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Assessing *FOXO1A* as a Potential Susceptibility Locus for Type 2 Diabetes and Obesity in American Indians

Yunhua L. Muller, **Robert L. Hanson**, **Gregory Wiessner**, **Lori Nieboer**, **Sayuko Kobes**, **Paolo Piaggi**, **Mahdi AbdusSamad**, **Chidinma Okani**, **William C. Knowler**, **Clifton Bogardus**, and **Leslie J. Baier**

Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Phoenix, AZ

Abstract

Objective—A prior genome-wide association study (GWAS) in Pima Indians identified variation within *FOXO1A* that modestly associated with early-onset (onset age<25years) type 2 diabetes (T2D). *FOXO1A* encodes the forkhead transcription factor involved in pancreatic β-cell growth and hypothalamic energy balance; therefore, *FOXO1A* was analyzed as a candidate gene for T2D and obesity in a population-based sample of 7710 American Indians.

Methods—Tag SNPs in/near *FOXO1A* (minor allele frequency≥0.05) were analyzed for association with T2D at early-onset (n=1060) and all ages (n=7710), and with insulin secretion (n=298). SNPs were also analyzed for association with maximum body mass index (BMI) in adulthood (n=5918), maximum BMI z-score in childhood (n=5350) and % body fat (n=555).

Results—An intronic SNP rs2297627 associated with early-onset T2D [OR=1.34(1.13-1.58), p=8.7×10^{-4}] and T2D onset at any age [OR=1.19 (1.09-1.30)**,** p=1×10^{-4}]. The T2D risk allele also associated with lower acute insulin secretion (β=0.88, as a multiplier, p=0.02). Another intronic SNP (rs1334241, D'=0.99, r^2=0.49 with rs2297627) associated with maximum adulthood BMI (β=1.02, as a multiplier, p=3×10^{-5}), maximum childhood BMI z-score (β=0.08, p=3 × 10^{-4}) and % body fat (β=0.83%, p=0.04).

Conclusions—Common variation in *FOXO1A* may modestly affect risk for T2D and obesity in American Indians.

Keywords

FOXO1A; Type 2 diabetes; obesity; Insulin secretion; American Indians

Corresponding author, Leslie J. Baier Ph.D., Diabetes Molecular Genetics Section, NIDDK, NIH, 445 North 5th street, Phoenix, AZ 85004 USA, Tel: (602) 440-6589, Fax: (602) 253-4140, ; Email: lbaier@phx.niddk.nih.gov

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Introduction

FOXO1A encodes the forkhead transcription factor, a downstream effector of insulin signaling, and plays crucial roles in pancreatic β-cell growth and hypothalamic energy balance. *FOXO1A* is widely expressed in various human tissues including pancreas, hypothalamus, adipose, kidney, liver and skeletal muscle (unpublished data). Over-expression of *foxo1a* in mouse β-cells blocks Pdx-1 transcription and leads to development of diabetes ([1-3]); whereas activation of *foxo1a* in the hypothalamus increases food intake and decreases energy expenditure via increased expression of Agouti-related protein and neuropeptide Y ([3,4]).

Pima Indians of Arizona have high prevalence and incidence rates of T2D and obesity ([5]). In a prior GWAS which utilized the Affymetrix 6.0 SNP array to identify susceptibility genes for early-onset T2D, 453,654 SNPs on the array were analyzed in 278 T2D cases (onset age<25 years) and 295 non-diabetic controls (≥45 years of age) ([6]). No SNP achieved genome-wide significance (p=4.9×10^{-7} in Pima Indians) in this small sample ([7]). SNPs with the strongest associations ([6]) or SNPs in biologic candidate genes with modest associations were further analyzed in follow-up studies. Since several SNPs in *FOXO1A* modestly associated with early-onset diabetes in our GWAS [*e.g.* rs2701896, OR=1.61(1.22-2.13), p=5×10^{-4}] and *FOXO1A* has been linked to risk of T2D and obesity in mouse models ([1-4]), *FOXO1A* was analyzed as a potential susceptibility locus for T2D and obesity in a population-based sample of 7710 American Indians.

