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Research article

Polymorphisms in the ANKS1B gene are associated with cancer, obesity and type 2 diabetes

Ke-Sheng Wang[1,*], Xuefeng Liu[2], Daniel Owusu[1], Yue Pan[3] and Changchun Xie[4]

[1] Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, Johnson City, TN, USA
[2] Department of Systems Leadership and Effectiveness Science, School of Nursing, University of Michigan, Ann Arbor, MI, USA
[3] Department of Public Health Sciences, Miller School of Medicine, University of Miami, Miami, FL, USA
[4] Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA

* **Correspondence:** Email: wangk@etsu.edu; Tel: +1 423 439 4481; Fax: +1 423 439 4606.

Abstract: Obesity and type 2 diabetes (T2D) are comorbidities with cancer which may be partially due to shared genetic variants. Genetic variants in the ankyrin repeat and sterile alpha motif domain containing 1B (ANKS1B) gene may play a role in cancer, adiposity, body mass index (BMI), and body weight. However, few studies focused on the associations of ANKS1B with obesity and T2D. We examined genetic associations of 272 single nucleotide polymorphisms (SNPs) within the ANKS1B with the cancer (any diagnosed cancer omitting minor skin cancer), obesity and T2D using the Marshfield sample (716 individuals with cancers, 1442 individuals with obesity, and 878 individuals with T2D). The Health Aging and Body Composition (Health ABC) sample (305 obese and 1336 controls) was used for replication. Multiple logistic regression analysis was performed using the PLINK software. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. We identified 25 SNPs within the ANKS1B gene associated with cancer, 34 SNPs associated with obesity, and 12 SNPs associated with T2D ($p < 0.05$). The most significant SNPs associated with cancer, T2D, and obesity were rs2373013 ($p = 2.21 \times 10^{-4}$), rs10860548 ($p = 1.92 \times 10^{-3}$), and rs7139028 ($p = 1.94 \times 10^{-6}$), respectively. Interestingly, rs3759214 was identified for both cancer and T2D ($p = 0.0161$ and 0.044, respectively). Furthermore, seven SNPs were associated with both cancer and obesity (top SNP rs2372719 with $p = 0.0161$ and 0.0206, respectively); six SNPs were associated with both T2D and obesity (top SNP rs7139028 with $p = 0.0231$ and 1.94×10^{-6}, respectively). In the Health ABC sample,

18 SNPs were associated with obesity, 5 of which were associated with cancer in the Marshfield sample. In addition, three SNPs (rs616804, rs7295102, and rs201421) were associated with obesity in meta-analysis using both samples. These findings provide evidence of common genetic variants in the ANKS1B gene influencing the risk of cancer, obesity, and T2D and will serve as a resource for replication in other populations.

Keywords: cancer; obesity; diabetes; ANKS1B; polymorphisms; meta-analysis; pleiotropic effect

1. Introduction

Obesity and type 2 diabetes (T2D) are associated with increased risks of developing cancer, and are both considerable public health burdens in the United States (US). Based on the 2009–2010 National Health and Nutrition Examination Survey (NHANES) data, the obesity prevalence in the US adults was 35.7% [1], with global projections of more than 1.12 billion obese individuals by 2030 [2]. The overall prevalence of T2D in the US was 7.7% based on the 2003–2004 NHANES [3] and 8.3% in 2011 (about 25.8 million) [4,5]. Worldwide, the incidence of T2D is rising rapidly. An estimated 284.6 million of people had diabetes in 2010 worldwide, and it is predicted that there will be 438.4 million in 2025, with about 90–95% being T2D [6]. In the US, it has been reported that more than 1.6 million new cancer cases were expected to be diagnosed in 2012 (Cancer Facts & Figures 2012, American Cancer Society). Based on the 2012 National Health Interview Survey (NHIS) data, the overall prevalence of cancer in the US is 8.6% (7.6% for males and 9.4% for females) [7]. The World Cancer Report 2014 reported that in 2012, the global incidence of cancer rose to an estimated 14 million new cases and the figure is expected to rise to an annual 19.3 million by 2025 [8].

It has been reported that obesity is associated with increasing rates of metabolic syndrome and T2D [9,10]; while overweight and obesity are associated with risk for some of the most common cancers [10]. Furthermore, meta-analyses of epidemiological data have linked diabetes to different types of cancers, along with an increased risk of cancer mortality, while obesity is associated with an increased risk of cancer and diabetes [11]. Observational studies have also demonstrated that obesity and diabetes are positively associated with an increased incidence of a number of cancers and cancer-related mortality [9,12,13]. However, the genetic mechanisms are still not clear.

