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SHORT COMMUNICATION

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Common variations in the *ALMS1* gene do not contribute to susceptibility to type 2 diabetes in a large white UK population

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Abstract *Aims/hypothesis:* Alström syndrome is a rare monogenic disorder characterised by retinal dystrophy, deafness and obesity. Patients also have insulin resistance, central obesity and dyslipidaemia, thus showing similarities with type 2 diabetes. Rare mutations in the *ALMS1* gene cause severe gene disruption in Alström patients; however, *ALMS1* gene polymorphisms are common in the general population. The aim of our study was to determine whether common variants in *ALMS1* contribute to susceptibility to type 2 diabetes in the UK population. *Methods:* Direct sequencing was performed on coding regions and intron/exon boundaries of the *ALMS1* gene in 30 unrelated probands with type 2 diabetes. The linkage disequilibrium (LD; D' and r^2) and haplotype structure were examined for the identified variants. The common (minor allele frequency [MAF] >5%) single-nucleotide polymorphisms tagging the common haplotypes (tagged SNPs [tSNPs]) were identified and genotyped in 1985 subjects with type 2 diabetes, 2,047 control subjects and 521 families. *Results:* We identified 18 variants with MAF between 6 and 38%. Three SNPs efficiently tagged three common haplotypes (rs1881245, rs3820700 and rs1320374). There was no association (all $p>0.05$) between the tSNPs and type 2 diabetes in the case–control study and minor alleles of the tSNPs were not over-transmitted to probands with type 2 diabetes in the family study. *Conclusions/interpretation:* Common variations in the *ALMS1* gene were not associated with type 2 diabetes in a large study of a white UK population.

Keywords *ALMS1* · Alström syndrome · Common variation · Haplotypes · Type 2 diabetes

Abbreviations ESM: Electronic Supplementary Material · LD: linkage disequilibrium · MAF: minor allele frequency · SNP: single-nucleotide polymorphism · TDT: transmission disequilibrium test · tSNP: tagged single-nucleotide polymorphism

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Introduction

The study of monogenic diabetes has led to the identification of a number of type 2 diabetes susceptibility genes. For instance, common variation in the *HNF4A* gene can predispose to type 2 diabetes in the general population [1]. Alström syndrome (OMIM 203800) [2] is a recessive form of monogenic diabetes characterised by retinal dystrophy, sensorineural deafness, cardiomyopathy and childhood-onset obesity, leading to type 2 diabetes (in 80% of Alström syndrome patients by the age of 16 years), insulin resistance, central adiposity and hypertriglyceridaemia

[3]. The phenotype suggests that mutations in the Alström gene lead to both obesity and diabetes, and obligate heterozygotes may be at increased risk of diabetes [2].

The *ALMS1* gene maps to chromosome 2p13, a region linked to type 2 diabetes [4]. It comprises 23 exons spanning over 224 kb of genomic DNA encoding a protein of 4,169 amino acids of unknown function [5]. Rare mutations in *ALMS1* segregate with Alström syndrome in affected families [5, 6].

We aimed to determine whether common variants of *ALMS1* are associated with type 2 diabetes in white individuals in the UK. We identified 18 common *ALMS1* variants and three common haplotypes in 30 subjects with type 2 diabetes, and then used case–control and family-based methods to test for association between the variants and type 2 diabetes.

Subjects and methods

Subjects

The populations used for the analysis and the inclusion and exclusion criteria have been described elsewhere [7]. Briefly, all case subjects were unrelated white UK citizens with type 2 diabetes, recruited from three sources: probands from type 2 diabetes sibships from the Diabetes UK Warren 2 repository (n=559), a new collection of individuals with type 2 diabetes from the Warren 2 repository (n=1,141) and a collection of subjects with young-onset type 2 diabetes (aged >18 and<45 years at diagnosis of type 2 diabetes) (n=285). The control subjects were white UK citizens recruited from two sources: parents from a consecutive birth cohort (the Exeter Family Study) (n=1,574) and a nationally recruited population-based control sample of blood donors without known diabetes from the European Collection of Cell Cultures (n=473). The families consisted of an affected proband with type 2 diabetes and both parents (n=402) (Warren 2 trios) or one parent and at least one unaffected sibling (n=119) (Warren 2 duos) [8]. The family study individuals were independent of the case–control study. The study was approved by local ethics committees and written consent was obtained for all subjects.

