Published in final edited form as:
Cancer. 2011 February 1; 117(3): 480–491. doi:10.1002/cncr.25612.

Glucose metabolism Gene Polymorphisms and Clinical Outcome in Pancreatic Cancer

Xiaoqun Dong, MD, PhD[1], **Hongwei Tang, MS**[1], **Kenneth R. Hess, PhD**[2], **James L. Abbruzzese, MD**[1], and **Donghui Li, PhD**[1]

[1]Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[2]Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Abstract

Background—Altered glucose-metabolism is the most common metabolic hallmark of malignancies. We tested the hypothesis that glucose-metabolism gene variations affect clinical outcome in pancreatic cancer.

Methods—We retrospectively genotyped 26 single nucleotide polymorphisms (SNPs) from 5 glucose-metabolism genes in 154 patients with localized disease and validated the findings in 552 patients with different stages of pancreatic adenocarcinoma. Association between genotypes and overall survival (OS) was evaluated using multivariable Cox proportional hazard regression models with adjustment for clinical predictors.

Results—*Glucokinase (GCK)* IVS1+9652C>T and *hexokinase (HK)2* N692N homozygous variants were significantly associated with reduced OS in the training set of 154 patients ($P < 0.001$). These associations were confirmed in the validation set of 552 patients and in the combined dataset of all 706 patients ($P \leq 0.001$). In addition, *HK2* R844K variant K allele was associated with a better survival in the validation set and the combined dataset ($P \leq 0.001$). When data was further analyzed by disease stage, *glutamine-fructose-6-phosphate transaminase (GFPT1)* IVS14-3094T>C, *HK2* N692N and R844K in patients with localized disease, and *GCK* IVS1+9652C>T in patients with advanced disease were significant independent predictors for OS ($P \leq 0.001$). Haplotype CGG of *GPI* and GCTATGG of *HK2* were associated with better OS, respectively, with a *P* value of 0.004 and 0.007.

Conclusions—We demonstrated that glucose-metabolism gene polymorphisms affect clinical outcome in pancreatic cancer. These observations support a role of abnormal glucose metabolism in pancreatic carcinogenesis.

Keywords

glucose-metabolism; pancreatic adenocarcinoma; single nucleotide polymorphism; overall survival; haplotype

Corresponding author: Dr. Donghui Li, Ph.D., Department of Gastrointestinal Medical Oncology, Unit 426, The University of Texas M. D. Anderson Cancer Center, 1550 Holcombe Blvd., Houston, TX, 77030-4009. Phone: 713-834-6690; Fax: 713-834-6153; dli@mdanderson.org.

Financial disclosures: None of the authors have financial disclosures.

Conflict of Interest Disclosures: The authors made no disclosures.

INTRODUCTION

A common property of malignant tumors is altered glucose metabolism. The 'Warburg effect' (aerobic glycolysis, a persistently high rate of glucose conversion into lactate even under normoxic condition), is a distinctive metabolic characteristic of malignancies that distinguishes them from normal cells [1]. Possibly this effect is an adaptation to intermittent hypoxia in pre-malignant lesions. Enhanced glycolysis at the expense of mitochondrial energy production causing microenvironment-acidosis triggers evolution to phenotypes resistant to acid toxicity, provide precursors for macromolecule biosynthesis and protect cells from excessive toxic reactive oxygen species [2]. Subsequent cell populations with intensified glycolysis and acid-resistance have a strong growth-advantage, which promotes malignant proliferation, unrestrained growth, and invasion [3]. On the basis of this prominent phenotype, positron emission tomography (PET) imaging has become a major method for cancer detection and surveillance. The worldwide clinical application of PET has resulted in a resurgence of interest in tumor metabolism [4]. PET using the glucose analogue tracer 2-[18F]-2-deoxy-D-glucose (FdG) has shown that most cancers profoundly strengthen glucose-uptake, which is dependent on glycolysis rate. FdG-uptake/trapping results from upregulation of glucose transporters and hexokinases (HK1/2) in pancreatic cancer [5, 6]. This is a marker that can be used to monitor cancer progression, the augmented glucose-uptake correlates with enhanced tumor aggression, advanced clinical stage, and poorer prognosis [7, 8].

Pancreatic cancer is the fourth leading cause of cancer mortality in the United States, with an estimated 42,470 new cases and 35,240 deaths in 2009[9]. Pancreatic cancer is one of the most difficult malignancies to treat, with a 5-year survival rate <5% [9]. Glucose intolerance and diabetes are common manifestations of pancreatic cancer. Whether and how genetic variations in glucose metabolism affect the clinical outcome of this disease is unknown.

