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Alström Syndrome: Mutation Spectrum of *ALMS1*

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ABSTRACT: Alström Syndrome (ALMS), a recessive, monogenic ciliopathy caused by mutations in *ALMS1*, is typically characterized by multisystem involvement including early cone-rod retinal dystrophy and blindness, hearing loss, childhood obesity, type 2 diabetes mellitus, cardiomyopathy, fibrosis, and multiple organ failure. The precise function of ALMS1 remains elusive, but roles in endosomal and ciliary transport and cell cycle regulation have been shown. The aim of our study was to further define the spectrum of *ALMS1* mutations in patients with clinical features of ALMS. Mutational analysis in a worldwide cohort of 204 families identified 109 novel mutations, extending the number of known *ALMS1* mutations to 239 and highlighting the allelic heterogeneity of this disorder. This study represents the most comprehensive mutation analysis in patients with ALMS, identifying the largest number of novel mutations in a single study worldwide. Here, we also provide an overview of all *ALMS1* mutations identified to date.

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KEY WORDS: ALMS1; Alström Syndrome; ciliopathy; SNV

Background

Alström Syndrome (ALMS; MIM# 203800, http://www.ncbi.nlm.nih.gov/omim) is a rare monogenic recessive disorder characterized by a complex array of clinical manifestations, typically beginning in the first year of life. Progressive retinal degeneration is usually noticed in infancy and hearing loss usually presents in the first decade. Obesity generally begins to develop in the first few years of life and the BMI of most young children is >95th centile. Height is normal in early childhood, but growth slows in adolescence and final adult height is usually <5th centile.

Hyperinsulinemia and type 2 diabetes develop in childhood. Dilated cardiomyopathy (DCM) can occur suddenly in infancy (first months of life) because of the aberrant differentiation of cardiomyocytes [Shenje et al., 2014; Luow et al., 2014]. Restrictive cardiomyopathy develops slowly in adolescents and adults [Smith et al., 2007]. Neurological disturbances can include absence seizures and febrile epilepsy. In the brain, myelin derangement and cortical reorganization have been documented [Manara et al., 2015]. Children with ALMS have frequent respiratory problems, and pulmonary fibrosis, chronic obstructive respiratory syndrome, and acute respiratory distress syndrome can develop in later years [Marshall et al., 2005]. There are multiple endocrine disturbances, including hypothyroidism, alterations in the insulin-like growth factor system, low testosterone in males and hyperandrogenism in females [Maffei et al. 2002, 2007]. Liver dysfunction begins with hepatosteatosis, and, in a subset of patients, can progress to hepatic fibrosis and cirrhosis. Renal failure develops slowly in late adolescence and adulthood [Izzi et al., 2011]. Fibrotic tissue has been observed in nearly all organs [Marshall et al., 2005]. The early changes in vision and hearing have tremendous impact on social development [Frölander et al., 2014]. Although delay of cognitive development is not a common feature of ALMS, delay in early fine and gross motor developmental milestones is seen in ~27% of affected children. There can be early learning difficulties, delays in language, or in gross or fine motor milestones, which tend, in most case, to resolve as the child ages. However, if one applies the WHO criteria for defining and assessing intellectual disabilities (ID), fewer than 10% of patients meet that criteria. [International Advisory Group for the Revision of ICD-10 Mental and Behavioural Disorders, *2011; personal communication-jdm*].

Diagnosis is difficult as affected children often present with only a subset of features early in the course of the disease [Marshall et al.,

Additional Supporting Information may be found in the online version of this article.

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2005, 2007a]. Additionally, there is interfamilial and intrafamilial clinical variability in the phenotypes, including age of onset, and the severity of the disease manifestations, which may be explained by the effects of genetic and/or environmental modifiers.

Located on chromosome 2p13, *ALMS1* spans 23 exons and encodes a predicted 461.2-kDa protein of 4169 amino acids (aa). Exon 1 harbors a polyglutamate tract (aa 13–29) consisting of a $(GAG)_NGAA(GAG)_3$ trinucleotide repeat, followed by a stretch of seven alanine residues (aa30–36) [Collin et al., 2002; Hearn et al., 2002]. Exon 8, a 6-kb exon, is also comprised of a large, variable tandem-repeat domain encoding 34 repeats of 45–50 amino acids.

Most pathogenic variants occur downstream from exon 7 and are almost all truncating mutations resulting in the early termination of ALMS1 and a non-functional protein (i.e., nonsense mutations that cause a stop codon or insertions and deletions of one or more nucleotides that cause a frameshift).