Sequencing

Common variation in the *ALMS1* gene was identified by direct sequencing of coding regions and exon/intron boundaries using 30 randomly selected unrelated patients with type 2 diabetes from the Warren parent–offspring trio collection [8]. Coding regions and exon/intron boundaries were divided into overlapping fragments and amplified by PCR (primers and conditions are available from the authors). The fragments were sequenced in both directions using the BigDye Terminator chemistry method either on an ABI 3730 sequencer (Applied Biosystems, Warrington, UK) or a CEQ 8000 genetic analyser (Beckman Coulter, Fullerton, CA, USA).

Genotyping

Genotyping was carried out by Kbiosciences (Hoddesdon, UK) using the TaqMan system (Applied Biosystems). Of the genotyped samples, 10% were duplicates and there was at least one negative control per 96-well plate. Genotyping accuracy was determined by the genotype concordance between duplicate samples and was greater than 99.6% for each of the tagged single-nucleotide polymorphisms (tSNPs). The genotyping success rate for each tSNP was as follows: case subjects, rs1881245, 96%, rs3820700, 98%, rs1320374, 95%; control subjects, rs1881245, 97%, rs3820700, 97%, rs1320374, 96%. There were no Mendelian inheritance errors in the families. All case, control

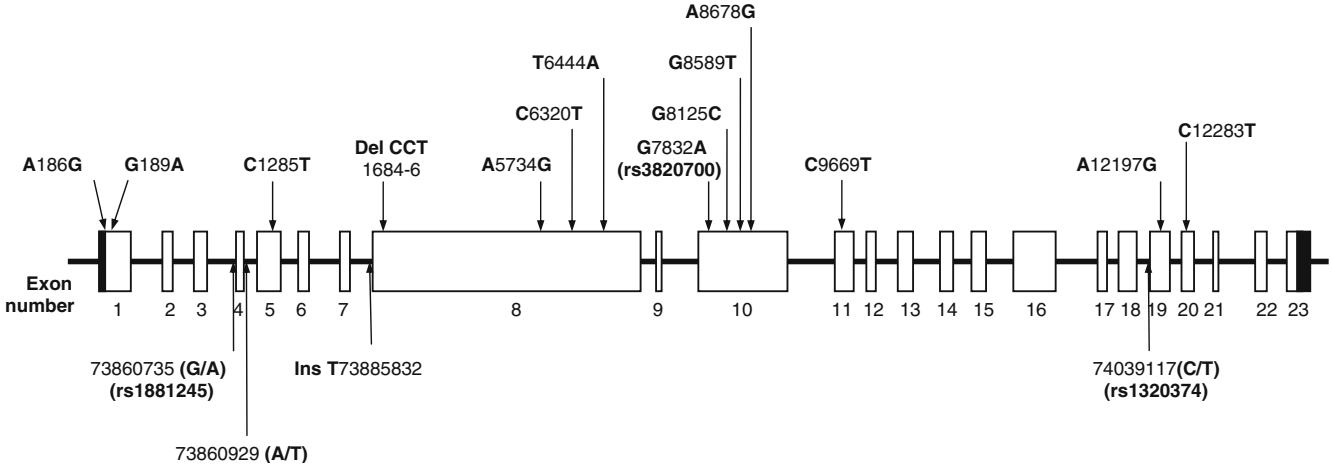

Fig. 1 Schematic representation of the *ALMS1* gene. Exons are shown in *white boxes* and the 3′ and 5′ untranslated regions in *black boxes*. The locations of variants identified by sequencing 30 unrelated type 2 diabetes probands are shown. Intronic variants are numbered according to chromosome 2 nucleotide position using genomic contig NT_022184 http://www.ncbi.nlm.nih.gov, last accessed in February 2006). The tagged SNPs are shown with their dbSNP rs identification numbers. *Ins*, insertion; *Del*, deletion