The ankyrin repeat and sterile alpha motif domain containing 1B (ANKS1B) gene (also known EB1, ANKS2, AIDA1 and cajalin-2) is located at 12q23.1 and expressed in adult testis and brain and fetal brain [14]. Expression of this gene has been shown to be elevated in patients with pre-B cell acute lymphocytic leukemia associated with t(1;19) translocation [14,15]. Through a genome-wide association study (GWAS) of four quantitative traits related to body size and adiposity (BMI, weight, waist circumference, and height) in a cohort of 1792 adult Filipino women from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), single nucleotide polymorphism (SNP) rs2373011 within the ANKS1B gene was associated with BMI ($p = 9.00 \times 10^{-6}$) and waist circumference ($p = 2.46 \times 10^{-6}$) [16]. Another study reported that some short tandem repeats (STRs) markers and SNPs and haplotypes of the EB1 gene were associated with genetic difference between diabetic patients with and without nephropathy [17]. The effect of ANKS1B gene on body weight was

also found in animal model [18]. Using a case-control design, three SNPs (rs1549102, rs11110099, and rs10745877) within ANKS1B were associated with lung cancer [19]. A recent study provided the first evidence that ANKS1B expression was associated with smoking-related clear cell renal cell carcinoma (ccRCC) development [20].

However, no study has focused on the associations of ANKS1B with the risk of obesity and T2D. This study explored the associations of 272 SNPs within ANKS1B gene with the risk of cancer, obesity and T2D using a Caucasian sample and confirmed the results of obesity using another sample.

2. Materials and Method

2.1. Samples

2.1.1. The Marshfield sample

The Marshfield sample is from the publicly available data from A Genome-Wide Association Study on Cataract and HDL in the Personalized Medicine Research Project Cohort-Study Accession: phs000170.v1.p1 (dbGaP). The primary goals of this project are to develop and validate electronic phenotyping algorithms, to accurately identify cases and controls while maintaining a positive predictive value (PPV) of > 95%, and to conduct a genome-wide association study that advances the understanding of two specific yet interrelated disease states, while simultaneously engaging the community in these research efforts. The details about these subjects were described elsewhere [21-23]. Obesity was determined as a body mass index (BMI) ≥ 30. Genotyping data using the ILLUMINA Human660W-Quad_v1_A are available for 3564 individuals (716 individuals with cancers, 1442 individuals with obesity and 878 individuals with T2D).

2.1.2. The Health ABC sample

The Health Aging and Body Composition (Health ABC) Study is a whole genome association study of visceral adiposity and is a NIA-sponsored cohort study of the factors that contribute to incident disability and the decline in function of healthier older persons, with a particular emphasis on changes in body composition in old age (dbGaP Study Accession: phs000169.v1.p1). The key components of Health ABC include a baseline examination, annual follow-up clinical examinations, and phone contacts every 6 months to identify major health events and document functional status between clinic visits. The details about these subjects were described elsewhere [23-27]. Obesity was determined as a body mass index (BMI) ≥ 30. The HABC dataset contains 1M Illumina Human SNPs for 1641 individuals (305 obese individuals and 1,336 controls).

2.2. Statistical analysis

Within the ANKS1B gene, 272 SNPs in the Marshfield sample and 310 SNPs in the Health ABC sample were available. Logistic regression analyses of cancer, obesity and T2D, adjusted for age and sex, were performed using PLINK v1.07 [28]. The asymptotic p-values were observed while the odds

ratio (OR) and standard error (SE) of OR were estimated. The additive model was applied. Hardy-Weinberg equilibrium was tested for all the SNPs in controls. Minor allele frequency (MAF) was determined for each SNP. In addition to obtaining nominal p-values, empirical p-values were generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK. In this procedure, pointwise estimate of an individual SNP's significance (empirical pointwise p-values) was calculated. Results of obesity from the two samples were directly meta-analyzed by combining the separate results of two samples (OR and standard error of OR) into one meta-analysis for overall effects. The basic meta-analysis function in PLINK was applied. Fixed-effect meta-analysis p-value and fixed-effect OR were reported. The between-study heterogeneity was tested by the χ^2-based Cochrane's Q statistic [29].

3. Results

3.1. Genotype quality control and descriptive statistics

We removed 4 of 272 SNPs with Hardy-Weinberg equilibrium test $p < 10^{-5}$ in the Marshfield sample. All 310 SNPs were in Hardy-Weinberg equilibrium in the controls of the Health ABC sample ($p > 0.001$). Participant characteristics for the two samples are presented in Table 1. The range of age was 46–90 years for the Marshfield sample and 69–80 years for the Health ABC sample. There were more females than males in both cases and controls in the Marshfield sample, and little more males than females in both cases and controls in the Health ABC sample.

Table 1. Descriptive characteristics of cases and controls in the Marshfield and Health ABC samples.