ALMS1 localizes to centrosomes and basal bodies of ciliated cells and is expressed in all tissues that are pathologically affected in patients with ALMS. Roles in cell cycle regulation and intraciliary transport [Hearn et al., 2005; Li et al., 2007; Knorz et al., 2010; Collin et al., 2012] have been suggested. Recently, ALMS1 has been shown to contribute to cell migration and extracellular matrix production [Zulato et al., 2011; Shenje et al., 2014; Louw et al., 2014], as well as in the endosomal trafficking of transferrin, GLUT4, and Notch1 [Collin et al., 2012; Favaretto et al., 2014; Leitch et al., 2014]. However, the precise molecular mechanisms underlying the multiple organ pathologies have not been fully elucidated.

The first *ALMS1* mutations identified were clustered in exons 8, 10, and 16 [Joy et al., 2002; Marshall et al., 2007b, 2011]. Therefore, early investigations preferentially sequenced these exons. The c.10775delC (p.Thr3592Lysfs*6) mutation in exon 16 was the most frequently identified, with a common founder suggested for persons of British descent [Marshall et al., 2007b]. Subsequently, the wider incorporation of automated sequencing to genotype patients with ALMS has uncovered additional mutations in exon 5 [Paisey et al., 2014; Casey et al., 2014], exon 11 [Taşdemir et al., 2012], exon 12 [Marshall et al., 2007b], exon 18 [Marshall et al., 2007b; Malm et al., 2008], exon 20 [Casey et al., 2014], and intronic regions [Bond et al., 2005; Aldahmesh et al., 2009; Sanyoura et al., 2014; Ozantürk et al., 2014].

Methods of Subject Ascertainment and Mutation Detection

Study Subjects

Our original cohort consisted of 239 patients from 204 unrelated families with a suspicion of ALMS who fulfilled the established clinical criterion for a diagnosis of ALMS [Marshall et al., 2007a] but without a molecular diagnosis. Subjects were recruited to The Jackson Laboratory (Bar Harbor, ME), to Padua University (Padua, Italy), to the University of Strasbourg (Strasbourg, France) or through the referral of Alström Syndrome International (ASI) over a period of more than twenty years. The study subjects were ethnically diverse, including multiple kindreds representing North America (59), South America (3), Caribbean (2), Northern Europe (10), Southern Europe (24), Western Europe (23), Eastern Europe (3), Southeastern Europe (25), Oceania (4), East Asia/Middle East (13), Southeast Asia (5), South Asia (6), West Asia (19), and North Africa (6).

Appropriate informed consent was obtained from adult patients and parents/guardians of minors. Clinical histories and medical records were obtained for all affected individuals, but the comprehensiveness of the records was variable from patient to patient. The research was approved by the Institutional Review Board of The Jackson Laboratory, the Padua University Hospital Ethics Committee, and the Ethics Committee of the Strasbourg University Hospital (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, CCPPRB).

Mutation Analysis Strategy

Genomic DNA was isolated from blood leukocytes using standard protocols, or DNA samples obtained in a clinical setting were submitted by referring physicians. We adopted a step-wise approach to identify causal variants for each patient. A detailed description of the mutation analysis strategy and methods can be found in Supp. Methods. Mutation nomenclature refers to GenBank reference sequence NM_015120.4; AC074008.5 for *ALMS1*. Nucleotide and amino acid numbering of mutation sites began at the start codon, ATG (Met) of the open reading frame, originally described by Collin et al. 2002 and Hearn et al. 2002.

Additionally, using VaRank [Geoffroy et al., 2015], we analyzed next generation sequencing data from 275 patients for rare or frequent SNVs (See Supp. Methods).

Criteria to Determine the Pathogenicity of New ALMS1 Mutations

Determination of pathogenicity was straight-forward in the case of likely truncating variants—frameshift indels, stop codons gained, and canonical splice site changes. A sequence variation was considered pathogenic when (1) it was not present in control databases (including dbSNP [Sherry et al., 2001]. EVS (Exome variant Server, NHLBI GO Exome Sequencing Project, http://evs.gs.washington.edu/EVS/) and, specifically for the TaGSCAN analysis, in at least 960 randomly selected controls from the Center for Pediatric Genomic Medicine's TaGSCAN database); (2) it altered a well-conserved amino acid, preferably in a conserved region [Larkin et al., 2007], and (3) it was judged significant in computational prediction tools for novel mutations: PolyPhen-2 analysis [Adzhubei et al., 2010], SIFT [Kumar et al. 2009], Mutation Taster [Schwarz et al., 2010], and Condel [Gonzales Perez and Lopes-Bigas, 2011].