Hexokinase 2 (HK2), glucokinase (GCK), glutamine-fructose-6-phosphate transaminase (GFPT1), glucose phosphate isomerase (GPI), O-linked N-acetylglucosamine (GlcNAc) transferase (OGT) are key enzymes involved in glucose metabolism. For its crucial role in determining the cell fate (survival or death) [10] glucose metabolism pathway has become a therapeutic target for cancer treatment [11], with clinical trials on HK2 inhibitors being conducted [12, 13]. We have previously shown that obesity and diabetes are associated with reduced overall survival in patients with pancreatic cancer [14, 15]. Whether genetic variations in glucose metabolism contribute to the poor clinical outcome of pancreatic cancer has never been explored. To test the hypothesis that genetic variation in glucose-metabolism genes is related to clinical outcome in pancreatic cancer, we evaluated 26 single nucleotide polymorphisms (SNPs) of *GCK*, *GFPT1*, *GPI*, *HK2* and *OGT* gene (Fig.1.) in reference to the overall survival (OS) and response to chemoradiotherapy in 706 patients with pancreatic cancer.

METHODS

Patient Recruitment and Data Collection

The 706 patients included 154 patients with resectable tumor who were enrolled in clinical trials of preoperative gemcitabine-based chemoradiation [16] and 552 patients who were recruited in a case-control study conducted at The University of Texas M. D. Anderson Cancer Center from February 1999 to May 2007, with follow-up to August 2009.[17] Patients were eligible for the current study if they had a diagnosis of pathologically confirmed pancreatic ductal adenocarcinoma and had an available DNA sample. All patients signed an informed consent for medical record review and DNA sample collection. The study was

approved by the institutional review board of M. D. Anderson Cancer Center and conducted in accordance with all current ethical guidelines.

We reviewed patients' medical records to collect demographic (age, sex and self-reported race) and clinical information on date of diagnosis, date of death or last follow-up, clinical tumor stage, tumor resection, tumor site, size and differentiation, performance status, serum markers for liver, kidney and pancreas functions, and serum carbohydrate antigen 19-9 (CA19-9) level at diagnosis. Clinical tumor staging followed the objective computed tomography (CT) criteria: A localized or potentially resectable tumor is defined as a tumor with no evidence of extra-pancreatic disease (extensive peri-pancreatic lymph node involvement), no involvement of the celiac axis and superior mesenteric artery, inferior vena cava, or aorta, or encasement or occlusion of the superior mesenteric vein–portal vein confluence. Tumor abutment and encasement of the SMV, in the absence of vessel occlusion or extension to the SMA was considered resectable. Locally advanced tumors are those unresectable but without distant metastasis. Tumor response to preoperative therapy was evaluated by CT at restaging in patients who had localized tumor and received preoperative chemoradiotherapy. Tumor margin and lymph node status were evaluated in patients with resected tumors only. Dates of death were obtained and cross-checked using the following sources: the M. D. Anderson Cancer Center tumor registry, inpatient medical records, or the United States Social Security Death Index (www.deathindexes.com/ssdi.html). OS time was calculated from the date of diagnosis to the date of death or last follow-up.

DNA Extraction, SNP Selection and Genotyping

DNA was extracted from peripheral lymphocytes using Qiagen DNA isolation kits (Valencia, CA). Seventeen tagging SNPs were selected using the SNPbrowser software (Applied Biosystems, www.allsnps.com/snpbrowser) with a cutoff of r^2=0.8 and a minor allele frequency (MAF) ≥10% in Caucasians from the HapMap Project database (www.hapmap.org). We also included nine coding SNPs (nonsynonymous or synonymous) or untranslated region (UTR) SNPs that have a MAF ≥ 5% in Caucasians. The genes, nucleotide substitutions, function, reference SNP identification numbers, and MAF of the 26 SNPs are described in Table 1. The protein sequences, structures, homology models, mRNA transcripts, and predicted functions for the SNPs were evaluated by F-SNP (Queen's University, Kingston, Ontario, Canada) [18]. Genotyping used the mass spectroscopy-based MassArray method (Sequenom, Inc, San Diego, CA). We randomly genotyped 20% of total samples in duplicate, showing 99.8% concordance. The inconsistent data were excluded from final analysis.

Statistical Analysis

The distribution of genotypes was tested for Hardy-Weinberg Equilibrium with the goodness-of-fit χ^2 test. Genotype and allele frequency of the SNP were determined by direct gene counting. Haplotype diversity and linkage disequilibrium index (Lewontin's D' and r^2) were calculated using SNPAlyze (DYNACOM Co., Ltd. Mobara, Japan). The median follow-up time was computed using censored observations only. The association between genotype/haplotype and OS was evaluated by Cox proportional hazard regression models. Hazard ratios (HR) and 95% confidence interval (CI) were calculated with adjustment of sex, race and any clinical factors that are significant predictors for OS in multivariate Cox regression models. The association of genotype with categorical variables such as sex, race, and tumor response to therapy was examined using Chi-square test and logistic regression model with adjustment for clinical factors. Statistical analysis used SPSS (SPSS Inc, Chicago, IL). The false discovery rate (FDR) was calculated using the Beta-Uniform Mixture method [19]. For 77 comparisons in a total of 26 SNPs (38 SNPs in dominant and 39 in recessive inheritance modes) for OS in all patients, we found a P value of 0.002

corresponded to an FDR of 5%. Thus, $P \leq 0.002$ in the genotype analysis was considered statistically significant.

