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SHORT COMMUNICATION

Reassessment of the putative role of *BLK*-p.A71T loss-of-function mutation in MODY and type 2 diabetes

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Abstract
Aims/hypothesis MODY is believed to be caused by at least 13 different genes. Five rare mutations at the *BLK* locus, including only one non-synonymous p.A71T variant, were reported to segregate with diabetes in three MODY families. The p.A71T mutation was shown to abolish the enhancing effect of BLK on insulin content and secretion from pancreatic beta cell lines. Here, we reassessed the contribution of *BLK* to MODY and tested the effect of *BLK*-p.A71T on type 2 diabetes risk and variations in related traits.
Methods BLK was sequenced in 64 unelucidated MODY samples. The *BLK*-p.A71T variant was genotyped in a

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French type 2 diabetes case–control study including 4,901 cases and 4,280 controls, and in the DESIR (Data from an Epidemiological Study on the Insulin Resistance Syndrome) and SUVIMAX (Supplementation en Vitamines et Mineraux Antioxydants) population-based cohorts (n=6,905). The variant effects were assessed by logistic and linear regression models.

Results No rare non-synonymous *BLK* mutations were found in the MODY patients. The *BLK* p.A71T mutation was present in 52 normoglycaemic individuals, making it very unlikely that this loss-of-function mutation causes highly penetrant MODY. We found a nominal association between this variant and increased type 2 diabetes risk, with an enrichment of the mutation in the obese diabetic patients, although no significant association with BMI was identified.

Conclusions/interpretation No mutation in *BLK* was found in our MODY cohort. From our findings, the *BLK*-p.A71T mutation may weakly influence type 2 diabetes risk in the context of obesity; however, this will require further validation.

Keywords *BLK* · Diabesity · Genetics · Low-frequency variant · Maturity-onset diabetes of the young · MODY · Mutation · Type 2 diabetes

Abbreviation

DESIR	Data from an Epidemiological Study on the Insulin Resistance Syndrome
HOMA-B	Homeostasis model assessment of pancreatic beta cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
SUVIMAX	Supplementation en Vitamines et Mineraux Antioxydants

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Introduction

MODY is a genetically and clinically heterogeneous form of non-autoimmune diabetes that is characterised by a highly penetrant autosomal-dominant mode of inheritance, an early age of onset (usually before 25 years) and a primary dysfunction of the pancreatic beta cells [1, 2].

So far, 13 MODY genes have been identified [3]. Among these, *BLK* (*MODY11*) encodes a non-receptor tyrosine-kinase of the SRC family of proto-oncogenes, which is present in many tissues and cells including pancreatic beta cells [4]. Five rare mutations were shown to segregate with diabetes in three MODY families: four mutations are located in non-coding regions (at the end of the non-coding 3′ untranslated region or outside the gene) and only one non-synonymous mutation (p.A71T) was found in the fourth exon of *BLK* [4]. The p.A71T mutation is part of a haplotype including two of the non-coding mutations [4]. It was shown that *BLK* overexpression in the MIN6 pancreatic beta cell line enhanced insulin content and insulin secretion in response to glucose [4]. These actions were greatly attenuated by the *BLK*-p.A71T mutation [4]. Since this report, no study has attempted to replicate these data, in particular to confirm *BLK* firmly as a MODY susceptibility gene in other cohorts.

In the present study, we investigated the role of *BLK* in MODY in two European cohorts of unelucidated MODY patients (MODY-X). Furthermore, we assessed the contribution of the *BLK*-p.A71T mutation to type 2 diabetes risk and to the variation of related traits, as we found that this mutation is not rare in the general European population.

Methods

Patients selected for BLK sequencing We studied 14 probands from MODY-X families recruited at the CNRS-UMR-8199 unit in Lille (France), who were diagnosed with diabetes before 36 years of age (10 of whom were diagnosed before 25 years of age). As previously published, these families are of French European origin, except one family from Mauritius [5]. We also studied 39 probands from Danish MODY-X families collected at the Steno Diabetes Center (Copenhagen, Denmark) (age at diagnosis 9–

25 years) [6] and 11 additional patients from families with a vertical transmission of diabetes (at least one family member presented with diabetes diagnosed before 25 years of age). The clinical features of these patients are reported in Table 1 of the electronic supplementary material (ESM).

Sequencing protocol *BLK* is located on human chromosome 8p23-p22 and encodes a 505-amino-acid protein (NM_001715.2; NP_001706.2 [NCBI36]). Genomic DNA was amplified by PCR with primers designed to cover the 13 exons and flanking intron–exon boundaries of *BLK*. A standard sequencing protocol was subsequently used [7].

Study cohorts The case–control study included 4,901 French type 2 diabetic individuals and 4,280 French normoglycaemic controls (age at examination ≥45 years) (ESM Table 2). Type 2 diabetic individuals were recruited by the CNRS-UMR-8199 unit (n=335), Corbeil-Essonnes Hospital (n=2,182), Diab2-Néphrogène study (n=1,997), DESIR (Data from an Epidemiological Study on the Insulin Resistance Syndrome) (n=304) and the SUVIMAX (Supplementation en Vitamines et Mineraux Antioxydants) study (n=83), as previously described [7]. Control individuals were recruited by the CNRS-UMR-8199 unit (n=249), the DESIR (n=3,118) and SUVIMAX (n=913) cohorts [7].