A mutation was considered novel if it was not present in the Human Gene Mutation Database (http://www.hgmd.org/) [Stenson et al., 2014], or the dbSNP database, or not previously published.

Database

All novel variants identified in the present study have been submitted to the Euro-WABB online database [Farmer et al., 2013], a locus-specific database (in the Leiden Open Variation Database format; https://lovd.euro-wabb.org/home.php?select_db=ALMS1) listed by the Human Genome Variation Society in the Locus specific Mutation Databases LSDB (www.HGVS.org). We encourage future authors of published reports of *ALMS1* mutations to include their findings within this database.

Pathogenic Variants

Novel ALMS1 pathogenic mutations identified in this study are shown in Table 1 and Figure 1. These, along with previously reported

Table 1. 109 Novel *ALMS1* Mutations

cDNA Variant	Predicted protein	#Exon	Total affected alleles[a]	Ethnicity/regional origin
c.454-5T>G		intron 2	1	Oceania
c.592C>T	p.Gln198*	3	1	N America
c.906del	p.Ile302Metfs*30	5	2	N Africa
c.967G>T	p.Glu323*	5	1	N America
c.1054C>T	p.Arg352*	5	1	N America
c.1613_1614delTC	p.Leu538Glnfs*12	8	1	N America
c.1628T>A	p.Leu543*	8	1	N America
c.1645delA	p.Thr549Leufs*5	8	1	Caribbean
c.1674delT	p.559Leufs*37	8	2	E Europe
c.1897dupT	p.Tyr633Leufs*9	8	1	W Europe
c.1903C>T	p.Gln635*	8	1	N America
c.2159delG	p.Arg720Lysfs*60	8	1	S Europe
c.2234C>G	p.Ser745*	8	1	N America
c.2265dupT	p.Glu756*	8	4	W Asia
c.2646dupT	p.Glu883*	8	1	N America
c.2676delT	p.Gly893Aspfs*41	8	1	S Europe
c.2708C>G	p.Ser903*	8	1	Oceania
c.2778dupT	p.Leu927Serfs*4	8	1	E Asia
c.2822T>A	p.Leu941*	8	3	W Europe
c.2930_2933dupGAGA	p.Ser979Argfs*13	8	1	W Europe
c.2967_2970delTGAC	p.Asp990Argfs*38	8	2	W Europe
c.3019delA	p.Arg1007Glufs*22	8	1	N America
c.3019dupA	p.Arg1007Lysfs*15	8	1	Oceania
c.3020delG	p.Arg1007Lysfs*22	8	1	S Europe
c.3066T>A	p.Tyr1022*	8	2	N Africa
c.3153C>A	p.Tyr1051*	8	1	S America
c.3220_3221delAG	p.Leu1075Glufs*10	8	1	E Asia
c.3300_3301delAA	p.Lys1103Alafs*16	8	2	SE Europe
c.3472C>T	p.Gln1158*	8	2	W Asia
c.3518C>G	p.Ser1173*	8	1	E Asia
c.3579C>G	p.Tyr1193*	8	2	N America
c.3583C>T	p.Gln1195*	8	2	S Asia
c.3754C>T	p.Gln1252*	8	1	Oceania
c.3876_3877dupGAAG	p.Lys1293Glufs*13	8	1	W Europe
c.4039C>T	p.Gln1347*	8	1	N America
c.4145_4146delACTC	p.Tyr1382Phefs*18	8	1	E Asia
c.4154A>G	p.Ser1383*	8	1	N Europe
c.4180C>T	p.Gln1394*	8	2	N America
c.4205dupT	p.Thr1403Asnfs*2	8	2	S Asia
c.4213G>T	p.Glu1405*	8	2	N America
c.4274T>A	p.Leu1425*	8	1	W Europe
c.4281_4282dupA	p.Thr1428Asnfs*11	8	1	N America
c.4363G>T	p.Glu1455*	8	1	E Asia
c.4477G>T	p.Glu1493*	8	1	S Europe
c.4605C>G	Tyr1535*	8	1	E Asia
c.4657delT	p.Ser1553Leufs*15	8	1	E Asia
c.4718C>G	p.Ser1573*	8	1	Oceania
c.4746C>A	p.Tyr1582*	8	2	E Asia
c.4823delA	p.Lys1608Argfs*9	8	1	N America
c.4823dupA	p.Thr1609Aspfs*2	8	1	W Europe
c.4891C>T	p.Gln1631*	8	2	W Europe N Africa
c.4917_4920delTAAA	p.Asn1639Lysfs*4	8	2	N America E Asia
c.5054_5060delTACCTGA	p.Leu1685*	8	1	W Europe
c.5135T>G	p.Leu1712*	8	2	S Europe
c.5277delC	p.Tyr1760Ilefs*20	8	1	N America
c.5455C>T	p.Arg1819*	8	2	SE Europe
c.5573_5574del	p. Ser1858Cysfs*5	8	1	Oceania
c.5575_5576delGT	p.Val1859Hisfs*4	8	1	W Asia
c.5929C>T	p.Gln1977*	8	1	N Africa
c.6116_6117delTC	p.Pro2040Ilefs*7	8	3	SE Europe E Asia
c.6169_6170dupAT	p.Leu2058Phefs*17	8	1	E Asia
c.6288T>A	p.Tyr2096*	8	2	SE Europe
c.6361A>T	p.Lys2121*	8	2	S Asia
c.6421_6427del	p.Ser2141Phefs*20	8	1	E Asia
c.6486_6489delAACT	p.Thr2163Argfs*4	8	2	SE Europe
c.6625_6626delA	p.Asn2209Ilefs*2	8	2	S America
c.6895delG	p.Val2299Trpfs*43	8	1	N Europe
c.7188_7192delTAGTG	p.Cys2396Trpfs*6	8	2	S America

