Evidence for Natural Selection and Ethnic Diversity at the Adenylate cyclase 3 Gene Associated with Obesity and Type 2 Diabetes

ISSEI YOSHIUCHI (✉ yoshiuchi@m9.dion.ne.jp)

Research article

Keywords: Selection, ADCY3, Obesity, T2DM, Evolution

Posted Date: April 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-18469/v1>

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Abstract

Background: Diabetes and obesity cause serious complications worldwide, including stroke and cardiovascular disease, and are a global health burden. Diabetes is strongly related with obesity and both are significantly heritable. The prevalence of diabetes and obesity are higher in African populations than in European and Asian populations. In human evolution, natural selection is a key process of genetic survival over generations. Thus, the selection for diabetes- and obesity-related genes is a key mechanism for survival during times of feast and famine. Loss-of-function variations in the adenylate cyclase 3 (ADCY3) gene are associated with obesity and diabetes, while mutations in ADCY3 are also associated with childhood obesity. ADCY3 -deficient mice showed severe obesity, impaired insulin sensitivity, and reduced physical activity. Here, we researched evidence for natural selection at ADCY3 .

Methods: We used a three-step genetic method to identify natural selection at ADCY3 using data on four populations from the 1000 Genomes Project and HapMap: Utah residents with Northern and Western European ancestry (CEU), the Yoruba in Ibadan, Nigeria (YRI), Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT). First we used Wright's F-statistics (Fst) as a measure of population differentiation to find ethnic diversity at ADCY3 . We then used a long-range haplotype (LRH) test to find significant long haplotypes, and then the integrated haplotype score (iHS) to find natural selection at ADCY3 .

Results: We observed high Fst values and significant ethnic diversity at four ADCY3 body mass index (BMI)-associated variations (rs7586879, rs6545814, rs11676272 and rs10182181) between the non-African and African populations. Both LRH and iHS also provided evidence for natural selection at ADCY3 .

Conclusions: These observations show evidence for natural selection and ethnic diversity at ADCY3 . Further exploration into the evolution of obesity- and Type 2 diabetes-associated genes is needed.

Background

Diabetes and obesity cause metabolic syndrome and coronary artery disease in particular. Approximately 30% of US adults have metabolic syndrome (1). The increased prevalence of this syndrome worldwide has become a global health burden.

Natural selection, as defined by Charles Darwin (2), is the key process of genetic survival over generations. Thus, selection for diabetes- and obesity-related genes is a key mechanism for survival during times of feast and famine (3). In adapting to historical environmental changes in food availability and lifestyle, there was evidence for natural selection of genes related to glucose (4,5), obesity and energy (6).

The prevalence of diabetes and obesity are higher in African populations than in European and Asian populations. In the 2013–2016 US National Health and Nutrition Examination Survey (NHANES) (<https://www.cdc.gov/nchs/data/databriefs/db319.pdf>), the prevalence of total diabetes among US adults was significantly higher in non-Hispanic black (17.9%) and Hispanic (19.8%) adults than in non-Hispanic white adults (12.4%). The prevalence of total diabetes among non-Hispanic Asians was 15.3%. In the 2015–2016 US NHANES (<https://www.cdc.gov/nchs/data/databriefs/db288.pdf>), the prevalence of total obesity was significantly higher among non-Hispanic black (46.8%) and Hispanic (47.0%) adults than among non-Hispanic white (37.9%) and non-Hispanic Asian (12.7%) adults. Significantly different incident rates of diabetes and obesity can be observed among ethnic groups worldwide.

Melanocortin-4 receptor (MC4R) gene is one of the major obesity- and Type 2 diabetes-associated genes (5,7,8,9), and one MC4R BMI-associated SNP (rs571312) (8) also showed the highest risk frequency (0.37) in the African population.

Melanocortin-3 receptor (MC3R) gene is also one of the obesity genes (6,10). Two MC3R childhood obesity-associated SNPs (rs3746619 and rs3827103) (6,10) also showed the highest risk frequencies (0.51 and 0.48) in the African population. This evidence may explain the differences in obesity prevalent among ethnicities.

Both diabetes and obesity are significantly heritable (11–14), with different incidences among populations. Loss-of-function variations in the adenylate cyclase 3 (ADCY3) gene are associated with obesity and diabetes (15–17). ADCY3 has the membrane-associated enzyme adenylate cyclase 3 that produces cAMP from ATP, and cAMP is linked with the metabolism of energy, fat, and insulin secretion (15–18).

ADCY3-deficient mice showed severe obesity, impaired insulin sensitivity and reduced physical activity (18).

