Doi: 10.32677/IJCR.2017.v03.i04.001 Case Report

Alström syndrome caused by deletion in *ALMS1* gene fixed in a Northern Pakistan recurrent haplotype

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Received - 17 July 2017 Initial Review - 10 August 2017 Published Online - 14 October 2017

ABSTRACT

Reduced genetic variability in isolated populations promotes the prevalence of long contiguous stretches of homozygosity (LCSH) that may carry deleterious mutations, manifesting recessive syndromes such as Alström syndrome (OMIM # 203800), caused principally by mutations in exons 8, 10, and 16 and deletions/insertions along the *ALMS1* gene. Here, Sanger sequencing of these exons and whole-genome copy-number variants/single-nucleotide polymorphisms (SNPs) microarray were used to characterize *ALMS1* gene in a 19-year-old Pakistani female with Alström syndrome. Sequencing did not reveal pathogenic alterations but described a set of homozygous polymorphisms. The microarray revealed these SNPs were included in an 8.24 Mb LCSH in 2p12.2p13 that contained a homozygous deletion including exons 13-16 of *ALMS1* gene. Therefore, reduced genetic variability in Pakistani population enhanced the inheritance of the homozygous deletion causing Alström syndrome. The comparison of the deletion with known deletions spanning exons 13-16, described on Middle Eastern patients, suggested a fixation of the deletion in a specific haplotype.

Key words: *ALMS1, Alström syndrome, Gross deletion, Haplotype, Long contiguous stretches of homozygosity, Reduced genetic variability*

Alström syndrome (OMIM # 203800) is a monogenic recessive disease characterized by a phenotype that begins its clinical manifestation during the 1st year of life [1]. Clinical signs include obesity, retinal degeneration developed during infancy and hearing loss during the first decade. Prevalence ranges 1:500,000-10,00,000 population, and it is, especially presented in populations with high consanguinity or reduced genetic variability [2]. This syndrome results primarily from mutations in *ALMS1* gene (OMIM #606844) [3]. Exons 8, 10 and 16 have been described as hotspots for nonsense, and frameshift mutations and exon 23 is affected by a great genetic variability [1,4]. Around 270 mutations have been reported and are mostly missense, nonsense, and small deletions. However, 6 gross deletions have been described [5-8].

ALMS1 is expressed in several tissues and participates in the centrosomes and basal bodies of ciliated cells formation, negatively affecting different processes when translation results in a truncated protein [4,5]. Although exact function is unknown, it has been related with ciliary function and intracellular transport [3]. For Alström syndrome, three divergent haplogroups, based on the distribution of polymorphisms through *ALMS1*, have been described with a peculiar worldwide distribution: Haplogroups D1 and D2 (derived) are nearly fixed in East Asian samples (98.9%), while Haplogroup A (ancestral) is common in the Americas (57.03%) but absent in East Asia (0.01%) [9]. Here, we report the genetic characterization of a female with Alström syndrome phenotype who carried a 38 kb homozygous deletion including exons 13-16 of *ALMS1*. This deletion was located in an 8.24 Mb long contiguous stretch of homozygosity (LCSH). Sanger sequencing of hotspot exons and microarray were used to elucidate the basis of the syndrome. The frequency of the haplotype that the patient had to determine the possibility of fixation of this deletion in Pakistani population was performed.

CASE REPORT

A 19-year-old Pakistani female, daughter of nonconsanguineous parents and sister of 5 siblings, was diagnosed with type 2 diabetes and hyperinsulinemia in the Endocrinology and Nutrition Unit at General University Hospital of Valencia (Spain). She had

secondary amenorrhea, hirsutism, and acanthosis nigricans, confirming polycystic ovaries syndrome. She presented nystagmus and early-onset blindness developed during the 1st year of life. She had retinal degeneration with macular thinning in both eyes and subcapsular cataracts in the right eye. Intellectual disability was suspected but difficult to confirm because of the sensorineural deficit and lack of school education. She did not have a history of deafness, but mild auditory impairment was confirmed by an otorhinolaryngology's examination.

Examination showed blood pressure 146/89 mmHg, height 149 cm, and weight 60.5 Kg (body mass index = 27.3 kg/m^2). Blood test highlighted elevated plasma glucose (144 mg/dl; normal range: 70-100), elevated insulin (52.5 µUI/ml; normal range: 2.0-23.0), elevated C-peptide (10.9 ng/ml; normal range: 1-3), and elevated testosterone (1.07 ng/ml; normal range: 0.1-0.75). Renal and hepatic functions were normal and echocardiogram did not show cardiomyopathy.

Having suspicion of Alström syndrome, Sanger sequencing of exons 8, 10, and 16 of *ALMS1* and intron-exon boundaries were performed. Analysis of exons 8 and 10 revealed 10 single-nucleotide polymorphisms (SNPs), all in homozygous state (Table 1). We evaluated their impact on the protein using Alamut mutation interpretation software v.2.0 (Interactive Biosoftware, Rouen, France), being all considered as benign. Regarding exon 16, we were unable to amplify any fragment.

