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Syndromic Obesity and Diabetes: Changes in Body Composition with Age and Mutation Analysis of *ALMS1* in 12 United Kingdom Kindreds with Alström Syndrome

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Context: Alström syndrome (AS) is a monogenic form of infancy-onset obesity and insulin resistance, caused by *ALMS1* mutations. The natural history of the insulin resistance is unknown, in particular how this relates to changes in body composition. It is also unclear how *ALMS1* mutations relate to the characteristic phenotype.

Objectives: Our objectives were to characterize body composition and metabolic parameters, to establish *ALMS1* mutation spectrum of United Kingdom AS patients, and to determine whether a genotype-phenotype correlation exists.

Design and Patients: We conducted a cross-sectional cohort study of 12 unrelated subjects with AS. Age-standardized body composition was assessed by anthropometry and dual-energy x-ray absorptiometry and insulin sensitivity by homeostasis model assessment. The exons and intron-exon boundaries of *ALMS1* were directly sequenced.

Setting: The study was performed during the annual Alström Syndrome UK multidisciplinary screening clinic.

Results: AS patients have early-onset obesity, but body mass index, waist circumference, and body fat from dual-energy x-ray absorptiometry were negatively correlated with age ($r = -0.37$, $P = 0.2$; $r = -0.84$, $P = 0.002$; and $r = -0.6$, $P = 0.05$). Despite this, insulin resistance increased, demonstrated by raised fasting insulin and fall in homeostasis model assessment insulin sensitivity with age ($r = -0.64$, $P = 0.02$). *ALMS1* mutations were identified in 10 of 12 patients, with a potential founder mutation in exon 16 present in five [np 10775del (C); Del3592fs/ter3597]. No genotype-phenotype correlation was observed.

Conclusions: We identified mutations in *ALMS1* in more than 80% of patients with no genotype-phenotype correlation. In AS, severe childhood obesity, waist circumference, and body fat decrease with age, whereas insulin resistance increases. The abdominal obesity, insulin resistance, diabetes, hypertriglyceridemia, and hypertension suggest that AS could represent a monogenic model for the metabolic syndrome. **(*J Clin Endocrinol Metab* 91: 3110–3116, 2006)**

ALSTRÖM SYNDROME (AS) (OMIN 203800) (1) is the autosomal recessive inherited association of retinal dystrophy, sensorineural deafness, and obesity. Other features include diabetes mellitus, hypertriglyceridemia, cardiomyopathy, hepatic disease, and urological abnormalities (2–5). The prevalence is less than 1:100,000, and heterozygote carriers may be at increased risk for deafness and type 2 diabetes (1).

Progressive cone-rod dystrophy causes nystagmus in infancy and blindness by adulthood (5); sensorineural deafness presents in childhood (2, 4). Other patients present with dilated cardiomyopathy in infancy (5, 6), and this can recur or present *de novo* later (2, 4). Obesity also develops in infancy, with diabetes mellitus in 70% of subjects in the second to third decade (2). Euglycemic hyperinsulinemic clamps confirm marked insulin resistance (7). The hyperinsulinemia is associated with acanthosis nigricans (8), hypertension, and hypertriglyceridemia (9), which can be severe and lead to pancreatitis (9, 10). Liver function abnormalities and nonalcoholic steatohepatitis are common (11). Other endocrine complications include hypogonadotropic hypogonadism in male patients and hypothyroidism. Global developmental delay with normal intelligence is common (2). Urological dysfunction due to detrusor-urethral dyssynergia occurs in 50% of patients in the second to third decade (12).

Cardiomyopathy accounts for most mortality in the first three decades of life; in the United Kingdom, death from cardiac failure occurred in 27% of Alström patients in the first or second decade (Alström Syndrome UK data). Renal failure is the commonest cause of death in older patients (2). Late fibrotic changes have been described at postmortem in several organs including the kidneys, liver, and lungs (2, 13).

There are few published data on body composition (2, 14). Rapid weight gain is reported in infancy with accelerated childhood growth but reduced final adult height. Body mass index (BMI) was raised in children compared with age-

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Abbreviations: AS, Alström syndrome; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; HOMA, homeostasis model assessment; SDS, SD score.

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matched controls, and adults remained obese, but BMI declined with the onset of other complications (2).

Mutations in the gene *ALMS1* on chromosome 2p13.1 were recently identified in patients (15, 16). *ALMS1* consists of 23 exons encompassing over 224 kb of genomic DNA encoding a polypeptide of 4169 amino acids, with a predicted molecular mass of 461.2 kDa. Computer predictions suggested a leucine zipper motif at amino acids 221–223, but no other known evolutionarily conserved sequence domains are apparent. The ALMS1 protein is of unknown function, is widely expressed in human and mouse tissues (15–17), and localizes to centrosomes and the base of cilia (18). The basic pathophysiology is thought to involve intracellular trafficking.