A loss-of-function variant of ADCY3, c.2433-1G > A, showed an increased risk of obesity and Type 2 diabetes in a Greenlandic population (17). This variant was a splice site variant and disrupted a splice-acceptor in exon 14 (17). There were two novel isoforms of ADCY3 in these variant carriers. One isoform showed exon 14 skipped transcript and the other isoform retained the intron between exon 13 and exon 14 (17). Variant carriers showed decreased ADCY3 RNA expression and the expression data of ADCY3 showed severely decreased AA carriers, while the GA carriers showed an intermediate expression levels (17). The AA carriers had higher body fat, a larger waist circumference and had higher levels of glucose after an oral glucose tolerance test (17).

The common loss-of-function missense variant (Ser107Pro) of rs11676272 in ADCY3 showed an association with increased body mass index (BMI) in populations with European ancestry (8), East Asian populations (15) and in children from British and Dutch populations (16). In a functional study, the rs11676272 risk G allele was associated with reduced expression of ADCY3 (16). This missense variant G (Ser107Pro) of rs11676272 was reported to disrupt the interaction between the two-helix bundle of ADCY3 and inhibit adenylyl cyclase activity (16). The missense variant of rs11676272 was also reported to be associated with fat mass in childhood (16).

The rs7586879 SNP in ADCY3 was also significantly associated with BMI in populations with African ancestry (19).

Meta analysis also identified the rs6545814 SNP in ADCY3 associated with BMI in East Asian populations (15).

Meta analysis identified the rs10182181 SNP in ADCY3 associated with overweight and BMI ($\text{BMI} \geq 30 \text{ kg/m}^2$) in populations with European ancestry (20).

Loss-of-function mutations in ADCY3 are associated with childhood obesity in Pakistani and European populations (21).

In this study, we examined evidence for natural selection at the ADCY3 by a three-step genetic method, including Wright's F-statistics (Fst) (22), the long-range haplotype (LRH) test (23) and the integrated haplotype score (iHS) (24,25), using data on four population from the 1000 Genomes Project and HapMap (26,27): Utah residents with Northern and Western European ancestry (CEU), the Yoruba in Ibadan, Nigeria (YRI), Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT).

Methods

Study Population

In the Fst test (22,28), we used the gene candidate approach and examined four BMI-associated single-nucleotide polymorphisms (rs7586879, rs6545814, rs11676272 and rs10182181) at ADCY3 (9,15,16,19,20) (Table 1). We used genotypes from the 1000 Genomes Project phase 3 data (26), accessed in July 2019, with four populations: Utah residents with Northern and Western European ancestry (CEU), the Yoruba population of Nigeria (YRI), Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT).

In the LRH (23), we used the complete data set of all chromosome 2 from HapMap release 24 (27) with sixty CEU, sixty YRI, and ninety East Asians from CHB and JPT.

Statistical methods

We carried out a 3-step genetic method with the Fst and LRH tests, and the iHS to identify natural selection at the ADCY3 in population data from the 1000 Genomes Project and HapMap (26,27).

Fst is calculated based on the variance of allele frequencies between populations. The results of Fst indicate the degree of ethnic diversity. Using Arlequin software (v. 3.1; <http://cmpg.unibe.ch/software/arlequin3/>) (29), we examined four BMI-associated single-nucleotide polymorphisms (rs7586879, rs6545814, rs11676272 and rs10182181) at ADCY3 (9,15,16,19,20) (Table 1). We compared F_{st}^{All} , $F_{st}^{CEU-YRI}$ and $F_{st}^{JPT+CHB-YRI}$ values with the 95th percentiles of the distributions (> 0.365 , > 0.406 and > 0.465 , respectively) (30). The ancestral alleles of these SNPs were found in dbSNP (<https://www.ncbi.nlm.nih.gov/projects/SNP/>, accessed in July 2019).

Table 1
Fst values for BMI associated snps at the ADCY3

Gene	SNP	Position	Risk/non risk	Clinical phenotype	Fst all	Fst ^{CEU-YRI}	Fst ^{JPT+CHB-YRI}
ADCY3	rs7586879 (intron)	25116977	T/C	BMI	0.36	0.58	0.46
	rs6545814 (intron)	25131316	G/A	BMI	0.32	0.49	0.43
	rs11676272Pro107Ser	25141538	G/A	BMI	0.34	0.50	0.49
	rs10182181intergenic region)	25150296	G/A	Overweight/BMI	0.34	0.49	0.48

We used the complete data set of all chromosome 2 from HapMap release 24 and the SWEEP 1.1 software (<http://www.broadinstitute.org/mpg/sweep/index.html>) (23) to analyze the long-range haplotype (LRH) test to find extreme long haplotypes in the region of the ADCY3 loci (Chr. 2, locus 25,042,030–25,155,000; <https://www.ncbi.nlm.nih.gov/>, accessed in July 2019), then compared these with the all chromosome 2 of each population, and detect the actual relative extended haplotype homozygosity (REHH).