	Non-Cancer[a]	Cancer	Non-Obesity	Obesity	Non-Diabetes	Diabetes	Non-Obesity[b]	Obesity
Number	2122	716	2848	1442	2686	878	1336	305
Sex, N (%)								
Males	852 (40%)	340 (47%)	1135 (40%)	623 (43%)	1051 (39%)	424 (48%)	700 (52%)	167 (55%)
Females	1270 (60%)	876 (53%)	1713 (60%)	819 (57%)	1635 (61%)	454 (52%)	636 (48%)	138 (45%)
Age, years								
Mean ± SD	67.1 ±11.8	71.1 ±10.3	65.1 ±11.3	65.2 ±10.4	65.4 ±11.4	69.2 ±10.6	73.9 ±2.8	73.4 ±2.8
Range	46–90	46–90	46–90	46–90	46–90	46–90	69–80	69–80

[a] Marshfield sample. [b] Health ABC sample.

3.2. Association with cancer

Single marker analysis showed that 25 SNPs were associated with cancer ($p < 0.05$) in the Marshfield sample (see supplement Table S1). The top seven SNPs ($p < 0.01$) are shown in Table 2. SNP rs2373013 revealed the strongest association with cancer (OR = 0.73, 95%CI = 0.61–0.86, $p = 2.21 \times 10^{-4}$), while the next best signal was rs1275649 ($p = 3.65 \times 10^{-3}$). All the seven SNPs in the Marshfield sample had empirical pointwise p-values $p < 0.05$ using a permutation procedure (Table 2).

Table 2. Top SNPs within ANKS1B gene associated with cancer (p< 0.01) in the Marshfield sample.

SNP	Position[a]	AL[b]	MAF[c]	HWE[d]	OR-Cancer[e]	P-Cancer[f]	EMP[g]	OR-Obesity[h]	P-Obesity[i]	OR-Diabetes[j]	P-Diabetes[k]
rs2373013	98447901	T	0.17	0.878	0.73 (0.61–0.86)	2.21E−04	2.0E−03	0.97 (0.85–1.1)	0.601	0.91 (0.79–1.06)	0.222
rs1275649	98508885	A	0.22	0.614	0.8 (0.69–0.93)	3.65E−03	5.0E−03	0.92 (0.82–1.03)	0.151	0.9 (0.78–1.03)	0.117
rs7137194	98396791	T	0.29	0.242	0.82 (0.71–0.94)	4.26E−03	7.0E−03	0.99 (0.89–1.1)	0.861	0.94 (0.83–1.06)	0.316
rs1398084	98394758	A	0.37	0.542	1.19 (1.05–1.34)	5.12E−03	5.0E−03	1.05 (0.95–1.16)	0.337	1.05 (0.94–1.17)	0.414
rs200788	97789837	G	0.44	0.93	1.18 (1.04–1.33)	7.93E−03	1.5E−02	1.0 (0.91–1.1)	0.986	0.94 (0.84–1.05)	0.26
rs4762219	97996650	A	0.26	0.73	1.2 (1.05–1.37)	8.87E−03	1.2E−02	1.06 (0.95–1.18)	0.313	1 (0.88–1.13)	0.992
rs7313721	98005215	A	0.26	0.73	1.2 (1.05–1.37)	9.16E−03	1.1E−02	1.06 (0.95–1.18)	0.292	1 (0.88–1.14)	0.97

[a] Physical position (bp);[b] Minor allele; [c] Minor allele frequency; [d] p-value for Hardy-Weinberg equilibrium test; [e] Odds ratio for cancer; [f] p-value for cancer based on logistic regression; [g] empirical p-value for cancer generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK; [h] Odds ratio for obesity; [i] p-value for obesity based on logistic regression; [j] Odds ratio for diabetes; [k] p-value for diabetes based on logistic regression.

3.3. Association with obesity and diabetes

We identified 34 SNPs associated with obesity and 12 SNPs associated with T2D ($p < 0.05$) in the Marshfield sample (see supplement Table S1). The most significant SNPs for obesity and T2D were rs7139028 (OR = 1.26, 95%CI = 1.15–1.39, $p = 1.94 \times 10^{-6}$) and rs10860548 (OR = 1.19, 95%CI = 1.19–1.33, $p = 0.00192$), respectively. Table 3 shows 13 SNPs associated with obesity with $p < 0.01$ in the Marshfield sample. Of them, six SNPs were also associated with T2D ($p < 0.05$). All the 13 SNPs in the Marshfield sample had empirical pointwise p-values $p < 0.05$ for obesity using a permutation procedure (Table 3).

Table 3. Top SNPs within ANKS1B gene associated with obesity (p< 0.01) in the Marshfield sample.