It is not clear how body composition in AS relates to insulin resistance, how many patients with AS have mutations in *ALMS1*, or whether the mutations correlate with phenotype. We therefore aimed to describe the clinical, body composition, and genetic characteristics of a cohort of United Kingdom patients with a clinical diagnosis of AS.

Subjects and Methods

Subjects were recruited from the Alström Syndrome UK society screening clinic. The Alström Syndrome UK society (www.Alstrom.org.uk) is an active patient support group representing British families with AS. At the annual clinic, patients have a multidisciplinary review, including cardiac, metabolic, ear, nose, and throat, and urological assessments. All participants had AS based on minimum clinical criteria of infantile retinal dystrophy, sensorineural deafness, and obesity (see Table 1 for full clinical characteristics of the subjects).

Subjects were recruited for additional measurement of metabolic parameters and body composition and for genetic analysis. All subjects gave written informed consent, and ethical approval was obtained from the local ethics committee.

A medical questionnaire was completed for each participant (birth details, medical history, medications and allergies, family history, and pubertal status); patients were examined, and hospital case notes were reviewed.

Metabolic measurements

Fasting blood samples were taken for the following measurements: glucose and lipids (Vitros 950 Dry Chemistry Analyser; Ortho Clinical Diagnostics, High Wycombe, UK); insulin was measured by a nonspecific insulin ELISA method (Mercodia Iso-Insulin assay; Diagenics Ltd., Milton Keynes, UK). This assay cross-reacts 56% with split proinsulin; an internal audit of 66 samples from patients with clinical signs of insulin resistance showed the lowest insulin level recorded was 120 pmol/liter (Wark G., unpublished data). This compares with the American Heart Association's guidelines for evaluating fasting plasma insulin levels in children: normal, less than 99 pmol/liter (<15 mU/liter); borderline high, 99–132 pmol/liter (15–20 mU/liter); high, more than 132 pmol/liter (>20 mU/liter) (19). These results were compared with published normal reference data for healthy white United Kingdom 5-yr-old children (boys, mean of 25 pmol/liter and SD of 18; girls, 34 pmol/liter and SD 19) (20) and white 14- to 16-yr-old adolescents (boys, 74 pmol/liter and SE of 5; girls, 84 pmol/liter and SE of 5) (21). C-peptide was measured by ELISA (Dako, Ely, UK) at the Regional Endocrine Laboratory, University Hospital Birmingham (fasting reference ranges quoted from this laboratory are 200–800 pmol/liter). Insulin sensitivity and β-cell function were calculated from fasting glucose and insulin measurements using the homeostasis model assessment (HOMA) (22, 23).

Body composition measurements

Weight was measured in light indoor clothing to the nearest 0.1 kg (Tanita Digital scales), and height was measured without shoes to the nearest 0.1 cm using a portable stadiometer (Leicester height measure; Seca, Birmingham, UK). SD scores (SDS) were calculated using the United Kingdom 1990 Growth Reference data (24). BMI was calculated as weight (kilograms) divided by height (meters) squared. Overweight and obesity were defined according to the International Obesity Task Force guidelines, which define overweight as a BMI equivalent to a BMI of 25 kg/m^2 at age 18 and obesity as a BMI equivalent to a BMI of 30 kg/m^2 at age 18 (25). These are equivalent to the 90th percentile (SDS for males of 1.3, for females 1.19) and 99th percentile (SDS for males of 2.37, for females 2.25), respectively. Waist circumferences were measured to the nearest 0.1 cm using a flexible nonstretchable tape measure. SDS were calculated using reference data for United Kingdom children (26) and United Kingdom young adults (27). Waist circumference measurements were also compared with adult cutoffs defined to identify those at high risk of visceral obesity and the metabolic syndrome (male normal, <94 cm; female normal, <80 cm) (2). Skinfold thickness was measured to the nearest 0.1 mm with a Holtain caliper at the following sites: biceps, triceps, suprailiac, and subscapular. All skinfold measurements were taken in triplicate on the nondominant side by the same person, and the average of three readings was used. Percent body fat was calculated from the skinfold thickness measurements using age-appropriate linear regression equations (28, 29).

Patients underwent dual-energy x-ray absorptiometry (DXA) to give measures of total body composition (GE Lunar Prodigy densitometer; GE Systems, Chalfont St. Giles, UK). Trunk thickness and body weight were used to ensure that each patient was scanned in the most appropriate acquisition mode. Scan acquisition was performed by trained personnel and analysis performed by a single trained operator (N.C.). Body composition was calculated from the whole-body scan to provide measures of lean and fat body mass. Values for total body fat percent derived from the DXA measurements were compared with Child Growth Foundation reference curves for United Kingdom children up to the age of 20 yr (30) or to the adult reference data supplied for the Prodigy densitometer.