(*Continued*)

Table 1. Continued

cDNA Variant	Predicted protein	#Exon	Total affected alleles[a]	Ethnicity/regional origin
c.7376_7379delATAG	p.Asp2459Alafs*18	8	2	W Europe
c.7378G>T	p.Glu2460*	8	1	W Europe
c.7571_7572delAT	p.His2524Argfs*11	9	1	S Europe
c.7577_7590delGTGGATACTCCATT	p.Cys2526Phefs*5	9	2	W Asia
c.7677G>C	p.Lys2559Asn	9	2	W Europe
c.7677+1G>T	p.?	9	1	SE Europe
5.7kb deletion[b]		intron 9	2	W Asia
c.7772dupT	p.Thr2592Asnfs*3	10	1	N America
c.7911dupC	p.Asn2638Glnfs*24	10	2	W Asia
c.8150_8151insT	p.Ser2719Phefs*7	10	2	W Asia
c.8352_8355delAGAA	p.Glu2785*	10	1	N America
c.8579C>A	p.Ser2860*	10	2	N America
c.8766C>A	p.Cys2922*	10	2	Caribbean
c.8995A>T	p.Gln2999*	10	1	N Europe
c.9379C>T	p.Gln3127*	10	1	S Europe
c.9517G>T	p.Glu3173*	10	2	SE Asia
c.9801T>A	p.Tyr3267*	12	1	W Europe
deletion of exon 13–16			1	W Asia
c.10124C>G	p.Ser3375*	14	2	N Africa
c.10265delC	p.Pro3422Glnfs*2	15	1	W Europe
c.10388-2A>G		intron 15	2	W Asia
c.10483delC	p.Gln3495Lysfs*14	16	1	N America
c.10535G>A	p.Trp3512*	16	1	N America
c.10561_10564delGACA	p.Asp3521Asnfs*25	16	2	S Europe
c.10609_10614delinsTCAAA	p.Asp3537Serfs*10	16	1	W Europe
c.10757dupA	p.Val3587Glyfs*15	16	1	W Europe
c.10828C>T	p.Gln3610*	16	1	W Europe
c.10830_10831insC	p.Arg3611Glnfs*7	16	1	S Europe
c.10898dupT	p.Leu3633Phefs*2	16	2	N Europe
c.10939_10945del	p.Val3647Lysfs*13	16	1	N Europe
deletion of exon 17		17	1	N America
c.11618_11619delCT	p.Ser3873Tyrfs*19	17	3	N America S Europe
c.11651_11652insGTTA	p.Asn3885Leufs*9	17	3	N America
c.11654_11657del	p.Asn3885Argfs*11	17	1	W Europe
c.11703delA	p.Lys3901Asnfs*8	18	1	S Europe
c.11812dupA	p.Met3938Asnfs*8	18	1	SE Europe
c.11881dupT	p.Ser3961Phefs*12	19	1	W Europe
c.12004G>T	p.Glu4002*	19	1	N America
c.12116_12117insT	p.Gln4039Serfs*10	19	1	N America
c.12365G>A	p.Arg4122Gln	21	1	N America