Methods

Subjects with longitudinal data on T2D and BMI

Subjects (characteristics given in Table S1) were derived from a longitudinal study of the etiology of T2D among the Gila River Indian Community in Arizona. Among them, 3625 are full-heritage Pima Indian and the remaining 4085, on average, are half Pima and three-quarters American Indian. Individuals (age≥5 years) participated in biennial measurements of BMI and a 75-g oral glucose tolerance test [data collected from 1965-2007, exams=5.2±3.8 (mean±SD); follow-up time=19±12 years, Figure S1]. Diabetes was determined according to the criteria of American Diabetes Association ([8]) (diabetic: n=2549; non-diabetic: n=5161). A subset of the sample including the prior GWAS sample was informative for analyses of early-onset diabetes, consisted of 500 cases with onset age<25 years and 560 non-diabetic controls who were ≥45 years old. BMI in adulthood is defined as the maximum BMI at age ≥15 years recorded from a non-diabetic examination (n=5918). Childhood BMI z-score is defined as the maximum sex- and age-specific z-score between the ages of 5 and 20 years scaled for Pima Indians (n=5350).

Subjects with quantitative metabolic trait data

A subset of non-diabetic and healthy adults (n = 555, Table S1) was also characterized in our Clinical Research Center for % body fat and insulin sensitivity ([9]), and some also had measures of the acute insulin response (AIR, n=298) ([9]), energy expenditure (n=423) ([10]) and *ad libitum* food intake (n=194) ([11]) as described elsewhere.

SNP identification and genotyping

Variation across a ~210.9 kb region encompassing *FOXO1A* (Chr13:41079801-41290734, ~50kb flanking *FOXO1A*) was identified from whole-genome sequence data (40x coverage) of 335 Pima Indians (Illumina, San Diego, CA). The 305 common SNPs (minor allele frequency, mAF ≥0.05) were captured by 17 tag SNPs with a pair-wise r^2 ≥0.8 (Haploview). Genotyping of tag SNPs utilized BeadXpress System (Illumina, San Diego, CA).

Statistical analysis

Statistical analyses were performed using the software of the SAS Institute (Cary, NC). A logistic regression analysis was used to assess the association of genotype with T2D. The model was fitted with the generalized estimating equations (GEE) to account for dependence among siblings. Genotype was analyzed as a numeric variable representing 0, 1 or 2 copies of a given allele. The association of quantitative traits with genotypes was analyzed by linear regression using the GEE procedure. Results were adjusted for covariates as indicated in the table/figure legends. The individual estimate of the proportion of European ancestry was derived by the method of Hanis et al. ([12]) from 45 informative markers with large differences in allele frequency between populations ([13]).

Results and Discussion

FOXO1A SNPs associate with T2D and BMI

Seventeen tag SNPs captured 305 SNPs (mAF≥0.05, r^2≥0.8) identified from whole-genome sequence data (Figure S2). All 17 SNPs were initially genotyped in 3625 full-heritage Pima Indians, and 9 tag SNPs with a nominal association for T2D or BMI (p<0.05) in the full-heritage sample were further genotyped in the remaining 4085 mixed-heritage American Indians. Association results of these 9 SNPs with T2D and BMI in all 7710 subjects are shown in Table 1.

After accounting for multiple testing at the *FOXO1A* gene level [17 tag SNPs and two phenotypes (T2D and BMI) require a p≤0.001 for significance], only rs2297627 which tags the early-onset T2D signal (rs2701896) in the GWAS (D'=0.97, r^2=0.93) associated with T2D with an onset at any age in American Indians [risk allele frequency (RAF)=0.59, OR=1.19 (1.09-1.30) per risk allele C**,** $p=1\times10^{-4}$, Figure 1a and Table 1]. There was no genotype × gender interaction at rs2297627 (p=0.30). Among the subset of individuals which could serve as a case/control sample for early-onset T2D (onset age <25 years), the risk allele C also associated with a higher risk for early-onset T2D [OR=1.34 (1.13-1.58), $p=8.7\times10^{-4}$; Figure 1b and Table 1). If the case-control sample was excluded, the SNP rs2297627 no longer associated with T2D in the remaining subjects [OR=1.04 (0.96-1.12), p=0.39], indicating that the association with T2D with onset at any age was largely driven by the association with early-onset T2D. The risk allele C also associated with an earlier age of T2D onset (Cox proportional hazards model: HRR=1.15(1.05-1.22), $p=5.7\times10^{-6}$; Figure 1c and Table 1). When 50% of the individuals developed T2D, the mean age of T2D onset in subjects homozygous for the risk allele (CC) was 5 years younger than that in subjects homozygous for the non-risk allele (TT). The diabetes risk allele C also associated with a lower acute insulin response (AIR: mean insulin increment from 3-5 min) during an IVGTT