Genetic analysis

DNA was extracted from peripheral leukocytes (Nucleon BACC2 kit, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The *ALMS1* gene was screened for mutations by PCR and direct sequencing of all the exons and intron-exon boundaries. Primer sequences are available from the authors. The PCR were performed in 30-μl volumes using 2 mM magnesium chloride, 2 mM dNTPs, and 1 U *Taq* polymerase (Invitrogen, Paisley, UK). A 10-μl aliquot of each reaction was cleaned by addition of 1.5 μl of both exonuclease I and shrimp alkaline phosphatase (Amersham). The sequencing reaction was performed in a 10-μl volume using 4 μl of cleaned PCR product, 1× ABI sequencing buffer, and 2 μl Big Dye terminator cycle sequencing mix (ABI Applied Biosystems, Warrington, UK). Products were resolved on a denaturing gel and analyzed on an ABI 3730 automated sequencer (ABI Applied Biosystems). All novel mutations were excluded and polymorphism prevalence was assessed in 50 normal controls by restriction digest or direct sequencing.

Results

Of 13 patients attending the annual Alström family screening clinic, 12 unrelated patients gave consent for the study; 11 underwent DXA scans (the younger sibling of family 10 did not take part in the study, and subject 4.1, aged 3 yr, declined a DXA scan). The median age of patients was 19 yr (range, 3–34 yr), and there were seven males. All patients were of white United Kingdom origin, except patient 3.1, who was of mixed African-Caribbean/white United Kingdom origin. Patients 6.1 and 10.1 had affected siblings. No parent was affected, and there was no consanguinity. Five families originated from the Yorkshire area of the United Kingdom (families 1, 4, 5, 6, and 7).

Clinical characteristics

The clinical and biochemical characteristics of the cohort are given in Table 1, and the body composition data are given

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TABLE 1. Clinical characteristics of United Kingdom Alström cohort

Subject	Age (yr)	Sex	Retinal dystrophy onset	Deafness onset	Cardiomyopathy onset	Diabetes mellitus	Acanthosis nigricans	Fasting glucose (mmol/liter)	Fasting insulin (pmol/liter) (normal, <32)	HOMA %S	HOMA %B	Fasting C-peptide (pmol/liter) (200–800)	Lipids		Hypertension	Other
													Cholesterol (mmol/liter) (normal, <5)	Triglyceride (mmol/liter) (normal, <1.8)		
4.1	3	M	Infancy	Age 3	Infancy	No	No	4.5	103	54.1	182.5				No	
1.1	7.9	M	Infancy	Infancy	Infancy	No	Yes	4.6	138	40.5	212.9	879	4.3	2.75	No	Mild developmental delay, NASH
2.1	11.4	F	Infancy	Age 7	Infancy	Yes	Yes	7.2	620	13.4	197.7	1339	4.3	5	Yes	
10.1	11.9	M	Infancy	Infancy	No	No	Yes	5.6	312	17.8	256.5	5265	6.3	3.53	No	
3.1	15.5	F	Infancy	Age 10	No	No	No	4.6	131	42.6	205.6	1669	4.8	2.09	Yes	
5.1	19.3	M	Infancy	Infancy	Infancy	Yes	No	4.7	215	26.4	275.7	2518	5	2.29	No	Hypogonadism
9.1	19.3	F	Infancy	Infancy	No	Yes	Yes	12.9	446	11.6	85	2632	5.9	2.1	Yes	NASH
11.1	19.8	F	Infancy	Age 5	No	No	No	5.5	134	39.8	147.7	1719	5.6	1.5	Yes	Detrusor dyssynergia
6.1	22.5	F	Infancy	Age 7	Infancy	Yes	Yes	8	473	13.1	166.6	3580	9	30	Yes	Detrusor dyssynergia
12.1	29.6	M	Infancy	Age 10	No	No	Yes	4	315	19.3	484.2	4563	4.3	1.8	Yes	Chronic renal failure, hypogonadism, Klinefelter
7.1	33	M	Infancy	Age 20	Adult	Yes	Yes	8.2	500	13.1	160.2	4350	3.8	4.84	Yes	Chronic renal failure. detrusor dyssynergia
8.1	34	M	Infancy	Infancy	Adolescent	Yes	Yes	15.4	400	10.1	69.7	2322	6.9	11	Yes	Chronic renal failure, hypogonadism, NASH

Hypertension was defined as a blood pressure of at least 130/85 mm Hg. M, Male; F, female; NASH, nonalcoholic hepatosteatosis; %S, insulin sensitivity; %B, β-cell function.

in Table 2. All patients developed cone-rod dystrophy in infancy; sensorineural deafness was present in all, presenting most commonly in infancy, but up to 20 yr at detection in patient 7.1. Seven patients had cardiomyopathy in infancy or early adulthood or both.