Mutation nomenclature refers to GenBank reference sequence NM_015120.4; AC074008.5 for *ALMS1*. Nucleotide and amino acid numbering of mutation sites began at the start codon, ATG (Met) of the open reading frame, originally described by Collin et al. [2002] and Hearn et al. [2002]. Ethnicity/regional origin was designated according to the United Nations *Composition of macro geographical (continental) regions, geographical sub-regions, and selected economic and other groupings*, http://millenniumindicators.un.org/unsd/methods/m49/m49regin.htm.

[a]Total number of alleles including this cohort and previous reports.

[b]Approximate genomic locations 73715902 in intron 9 to 73721624 in intron 10.

ALMS1 mutations, ethnicities, and references are listed together in Supp. Table S1. There are now 239 disease-causing mutations in *ALMS1* identified in many diverse ethnic groups. The majority (96%) of the mutations are nonsense and frameshift variations (insertions or deletions) that are predicted to cause premature protein truncation. There is no evidence for triallelic or complex modes of inheritance.

Additionally, deletions removing entire exons and splice-site mutations [Bond et al., 2005; Sanyoura et al., 2014], an *Alu* transposon insertion [Taşkesen et al., 2012] and one balanced translocation [Hearn et al., 2002] have been reported.

Several mutations were found in multiple ethnicities and 3 or more regional origins: c.4156dupA, c.4937C>A, c.5145T>G, c.8164C>T, c.8394_8395insA, c.8782C>T, c.9900dupC, c.10483C>T, c.10790_10791delTG, c.10849G>T, c.10885C>T, c.10975C>T, c.11316_11319delAGAG, c.11385delT, and c.11416C>T (Supp. Table S1). The mutation c.10775delC remains the most common with 24 alleles identified, however, all individuals carrying that particular allele were from the UK or North America.

We identified 109 novel mutations in *ALMS1* (Table 1; Fig. 1) in exons 3, 5, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, and 21 as well as introns 2, 9, and 15. Additionally, four indel and splice mutations in *ALMS1* were detected: c.454-5T>G, c.7677+1G>T, c.10388-2A>G, and c.10609_10614delinsTCAAA.

Several large deletions were also observed. A homozygous 5.7 kb deletion with approximate genomic locations 73715902 in intron 9 to 73721624 in intron 10 was seen in a kindred of Middle Eastern heritage. Another patient of Middle Eastern descent harbored a heterozygous deletion of exons 13–16. Lastly, a patient of German heritage harbored a heterozygous deletion of exon 17. Most of the novel mutations were private mutations identified in only one family (100/109), suggesting that the mutation spectrum is far from being saturated in spite of numerous *ALMS1* mutation reports.

Thirty kindreds carried novel homozygous mutations, consistent with the large number of consanguineous unions, whereas 82 were compound heterozygotes. In four kindreds, only one heterozygous disease-causing allele was identified after complete NGS analysis