The integrated haplotype score (iHS) (24) used to find evidence of natural selection was obtained using Haplotter (<http://haplotter.uchicago.edu/>) in April 2018. We used the gene candidate approach and tested the SNPs of the ADCY3 locus. There were 111 SNPs of this ADCY3 locus in the CEU population, there were 130 SNPs of this locus in the YRI population and 104 SNPs of this locus in the East Asian population (Table 3). Significant scores (iHS >2.5 or iHS <−2.5) show the highest or lowest 1% of the distribution for all SNPs respectively (24,25).

Results

Fst test

We examined four BMI-associated SNPs (rs7586879, rs6545814, rs11676272 and rs10182181) of ADCY3 (Table 1, Supplementary Table 1) using the Fst test. Frequencies of these four BMI-associated SNPs (rs7586879, rs6545814, rs11676272 and rs10182181) showed the highest risk frequencies (0.90, 0.89, 0.94 and 0.94, respectively) in the YRI populations, while the frequencies of two SNPs (rs7586879 and rs6545814) showed the lowest risk frequencies (0.35 and 0.35, respectively) in the CEU populations and the frequencies of two SNPs (rs11676272 and rs10182181) showed the lowest risk frequencies (0.42 and 0.43, respectively) in the CHB populations.

Average Fst^{All}, average Fst^{CEU-YRI}, average Fst^{JPT-YRI} and average Fst^{CHB-YRI} values were 0.15, 0.15, 0.19 and 0.19, respectively (31). We compared Fst^{All}, Fst^{CEU-YRI} and Fst^{JPT+CHB-YRI} values for these BMI-associated SNPs with the 95th percentiles of the distributions (> 0.365, > 0.406 and > 0.465, respectively) (30) and identified high Fst^{CEU-YRI} values for rs7586879:0.58, rs6545814:0.49, rs11676272:0.50 and rs10182181:0.49 and high Fst^{JPT+CHB-YRI} values for rs11676272:0.49 and rs10182181:0.48 (Table 1). We found significant ethnic diversity between non-African (European and/or Asian populations) and African populations at these four ADCY3 BMI-associated SNPs. The risk alleles for these four ADCY3 BMI-associated SNPs are all ancestral alleles (Supplementary Table 1).

LRH test

We examined the ADCY3 region and found six significant core haplotypes (REHH percentile, 99.6–99.9) in the YRI population (Table 2). There were 74 haplotypes of this ADCY3 locus in the CEU population, 109 haplotypes of this locus in the YRI population and 68 haplotypes of this locus in the East Asian population.

Table 2
Core Haplotypes at the LRH test in the *ADCY3*

Ethnic	Associated SNP	Start position	Distance	Frequency	EHH	REHH	Max REHH	Percentile	P-value
CEU	rs7586879C (Obesity non-risk allele)	25109302	13550	0.12	0.42	6	6	89.0 th	0.12
YRI	rs11676272G, rs10182181G (Obesity risk alleles)	25138040	12256	0.66	0.17	6	6	99.9 th	0.0003
		25090236	9121	0.47	0.30	8	15	99.9 th	0.0007
		25126046	6146	0.44	0.35	7	7	99.9 th	0.002
		rs753529G	25054261	0.73	0.22	3	6	99.9 th	0.002
		rs7586879T (Obesity risk allele)	25112885	0.39	0.47	9	9	99.9 th	0.003
		25100328	10634	0.41	0.27	4	9	99.6 th	0.010
East Asian	rs6545814A, rs11676272A, rs10182181A (Obesity non-risk alleles)	25130462	19834	0.52	0.14	4	5	92.8 th	0.10
East Asian including CHB and JPT									

Interestingly, we observed that one core major haplotype with two BMI risk alleles (rs11676272G and rs10182181G) and the other core major haplotype with one BMI risk allele (rs7586879T) in the YRI population showed evidence for selection (REHH percentile, 99.9). We also found that the core major haplotype with one BMI-related SNP (rs753529G) in the YRI population showed evidence for selection (REHH percentile, 99.9). This rs753529 intron SNP was reported to be associated with BMI in Chinese obesity (32).

iHS

Significantly high scores ([iHS] > 2.5) presented positive selection. There were 111 SNPs of this *ADCY3* locus in the CEU population, 130 SNPs of this locus in the YRI population and 104 SNPs of this locus in the East Asian population (Table 3). Fourteen SNPs had a positive score ([iHS] score, 2.51–3.62) in the YRI population (Table 3). These 14 SNPs include 11 intron SNPs, one silent mutation, and two 3' UTR SNPs. These SNPs may be within a gene regulatory region in *ADCY3*. Interestingly, these SNPs include BMI-associated rs753529 SNP (32) with a high score of 2.86.