RESULTS

Patients' Characteristics

The patients' demographics and clinical predictors for OS are summarized in Table 2. There were 333 patients with localized disease, 211 with locally advanced disease, and 162 with metastatic disease. Of the 333 patients with localized tumor, 275 (83%) had tumor resection. Of the 706 patients, 138 (19.5%) were alive at the end of the study, with a median follow-up time of 46.0 months. The median survival time (MST) for the entire patient population was 17.2 months (95% CI, 15.8–18.5). Advanced tumor stage, unresected tumor, an elevated CA19-9 serum level or biochemical index, or poor performance status remained as significant predictors for worse OS in multivariate Cox regression models (data not shown).

Genotype Distribution and Allele Frequencies

The observed allele frequencies in this study population were comparable to the previously reported allele frequencies in the general population (Table 1). The distribution of 26 SNPs followed Hardy-Weinberg equilibrium ($P > 0.05$) except for *OGT* IVS18-424A>G ($P = 0.001$). Linkage disequilibrium data of the 26 SNPs are described in Table 3. There were significant sex and racial differences in the genotype distributions, e.g. the *HK2* N692N CC genotype frequency was 22.4% for men but 10.3% for women ($P<0.001$), and the *HK2* R844K GG genotype frequency was 63.9%, 53.5%, and 25.9% for whites, Hispanics and blacks, respectively ($P<0.001$) (Data for other SNPs are not shown). Therefore, sex and race were included in all Cox regression models.

Associations of Genotype with Overall Survival

The association of each genotype with OS was first analyzed in a relatively homogenous population of 154 patients who had resectable tumor and were treated on protocol for preoperative chemoradiotherapy. SNPs with a P value < 0.05 in the multivariate Cox regression models are listed in Table 4. Of the 26 SNPs evaluated, *GCK* IVS1+9652 C>T and *HK2* N692N homozygous mutants were significantly associated with OS at the level of 5% FDR ($P < 0.002$). The significant associations of both SNPs with OS were confirmed in the validation set of 552 patients (Table 4). In addition, the homozygous K variant of the nonsynonymous SNP *HK2* R844K was significantly associated with a better OS in the validation set ($P = 0.001$). When data of the training set and the validation set was pooled to increase power, the significant associations of *GCK* IVS1+9652C>T, *HK2* N662N and R844K genotype with OS all remained highly significant.

Next we analyzed the association of each genotype and OS by disease stage. In a total of 333 patients with localized disease, *GFPT1*-3094T>C, *HK2* N692N and R844K were significantly associations with OS (Table 5). The *GCK* IVS1+9652C>T and *HK2* N692N genotype showed some associations with OS among the 211 patients with locally advanced disease but neither reached the significance level ($P = 0.027$ and 0.013). Among the 162 metastatic patients, *GCK* IVS1+9652C>T was the only SNP had significant association with OS ($P < 0.001$). When data was pooled from patients with locally advanced and metastatic disease, *GCK* IVS1+9652C>T remained as the sole significant genetic predictor for OS ($P \leq 0.001$).

Associations of Haplotype Diversity with OS

Haplotype frequencies and their associations with OS are described in Table 6. The *GPI* IVS6-378T>C, IVS9+2363C>G and G163G CGG haplotype was associated with a better OS ($P = 0.004$) and the CCG haplotype with a worse OS ($P = 0.01$). The different associations with OS of these two haplotypes were obviously determined by the IVS9+2363C>G genotype. Two haplotypes of *HK2* gene, i.e. GCTATGG and ATTACAT were associated with a better or worse OS with a *P* value of 0.007 and 0.03, respectively, in multivariate Cox regression (Table 6). Two other haplotypes, GCCGCAT and ATTGCAT, showed non-significant associations with OS ($P = 0.055$ and 0.06). Apparently, haplotypes containing CAT of N692N, L766L, and Ex18+407T>G (3'UTR SNP) all conferred a worse OS.