To elucidate the alteration in exon 16, the genomic characterization was performed using a SNPs/copy-number variants (CNVs) CytoScan HD array (Affymetrix Inc., Santa Clara, CA, USA). Microarray formula obtained was arr [hg19] 2p13.1(73,772,595-73,811,051)×0. Homozygous deletion of 38 kb encompassed exons 13-16 of *ALMS1*, and it was included in an 8.24 Mb LCSH, arr[hg19]2p12.2p13(71,998,679-80,247,986)hmz that contained the polymorphisms in exons 8 and 10 (Fig. 1 and Table 1). LCSH regions estimated the consanguinity percentage in 3.71%, manifesting a blood relationship or reduced genetic variability in Pakistani population. Analyzing the pedigree, no more cases of Alström syndrome were diagnosed.

Finally, to study the possibility of deletion fixation because of reduced genetic variability in Pakistani population for *ALMS1* and using LDlink [10], we estimated the linkage disequilibrium blocks of the analyzed SNPs. LDmatrix showed that all SNPs except for rs2037814 (r^2=0.076) formed a block of linkage disequilibrium (r^2=0.885-1) for Pakistani population. The rs372423387 was excluded because it does not have a reported frequency. After

Table 1: Homozygous SNPs carried by the patient on exons 8 and 10 of *ALMS1* gene. Combination of SNPs segregating with the deletion described in this study

RefSeq NM_015120.4	Protein NP_055935.4	Reference	Exon	Genotype[a]	Change	MAF[b] (%)
c. 1577_1579del	p.Pro526del	rs372423387	8	-	-	-
c. 2018T>G	p.Val673Gly	rs2037814	8	G	T>G	21
c. 4182A>G	p.Gln1394Gln	rs6546836	8	G	A>G	22
c. 4247G>C	p.Gly1416Ala	rs6546837	8	C	G>C	22
c. 5629A>G	p.Ile1877Val	rs6546838	8	G	A>G	22
c. 6215T>C	p.Ile2072Thr	rs10496192	8	C	T>C	21
c. 6339T>A	p.Ser2113Arg	rs6724782	8	A	T>A	22
c. 6857G>C	p.Arg2286Pro	rs6546839	8	C	G>C	23
c. 8484G>T	p.Arg2828Ser	rs2056486	10	T	G>T	22
c. 8573A>G	p.Asn2858Ser	rs10193972	10	G	A>G	22

[a]Genotype: SNPs combination linked to the North Pakistan ancestral deletion of exon 13-16 in the *ALMS1* gene. The patient carried this haplotype in homozygous state.
[b]MAF: Minimum allele frequency in Punjabi in Lahora population., Pakistan SNPs: Single nucleotide polymorphisms

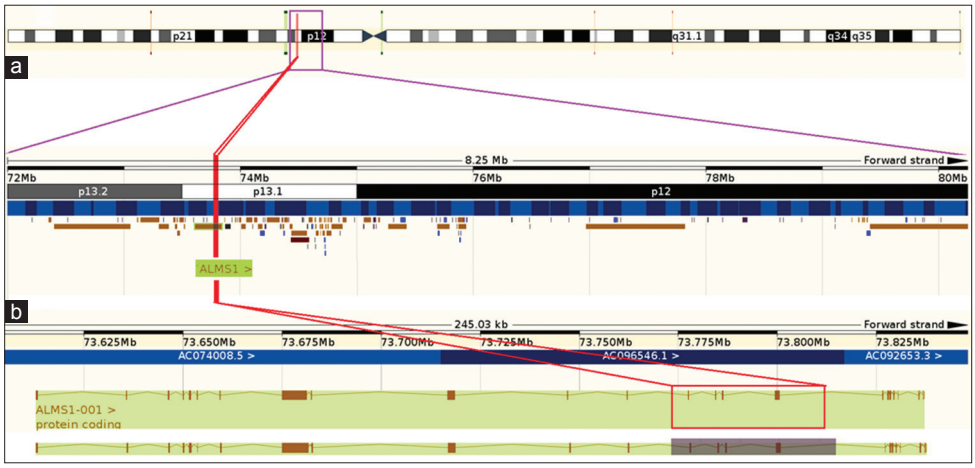

Figure 1: Image obtained from ensemble (GRCh37.p13) of the identified deletion. (a) Regions included in the long contiguous stretches of homozygosity (purple) and the 38 kb deletion (red) carried by the patient. (b) Comparison of deletions identified in the studied patient (red) and the familial deletion described by Nikopoulos and collaborators [8] (gray)

knowing the SNPs inherited together, we calculated the haplotype frequency with LDhap. For Punjabi in Lahore, Pakistan, the haplotype frequency of rs6546836, rs6546837, rs6546838, rs10496192, rs6724782, rs6546839, rs2056486, and rs10193972 combination was 20.8%, while the frequency of this haplotype is 7.8% in Africa, 17.6% in the Ad Mixed American population, 16% in Europe, 12.8% in South Asia, and do not exist in East Asia. The worldwide frequency estimated was 10.3%. Therefore, Pakistani population had the highest frequency for this haplotype even higher than South Asian population, where it is included exposing the reduced genetic variability.