Table 1 Clinical characteristics of the study subjects

	Cases			Controls		Families	
	Warren 2 probands	Warren 2 cases	Young-onset type 2 diabetes	Exeter Family Study	ECACC	Warren 2 family trios[d]	Warren 2 duos[d]
Number (*n*)	559	1,141	285	1,574	473	402	119
Male (%)	54	61	55	49	52[c]	58	58
Age at diagnosis[a] (years)	56 (50–61)	52 (45–57)	40 (35–44)	NA	NA	40 (35–45)	45 (39.5–49)
BMI (kg/m^2)	28.1 (25.3–31.5)	30.7 (27.3–35.1)	32.2 (27.8–36.6)	26.2 (24.0–28.9)[b]	NA	32.2 (28.4–37.5)	32.2 (28.2–36.2)
Treatment (diet/OHA/insulin) (%)	18/67/15	8/64/27	9/53/38	NA	NA	21/64/16	15/57/28

Only subjects with genotype data for at least one SNP are included
Data are median (interquartile range)
Clinical details were not available for ECACC population control samples
Control study populations were not on treatment for diabetes
ECACC Population-based control sample of blood donors from the European Collection of Cell Cultures, *OHA* oral hypoglycaemic agents
[a]Age at diagnosis for case subjects, age at time of study for control subjects
[b]BMI measurement for men only because women were pregnant at the time of the study
[c]Percentage of males was determined by XY PCR
[d]Only probands

and family cohorts were in Hardy–Weinberg equilibrium (χ^2 test, $p>0.05$), except for the Warren 2 case subjects for SNP rs3820700 (χ^2 test, $p=0.04$). Given the other quality control results and the similarity of linkage disequilibrium (LD) between the SNPs in all our cohorts (and the HapMapII data, where the D' between SNPs is 1), we suggest that the mild deviation from Hardy–Weinberg equilibrium was due to chance variation and multiple testing rather than genotyping error.

Statistical analysis

We performed analysis of the LD (D' and r^2) and haplotype structure of the *ALMS1* gene using the Haploview program http://www.broad.mit.edu/mpg/haploview/index.php, version 3.2, last accessed in February 2006). For the case–control analysis, odds ratios with 95% CIs and p values were determined using χ^2 tests. For the family data we used the transmission disequilibrium test (TDT)/sibTDT of Spielman and Ewens [9]. Family trios were excluded from the analysis if the genotype data for parents were missing. The trios were also analysed using the TRANSMIT program http://www-gene.cimr.cam.ac.uk/clayton/software/transmit.txt), and the results were very similar to those obtained by the first method.

Results

We identified 18 variants across the *ALMS1* gene with a minor allele frequency (MAF) between 6 and 38% (Fig. 1). These included three intronic and 13 coding SNPs (four synonymous and nine non-synonymous). We also identified two novel insertion/deletion variants (not previously reported in the SNP databases [dbSNP], http://www.ncbi.

Table 2 Association of the *ALMS1* tagged SNPs based on 1985 case subjects with type 2 diabetes and 2,047 control subjects

					Allele frequency (*n*)			
tSNP	Genotype frequency (*n*)			*p* value	Allele 1	Allele 2	Odds ratio (95% CI)	*p* value
rs1881245	GG	GA	AA		G	A		
Case	58.2 (1,112)	36.2 (691)	5.6 (107)	0.76	76.3 (2,915)	23.7 (905)	1.03 (0.93–1.14)	0.57
Control	57.7 (1,144)	36.2 (717)	6.1 (122)		75.8 (3,005)	24.2 (961)		
rs3820700	GG	GA	AA		G	A		
Case	74.9 (1,461)	22.8 (444)	2.3 (45)	0.92	86.3 (3,366)	13.7 (534)	1.00 (0.88–1.14)	0.98
Control	74.7 (1,490)	23.1 (461)	2.2 (43)		86.3 (3,441)	13.7 (547)		
rs1320374	CC	CT	TT		C	T		
Case	39.4 (745)	47.1 (889)	13.5 (255)	0.70	63.0 (2,379)	37.0 (1,399)	1.01 (0.96–1.16)	0.81
Control	39.7 (780)	46.0 (903)	14.3 (281)		62.7 (2,463)	37.3 (1,465)		

tSNP tagged SNP

Table 3 TDT/sibTDT analysis of *ALMS1* tagged SNPs

tSNP	Observed transmission	Expected transmission	Z_{max} score[a]	*p* value[b]
rs1881245	179	179	−0.04	0.9
rs3820700	160	157	0.38	0.71
rs1320374	228	233	0.48	0.63