SNP	Position[a]	AL[b]	MAF[c]	HWE[d]	OR-Cancer[e]	P-Cancer[f]	OR-Obesity[g]	P-Obesity[h]	EMP[i]	OR-Diabetes[j]	P-Diabetes[k]
rs7139028	97877414	T	0.41	0.338	1.09 (0.97–1.23)	0.164	1.26 (1.15–1.39)	1.94E−06	1.0E−03	1.14 (1.02–1.27)	0.0231
rs1523097	97901771	G	0.42	0.259	1.06 (0.94–1.2)	0.332	1.23 (1.12–1.36)	1.77E−05	1.0E−03	1.15 (1.03–1.28)	0.0155
rs4762543	97940543	A	0.41	0.22	1.08 (0.96–1.22)	0.202	1.22 (1.11–1.34)	5.98E−05	1.0E−03	1.15 (1.03–1.29)	0.012
rs10459194	97957421	C	0.33	0.581	1.03 (0.91–1.17)	0.662	1.2 (1.09–1.33)	2.9E−04	1.0E−03	1.11 (0.99–1.24)	0.086
rs1403506	97874831	C	0.22	0.392	1.06 (0.91–1.22)	0.454	1.22 (1.09–1.37)	6.49E−04	1.0E−03	1.11 (0.97–1.27)	0.117
rs201412	97814444	A	0.08	0.33	1.01 (0.81–1.26)	0.941	0.75 (0.63–0.9)	2.16E−03	3.0E−03	0.82 (0.67–1.02)	0.0685
rs7959046	97936953	A	0.23	0.309	1.04 (0.9–1.2)	0.625	1.19 (1.06–1.34)	2.51E−03	2.0E−03	1.18 (1.04–1.35)	0.0118
rs2638557	98214354	C	0.13	0.169	0.96 (0.8–1.15)	0.679	0.8 (0.69–0.93)	2.95E−03	3.0E−03	1.02 (0.86–1.2)	0.853
rs869032	97922148	T	0.23	0.701	1.05 (0.91–1.21)	0.534	1.19 (1.06–1.33)	3.01E−03	2.0E−03	1.18 (1.04–1.34)	0.0122
rs7301050	97942018	A	0.22	0.289	1.1 (0.95–1.27)	0.221	1.19 (1.05–1.33)	4.33E−03	4.0E−03	1.15 (1.01–1.31)	0.0379
rs4762523	97818630	G	0.30	0.06	0.98 (0.86–1.11)	0.737	0.86 (0.78–0.95)	4.52E−03	2.0E−03	0.96 (0.85–1.08)	0.507
rs2638559	98115032	G	0.46	0.569	1.08 (0.96–1.21)	0.226	1.14 (1.04–1.25)	6.53E−03	6.0E−03	1.03 (0.93–1.15)	0.553
rs2712662	98123521	G	0.37	0.638	1.08 (0.96–1.22)	0.217	1.14 (1.04–1.26)	7.64E−03	7.0E−03	1.06 (0.95–1.19)	0.277

[a] Physical position (bp);[b] Minor allele; [c] Minor allele frequency; [d] p-value for Hardy-Weinberg equilibrium test; [e] Odds ratio for cancer;

[f] p-value for cancer based on logistic regression; [g] Odds ratio for obesity; [h] p-value for obesity based on logistic regression; [i] empirical p-value for obesity generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK p-value for obesity based on logistic regression; [j] Odds ratio for diabetes; [k] p-value for diabetes based on logistic regression.

3.4. Shared SNPs among cancer, obesity, and diabetes

Table 4 shows that eight cancer-associated SNPs also contributed to obesity or T2D in the Marshfield sample. SNP rs3759214 was associated with both cancer and T2D (OR = 0.35, 95%CI = 0.15–0.82, p = 0.0161 and OR = 0.5, 95%CI = 0.25–0.98, p = 0.044, respectively). There were seven SNPs associated with both cancer and obesity (top SNP rs2372719 with OR = 1.16, 95%CI = 1.03–1.31, p = 0.0161 and OR = 1.12, 95%CI = 1.02–1.24, p = 0.0206, respectively).

Table 4. SNPs within ANKS1B gene associated with cancer and obesity or diabetes (p < 0.05) in the Marshfield sample.

SNP	Position[a]	AL[b]	MAF[c]	HWE[d]	OR-Cancer[e]	P-Cancer[f]	OR-Obesity[g]	P-Obesity[h]	OR-Diabetes[i]	P-Diabetes[j]
rs616804	97862475	T	0.09	0.332	0.75 (0.61–0.94)	0.0115	1.18 (1.01–1.38)	0.049	1.07 (0.89–1.28)	0.501
rs3759214	97797763	G	0.01	1.00	0.35 (0.15–0.82)	0.0161	1.08 (0.39–2.95)	0.885	0.5 (0.25–0.98)	0.044
rs2372719	98025746	C	0.38	0.243	1.16 (1.03–1.31)	0.0161	1.12 (1.02–1.24)	0.0206	1.08 (0.96–1.21)	0.18
rs2372641	98004347	A	0.38	0.245	1.16 (1.03–1.31)	0.0176	1.12 (1.02–1.24)	0.0213	1.09 (0.97–1.22)	0.151
rs10745842	98018970	C	0.38	0.179	1.16 (1.03–1.31)	0.0182	1.12 (1.01–1.23)	0.0282	1.08 (0.96–1.21)	0.197
rs4762559	98022771	G	0.38	0.194	1.16 (1.03–1.31)	0.0186	1.12 (1.01–1.23)	0.0257	1.08 (0.96–1.2)	0.207
rs2372731	98043575	T	0.39	0.383	1.14 (1.01–1.29)	0.0345	1.11 (1.01–1.22)	0.04	1.07 (0.96–1.2)	0.226
rs3794799	97971270	G	0.38	0.375	1.14 (1.01–1.28)	0.0429	1.12 (1.02–1.24)	0.0218	1.1 (0.99–1.24)	0.088