Table 3
Variables extracted from waveforms

Ethnic	<u>N of SNPs with [iHS] > 2.5 (Total N of SNPs)</u>
	Max [iHS] (SNP)
CEU	0 (111)
	1.46 (rs6733224)
YRI	14 (130)
	3.62 (rs1044040)
East Asian	0 (104)
	1.89 (rs2241759)
East Asian including CHB and JPT	

Discussion

The prevalence of diabetes and obesity are increasing worldwide. Significantly different incident rates of diabetes and obesity can be observed among ethnic groups worldwide. The prevalence of diabetes and obesity are higher in African populations than in European and Asian populations. Interestingly, frequencies of four body mass index-associated SNPs (rs7586879, rs6545814, rs11676272 and rs10182181) at ADCY3 showed the highest risk frequencies (0.90, 0.89, 0.94 and 0.94, respectively) in the African population. This evidence may explain the differences in obesity prevalent among ethnicities.

As defined by Charles Darwin in *On the Origin of Species* (2), natural selection is a key process to ensure genetic survival over generations. In human history, significantly important genes may have been the targets for selection for surviving different food supplies and geographies over more than 10,000 years. These selected genes could present significant historical footprints in the current human genome.

One genetic selection analysis of 65 loci associated with type 2 diabetes showed that positive selection did not have a powerful influence on the prevalence of type 2 diabetes risk alleles (33). One more genetic selection analysis of genes associated with type 2 diabetes and obesity presented only some evidence for selection at specific loci (30). The other genetic selection studies showed natural selection at the genes associated with type 2 diabetes (25,34,35).

Using the genotypes of selected diabetes and obesity genes could be helpful for decisions about clinical and hospital medication for health care of patients with diabetes and obesity.

The ADCY3 gene maps to chromosome 2p23.3 and is expressed in broad tissues including adipocyte, hypothalamus, skeletal muscle, and pancreatic islet (16). Prevention of diet-induced obesity was observed in ADCY3-gain-of-function mice. The common loss-of-function variant (Ser107Pro) of rs11676272 (A to G) showed an association with increased BMI (9,15,16) and the rs11676272 risk G allele was associated with reduced expression of ADCY3 (16). The missense variant of rs11676272 was also reported to be associated with fat mass in childhood (16).

The rs7586879 SNP in ADCY3 was also significantly associated with BMI in populations with African ancestry (19). Meta analysis also identified the rs6545814 SNP in ADCY3 associated with BMI in East Asian populations (15). Meta analysis identified the rs10182181 SNP in ADCY3 associated with overweight and BMI ($\text{BMI} \geq 30 \text{ kg/m}^2$) in populations with European ancestry (20).

In this study, we detected high $F_{\text{st}}^{\text{CEU-YRI}}$ values for rs7586879:0.58, rs6545814:0.49, rs11676272:0.50 and rs10182181:0.49, and identified high $F_{\text{st}}^{\text{JPT+CHB-YRI}}$ values for rs11676272:0.49 and rs10182181:0.48 (Table 1). These four ADCY3 BMI-associated SNPs showed significant ethnic diversity between non-African (European and/or Asian populations) and African populations. Both LRH and iHS analyses showed natural selection at ADCY3 (Tables 2, 3). The observed one core major haplotype with two BMI risk alleles (rs11676272G and rs10182181G) and the other core major haplotype with one BMI risk allele (rs7586879 T) provided evidence for selection (both REHH percentile, 99.9, and p-value, 0.0003 and 0.003, respectively) in the African population (Table 2). The prevalence of diabetes and obesity are higher in African populations than in European and Asian populations. The differences in risk allele frequencies caused significant differences in the genetic risk and prevalence of diabetes and obesity, which were higher in African populations than in European and Asian populations.

While complex, the findings on important genes that underwent natural selection, genetic drift, or migration may be significant for clinical and hospital medications.

Conclusions

We detected natural selection and ethnic diversity at ADCY3 and these results are significant for clinical prescriptions of medicine for patients with obesity and diabetes. Future studies should further examine the evolution of diabetes- and obesity-related genes.

Declarations

Conflict of interest:

None

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Acknowledgements

We thank Kyoko Yoshiuchi for secretarial contribution.

Prior Presentation We presented parts of this project at the 68th Annual Meeting of The American Society of Human Genetics, San Diego, USA, in October 2018.

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