in full-heritage Pima Indians with normal glucose tolerance (β=0.88, as a multiplier, p=0.02; Figure 1d and Table S1). Pancreatic β-cell function was also assessed using the corrected insulin response at 30-min [$I_{30}/(G_{30} \times (G_{30} - 70))$] estimated from a 75g-OGTT ([14]) in the same subjects who had AIR measurements. SNP rs2297627 did not associate with the corrected insulin response at 30-min (β=0.95, as a multiplier, p=0.33). Among 555 non-diabetic American Indians who had insulin sensitivity assessed by a hyperinsulinemic, euglycemic clamp, rs2297627 also did not associate with the rate of glucose disposal (p=0.21, Table S2). These results suggest that *FOXO1A* variation may impair insulin secretory function, thus lead to increased risk for early-onset T2D.

Weaker associations with T2D were observed with 4 other tag SNPs: rs7328015, rs3751437, rs1334241, and rs34733279 (D82N) ($p \leq 0.05$, Table 1). These SNPs were in high linkage disequilibrium (LD) with rs2297627 (D'≥0.92, $r^2 \geq 0.09$, Figure S2b), and conditional analyses demonstrated that the weaker signals were explained by the stronger association of rs2297627 ($p > 0.05$ conditioning on rs2297627). Since rs7328015 and rs2297627 were in moderate r^2 of 0.62, we conducted a haplotype analysis for these two SNPs (Figure S3). The risk allele (C) at rs2297627 had a stronger effect on diabetes when it occurred with the A allele at rs7328015 [AF=0.03, OR=1.39(1.04-1.83), p=0.02) as compared to the C allele at rs7328015 [AF=0.56, OR=1.15(1.05-1.26), p=0.0019].

Tag SNPs were also analyzed for associations with maximum BMI (Table 1) and % body fat (Table S2). A mixed model analysis was also performed using longitudinal data on BMI from multiple exams; the associations with multiple measures of BMI were comparable to the associations with a single measure of the maximum BMI (Table 1). Rs1334241 associated with maximum BMI in adulthood (RAF=0.41, β=1.02 per risk allele A, as a multiplier, $p=3\times10^{-5}$; p for genotype × gender interaction=0.69, Figure 2a and Table 1) and maximum BMI z-score in childhood (β=0.08, $p=3\times10^{-4}$; Figure 2b and Table 1). The BMI risk allele also associated with higher % body fat (β=0.83%, p=0.04; Figure 2c and Table S2). However, in our limited sample, there was no evidence to support that the associations with these obesity-related phenotypes were driven by an effect on 24-h energy expenditure as assessed in a respiratory chamber (n=423, p=0.81) or *ad libitum* energy intake from vending machine studies (n=194, p=0.51). Weak associations of SNPs with BMI ($p < 0.05$, Table 1) were no longer significant after conditioning on rs1334241.

Tag SNPs rs2297627 and rs1334241 (D'=0.99 and r^2=0.49, Figure S2b) associated with T2D and BMI, respectively. To determine whether the T2D association is driven by BMI, we repeated the analysis including BMI as a covariate. After accounting for BMI, the association of rs2297627 with T2D remained unchanged ($p=2\times10^{-4}$), suggesting that this T2D association is independent of BMI. In contrast, the BMI associated SNP rs1334241 had a nominal association with T2D (p=0.02), but this T2D association was weakened after accounting for BMI (p=0.13).