[a] Physical position (bp);[b] Minor allele; [c] Minor allele frequency; [d] p-value for Hardy-Weinberg equilibrium test; [e] Odds ratio for cancer; [f] p-value for cancer based on logistic regression; [g] Odds ratio for obesity; [h] p-value for obesity based on logistic regression; [i] Odds ratio for diabetes; [j] p-value for diabetes based on logistic regression.

3.5. Replication study and meta-analysis

In the Health ABC sample, 18 SNPs were associated with obesity (p < 0.05) (Table 5). Five SNPs were associated with cancer in the Marshfield sample. Three SNPs (rs616804, rs7295102, and rs201421) were associated with obesity in the meta-analysis of the Marshfield and the Health ABC samples (p = 0.00479, 0.00152 and 0.00799, respectively).

Table 5. SNPs within ANKS1B gene associated with obesity in the Health ABC sample ($p < 0.05$).

SNP	Position[a]	AL[b]	MAF[c]	HWE[d]	OR-Cancer[e]	P-Cancer[f]	OR-Obesity[g]	P-Obesity[h]	EMP[i]	OR-Meta[j]	P-Meta[k]	Q[l]
rs616804	97862475	T	0.08	0.995	0.75 (0.61–0.94)	0.0115	1.58 (1.19–2.10)	1.76E–03	4.99E–03	1.34	4.79E–03	0.109
rs1921023	98887763	A	0.37	0.429	1.0 (0.88–1.13)	0.962	1.27 (1.06–1.52)	9.22E–03	6.99E–03	1.10	0.125	0.0331
rs7295102	97916299	G	0.04	0.124	0.94 (0.72–1.23)	0.653	1.66 (1.13–2.43)	9.93E–03	1.10E–02	1.52	1.52E–03	0.545
rs3851623	98833395	A	0.36	0.43	1.0 (0.88–1.13)	0.996	1.26 (1.05–1.51)	0.0112	0.015	1.11	0.108	0.049
rs1275650	98508721	C	0.43	0.526	1.16 (1.03–1.31)	0.0171	0.80 (0.66–0.95)	0.0132	0.019	0.96	0.556	0.0042
rs1995991	98445422	C	0.38	0.093	1.14 (1.01–1.29)	0.0383	0.79 (0.65–0.95)	0.0136	0.015	0.99	0.858	0.0016
rs10778034	98882131	T	0.37	0.514	–	–	–	0.0159	0.021	–	–	–
rs7132927	98821773	C	0.42	0.719	0.95 (0.84–1.08)	0.443	1.23 (1.03–1.47)	0.0208	0.027	1.09	0.138	0.076
rs7963120	97781085	C	0.09	0.697	1.0 (0.83–1.21)	0.985	1.36 (1.04–1.79)	0.0257	0.028	1.09	0.356	0.229
rs201421	97742374	C	0.26	0.581	1.01 (0.88–1.15)	0.937	1.24 (1.03–1.51)	0.0264	0.024	1.19	7.99E–03	0.578
rs7138269	98824160	T	0.27	0.34	0.98 (0.86–1.13)	0.801	1.24 (1.02–1.50)	0.0309	0.034	1.09	0.165	0.097
rs17471850	98452219	G	0.14	0.64	1.06 (0.90–1.24)	0.486	1.29 (1.02–1.64)	0.0345	0.034	1.07	0.439	0.031
rs809794	98505657	G	0.47	0.895	0.86 (0.76–0.97)	0.0143	1.21 (1.01–1.44)	0.0364	0.036	1.06	0.348	0.0048
rs12298165	98454031	G	0.19	0.03	1.04 (0.90–1.21)	0.558	1.24 (1.01–1.53)	0.0409	0.033	1.05	0.515	0.027
rs2373011	98485480	C	0.43	0.626	–	–	–	0.0416	0.044	–	–	–
rs17471231	98425199	C	0.14	0.369	1.11 (0.95–1.3)	0.195	1.28 (1.01–1.63)	0.0438	0.039	1.05	0.534	0.03
rs2373016	98420463	A	0.40	0.145	0.88 (0.78–0.99)	0.0474	1.20 (1.01–1.43)	0.0452	0.047	1.03	0.689	0.018
rs7313882	98424703	T	0.17	0.437	–	–	–	0.0487	0.042	–	–	–