Associations of Genotype with Other Clinical Parameters

The association between each genotype and tumor response to therapy was evaluated in 261 patients who had resectable tumor and received preoperative chemoradiotherapy. *HK2* N692N and R844K genotype showed associations with tumor response (Table 7). Interestingly, the genotype distribution of these two SNPs was significantly different by disease stage and tumor resection status. For example, the *HK2* N692N CC genotype was detected in 12.6%, 19.0%, and 25.9% of patients with localized, locally advanced and metastatic disease ($P < 0.001$, χ^2 test). The *HK2* R844K GG genotype was present in 52.9% of the patients with localized disease and 69.7% of the patients with advanced disease ($P < 0.001$, χ^2 test).

DISCUSSION

We identified glucose-metabolism gene variations associated with clinical outcome in pancreatic cancer. *GCK* IVS1+9652C>T; *HK2* N692N, and R844K in all patients, *GFPT1* IVS14-3094T>C, *HK2* N692N and R844K in patients with localized tumor, and *GCK* IVS1+9652C>T in patients with advanced diseases were significant independent predictors for OS. We also found a significant association of *HK2* N692N and R844K genotype with disease stage, tumor resection status and response to preoperative chemoradiotherapy. These data support a role of glucose-metabolism gene polymorphisms in modifying the clinical outcome in pancreatic cancer.

Hexokinases catalyze the phosphorylation of glucose to glucose-6-phosphate. This is the first and rate-limiting step in glucose metabolism. HK2 localizes to the outer membrane of the mitochondria and is the major hexokinase isoform expressed in cancer cells.[20] HK2 expression is insulin-responsive and responsible for the accelerated glycolysis in cancer cells [21]. Overexpression of HK2 in tumor tissues has been correlated to poor prognosis in breast cancer and liver cancer but not in pancreatic cancer [7, 22, 23], although the negative finding in pancreatic cancer could be partially explained by the heterogeneity of the study population [23]. We observed two *HK2* SNPs R844K and N692N, significantly associated with OS, tumor stage, tumor resection status and tumor response to therapy. *HK2* R844K, an evolutionary conservative SNP, K variant, is computationally predicted to deleteriously affect protein coding and RNA splicing which change the solvent accessibility and hydrophobicity of the protein [18]. The K variant thus, may confer a dysfunctional low enzymatic activity of HK2, impose restraint on glycolysis rate, dampen tumor progression due to lack of energy supply. Indeed, a better response to therapy, a higher tumor resection rate, and a longer OS was observed among patients carrying the K variant allele (GA/AA genotype). The functional significance of the synonymous SNP *HK2* N692N is unknown. By computational prediction, such SNP may result in altered conformation, substrate affinity, and mRNA splicing [18]. Whether such changes result in a higher enzyme activity,

which may explained the association of the variant allele with reduced OS, needs further investigation. We observed the *HK2* N692N CC frequency in men was higher than that in women, we inferred CC represents higher enzyme activity, whether the genotype difference contributes to previous report that men had a higher HK enzyme activity than women needs further investigation [24]. We also observed that haplotypes containing variant alleles of *HK2* N692N, L766L, and Ex18+407T>G (3'-UTR SNP) were associated with worse OS. It is possible that these genotypes/haplotypes conferred a higher level/activity of HK2 that contribute to a higher rate of glycolysis, accelerated tumor progression, and reduced OS.

We found three intronic SNPs were associated with OS, i.e. *GCK* IVS1+9652 TT genotype in patients with advanced diseases, *GFPT1* IVS14-3094T>C in all patients and in patients with localized tumors, and a *GPI* haplotype containing the IVS9+2363C>G G allele in all patients. GCK is another member of the hexokinase family, catalyzing the ATP-dependent phosphorylation of glucose. Unlike HK2, GCK activity is not inhibited by its product glucose-6-phosphate but remains active while glucose is abundant. GCK plays a role in maintaining glucose homeostasis as the glucose-sensor and glycolysis pacemaker involved in regulating insulin secretion [25]. We speculate that there is an increased demand for glucose phosphorylation in advanced tumors because of the rapid cell growth, so GCK is required to maintain a constantly active glucose metabolism. *GFPT1* gene encoding the glutamine-fructose-6-phosphate transaminase, the first and rate-limiting enzyme of hexosamine biosynthesis pathway (HBP) controls the glucose flux into HBP. HBP is responsible for shuttling glucose to cellular glycosylation events, e.g., promoting N-linked glycosylation of Wnt-related proteins [26]. Glucose flux into HBP initiating post-translational modifications of cytoplasmic and nuclear proteins that regulate signal transduction, transcription, and protein degradation [27]. GPI catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate, and plays a central role in glycolysis and gluconeogenesis. GPI can guide the glucose flow to the pentose phosphate pathway to produce NADPH and pentose [28]. GPI also functions as an autocrine motility factor, secreted from the tumor cells to promote cell migration, progression and metastasis and to help the cells survive and proliferate under hypoxic and nutrient-deprived conditions [29]. Although the functional significance of these intron SNPs is unknown, the variant alleles may affect the binding of transcriptional factors to the gene, thus upregulate the mRNA and protein expression [18]. The possibility that these SNPs are in linkage with unidentified functional SNPs could not be excluded.