Fasting hyperinsulinemia based on American Heart Association guidelines (19) was present in 10 of 12 patients (>132 pmol/liter). The four youngest patients, subjects 4.1, 1.1, 2.1, and 10.1, had insulin levels more than 3 SD more than age-matched norms. All the subjects had C-peptide greater than the upper lab reference range of 800. Eight subjects had acanthosis nigricans, and six had diabetes mellitus. Hypertriglyceridemia was present in 10 patients and hypercholesterolemia in six. Eight patients were hypertensive or on antihypertensive medication. Five of seven of the adult patients had the metabolic syndrome according to the current International Diabetes Federation consensus definition (evidence of central obesity (waist circumference ≥ 94 cm in males and ≥80 cm females), together with two other components (fasting plasma glucose ≥ 5.6 mmol/liter, blood pressure ≥ 130/85 mm Hg, triglycerides ≥ 1.7 mmol/liter, and high-density lipoprotein cholesterol ≤ 0.9 mmol/liter in males and ≤1.1 mmol/liter females) (26).

Body composition and metabolic characteristics

The children in this cohort had height SDS above the mean, but the adults were below the mean. All subjects were overweight, and 11 of 12 were obese according to the International Obesity Taskforce cutoffs (25). All the adults had waist circumferences above the International Diabetes Federation definition cutoffs for central obesity, and for all subjects, the waist circumference was significantly above age-related cutoffs. This was more marked in the younger subjects because waist circumference SDS was strongly negatively correlated with age ($r = -0.84$, $P = 0.002$). There was also a trend toward decreasing BMI SDS with age ($r = -0.37$, $P = 0.2$). The relationship with waist circumference remained after controlling for BMI ($r = -0.86$, $P = 0.003$), confirming that this observation was not just a reflection of change in body weight.

Body fat calculated from skinfold thickness correlated poorly with that measured on DXA scan, implying that skinfold thickness equations may not be valid for use in calculating body fat in AS patients (skinfolds *vs.* DXA $r = 0.39$, $P = 0.34$). However, body fat calculated from bioimpedance data correlated well with DXA data (bioimpedance *vs.* DXA $r = 0.9$, $P = 0.003$) (data not shown). DXA estimates of body fat (DXA percent body fat) were used for subsequent analyses. All patients had DXA percent body fat in the top quartile for age and sex. The falls in waist circumference SDS and BMI SDS with age were accompanied by a fall in DXA percent body fat with age ($r = -0.6$, $P = 0.05$). This relationship persisted after controlling for BMI. There was no significant sex difference in the median percentage body fat (males 38.4% *vs.* females 34.7%, $P = 0.9$). Because normal ranges for percent body fat are higher in females (30, 31), this meant that the male subjects generally had proportionately higher total fat levels. However, the decrease in body fat seen with age

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TABLE 2. Body composition data on United Kingdom Alström cohort

Subject	Age (yr)	Sex	Height (m)	Weight (kg)	BMI (kg/m^2)	Waist (cm)	Biceps skinfold (mm)	Triceps skinfold (mm)	Subscapular skinfold (mm)	Suprailiac skinfold (mm)	Body fat % (from skinfold)	Total body fat % (DXA)	Trunk fat % (DXA)	Lean mass % (DXA)
4.1	3	M	0.97 (1.30)	24.0 (4.89)	25.4 (4.78)									
1.1	7.9	M	1.39 (2.17)	51.0 (3.48)	26.4 (3.43)	89 (9.87)						38.5, >98th percentile[a]	36.9	60
10.1	11.4	M	1.55 (1.43)	66.3 (2.69)	27.6 (2.80)	95 (5.29)	21	20	33	34	43.4	42.1, >98th percentile[a]	39.1	56.3
2.1	11.9	F	1.53 (0.57)	52.7 (1.40)	22.5 (1.48)	80 (3.65)	11.9	15.1	17.3	14.4	30.6	31.6, 91st −98th percentile[a]	32	65.9
3.1	15.5	F	1.53 (−1.56)	70.4 (1.66)	30.1 (2.47)	88 (4.69)						34.7, 91st-98th percentile[a]	32.4	63.2
5.1	19.3	M	1.59 (−2.56)	90.5 (1.94)	35.8 (3.12)	103 (4.66)	11.3	17.2	34.7	35	27.6	40.8, >98th percentile[a]	40.8	57.7
9.1	19.3	F	1.59 (−0.77)	73.4 (1.61)	29.0 (2.04)	95 (5.26)	16.8	11.2	27.9	19.7	30.5	36.0, 91st-98th percentile[a]	34.8	61.8
11.1	19.8	F	1.59 (−0.78)	87.6 (2.64)	34.6 (2.93)	100 (6.17)	21.5	38.1	40	23.5	38.6	44.8, >98th percentile[a]	41.2	53.4
6.1	22.5	F	1.55 (−1.46)	70.4 (1.32)	29.3 (1.98)	89	18.2	17.4	39	31.5	37.8	33.8, 76th percentile[b]	38.5	63.9
12.1	29.6	M	1.33 (−6.27)	72.4 (0.63)	40.9 (3.66)	95 (0.27)	18.1	17.2	40	23.3	27.3	38.3, >98th percentile[b]	36.3	60
7.1	33	M	1.56 (−2.95)	69.8 (0.38)	26.7 (1.73)	98 (0.55)	16.4	8.7	39.4	26	27.9	24.2, 85th percentile[b]	30.3	73.6
8.1	34	M	1.60 (−2.08)	83.8 (1.59)	32.7 (2.80)	106 (1.29)	20.5	21.1	39.7	21.5	29.3	23.3, 78th percentile[b]	28	74.6