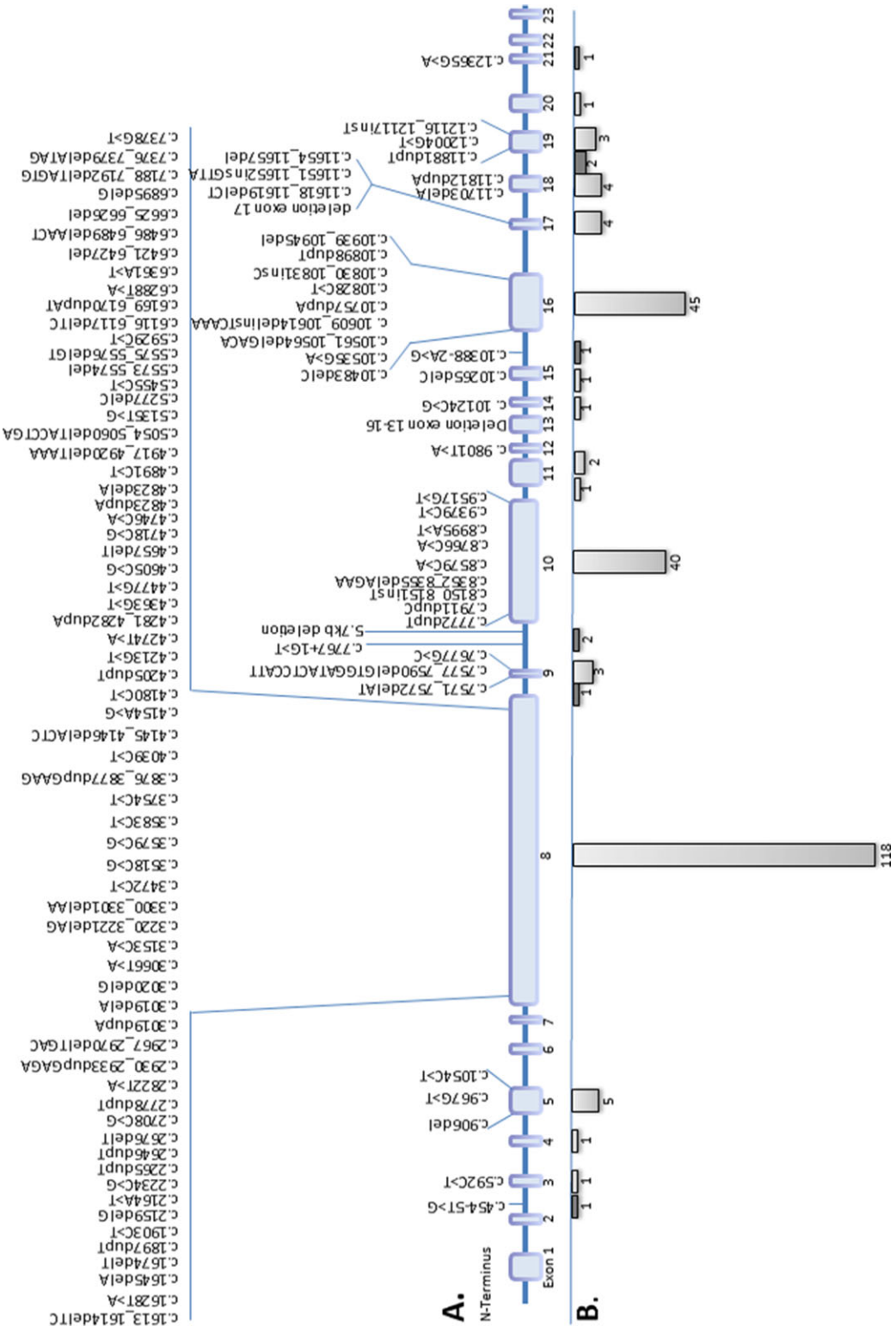

Figure 1. Novel *ALMS1* mutations identified in this report and total previously reported. **A**: Exonic structure and location of novel *ALMS1* mutations. **B**: Total number of reported substitution, deletion, insertion, and splice-site mutations per exon, including novel mutations and those previously reported in Supp. Table S1. Intronic regions where mutations of unknown significance, but predicted to alter splicing have been identified are shaded in dark gray.

of *ALMS1*. No apparent difference in phenotypic expression was observed in patients in whom only one heterozygous mutation has been identified compared with patients with two *ALMS1* mutations (data not shown).

Twenty two of the patients who presented with features suggestive of ALMS received clear alternative diagnoses either subsequently identified by the referring physicians or using the TAGScan diagnostic panels (Supp. Table S2). Deleterious mutations were identified in Usher Syndrome type 2A (*USH2A* (4), Bardet Biedl Syndrome *(BBS1* (5), *BBS2* (1), *BBS4* (1), *BBS5* (1), *BBS9* (2), *BBS10* (2), *BBS12* (2), Dedicator of Cytokinesis (*DOCK7*) (2), Myosin heavy chain 7B (*MYH7B*) (1), and Congenital stationary night blindness, *CSNBAD3* (1). Although the NGS protocols utilized in this study enrich all exons of *ALMS1* and detect nucleotide changes at over 95% of the *ALMS1* coding domain, there remain 21 families (10%) in whom we have not yet been able to identify mutations in *ALMS1*.