OGT catalyzes the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues. O-linked glycosylation plays a role in controlling gene expression, fuel metabolism, cell growth, differentiation, and cytoskeleton organization [30]. We did not observe any significant association of the OGT genotype/haplotype with OS, partly because only 2 SNPs were examined in this study. Further study of this gene is warranted when additional SNPs are revealed by DNA sequencing.

The strength of our study includes detailed clinical information, a large sample size, a two-step design and a hypothesis-driven gene-selection. The limitations of the study include the limited number of genes and SNPs evaluated and the potentially false-positive findings owing to multiple comparisons. To keep the FDR < 5%, we applied a *P* value of 0.002 as the significance level in the genotype analysis. However, the frequencies of most homozygotes with major effects on clinical outcome are relatively low, so the possibility that these observations are by chance alone cannot be excluded. Additional studies with larger samples in different patient populations are required to confirm these findings. Furthermore, demonstrating the functional significances of these gene traits is pivotal in understanding their role in pancreatic cancer. Nevertheless, our findings provided supporting evidence for the importance of glucose-metabolism pathway in pancreatic cancer. Whether these genetic

markers have a potential value in predicting response to glucose-metabolism-targeted therapy in pancreatic cancer is under current investigation.

Acknowledgments

Grant Support: National Institutes of Health (NIH) RO1 grant CA098380 (to D.L.), SPORE P20 grant CA101936 (to J.L.A.), NIH Cancer Center Core grant CA16672, Lockton Research Funds (to D.L.).

REFERENCES

1. Warburg O. On the origin of cancer cells. Science. 1956; 123:309–314. [PubMed: 13298683]
2. Vousden KH, Ryan KM. p53 and metabolism. Nat Rev Cancer. 2009; 9:691–700. [PubMed: 19759539]
3. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer. 2004; 4:891–899. [PubMed: 15516961]
4. Gambhir SS. Molecular imaging of cancer with positron emission tomography. Nat Rev Cancer. 2002; 2:683–693. [PubMed: 12209157]
5. Higashi T, Saga T, Nakamoto Y, et al. Relationship between retention index in dual-phase (18)F-FDG PET, and hexokinase-II and glucose transporter-1 expression in pancreatic cancer. J Nucl Med. 2002; 43:173–180. [PubMed: 11850481]
6. Natsuizaka M, Ozasa M, Darmanin S, et al. Synergistic up-regulation of Hexokinase-2, glucose transporters and angiogenic factors in pancreatic cancer cells by glucose deprivation and hypoxia. Exp Cell Res. 2007; 313:3337–3348. [PubMed: 17651733]
7. Peng SY, Lai PL, Pan HW, et al. Aberrant expression of the glycolytic enzymes aldolase B and type II hexokinase in hepatocellular carcinoma are predictive markers for advanced stage, early recurrence and poor prognosis. Oncol Rep. 2008; 19:1045–1053. [PubMed: 18357395]
8. Sperti C, Pasquali C, Chierichetti F, et al. 18-Fluorodeoxyglucose positron emission tomography in predicting survival of patients with pancreatic carcinoma. J Gastrointest Surg. 2003; 7:953–959. discussion 959-60. [PubMed: 14675704]
9. Jemal A, Siegel R, Ward E, et al. Cancer statistics 2009. CA Cancer J Clin. 2009; 59:225–249. [PubMed: 19474385]
10. Hammerman PS, Fox CJ, Thompson CB. Beginnings of a signal-transduction pathway for bioenergetic control of cell survival. Trends Biochem Sci. 2004; 29:586–592. [PubMed: 15501677]
11. Cao X, Bloomston M, Zhang T, et al. Synergistic antipancreatic tumor effect by simultaneously targeting hypoxic cancer cells with HSP90 inhibitor and glycolysis inhibitor. Clin Cancer Res. 2008; 14:1831–1839. [PubMed: 18347186]
12. Kurtoglu M, Maher JC, Lampidis TJ. Differential toxic mechanisms of 2-deoxy-D-glucose versus 2-fluorodeoxy-D-glucose in hypoxic and normoxic tumor cells. Antioxid Redox Signal. 2007; 9:1383–1390. [PubMed: 17627467]
13. Hulleman E, Kazemier KM, Holleman A, et al. Inhibition of glycolysis modulates prednisolone resistance in acute lymphoblastic leukemia cells. Blood. 2009; 113:2014–2021. [PubMed: 18978206]
14. Li D, Morris JS, Liu J, et al. Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. JAMA. 2009; 301:2553–2562. [PubMed: 19549972]
15. Li D, Hassan MM, Abbruzzese JL. Obesity and Survival Among Patients With Pancreatic Cancer--Reply. JAMA. 2009; 302:1752-a53. [PubMed: 19861663]
16. Dong X, Jiao L, Li Y, et al. Significant associations of mismatch repair gene polymorphisms with clinical outcome of pancreatic cancer. J Clin Oncol. 2009; 27:1592–1599. [PubMed: 19237629]
17. Dong X, Javle M, Hess KR, et al. Insulin-like Growth Factor Axis Gene Polymorphisms and Clinical Outcome in Pancreatic Cancer. Gastroenterology.
18. Lee PH, Shatkay H. F-SNP: computationally predicted functional SNPs for disease association studies. Nucleic Acids Res. 2008; 36:D820–D824. [PubMed: 17986460]