Numbers in *parentheses* are SDS. F, Female; M, male.
[a] Child body fat age percentile values from Ref. 30.
[b] Prodigy machine adult reference data based on United Kingdom population.

meant that the two oldest subjects had body fat levels only slightly above the normal range.

Insulin sensitivity as assessed by HOMA decreased with age ($r = -0.64$, $P = 0.02$) despite the decrease in waist circumference and DXA percent body fat.

Mutation analysis data

The *ALMS1* cDNA sequence (GenBank NM_15120.2) was used in this study. Sequences obtained from patients were compared with the *ALMS1* sequence and with normal control chromosomes. The A of the ATG of the initiator Met codon was denoted as nucleotide +1. Sequencing of exons 1–23 revealed mutations in 10 of 12 patients (Table 3). However, in two subjects (patients 10.1 and 5.1), only one mutation was identified. The majority of the mutations were nonsense or frameshift mutations and suggested *ALMS1* loss-of-function mutations as the cause of AS. In total, five novel mutations were identified in our cohort, of which three were predicted to give rise to truncated protein products; two of these were frameshifts followed by premature truncations, and one was a nonsense mutation (Table 3). Missense mutations were present as heterozygous mutations in two families: 1788 Asn[N]→Asp[D] (AAT→GAT) in patient 1.1 and 2946 Asn[N]→Lys[K] (AAC→AAG) in patient 2.1. None of the mutations were present in 100 control alleles.

Phenotype-genotype relationships

Relationship with clinical features was examined for both mutation positions within the gene, *e.g.* exons 8, 10, and 16, and with the type of mutation, *e.g.* frameshift, nonsense, or missense. Individuals with detected mutations were compared with those in whom mutations were not detected. No significant relationship could be observed between the clinical features and mutation positions, type, or combinations. There was no difference in clinical severity between patients with no, one, or two mutations identified.

Evidence for a founder effect

As can be seen, the deletion in exon 16 (10775delC) was identified in five of our cohort, who all either resided or originated from Yorkshire, United Kingdom. These patients had the same single-nucleotide polymorphism haplotype (data not shown), suggesting the probability of a common founder effect in these individuals.

Discussion

To our knowledge, this is the first systematic study on insulin sensitivity and body composition in AS. We also report mutation data from the largest United Kingdom cohort to date. Our cohort is likely to be representative of white United Kingdom patients with AS, of whom there are about 30 known cases. However, there may be ethnic differences with other United Kingdom patients of Pakistani origin (6). All patients in our cohort were overweight or obese, but the BMI SDS was lower in the older patients, suggesting that the obesity ameliorated with age. This finding is in agreement with others, who suggested that obesity moderated with the onset of other clinical complications (2). Our data extend

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TABLE 3. Mutations identified in the *ALMS1* gene

Family no.	Exon	Nucleotide change	Amino acid change	Type of mutation
4.1	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation (15, 16)
	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation
1.1	8	5362A→G	N1788D	Missense (novel)
	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation
10.1	16	11107C→T	R3703X	Nonsense (6)
	ND	ND	ND	ND
2.1	8	6590del (A)	Del2197fs/ter2206	Frameshift/truncation (novel)
	10	8838C→G	N2946K	Missense (novel)
3.1	8	2225ins (A)	Ins742fs/ter743	Frameshift/truncation (novel)
	16	10975C→T	R3659X	Nonsense (novel)
5.1	16	11449C→T	Q3817X	Nonsense (15)
	ND	ND	ND	ND
9.1	10	8008C→T	R2637X	Nonsense (6)
	16	10885C→T	R3629X	Nonsense (6)
11.1	ND	ND	ND	ND
	ND	ND	ND	ND
6.1	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation
	16	10992G→A	W3664X	Nonsense (16)
12.1	ND	ND	ND	ND
7.1	16	10483C→T	Q3495X	Nonsense (16)
	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation
8.1	16	10775del (C)	Del3592fs/ter3597	Frameshift/truncation
	16	11416C→T	R3806X	Nonsense (6)

The references show which mutations have been previously reported. ND, Mutation not detected.

these observations by showing that waist circumference SDS and DXA percent body fat also decrease with age independently of BMI changes. Paradoxically, insulin resistance increases with age.