[a] Physical position (bp);[b] Minor allele; [c] Minor allele frequency; [d] p-value for Hardy-Weinberg equilibrium test; [e] Odds ratio for cancer in the Marshfield sample; [f] p-value for cancer based on logistic regression; [g] Odds ratio for obesity in the Health ABC sample; [h] p-value for obesity based on logistic regression in the Health ABC sample; [i] empirical p-value for obesity generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK; [j] Odds ratio for the meta-analysis of the Marshfield and the Health ABC samples; [k] p-value for the meta-analysis of obesity; [l] p-value based on the χ_2-based Cochrane's Q statistic.

4. Discussion

In this study, we found statistically significant associations of polymorphisms within the ANKS1B gene with cancer, obesity, and T2D. The most significant SNPs in the Marshfield sample for cancer, T2D, and obesity were rs2373013, rs10860548, and rs7139028, respectively. Interestingly, rs3759214 was linked with both cancer and T2D while seven SNPs were linked with both cancer and obesity. Furthermore, six SNPs were associated with both T2D and obesity. In the Health ABC sample, five cancer-related SNPs in the Marshfield sample were replicated. Finally, three SNPs were associated with obesity in meta-analysis using both the Marshfield and the Health ABC samples.

Previous studies have reported that expression of ANKS1B gene may be elevated in patients with pre-B cell acute lymphocytic leukemia associated with t(1;19) translocation [14,15]. A recent study demonstrated that it may play a role in a smoking-related molecular alteration in cancer [20]. Lin et al. (2012) reported that three SNPs (rs1549102, rs11110099, and rs10745877) of ANKS1B were associated with lung cancer using a case-control design [19]. In the present study, we identified 25

SNPs in the Marshfield sample associated with cancer ($p < 0.05$). However, we did not find associations of rs1549102, rs11110099 and rs10745877 with cancer in the Marshfield sample. It may due to the fact that the outcome in Lin et al. (2012) was restricted to lung cancer, while cancer status definition in the Marshfield sample was very broad. On the other hand, our results suggested that ANKS1B might play a role in various cancers.

To our knowledge, the present study is the first attempt to evaluate the associations of ANKS1B with obesity and T2D. The SNP rs2373011 within ANKS1B gene was associated with obesity in the Health ABC sample ($p = 0.0416$). Similarly, the same SNP was previously reported to be linked to BMI ($p = 9.00 \times 10^{-6}$) and waist circumference ($p = 2.46 \times 10^{-6}$) among Filipino women [16]. Another study reported that the polymorphisms of ANKS1B gene were associated with genetic difference between diabetic patients with and without nephropathy [17]. A more recent study revealed the effect of ANKS1B gene on body weight in an animal model [18]. We have identified 34 SNPs associated with obesity and 12 SNPs associated with T2D in the Marshfield, and 15 SNPs associated with obesity in the Health ABC sample. Interestingly, there are six SNPs associated with both T2D and obesity in the Marshfield sample. Furthermore, there are seven SNPs associated with both cancer and obesity in the Marshfield sample while five SNPs are associated with cancer in the Marshfield sample and with obesity in the Health ABC sample. The present study showed that the ANKS1B gene might play a role in the cancer, obesity and T2D.

Epidemiology studies have shown that obesity is associated with increasing rates of the metabolic syndrome and T2D [9-11]; while overweight, obesity and T2D are associated with increased risk of developing many different types of cancers [9-13,30,31]. However, the biological mechanisms underlying the relationship among obesity, T2D, and cancer are not well understood. Insulin resistance is common in obese individuals and is believed to be a key factor in the pathogenesis of the metabolic syndrome. For example, insulin, insulinlike growth factor 1, and insulinlike growth factor 2 signaling through the insulin receptor and the insulinlike growth factor 1 receptor can induce tumorigenesis, accounting to some extent for the link between diabetes, obesity, and cancer [30,31]. It has been suggested that such mechanisms may include modulation of energy balance and calorie restriction, growth factors, multiple signaling pathways, and inflammatory processes. For example, the PI3K/Akt/mTOR cascade, one of the signaling pathways linking obesity and cancer, is a target of many of the obesity-associated factors and regulates cell proliferation and survival [32]. Another study proposed that obesity, particularly abdominal obesity, is associated with insulin resistance and the development of dyslipidemia, hyperglycemia, and ultimately T2D. Although many metabolic abnormalities occur with obesity and T2D, insulin resistance and hyperinsulinemia appear to be central to these conditions and may contribute to dyslipidemia and alter levels of circulating estrogens and androgens; while hyperinsulinemia and dyslipidemia may contribute to cancer development [9]. Other mechanisms have been proposed such as hyperglycemia, hyperinsulinemia, hypertriglyceridemia, inflammatory cytokines, adipocytokines, and IGF-1 [31].