19. Pounds S, Cheng C. Improving false discovery rate estimation. Bioinformatics. 2004; 20:1737–1745. [PubMed: 14988112]

20. Mathupala SP, Ko YH, Pedersen PL. Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin Cancer Biol. 2009; 19:17–24. [PubMed: 19101634]

21. Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem. 2001; 276:43407–43412. [PubMed: 11557773]

22. Palmieri D, Fitzgerald D, Shreeve SM, et al. Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. Mol Cancer Res. 2009; 7:1438–1445. [PubMed: 19723875]

23. Lyshchik A, Higashi T, Hara T, et al. Expression of glucose transporter-1, hexokinase-II, proliferating cell nuclear antigen and survival of patients with pancreatic cancer. Cancer Invest. 2007; 25:154–162.

24. Green HJ, Fraser IG, Ranney DA. Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. J Neurol Sci. 1984; 65:323–331.

25. Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. Diabetes. 1990; 39:647–652. [PubMed: 2189759]

26. Sethi JK, Vidal-Puig AJ. Wnt signalling at the crossroads of nutritional regulation. Biochem J. 2008; 416:e11–e13. [PubMed: 18990086]

27. Marshall S. Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. Sci STKE. 2006; 2006:re7. [PubMed: 16885148]

28. Tong X, Zhao F, Thompson CB. The molecular determinants of de novo nucleotide biosynthesis in cancer cells. Curr Opin Genet Dev. 2009; 19:32–37. [PubMed: 19201187]

29. Niizeki H, Kobayashi M, Horiuchi I, et al. Hypoxia enhances the expression of autocrine motility factor and the motility of human pancreatic cancer cells. Br J Cancer. 2002; 86:1914–1919. [PubMed: 12085186]

30. Butkinaree C, Park K, Hart GW. O-linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. Biochim Biophys Acta. 2009