DISCUSSION

The majority of variants causing Alström syndrome are found in exons 8, 10 and 16 of *ALMS1*. The first approach to diagnose the patient was the Sanger sequencing for those exons, which revealed 10 homozygous SNPs located in a LCSH segment carried by the patient. This combination of SNPs may infer the gross deletion reported here and could facilitate the diagnosis of future patients of Pakistan.

Previous articles defined the existence of strong population structure across an approximately 800 kb region centered on *ALMS1*, and evaluated how unusual the worldwide distribution of *ALMS1* allele frequency variation is relative to the rest of the genome. Different alleles of *ALMS1* would provide serial founder effects [9]. The sequence centered on *ALMS1* denotes the highest average r^2 (0.7), even more among the central Asian population, and SNPs located up and downstream of *ALMS1* show markedly lower linkage disequilibrium values. Its many anomalous patterns of genetic variation such as extensive population structure are evidenced with highly differentiated SNPs located in exons 5, 8, and 10.

According to Human Gene Mutation Database (http://www.hgmd.cf.ac.uk), six patients with Alström syndrome have been identified carrying large deletions removing exons [5-8]. Three carried deletions spanning exons 13-16. In one case, sequencing of all exons of *ALMS1* with the Targeted Gene Sequencing and Custom Analysis was used to identify it [6]. In the other two cases, series of polymerase chain reactions (PCRs) in introns 12 and 16 were used [8]. Here, the 38 kb homozygous deletion was identified using PCR, Sanger sequencing and SNPs/CNVs microarray on a Pakistani patient.

This homozygous deletion was compared to the heterozygous deletion including exons 13-16 formerly identified on a Middle Eastern patient (Fig. 1) [6] and two homozygous deletions described on two consanguineous siblings [8] in Pakistan. Although fine mapping of deletion and geographic area of the patient is needed, these deletions seem to be regions that are identical by descent, autozygous chromosome segments that were passed on through generations. The resemblance between these cases endorses the founder effect proposed by Nikopoulos et al. [8]. Five of the six known large deletions in the *ALMS1* are carried by individuals from the Middle East. These findings are consistent with consanguinity modulating the genetic landscape of the population [2].

In our patient, consanguinity calculation resulted in 3.71% and the pedigree did not indicate a close familiar relationship between her parents. This finding and comparison with the known large deletions spanning exons 13-16 suggested our patient inherited the 8 Mb LCSH regions including the deletion that causes Alström syndrome due to reduced genetic variability in Pakistan. It is possible that the deletion she carried could have been fixed in the reported haplotype in Pakistani population. Furthermore, it is important to remark that this haplotype is formed in an especial genomic region because despite it is composed by 8 SNPs with low allelic frequency (around 22%), it presented a strong linkage disequilibrium, even forming a haplotype with high frequency in Pakistan (20.8%), twice as much than worldwide (10.3%).

Although previous studies were not able to determine a genotype-phenotype correlation for Alström syndrome [11], researchers propose diabetes and obesity result from lack of functional protein in the pancreas and in the brain. Another association study suggested mutations in exon 16 cause onset retinal degeneration, urologic dysfunction, and diabetes and dilated cardiomyopathy, while variants in exon 8 would cause delay or renal failure [4]. Our patient carried a deletion of exons 13-16 and more studies are required to understand how the variants contribute to the severity. Comparing the studied patient and the formerly described consanguineous family carrying a similar deletion [8], differences on the clinical signs were found: Obesity, high blood pressure, acanthosis nigricans, altered testosterone levels, and blindness are common; renal failure, hepatomegaly, and cardiomyopathy are Alström syndrome signs developed on the consanguineous Pakistani family [8] but not in this patient.

CONCLUSION

In summary, we have identified a new case of Alström syndrome caused by homozygous deletion encompassing exons 13-16 of *ALMS1* that would indicate the fixation of this deletion in a specific haplotype with peculiar distribution in the worldwide being the highest frequency in Pakistan. Clinical signs in the patient are obesity, diabetes, high blood pressure, acanthosis nigricans, blindness, mild auditory defect, and altered testosterone levels.

ACKNOWLEDGMENTS

David Albuquerque is recipient of a post-doctoral fellowship (SFRH/BPD/109043/2015) from Fundação para a Ciência e a Tecnologia (Portugal).

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Funding: None; Conflict of Interest: None Stated.

How to cite this article: Monzó C, Gimeno-Ferrer F, Ferrer García JC, Amadoz A, Albuquerque D, Barros Angueira F, Marcaida G, Rodríguez-López R. Alström syndrome caused by deletion in *ALMS1* gene fixed in a Northern Pakistan recurrent haplotype. Indian J Case Reports. 2017;3(4):171-174.