Previous findings and our present results suggested that ANKS1B was associated with cancer, obesity and diabetes. The ANKS1B gene encodes a multi-domain protein that is predominantly expressed in brain and testis [14]; while ANKS1B is involved in signals that regulate normal proliferation or differentiation of cells and therefore may play a role in cancer development [14,20,33]. In addition, the ANKS1B gene may be involved in some signaling pathways and multiple protein-protein interactions [14,34]. For example, ANKS1B is one of the genes in the apoptotic-pathway and was found to be associated with lung cancer [19]. However, the direct

evidence of the role of ANKS1B in both obesity and T2D remain lacking. Previous studies showed that obesity may increase risk of cancer and diabetes, potentially caused by intracellular signaling pathways that involve in many essential cellular processes such as proliferation, differentiation, and apoptosis [35-40]. Therefore, ANKS1B may be one of the mechanisms linking obesity and T2D to cancer.

There are several strengths in this study. First, our sample sizes were relatively large and the two samples were relatively ethnically homogeneous. Second, we examined 272 SNPs in the Marshfield sample for discovery and used 310 SNPs in the Health ABC sample to replicate our findings. Third, we implemented a meta-analysis to increase precision in estimating effects by combining two study samples. Fourth, we detected pleiotropic effects of ANKS1B gene on three complex diseases—cancer, obesity and T2D.

We also realized some limitations in this study. First, the definition of cancer status in the Marshfield sample was broad (including any diagnosed cancer omitting minor skin cancer). It will be interesting to investigate the association of ANKS1B with single cancer type. Second, this study focused on obesity with BMI $\geq$ 30. It will be more informative to compare subtypes of obesity such as Class I obesity (BMI = 30–34.9), Class II obesity (BMI = 35–39.9), and Class III obesity (BMI $\geq$ 40). Third, our current findings might be subject to type I error. Therefore, these findings need to be replicated in additional samples.

5. Conclusion

These findings provide the first evidence of genetic variants in the ANKS1B gene influencing the risk of cancer, obesity and T2D and will serve as a resource for replication in other populations. Future functional study of this gene may help to better characterize the genetic architecture of these three comorbidities.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Supplementary

Associations of each SNP within the ANKS1B gene with cancer, obesity and diabetes in the Marshfield sample (Table S1, provided as a separate file).

References

1. Flegal KM, Carroll MD, Kit BK, et al. (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 307: 491-497.
2. Kelly T, Yang W, Chen CS, et al. (2008) Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 32: 1431-1437.
3. Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, et al. (2008) Arsenic exposure and prevalence of type 2 diabetes in US adults. *JAMA* 300: 814-822.
4. Center for Disease Control and Prevention (2011) National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States. U.S. Department of Health and Human Services, Center for Disease Control and Prevention, Atlanta, GA.
5. Shin JK, Chiu YL, Choi S, et al. (2012) Serious psychological distress, health risk behaviors, and diabetes care among adults with type 2 diabetes: the California Health Interview Survey 2007. *Diabetes Res Clin Pract* 95: 406-414.
6. IDF (2009) IDF Diabetes Atlas, 4th edn. International Diabetes Federation, Brussels.
7. Wang KS, Liu X, Wang L, et al. (2014) Associations of anxiety and psychological distress with cancer in the US Adults: Results from the 2012 National Health Interview Survey. *American Journal of Cancer Epidemiology and Prevention* 2: 20-31.
8. Gulland A (2014) Global cancer prevalence is growing at "alarming pace," says WHO. *BMJ* 348: g1338.
9. Ferguson RD, Gallagher EJ, Scheinman EJ, et al. (2013) The epidemiology and molecular mechanisms linking obesity, diabetes, and cancer. *Vitam Horm* 93: 51-98.
10. Hjartåker A, Langseth H, Weiderpass E (2008) Obesity and diabetes epidemics: cancer repercussions. *Adv Exp Med Biol* 630: 72-93.
11. Garg SK, Maurer H, Reed K, et al. (2014) Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes Obes Metab* 16: 97-110.