All participants with type 2 diabetes or normoglycaemia were defined as such according to the 2003 American Diabetes Association criteria.

Non-diabetic participants in the DESIR (n=4,760) and SUVIMAX (n=1,758) cohorts were also analysed for metabolic quantitative traits (ESM Table 2).

All studies were approved by local ethics committees and were performed according to the principles of the Helsinki Declaration II. Written informed consent was obtained from all participants.

Genotyping of the p.A71T variant Genotyping was performed using the high-resolution melting method on a LightCycler 480 PCR System (Roche Diagnostics, Meylan, France), as previously described [7]. Positive signals were confirmed by sequencing, with a concordance rate of 99% between genotyping and sequencing results. A genotype call rate of at least 96% was obtained in each cohort. No deviation from Hardy–Weinberg equilibrium (p>0.05) was detected in any of the studied populations.

Statistical analyses The association between the p.A71T variant and the risk of type 2 diabetes or the variation of related metabolic traits was assessed using logistic or linear regression models, respectively, which were adjusted for age, sex and BMI (when appropriate) under a dominant model. By applying a Bonferroni correction, a p value below $5.6\times10^{-3}=0.05/(6_{[\text{metabolic traits}]}+3_{[\text{type 2 diabetes case–controls}]})$ was considered significant, and between 0.05 and 5.6×10^{-3} was considered nominal (trend of association).

We assessed the power of our study using QUANTO software. The statistical power to detect an OR of 1.45 was 86% in the overall case–control analysis (all cases, n=4,901) and 80% when the cases were stratified according to BMI <30 or ≥30 kg/m^2. In the non-diabetic participants in the DESIR (n=4,760) and SUVIMAX (n=1,758) cohorts, the statistical power to detect an effect on BMI variation between 2 and 5 kg/m^2 was higher than 99%. All statistical analyses were performed with SPSS (version 14.0,) and QUANTO (version 1.2.4, http://hydra.usc.edu/GxE/) software.

Homeostasis model assessment of pancreatic beta cell function (HOMA-B) and insulin resistance (HOMA-IR) were calculated as previously described [8].

Results

No rare non-synonymous *BLK* mutations were found in French and Danish MODY patients (n=64); in particular, the c.211G>A/p.A71T mutation was not identified. Of note, the previously identified extragenic or non-coding mutations were not screened in these patients, because, first, two of these variants were reported to be unequivocally transmitted together with the missense p.A71T mutation; and, second, the effect of these mutations on pancreatic beta cell function was not investigated, contrary to the p.A71T mutation [4]. The 1000 Genomes Project and the NHLBI Exome Sequencing Project listed the *BLK* c.211G>A/p.A71T mutation as a low-frequency variant in Europeans (rs55758736; minor allele frequency 0.012–0.013). We genotyped this variant in the DESIR French general population (n=5,064) and found 58 carriers of the A allele, including 52 normoglycaemic carriers (age at examination 31–65 years). We therefore concluded that the p.A71T variant was unlikely to cause MODY, which is a highly penetrant disorder.

The c.211G>A/p.A71T variant was further genotyped in French type 2 diabetic individuals (n=4,901) and control samples (n=4,280). We found a nominal effect of the A allele on increased type 2 diabetes risk (OR 1.47 [95% CI 1.03, 2.11], p=0.035; Table 1). As most of the carriers of the *BLK* c.211G>A/p.A71T mutation in the MODY family reported by Borowiec et al were overweight or obese [4], we stratified the diabetic individuals according to BMI (BMI <30 kg/m^2, n=2,456; BMI ≥30 kg/m^2, n=2,445). We found an enrichment of the A allele in obese diabetic participants only (OR 2.44 [95% CI 1.32, 4.49], $p=4.29\times10^{-3}$; Table 1), in contrast to non-obese diabetic participants (p=0.145; Table 1).

Despite high statistical power, no nominal or significant association was found between the c.211G>A/p.A71T

Table 1 Effect of the *BLK* c.211G>A/p.A71T variant on type 2 diabetes risk

Group	A allele frequency (%)	*n*	Genotype counts (%)			OR[a] (95 % CI)	p_{dom}
			GG	GA	AA		
Controls	0.95	4,280	4,198 (98.08)	82 (1.92)	0 (0.00)	Ref.	Ref.
All type 2 diabetes	1.27	4,901	4,779 (97.51)	120 (2.45)	2 (0.04)	1.47 (1.03, 2.11)	0.035
Non-obese type 2 diabetes (BMI <30 kg/m^2)	1.22	2,456	2,396 (97.56)	60 (2.44)	0 (0.00)	1.32 (0.91, 1.93)	0.145
Obese type 2 diabetes (BMI ≥30 kg/m^2)	1.31	2,445	2,383 (97.46)	60 (2.46)	2 (0.08)	2.44 (1.32, 4.49)	4.29×10^{-3}