In some cases, where no clear pathogenic *ALMS1* mutations are found, rare missense variations pose a problem in genetic counseling, as their impact is still unclear. The length of the resulting protein sequence is preserved but the functional consequences of most missense variants that result in a single amino acid residue change in ALMS1 protein is not known. Although missense mutations can render the resulting protein nonfunctional by affecting splice sites or the binding with other proteins, we cautiously questioned some previously reported missense variants given their uncertain pathogenicity. The latest data available for variation frequency such as EVS or ExAC (Exome Aggregation Consortium, http://exac.broadinstitute.org, accessed March 2015) providing an accumulating allele count for several thousand individuals allows for a more critical analysis.

Marshall et al. (2007b) described 8 missense variants: p.K1718N, p.S2216R, p.T2458I, p.R2589W, p.V2996G, p.E3500V, p.S3505C, and p.I3888T that were predicted to be disease-causing on the basis of SIFT prediction, a BLOSUM45 matrix score, absence of the variant in the general population, and a conserved identity score. All of these variations are either not in the current databases or occur with a very low allele frequency (<0.01%). Previously, others have identified heterozygous missense alterations in *ALMS1* (ExAC allele frequencies in parenthesis when available), p.V424I and p.H3882Y (0.15%) [Joy et al., 2002], p.Asn1788Asp (1.443%) and p.Asn2946K (~1%) [Minton et al., 2006], p.S3707C (0.14%) [Sathya Priya et al., 2015] and p.S2102L (2.56%) [Redin et al., 2012] were identified as potentially disease-causing. However, given that several of these alleles are common in the general population, they should not be included in the cohort of pathogenic *ALMS1* mutations without a more thorough analysis.

Single-Nucleotide Non-Pathogenic Variants

We also identified 194 SNVs that were unlikely to be pathogenic by assessing their frequency in our cohort of 275 patients and the functional significance of these variants based on the conserved identity of the protein and the severity of the resulting amino acid substitution (Supp. Table S3). It is interesting to note that a frequent SNV in exon 8 (inframe insertion/deletion of a CTC, present in >60% of our patients) can be, under certain circumstances, wrongly interpreted by NGS pipelines (mainly due to bad alignments) and lead to false positive SNVs including frameshifts (i.e., c.1574dup, p.Pro526Serfs*11, representing 25 alleles in our cohort). Therefore, we recommend careful examination of any variant identified closely neighboring this SNV (Supp. Fig. S1).

Clinical Relevance

Phenotypic variation, such as differences in visual acuity and hearing loss or presence versus absence of cardiomyopathy, and varying hepatic manifestations, is observed in siblings or in unrelated families with the same mutations. This suggests that, besides some possible variability due to the mutation itself, there is interplay between a multitude of potential genetic modifiers, environmental or infectious exposures, and stochastic events leading to the wide variations in age of onset and severity of the ALMS phenotypic spectrum [Collin et al., 2002]. Zumsteg et al. (2000) speculated that a gene encoding a major modifier of plasma cholesterol levels could be responsible for the phenotypic heterogeneity in lipid metabolism and T2DM in a study of three age-adjusted siblings with ALMS. In three small genotype–phenotype correlation studies, t'Hart et al. (2003), Bond et al. (2005), and Patel et al. (2006) found no association between the location or type of *ALMS1* mutations and type 2 diabetes, BMI, or the occurrence of DCM, respectively. Another study suggested a correlation between renal disease and the genomic location of *ALMS1* mutation sites. Subjects harboring mutations only in exon 8 appeared to have delayed and milder renal complications, perhaps due to tissue-specific expression of different splice isoforms [Marshall et al., 2007b]. In a recent study of the general population Ichihara et al. found a correlation between the polyglutamine repeat in *ALMS1* and early onset myocardial infarct [Ichihara et al., 2013].

Feasibility of Genotype–Phenotype Correlations

Today, in spite of advances in our knowledge of the spectrum of *ALMS1* mutations, we do not yet have evidence for prognostic predictions based on genotype. Since ALMS is a rare recessively inherited disorder, enumerating individual mutant alleles in patients with various phenotypes has limited value. Indeed, it is the combination of mutated alleles that is important in defining the phenotype. Therefore, to avoid misinterpretation, it would be necessary to include only the homozygous mutations. The majority of mutations identified are not found in homozygosity, thus reducing the number of patients suitable for such analysis.

Additionally, our cohort is very large and ethnically diverse, and patients received inconsistent clinical care. Often a particular phenotype, for instance age of vision loss, diabetes onset, or cardiomyopathy has not been examined in many of these patients.