Our findings may indicate a change in body fat distribution with age to more metabolically active fat stores. However, it may be secondary to weight loss because of lifestyle changes after the onset of diabetes. Alternatively, with onset of diabetes, hyperglycemia *per se* may contribute to insulin resistance. Other authors suggested that the onset of additional complications ameliorated obesity (2). We tested whether presence of infantile or adult-onset cardiomyopathy, chronic renal failure, or diabetes was associated with any difference in BMI SDS. We found that infantile-onset or adult-onset cardiomyopathy and presence of chronic renal failure were not associated with differences in BMI SDS. We also hypothesized that BMI SDS may apparently decrease with age because of a survival advantage for those with lower BMI. However, analysis of BMI data from AS patients who have died did not show any difference in BMI SDS compared with the study population (Paisey, R., unpublished data). Therefore, there is not strong support from our data for the hypothesis that onset of complications is responsible for the changes observed. Additional study of body composition with cross-sectional imaging techniques would be required to demonstrate alterations in body fat distribution.

HOMA insulin sensitivity has previously been shown to correlate strongly with insulin sensitivity measured by other methods including hyperinsulinemic-euglycemic clamp and the frequently sampled iv glucose tolerance test (32, 33). It has also been validated for use in both prepubertal and obese children, and it correlates similarly with clamp data in white children (34). DXA has been evaluated against four-compartment models, and studies have shown that it overestimates body fat by 1–4%, depending on age, sex, and degree of obesity (35). The good correlation between DXA fat measurements and four-compartment measurements, with such a small magnitude of error, make it a convenient and suitable method for studying patients in the clinical setting (36). Body fat calculated from skinfolds did not correlate well with that measured on DXA scan, implying that the skinfold thickness equations used are not valid in AS.

One problem encountered in this study was lack of availability of population control data for the wide age range of the subjects studied. Most population cohorts have an older average age reflected in higher mean waist circumference and body fat values. We accept that there are some limitations to the values used. The use of waist circumference norms for 17-yr-old subjects to calculate waist circumference SDS in 19-yr-old subjects may overestimate the SDS for the older subjects. We were not able to find a suitable young adult cohort for percent body fat and so have quoted the Prodigy densitometer reference data, which may underestimate the percentiles for young adults. Contemporary United Kingdom skinfold data across the whole childhood age range are not published. Similarly, there are limited published data available to age standardize fasting insulin. However, we were able to use United Kingdom data from healthy 5-yr-old children (20) and adolescents (21) to show that the Alström subjects were insulin resistant from an early age.

Direct sequencing of the *ALMS1* gene identified 13 different mutations in 10 of 12 patients, of which five were novel, including three protein truncation mutations and two missense mutations. There were no obvious phenotypic differences in the two patients in whom we did not detect mutations. It is possible that they harbor mutations in the promoter or intronic regions. Alternatively, it is possible that a second AS gene exists. To identify any possible mutation hotspots in the ALMS1 protein sequence, we combined our data with results from four other reports (6, 15, 16, 37). The frequency of mutations was plotted against amino acid residues (Fig. 1). The deletion in exon 16 (10775delC) is the

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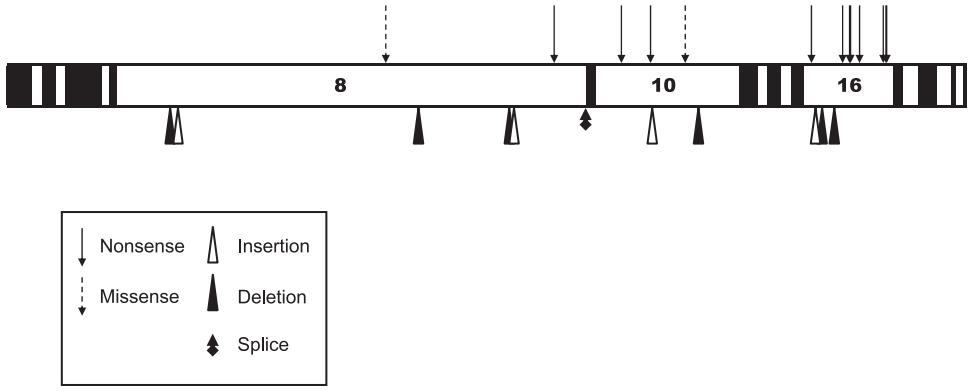

FIG. 1. Mutations of the *ALMS1* gene. All the demonstrated mutations of *ALMS1* cluster within exons 8, 10, and 16 (6, 15, 16, 37, and the present study). Exon 16 alone holds approximately half of the known mutations (11 of 23) and represents a potential mutational hotspot.

commonest mutation, both in our cohort and from previous reports. This mutation was identified in five of our cohort, who all either resided or originated from Yorkshire, United Kingdom. These patients had the same single-nucleotide polymorphism haplotype, suggesting the probability of a common founder effect in these individuals. It is also possible that the frequency of this mutation is falsely elevated because of the same Alström patients being reported in different publications. There were no significant genotype/phenotype correlations in our cohort; this may be a result of small numbers in our study. However, heterogeneity has been observed between members of the same sibship who clearly share both genetic and environmental background in the United Kingdom (37).