[a]OR was assessed by logistic regression adjusted for age, sex and BMI, under a dominant model

variant and BMI variation in 6,518 non-diabetic participants (p=0.576; Table 2). Surprisingly, in the 4,760 non-diabetic participants from DESIR, the reported MODY A allele was associated with decreased HbA_{1c} levels ($\beta_{[SE]}=-0.139_{[0.050]}$%, $p=5.27\times10^{-3}$; Table 2) and an increase in beta cell function modelled by HOMA-B ($\beta=0.185_{[0.066]}$, $p=5.37\times10^{-3}$; Table 2), which does not fit with a MODY phenotype. No association was found for fasting glucose levels, fasting insulin levels or HOMA-IR (Table 2).

Discussion

In this study, we identified numerous elderly normoglycaemic carriers of the *BLK*-p.A71T mutation, which does not fit with either rarity and high penetrance of a MODY gene mutation or the usual clinical features [3, 9, 10]. Furthermore, we did not find any other coding mutations in two European cohorts that included 64 MODY patients.

We observed a weak effect of the loss-of-function *BLK*-p.A71T mutation on increased type 2 diabetes risk, with an enrichment of the variant in obese diabetic cases, although no association with BMI was found. The *BLK*-p.A71T variant may therefore be 'diabetogenic' through obesity-related mechanisms, as previously described for some more frequent genetic variants such as the *ENPP1*-K121Q, *PPARG*-P12A or *ADIPOQ* polymorphisms [11–14]. Twelve out of 13 carriers of the p.A71T variant in the MODY family reported by Borowiec et al were overweight, of whom eight were obese [4]. The only carrier presenting with a normal BMI was non-diabetic at age 71 years [4]. This is in line with our present results and may reflect a modulation of the p.A71T variant effect by adiposity on type 2 diabetes risk.

Of note, the meta-analyses of European genome-wide association studies performed by the DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium, the Meta-Analyses of Glucose and Insulin-related traits

Table 2 Effect of the *BLK* c.211G>A/p.A71T variant on the variation of metabolic traits in non-diabetic participants in the DESIR and SUVIMAX studies

Metabolic traits	*n*	Mean/median data level by genotype			β[a] (SE)	p_{dom}
		GG	GA	AA		
BMI (kg/m^2)	6,518	24.38±3.69	23.93±3.79	NA	−0.189 (0.34)	0.576
Fasting glucose (mmol/l)	4,760	5.28±0.53	5.15±0.58	NA	−0.108 (0.07)	0.102
Fasting insulin (pmol/l)	4,760	39.2 (28.6;55.8)	40.4 (26.5;61.4)	NA	0.034 (0.06)	0.587
HOMA-B	4,760	68.1 (48.9;94.6)	73.2 (53.7;107.4)	NA	0.185 (0.07)	5.37×10^{-3}
HOMA-IR	4,760	9.1 (6.5;13.5)	9.2 (5.8;14.1)	NA	0.011 (0.07)	0.865
HbA_{1c} (%)	4,760	5.43±0.40	5.27±0.46	NA	−0.139 (0.05)	5.27×10^{-3}
HbA_{1c} (mmol/mol)	4,760	34.10±4.60	32.24±5.22	NA	−1.589 (0.58)	5.27×10^{-3}

Data are presented as mean±standard deviation or median (interquartile range)

Data for fasting serum insulin, HOMA-B and HOMA-IR were $\log_e$−transformed before statistical analysis

[a]Per A allele effect size: coefficient β from dominant linear regression models adjusted for age, sex and BMI, except for the analysis of BMI that was adjusted for age and sex

NA, not applicable

Consortium (MAGIC) or the Genetic Investigation of ANthropometric Traits (GIANT) Consortium, did not show any evidence of associations between variants at the *BLK* locus and risk of type 2 diabetes or variation of metabolic traits including BMI, fasting glucose, fasting insulin, HbA_{1c} or HOMA-B (ESM Figure 1). Our present results on the contribution of the *BLK*-p.A71T variant to the risk of type 2 diabetes or the variation of HbA_{1c} or HOMA-B are therefore likely to be nominal associations only.

In conclusion, we demonstrated that the loss-of-function *BLK*-p.A71T mutation is very unlikely to cause MODY. Instead, it may modestly influence type 2 diabetes risk through an interaction with obesity, although this will require further validation in additional studies.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement AB and MV conceived and designed the study, and wrote the paper; AB, LY and MV performed the genetic analyses and interpreted the data; AD and IE performed the sequencing and analysed the data; JP and EV performed the genotyping and analysed the data; APG, EAA, SC, SHer, SHad, GC, OL, BB, MM, OP, TH and PF contributed to cohort-study samples and clinical data; PF reviewed the manuscript and contributed to the discussion. LY, AD, IE, JP, EV, APG, EAA, SC, SHer, SHad, GC, OL, BB, MM, OP and TH revised the final version of the manuscript. MV is the guarantor of the manuscript. All authors have read and approved the final version of the article.

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