12. Cohen DH, LeRoith D (2012) Obesity, type 2 diabetes, and cancer: the insulin and IGF connection. *Endocr Relat Cancer* 19: F27-45.
13. Forte V, Pandey A, Abdelmessih R, et al. (2012) Obesity, Diabetes, the Cardiorenal Syndrome, and Risk for Cancer. *Cardiorenal Med* 2: 143-162.
14. Fu X, McGrath S, Pasillas M, et al. (1999) EB-1, a tyrosine kinase signal transduction gene, is transcriptionally activated in the t(1;19) subset of pre-B ALL, which express oncoprotein E2a-Pbx1. *Oncogene* 18: 4920-4929.
15. Casagrande G, te Kronnie G, Basso G (2006) The effects of siRNA-mediated inhibition of E2A-PBX1 on EB-1 and Wnt16b expression in the 697 pre-B leukemia cell line. *Haematologica* 91: 765-771.
16. Croteau-Chonka DC, Marvelle AF, Lange EM, et al. (2010) Genomewide association study of anthropometric traits and evidence of interactions with age and study year in Filipino women. *Obesity* 19: 1019-1027.
17. Pei D, Huang YJ, Hsieh CH, et al. (2010) The genetic background difference between diabetic patients with and without nephropathy in a Taiwanese population by linkage disequilibrium mapping using 382 autosomal STR markers. *Genet Test Mol Biomarkers* 14: 433-438.
18. Parker CC, Cheng R, Sokoloff G, et al. (2011) Fine-mapping alleles for body weight in LG/J × SM/J F_2 and F(34) advanced intercross lines. *Mamm Genome* 22: 563-571.
19. Lin J, Lu C, Stewart DJ, et al. (2012) Systematic evaluation of apoptotic pathway gene polymorphisms and lung cancer risk. *Carcinogenesis* 33: 1699-1706.
20. Eckel-Passow JE, Serie DJ, Bot BM, et al. (2014) ANKS1B is a smoking-related molecular alteration in clear cell renal cell carcinoma. *BMC Urol* 14: 14.
21. McCarty CA, Wilke RA, Giampietro PF, et al. (2005) Marshfield clinic personalized medicine research project (PMRP): design, methods and recruitment for a large population-based biobank. *Personalized Med* 2: 49-79.
22. McCarty CA, Peissig P, Caldwell MD, et al. (2008) The marshfield clinic personalized medicine research project: 2008 scientific update and lessons learned in the first 6 years. *Personalized Med* 5: 529-542.
23. Wang KS, Liu X, Zheng S, et al. (2012) A novel locus for body mass index (BMI) on 5p15.2: a meta-analysis of two genome-wide association studies. *Gene* 500: 80-84.
24. Harris TB, Visser M, Everhart J, et al. (2000) Waist circumference and sagittal diameter reflect total body fat better than visceral fat in older men and women. The health, aging and body composition study. *Ann N Y Acad Sci* 904: 462-473.
25. Cauley JA, Danielson ME, Boudreau RM, et al. (2007) Inflammatory markers and incident fracture risk in older men and women: the health aging and body composition study. *J Bone Miner Res* 22: 1088-1095.
26. Qi Q, Menzaghi C, Smith S, et al. (2012) Genome-wide association analysis identifies TYW3/CRYZ and NDST4 loci associated with circulating resistin levels. *Hum Mol Genet* 21: 4774-4780.
27. Wang KS, Wang L, Liu X, et al. (2013) Association of HS6ST3 gene polymorphisms with obesity and triglycerides: gene-gender interaction. *J Genet* 92: 395-402.
28. Purcell S, Neale B, Todd-Brown K, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559-575.

29. Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* 21: 1539-1558.
30. Gallagher EJ, Fierz Y, Ferguson RD, et al. (2010) The pathway from diabetes and obesity to cancer, on the route to targeted therapy. *Endocr Pract* 16: 864-873
31. Gallagher EJ, LeRoith D (2013) Epidemiology and molecular mechanisms tying obesity, diabetes, and the metabolic syndrome with cancer. *Diabetes Care* 36 Suppl 2: S233-239
32. Vucenik I, Stains JP (2012) Obesity and cancer risk: evidence, mechanisms, and recommendations. *Ann N Y Acad Sci* 1271: 37-43.
33. Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. *Nature* 411: 342-348.
34. Smirnova E, Shanbhag R, Kurabi A, et al. (2013). Solution structure and peptide binding of the PTB domain from the AIDA1 postsynaptic signaling scaffolding protein. *PLoS One* 8: e65605.
35. Bianchini F, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. *Lancet Oncol* 3: 565-574.
36. Wang YW, Jones PJ (2004) Conjugated linoleic acid and obesity control: efficacy and mechanisms. *Int J Obes Relat Metab Disord* 28: 941-955.
37. Bost F, Aouadi M, Caron L, et al. (2005) The role of MAPKs in adipocyte differentiation and obesity. *Biochimie* 87: 51-56.
38. Barb D, Pazaitou-Panayiotou K, Mantzoros CS (2006) Adiponectin: a link between obesity and cancer. *Expert Opin Investig Drugs* 15: 917-931
39. Saxena NK, Sharma D (2013) Multifaceted leptin network: the molecular connection between obesity and breast cancer. *J Mammary Gland Biol Neoplasia* 18: 309-320.
40. Riondino S, Roselli M, Palmirotta R, et al. (2014) Obesity and colorectal cancer: role of adipokines in tumor initiation and progression. *World J Gastroenterol* 20: 5177-5190.

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