Replication of FOXO1A SNPs in other studies

The association of rs2297627 with T2D in American Indians directionally replicates that reported in 5957 Finnish men [870 T2D and 5087 non-diabetic, OR=1.38 (1.10-1.72), p=0.005] ([15]), while the association of rs2297627 with insulin secretion in American

Indians also directionally replicates that reported in 941 non-diabetic Germans (p=0.04) ([15]). In contrast, rs2297627 did not associate with T2D (OR=1.01 [0.97-1.05], p=0.66, n=65812, mAF=0.32) in the large GWAS meta-analysis of Caucasians conducted by the DIAbetes Genetics Replication and Meta-analysis (DIAGRAMv3) Consortium ([16]). No association between rs1334241 and BMI was detected in the large GIANT (Genetic Investigation of ANthropometric Traits) meta-analysis of Caucasians (p=0.14, n=339109, mAF=0.28) ([17]). The lack of association of *FOXO1A* SNPs with T2D or BMI in these large consortia suggests that a causal variant could be in higher LD with rs2297627 or rs1334241 in American Indians as compared to Caucasians. Alternatively, our associations may represent false positives.

In conclusion, our study identified modest associations for two *FOXO1A* tag SNPs. Rs2297627 associated with T2D ($p=1\times10^{-4}$), age of onset for T2D ($p=5.7\times10^{-6}$) and insulin secretion (p=0.02), while rs1334241 associated with BMI in adulthood ($p=3\times10^{-5}$) and BMI z-score in childhood ($p=3\times10^{-4}$). Although none of the variants achieved genome-wide significance, the role of FOXO1A in T2D/obesity is implicated in mouse models, thus applying such stringent statistical criteria may be overly conservative. Further replication and systematic functional analyses of *FOXO1A* SNPs are needed to better understand its role in T2D and obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is already known about this subject?

1. *FOXO1A* has been linked to risk of T2D and obesity in mouse models.
2. Variation in *FOXO1A* modestly associates with T2D and insulin secretion in some human studies.

What does your study add?

1. Variation in *FOXO1A* modestly associated with age of T2D onset in Pima Indians, with its largest effect at onset <25 years, implicating that *FOXO1A* may affect T2D risk in early-life, perhaps through insulin secretion.
2. Variation in *FOXO1A* also modestly associated with BMI in American Indian adults and children, as well as % body fat, which supports a potential role of FOXO1A in human obesity.
3. Our longitudinal data with repeated measures as well as extensively characterized metabolic phenotypes may provide a comprehensive assessment for the effect of *FOXO1A* on risk for T2D and/or obesity. Although *FOXO1A* SNPs have been shown to associate with T2D/BMI in some small studies, large studies did not replicate these associations. Therefore, additional studies are required to understand the basis of this inconsistency.

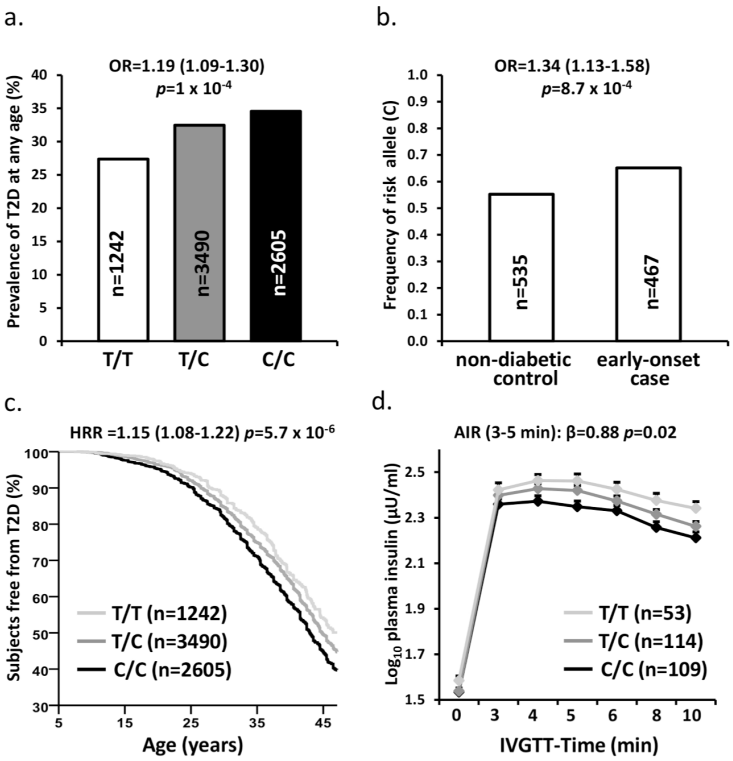

Figure 1. Association data for rs2297627

Association with prevalence of T2D at any age (a); risk allele frequency distribution in early-onset T2D case and control groups (b); Kaplan-Meier survival curve for time to T2D onset (c) and plasma insulin concentrations in response to a 25-g intravenous glucose bolus (IVGTT) (d).