ALMS is extremely complex. Most of the features emerge as the children grow. Therefore, when looking for the presence or absence of a particular phenotype, the age of the patient needs to be considered. For example, the average age of onset for diabetes is 16 years [Marshall et al., 2005], so only those patients over the mean age should be included. Likewise, the age of onset of the restrictive cardiomyopathy ranges from 12 to 40 years. Typically renal disease does not emerge until late adolescence and develops gradually. This limits the number of patients to evaluate with any degree of certainty for each phenotype.

In summary, the possibility of genotype–phenotype correlations should certainly be studied carefully, but further analysis with larger numbers of patients is required. The limitations in current genotype–phenotype studies demonstrate that another major challenge is to determine those factors, other than *ALMS1* mutations, contributing to the phenotypic diversity in ALMS.

Diagnostic Relevance

This study brings the total number of reported *ALMS1* disease causing variants to 239. Out of our cohort of 204 families (408 alleles), 357 alleles were identified, achieving a mutation detection rate of 88%, which improves upon previous studies in which mutations were identified in 70%–75% of Alström families. The large number of private mutations unique to a single kindred underscores the allelic heterogeneity in the disorder.

There is a strong clustering of disease-causing variants in exon 8 (118/239, 49%), exon 10 (40/239, 17%), and exon 16 (45/239, 19%), continuing to support the observation suggesting that these regions represent mutational hotspots for *ALMS1*. This may be due, in part, to the relative size, although this does not seem to be true for exon 16 [Marshall et al., 2011]. It likely also reflects the screening strategy employed in early studies, in which exons 16, 10, and part of 8 were preferentially selected for complete sequencing. However, an explanation for the disproportionate clustering in these exons is not fully apparent given the extensive use of NGS, which genotypes all coding domain nucleotides.

Certainly, ethnicity contributes significantly to the distribution of mutations, and some generalizations can be made. Among non-consanguineous populations of America and Europe, the prevalence of ALMS is estimated to be approximately 1:1000000 [Marshall et al., 2011]. However, evidence exists that the incidence of ALMS increases within populations of high consanguinity or in those that are geographically isolated [Marshall et al., 1997; Aldahmesh et al., 2009; Ozantürk et al., 2014].

Several recurrent mutant alleles have been identified in Northern European populations, including c.10775delC (24/557), c.10483C>T (17/557, and c.11449C>T (15/557), c.11316_11319delAGAG (15/557), and c.11416C>T (10/557). In addition, c.8177_8187, c.8164C>T, and c.10945G>T are common in West Asian/Middle Eastern kindreds. Specifically, c.10945G>T makes up approximately 8.2% of the mutant alleles in this population, c.8164C>T and c.8177_8187 each make up approximately 11%. The common alleles in Europe and the Americas are not generally seen in Chinese and Japanese cohorts. The following variants occurred more than once only in East Asian families: c.6169_6170dupAT, c.10831_10832delAG, and c.11116_11134del19. Therefore, screening for these mutations in patients of West and East Asian descent is a logical first step in mutation detection. Among other European and North/South American patients, however, this strategy fails to identify most of the rare mutant alleles, and whole gene sequencing is required for accurate genotyping.

So far, no disease-causing mutations have been identified in exons 1, 2, 6, 7, 13, 22, and 23. Variants in these other exons may result in different phenotypes or in no phenotype at all, or alternatively, have deleterious effects on fetal viability.

In four families only one heterozygous disease-causing variant was identified, and in 21 families no disease-causing variant was identified after complete NGS analysis of *ALMS1*. The "missing" mutated *ALMS1* alleles in the 21 families (presumably 42 alleles) and the four families with only one allele identified (four alleles) may harbor other types of molecular lesions including large deletions, chromosomal translocations, promoter/enhancer defects, or splice site alterations, intronic nucleotide variants more than 20 nucleotides from the intron-exon boundary, or variants in a non-exonic regulatory motif, which we have not identified in our screening.

Whole genome sequencing with structural variant detection may identify these missing alleles. Alternatively these patients may have mutations in another disease gene which might be identified through exome or genome sequencing.

Future Prospects

New advances in high throughput genome sequencing will aid in more effective mutational screening for early clinical diagnosis by documenting the high number of private mutations.

Given the emerging importance of disease modifying alleles on the diverse phenotypic expression of other ciliopathies, identifying modifying loci will help elucidate the molecular pathways through which ALMS1 acts. Future studies of discordant sib pairs as well as studies in the mouse models [Collin et al., 2005] are required to understand the disease mechanisms in ALMS.

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