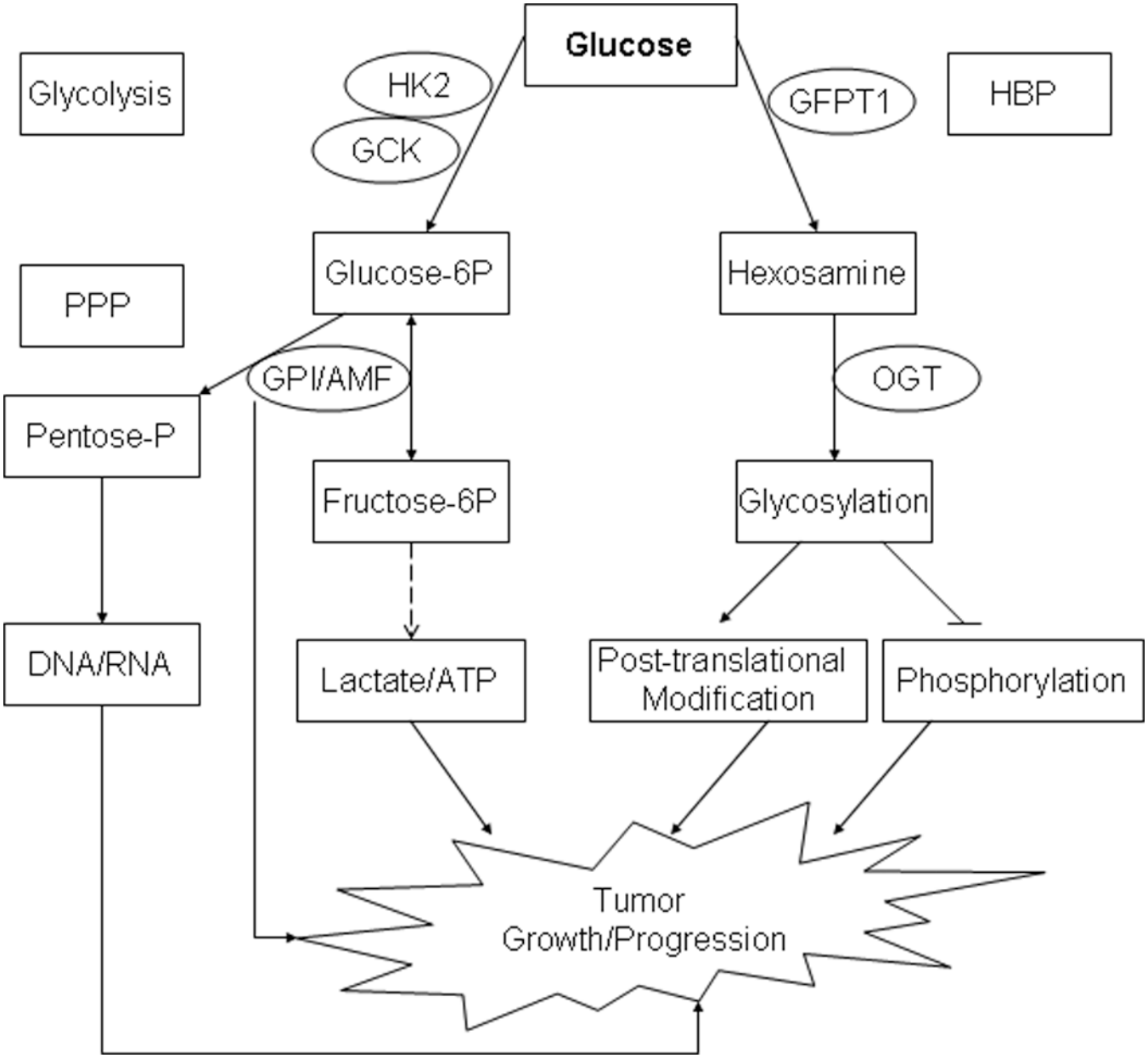

Fig. 1.

Selected glucose metabolic genes and their potential roles in tumor development. PPP: pentose phosphate pathway. Hexokinases (HK) 2 and GCK/HK4 phosphorylate glucose to produce glucose-6-phosphate (Glucose-6P), the first step in most glucose metabolism pathways including glycolysis [25]. GPI (phosphoglucose isomerase) catalyzes the reversible isomerization of Glucose-6P and fructose-6P, and guide the glucose flow to the pentose phosphate pathway (PPP) [28]. GPI also functions as an autocrine motility factor (AMF), secreted from the tumor cells to promote progression [29]. GFPT1 is the first and rate-limiting enzyme of the hexosamine biosynthesis pathway (HBP) and controls the flux of glucose into the hexosamine pathway. OGT is a glycosyltransferase that catalyzes the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues. O-linked glycosylation plays a role in controlling gene expression, fuel metabolism, cell growth, differentiation, and cytoskeleton organization [30].

Table 1

SNPs Examined and Allele Frequency

Gene	Chromosome	SNP	Function	RS#	Allele Frequency	
					Observed	Reported†
GCK	7p15.3-p15.1	−515G>A	Promoter	rs1799884	0.194	0.203
		IVS1-11823G>A	Intron	rs2908292	0.209	0.2
		IVS1+6037T>C	Intron	rs758985	0.175	0.175
		IVS1+9652C>T	Intron	rs2300586	0.165	0.158
		IVS1+11382G>A	intron	rs2244164	0.445	0.45
		IVS3-1489C>T	Intron	rs2268572	0.184	0.175
		IVS6+87A>C	intron	rs2268573	0.479	0.457
GFPT1	2p13	IVS12-1764C>T	Intron	rs4625963	0.147	0.167
		IVS14-3094T>C	Intron	rs12466648	0.173	0.186
		Ex19-115G>T	3'UTR	rs2667	0.413	0.408
		*4058A>G	3'Flank	rs13751	0.39	0.408
GPI	19q13.1	IVS6-378T>C	Intron	rs8191376	0.072	0.07
		IVS9+2363C>G	Intron	rs7248411	0.177	0.181
		Ex6+3A>G	G163G	rs1801015	0.07	0.103
HK2	2p13	IVS1-6165G>A	Intron	rs680545	0.446	0.45
		IVS1+7072C>T	Intron	rs35902493	0.352	0.38
		IVS2+3581C>T	Intron	rs3771781	0.415	0.48
		Ex1+318A>G	5'UTR	rs656489	0.474	0.45
		Ex4+51A>T	Q142H	rs2229621	0.157	0.233
		Ex7+62T>C	D251D	rs2229622	0.216	0.237
		Ex15+41C>T	N692N	rs2229626	0.398	0.408
		Ex16-78A>G	L766L	rs10194657	0.373	0.35
		Ex17-79G>A	R844K	rs2229629	0.21	0.2
		Ex18+407T>G	3'UTR	rs943	0.458	0.455
OGT	Xq13	IVS8-72G>A	Intron	rs3736670	0.151	0.142
		IVS18-424A>G	Intron	rs6525489	0.43	0.45

SNP, single nucleotide polymorphism; RS#, reference SNP identification number; UTR, untranslated region.