The *p* value and OR for T2D at any age are adjusted for age, sex, birth year and heritage. The *p* value and OR for early-onset T2D (case-control analysis) are adjusted for sex and birth year and heritage. Cox proportional hazards model is used to determine the hazard rate ratio (HRR) for association with age of T2D onset including sex, birth-year, American/European ancestry and self-reported Pima heritage as covariates. Data for plasma insulin and glucose concentrations are given as adjusted mean±SE by genotypes. AIR (mean insulin increment from 3-5 min) is $\log_{10}$-transformed before analyses to approximate a normal distribution, and beta is exponentiated to obtain the effect per risk allele, expressed as a multiplier. The *p* value and beta for AIR are adjusted for age, sex, % body fat and rate of glucose disappearance during insulin-stimulation. The *p* value and beta for plasma glucose concentrations are adjusted for age, sex and % body fat.

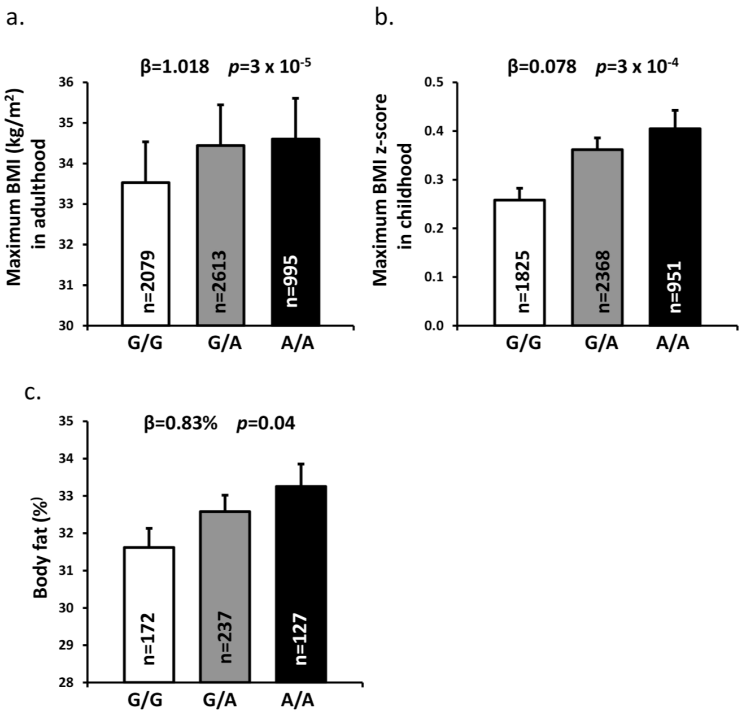

Figure 2. Association data for rs1334241

Maximum BMI in adulthood (a), maximum BMI z-score in childhood (b) and % body fat in adulthood (c).

Adult BMI is the maximum BMI (kg/m^2) from a non-diabetic examination recorded at age ≥15 years. Childhood BMI z-score is the maximum sex- and age-specific z-score identified between the ages of 5 and 20 years. Data are given as adjusted mean±SE by genotypes. Adult BMI is $\log_e$–transformed before analyses to approximate a normal distribution, and beta is exponentiated to obtain the effect estimate for each risk allele, expressed as a multiplier. Beta and *p* values for adult BMI and childhood BMI z-score are adjusted for age, sex, birth year and heritage. Beta and *p* values for % body fat are adjusted for age, sex and heritage.

Table 1

Associations of 9 tag SNPs in *FOXO1A* with T2D and BMI in American Indians.