In conclusion, we describe the clinical and genetic characteristics of a cohort of United Kingdom subjects with AS. We confirm the clinical features of the syndrome and show that in our patients, severe childhood obesity, waist circumference, and body fat decrease compared with age-standardized norms, whereas insulin resistance increases. We identified mutations in *ALMS1* in more than 80% of patients with no genotype-phenotype correlation and suggest that a screening strategy in affected families should target exons 8, 10, and 16. The abdominal obesity, insulin resistance, diabetes, hypertriglyceridemia, and hypertension suggest that AS could represent a monogenic model for the metabolic syndrome.

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References

1. **Alström CH, Hallgren B, Nilsson LB, Asander H** 1959 Retinal degeneration combined with obesity, diabetes mellitus and neurogenous deafness: a specific syndrome (not hitherto described) distinct from the Laurence-Moon-Bardet-Biedl syndrome. A clinical, endocrinological and genetic examination based on a large pedigree. Acta Psychiatr Neurol Scand Suppl 129:1–35
2. **Marshall JD, Bronson RT, Collin GB, Nordstrom AD, Maffei P, Paisey RB, Carey C, Macdermott S, Russell-Eggitt I, Shea SE, Davis J, Beck S, Shatirishvili G, Mihai CM, Hoeltzenbein M, Pozzan GB, Hopkinson I, Sicolo N, Naggert JK, Nishina PM** 2005 New Alström syndrome phenotypes based on the evaluation of 182 cases. Arch Intern Med 165:675–683
3. **Marshall JD, Ludman MD, Shea SE, Salisbury SR, Willi SM, LaRoche RG, Nishina PM** 1997 Genealogy, natural history, and phenotype of Alström syndrome in a large Acadian kindred and three additional families. Am J Med Genet 73:150–161
4. **Michaud JL, Heon E, Guilbert F, Weill J, Puech B, Benson L, Smallhorn JF, Shuman CT, Buncic JR, Levin AV, Weksberg R, Breviere GM** 1996 Natural history of Alström syndrome in early childhood: onset with dilated cardiomyopathy. J Pediatr 128:225–229
5. **Russell-Eggitt IM, Clayton PT, Coffey R, Kriss A, Taylor DSI, Taylor JFN** 1998 Alström syndrome. Report of 22 cases and literature review. Ophthalmology 105:1274–1280
6. **Bond J, Flintoff K, Higgins J, Scott S, Bennet C, Parsons J, Mannon J, Jafri H, Rashid Y, Barrow M, Trembath R, Woodruff G, Rossa E, Lynch S, Sheilds J, Newbury-Ecob R, Falconer A, Holland P, Cockburn D, Karbani G, Malik S, Ahmed M, Roberts E, Taylor G, Woods CG** 2005 The importance of seeking *ALMS1* mutations in infants with dilated cardiomyopathy. J Med Genet 42:e10
7. **Satman I, Yilmaz MT, Gursoy N, Karsidag K, Dinccag N, Ovali T, Karadeniz S, Uysal V, Bugra Z, Okten A, Devrim S** 2002 Evaluation of insulin resistant diabetes mellitus in Alström syndrome: a long-term prospective follow-up of three siblings. Diabetes Res Clin Pract 56:189–196
8. **Alter CA, Moshang Jr T** 1993 Growth hormone deficiency in two siblings with Alström syndrome. Am J Dis Child 147:97–99
9. **Paisey RB, Carey CM, Bower L, Marshall J, Taylor P, Maffei P, Mansell P** 2004 Hypertriglyceridaemia in Alström 's syndrome: causes and associations in 37 cases. Clin Endocrinol (Oxf) 60:228–231
10. **Wu WC, Chen SC, Dia CY, Yu ML, Hsieh MY, Lin ZY, Wang LY, Tsai JF, Chang WY, Chuang WL** 2003 Alström syndrome with acute pancreatitis: a case report. Kaohsiung J Med Sci 19:358–361
11. **Connolly MB, Jan JE, Couch RM, Wong LT, Dimmick JE, Rigg JM** 1991 Hepatic dysfunction in Alström disease. Am J Med Genet 40:421–424
12. **Hopkinson I, Marshall JD, Paisey RB, Carey C, MacDermott S** Updated 7 February 2005 Alström syndrome. In: GeneReviews at Gene Tests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle, WA. Available at http://www.genetests.org/query?dz=alstrom
13. **Hoffman JD, Jacobson Z, Young TL, Marshall JD, Kaplan P** 2005 Familial variable expression of dilated cardiomyopathy in Alström syndrome: a report of four sibs. Am J Med Genet A 135:96–98
14. **Tai TS, Lin SY, Sheu WH** 2003 Metabolic effects of growth hormone therapy in an Alström syndrome patient. Horm Res 60:297–301
15. **Collin GB, Marshall JD, Ikeda A, So WV, Russell-Eggitt I, Maffei P, Beck S, Boerkoel CF, Sicolo N, Martin M, Nishina PM, Naggert JK** 2002 Mutations in *ALMS1* cause obesity, type 2 diabetes and neurosensory degeneration in Alström syndrome. Nat Genet 31:74–78
16. **Hearn T, Renforth GL, Spalluto C, Hanley NA, Piper K, Brickwood S, White C, Connolly V, Taylor JF, Russell-Eggitt I, Bonneau D, Walker M, Wilson DI**