The TDT results are presented for the minor allele at each tSNP
tSNP tagged SNP
[a]Z_{max} score from the TDT/sibTDT analysis
[b]Two-tailed

nlm.nih.gov/SNP/, last accessed in February 2006), an in-frame CCT deletion in exon 8 resulting in a proline amino acid deletion and a T insertion 64 bp upstream of exon 8. We found all of the validated common (MAF >5%) dbSNPs in the regions we sequenced. Table 1 of the Electronic Supplementary Material (ESM) provides information on the variants.

We examined the LD structure for the identified variants using the Haploview program. In the subsample of 30 probands, three haplotypes that were tagged by three SNPs occurred at a frequency greater than 5% and accounted for 75% of all haplotypes. The three common haplotypes defined by the three tSNPs (rs1881245, rs3820700 and rs1320374) were: G, G, C (54%), G, A, T (13%) and A, G, T (8%).

We used the HapMapII project CEPH (Utah residents with ancestry from northern and western Europe) trio data http://www.HapMap.org, last accessed in February 2006) to see how well our three tSNPs captured the common variation across the *ALMS1* gene.

Overall, there was very good correlation between the SNPs across the *ALMS1* gene from the HapMapII data (ESM Fig. 1). We examined the extent of the LD using the Tagger program http://www.broad.mit.edu/mpg/tagger/, last accessed in February 2006); our three tSNPs captured almost all the common variation (MAF >5%) in the *ALMS1* region from HapMapII (captured 92% [137/149 SNPs] with r^2 >0.8 and mean r^2=0.975).

We genotyped the three tSNPs and performed association analysis in 1985 case subjects with type 2 diabetes, 2,047 population control subjects without type 2 diabetes and 521 families. The clinical characteristics of these subjects are presented in Table 1. There were no significant differences (p>0.05) in the genotype or allele frequencies for the tSNPs between the three groups that made up the case subjects and the two groups that made up the controls (the analysis is shown in ESM Table 2). Therefore, the case and control groups were combined for analysis. There were no significant differences (p>0.05) in the genotype or allele frequencies between the case and control groups for any of the tSNPs (Table 2). Table 3 shows the results of the family-based analysis using the TDT/sibTDT method [9]. There was no significant overtransmission of the minor alleles for the tSNPs in 521 families.

Discussion

This is the first large population-based case–control and family-based association study investigating common variation in the *ALMS1* gene and type 2 diabetes. The HapMapII data show very good correlation between most of the SNPs across *ALMS1* and this extends approximately 77 kb from the 5′ end and 11 kb from the 3′ end of the gene. Our three tSNPs captured 92% (mean r^2=0.975) of the common variation in the *ALMS1* region from the HapMapII data. However, we found no evidence of association of variation in *ALMS1* with type 2 diabetes in the case–control and family-based studies.

Our results confirm the findings of a previous small case–control study looking for association between *ALMS1* and type 2 diabetes [10]. This group studied the gene variants D2672H, R2826S and R4029K in 188 type 2 diabetes patients and 167 age-matched normoglycaemic controls. Genotype and allele frequencies did not differ between patients and control subjects for gene variants (p>0.2). However, this study had less power and did not involve a full SNP analysis in type 2 diabetes patients across the *ALMS1* gene. Our final analysis included over 4,000 case–control subjects and 521 families and therefore had substantial power to detect odds ratios of 1.22–1.36 that are comparable to proven type 2 diabetes susceptibility genes, such as *PPARG*.

ALMS1 is widely expressed in tissues, including the heart and pancreas, and has been localised to centrosomes and the base of cilia [11]. The function of *ALMS1* is not known, but it is thought to be involved in microtubule organisation and intracellular transport. This may have implications for understanding mechanisms of insulin secretion and the development of diabetes in the Alström syndrome. However, we have found no evidence of association between common variations of the *ALMS1* gene and type 2 diabetes in the general population.

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Duality of interest There is no duality of interest.

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