		Allele	**T2D Onset at any age (n=7710)**			**Early-onset T2D Onset age<25 years (n=1060)**		**Age of T2D onset (n=7710)**		**Maximum BMI Adulthood (n=5918)**		**Multiple BMI Adulthood (n=5918)**		**Maximum BMI z-score Childhood (n=5350)**	
SNP	Location	R/N	RAF	OR (95% CI)	*p*	OR (95% CI)	*p*	HRR (95% CI)	*p*	Beta	*p*	Beta	*p*	Beta	*p*
rs2701879	3′flanking	C/T	0.22	1.08 (0.98-1.19)	0.14	1.40 (1.14-1.72)	**0.001**	1.07 (1.00-1.14)	0.04	0.987	0.005	0.990	0.05	−0.016	0.52
rs4943788	3′flanking	C/T	0.93	1.12 (0.95-1.32)	0.17	1.63 (1.18-2.24)	0.003	1.09 (0.98-1.21)	0.12	1.006	0.46	1.012	0.14	0.126	**8.5×10^{-4}**
rs9594421	3′flanking	A/T	0.41	0.97 (0.89-1.06)	0.57	1.13 (0.96-1.34)	0.15	1.00 (0.95-1.06)	0.93	1.010	0.02	1.009	0.04	0.006	0.77
rs7328015	3′flanking	C/A	0.69	1.14 (1.04-1.24)	0.004	1.23 (1.03-1.46)	0.02	1.12 (1.05-1.19)	**2.3×10^{-4}**	1.001	0.75	1.005	0.30	0.032	0.14
rs9603768	3′flanking	A/G	0.29	1.01 (0.92-1.11)	0.81	1.09 (0.92-1.29)	0.33	1.02 (0.96-1.08)	0.60	0.971	0.009	0.991	0.03	−0.002	0.93
rs3751437	3′UTR	T/C	0.93	1.21 (1.00-1.46)	0.05	1.45 (1.02-2.08)	0.04	1.14 (1.01-1.29)	0.04	1.021	0.02	1.021	0.01	0.076	0.05
rs1334241	intron	A/G	0.41	1.10 (1.01-1.20)	0.02	1.03 (0.88-1.22)	0.70	1.07 (1.02-1.14)	0.01	1.018	**3×10^{-5}**	1.015	**7×10^{-4}**	0.078	**3×10^{-4}**
rs2297627	intron	C/T	0.59	1.19 (1.09-1.30)	**1×10^{-4}**	1.34 (1.13-1.58)	**8.7×10^{-4}**	1.15 (1.08-1.22)	**5.7×10^{-6}**	1.007	0.10	1.007	0.10	0.046	0.03
rs34733279	D82N	A/G	0.16	1.15 (1.02-1.29)	0.02	1.48 (1.18-1.86)	**6.1×10^{-4}**	1.13 (1.05-1.22)	0.002	0.984	0.007	0.989	0.06	−0.038	0.20

Data are given for the combined sample of full-heritage Pima Indians and mixed-heritage American Indians (n as indicated). R: risk allele; N: non-risk allele. RAF: risk allele frequency. The risk allele is defined as the allele with higher risk for early-onset T2D. Odds ratios (OR) for T2D are given per copy of this allele. OR and *p* value for T2D at any age are adjusted for age, sex, birth year and heritage. OR and *p* value for early-onset T2D (case-control analysis) are adjusted for sex, birth year and heritage. Cox proportional hazards model was used to determine the hazard rate ratio (HRR) for association with age of T2D onset including sex, birth-year, American/European ancestry and self-reported Pima heritage as covariates (baseline for follow-up: 5 years). The proportional hazards assumption is met since evaluation of the three genotypic curves in the log-minus-log shows that they are largely parallel. Adult BMI is the maximum BMI (kg/m^2) from a non-diabetic examination recorded at age ≥15 years. Childhood Max-BMI z-score is the maximum sex and age specific z-score identified between the ages of 5 and 20 years. A mixed model analysis was also performed using all longitudinal non-diabetic BMI measurements from each individual at age ≥15 years (range:1-17 exams between the years 1965 to 2004). A “PROC MIXED” model was fitted that included genotype as a fixed effect along with age, sex, birth-year and heritage as covariates. The model also included random effects representing sibship and individual (to account for multiple examinations within an individual) as previously described ([18]). Adult BMI (maximum and multiple) is $\log_e$–transformed before analyses to approximate a normal distribution, and beta is exponentiated to obtain the effect estimate for each risk allele, expressed as a multiplier. Beta and *p* value for BMI and BMI z-score are adjusted for age sex, birth year and heritage. Bold: p≤0.001 indicates significance after accounting for multiple testing of 17 tag SNPs and two phenotypes (T2D and BMI) at the FOXO1A gene level.