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2002 Mutation of *ALMS1*, a large gene with a tandem repeat encoding 47 amino acids, causes Alström syndrome. Nat Genet 31:79–83
17. **Collin GB, Cyr E, Bronson R, Marshall JD, Gifford EJ, Hicks W, Murray SA, Zheng QY, Smith RS, Nishina PM, Naggert JK** 2005 *Alms1*-disrupted mice recapitulate human Alström syndrome. Hum Mol Genet 14:2323–2333
18. **Hearn T, Spalluto C, Phillips VJ, Renforth GL, Copin N, Hanley NA, Wilson DI** 2005 Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. Diabetes 54:1581–1587
19. **Williams CL, Hayman LL, Daniels SR, Robinson TN, Steinberger J, Paridon S, Bazzarre T** 2002 Cardiovascular health in childhood: a statement for health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. Circulation 106:143–160
20. **Wilkin TJ, Metcalf BS, Murphy MJ, Kirkby J, Jeffery AN, Voss LD** 2002 The relative contributions of birth weight, weight change, and current weight to insulin resistance in contemporary 5-year-olds: the EarlyBird Study. Diabetes 51:3468–3472
21. **Ehtisham S, Crabtree N, Clark P, Shaw N, Barrett T** 2005 Ethnic differences in insulin resistance and body composition in United Kingdom adolescents. J Clin Endocrinol Metab 90:3963–3969
22. **Levy JC, Matthews DR, Hermans MP** 1998 Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21:2191–2192
23. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment (HOMA): insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
24. **Cole TJ, Freeman JV, Preece MA** 1995 Body mass index reference curves for the UK, 1990. Arch Dis Child 73:25–29
25. **Cole TJ, Bellizzi MC, Flegal KM, Dietz WH** 2000 Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320:1240–1243
26. **McCarthy HD, Jarrett KV, Crawley HF** 2001 The development of waist circumference percentiles in British children aged 5.0–16.9 y. Eur J Clin Nutr 55:902–907
27. **Knight B, Shields BM, Hattersley AT** 2006 The Exeter Family Study of Childhood Health (EFSOCH): study protocol and methodology. Paediatr Perinat Epidemiol 20:172–179
28. **Durnin JV, Womersley J** 1974 Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32:77–97
29. **Siri WE** 1993 Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition 9:480–491; discussion 480:492
30. **McCarthy HD, Cole TJ, Fry T, Jebb S, Prentice A** 2006 Body fat reference curves for children. Int J Obes 30:598–602
31. **Ogle GD, Allen JR, Humphries IR, Lu PW, Briody JN, Morley K, Howman-Giles R, Cowell CT** 1995 Body-composition assessment by dual-energy x-ray absorptiometry in subjects aged 4–26 y. Am J Clin Nutr 61:746–753
32. **Bonora E, Targher G, Zenere MB, Saggiani F, Cacciatori V, Tosi F, Travia D, Zenti MG, Branzi P, Santi L, Muggeo M** 1996 Relationship of uric acid concentration to cardiovascular risk factors in young men: role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. Int J Obes Relat Metab Disord 20:975–980
33. **Wallace T, Levy J, Matthews D** 2004 Use and abuse of HOMA modelling. Diabetes Care 27:1487–1495
34. **Gungor N, Saad R, Janosky J, Arslanian S** 2004 Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr 144:47–55
35. **Roemmich JN, Clark PA, Weltman A, Rogol AD** 1997 Alterations in growth and body composition during puberty. I. Comparing multicompartment body composition models. J Appl Physiol 83:927–935
36. **Sopher AB, Thornton JC, Wang J, Pierson Jr RN, Heymsfield SB, Horlick M** 2004 Measurement of percentage of body fat in 411 children and adolescents: a comparison of dual-energy x-ray absorptiometry with a four-compartment model. Pediatrics 113:1285–1290
37. **Titomanlio L, De Brasi D, Buoninconti A, Sperandeo MP, Pepe A, Andria G, Sebastio G** 2004 Alström syndrome: intrafamilial phenotypic variability in sibs with a novel nonsense mutation of the *ALMS1* gene. Clin Genet 65:156–157

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