de Barsy syndrome molecularly confirmed by identification of two novel mutations in the *ALDH18A1* gene: p.Glu100* and p.Arg724His (rs773714478) in both brothers. The p.Glu100* variant has not been reported before and it is predicted to cause absence of the protein. The p.Arg724His has been found with very low frequency (0.000016) but not in association with human disease. The p.Arg724His variant is likely to significantly decrease the protein function as it has been scored as pathogenic by CADD, MetaSVM, Polyphen2, MutationAssessor, SIFT and MutationTaster (D).

Both patients reported here had very similar disease presentation including birth hypotonia, feeding and sucking problems, progeria-like appearance, spasticity with severe athetoid movements and anxiety, hypoplasia of corpus callosum, cataracts needed surgical procedure, bilateral hearing impairment and abnormal ECG with severe seizures [1-9]. Biochemical studies performed in serum in the second affected sib showed normal level of ammonia whereas concentration of arginine, cytruline, homocysteine, PHE and ornitine were decreased. These symptoms are typical of de Barsy syndrome however; the disease in our patients was relatively severe leading to early death of older sibling. It is likely that the severity of the disease is related to the strong damaging effect of the observed *ALDH18A1* mutations [7-9,15,16]. In the second sibling seizures were treated by two epileptic drugs: valproic acid, phenobarbitalum as well as clobazam and biperideni lactas because of tremor, slowness of movement and impaired motor development. Moreover, arginine and cytriline supplementation as well as normal kaloric and protein diet were applied.

In addition to describing novel *ALDH18A1* mutations our results extend the knowledge on the profile of pathogenicity of *PDK3* variants. *PDK3* encodes pyruvate dehydrogenase kinase isoform 3 and recently, based on a single family, the p.R158H missense mutation in this gene has been described as causing X-linked dominant Charcot-Marie-Tooth disease-6. The prosed pathogenic mechanism was associated with increased enzyme activity conferred by the mutation (CMTX6; OMIM: 300905) (PubMed: 23297365). According to the ExAC database the *PDK3* has relatively low tolerance of loss of function mutations (11 expected, one observed, pLI=0.88) thus raising a possibility that loss of PDK3 function may also be pathogenic. We found the *PDK3* p.Leu71* mutation in our proband and his mother but not his affected brother. The p.Leu71* mutation is predicted to cause nonsense mediated decay of mRNA thus leading to loss of function. The lack of any neurological symptoms in the mother indicates that loss of *PDK3* function is most likely not pathogenic in heterozygous females. Likewise, the lack of differences in diseases phenotype between the affected brothers discordant for the *PDK3* p.Leu71* suggests that the mutation does not have a strong effect in hemizygous males, at least early in life.

In conclusion, our report broadens the spectrum of pathogenic recessive mutations causing *ALDH18A1* associated de Barsy syndrome. In addition, we provide evidence for non-pathogenicity of loss of function variants in *PDK3*.

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Conflicts of Interest

There are no conflicts of interest.

Contributors RS: studied the patients, collected the samples, conceived the manuscript, supervised whole work, KS: studied the patients, helped in the clinical work, KK, TP: performed of the metabolic tests, AP, JK, GK: performed of the NGS study and bioinformatics processing of NGS raw data, MS: critically revised the manuscript, RP: supervised experimental work, analyzed the processed NGS data and critically revised the manuscript. Patient consent obtained. Competing interests none. Ethics approval Ethics approval was granted by the Institutional Review Board of Warsaw Medical University.

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