† The reported minor allele frequency was from NCBI dbSNP database.

Table 2

Linkage Disequilibrium in the 26 SNPs Examined in All Patients

GCK	**−515G>A**	**IVS1-11823G>A**	**IVS1+6037T>C**	**IVS1+9652C>T**	**IVS1+11382G>A**	**IVS3-1489C>T**	**IVS6+87A>C**
−515G>A							
IVS1-11823G>A	0.4157						
IVS1+6037T>C	−0.8878	−0.5018					
IVS1+9652C>T	−0.7179	−1	−0.5313				
IVS1+11382G>A	−0.5025	−0.9529	0.6206	−0.8828			
IVS3-1489C>T	−0.4924	−0.5276	−0.6385	0.8326	−0.8324		
IVS6+87A>C	−0.4134	−0.6997	0.533	−0.7679	0.7684	−0.8917	

GFPT1	IVS12-1764C>T	IVS14-3094T>C	Ex19-115G>T	*4058A>G
IVS12-1764C>T				
IVS14-3094T>C	−0.7469			
Ex19-115G>T	0.945	−0.5998		
*4058A>G	0.91	−0.7001	0.8904	

GPI	IVS6-378T>C	IVS9+2363C>G	G163G
IVS6-378T>C			
IVS9+2363C>G	0.7961		
G163G	0.9285	0.775	

HK2	IVS1-6165G>A	IVS1+7072C>T	IVS2+3581C>T	Ex1+318A>G	Ex4+51A>T	Ex7+62T>C	Ex15+41C>T	Ex16-78A>G	Ex17-79G>A
IVS1-6165G>A									
IVS1+7072C>T	−0.4413								
IVS2+3581C>T	0.1438	−0.3931							
Ex1+318A>G	−0.0719	−4.62E-03	−0.0611						
Ex4+51A>T	0.0272	0.0772	0.0309	−3.07E-03					
Ex7+62T>C	0.1307	4.49E-03	−0.0983	0.0262	0.9261				
Ex15+41C>T	−2.02E-03	0.0337	−0.0651	0.0172	0.2625	0.1285			

GCK	**−515G>A**	**IVS1-11823G>A**	**IVS1+6037T>C**	**IVS1+9652C>T**		**IVS1+11382G>A**	**IVS3-1489C>T**		**IVS6+87A>C**
Ex16-78A>G	−0.1114	0.0501	−0.055	0.0334	−0.5114	−0.3481	0.2414		
Ex17-79G>A	0.0564	5.33E-03	−0.2623	4.72E-03	0.1457	0.2392	−0.5486	0.0218	
Ex18+407T>G	−0.0167	0.0282	−0.0639	0.0185	−0.6188	−0.4502	0.3841	0.6971	−0.1156

OGT	IVS8-72G>A	IVS18-424A>G
IVS8-72G>A		
IVS18-424A>G	0.6425	

Table 3

Patients' Characteristics and Clinical Predictors for Overall Survival

Variable	No. of Patients	No. of Deaths	MST (months)	$P_{log\text{-}rank}$
Gender				.48
Female	281	235	17.5	
Male	425	333	16.8	
Age (years)				.22
≤ 50	90	80	16.4	
51–60	181	142	18.4	
61–70	272	213	17.2	
> 70	163	133	16.5	
Race				.63
White	624	502	16.6	
Hispanic	43	35	16.8	
Black	27	21	20.2	
Other	12	10	17.3	
Clinical Stage				<.001
Localized	333	237	28.5	
Locally Advanced	211	188	14.7	
Metastatic	162	143	9.2	
Performance Status				<.001
0	148	106	31.0	
1	483	394	16.0	
2–3	75	68	9.5	
Tumor Size (cm)				<.001
≤ 2	134	94	27.5	
> 2	572	474	15.3	
Tumor Site				.001
Head	5117	411	18.2	
Non-head	195	157	13.7	
CA19-9 (U/ml)				<.001
≤ 47	161	107	32.1	
48–500	291	237	18.0	
500–1000	73	60	14.5	
> 1000	181	164	10.6	
Tumor Differentiation[†]				<.001
Well to Moderate	355	261	26.2	
Poorly	139	120	12.0	
Tumor Response to Therapy[‡]				<.001
PR/SD	225	146	35.8	
PD	36	35	9.3	
Tumor Resection				<.001

Variable	No. of Patients	No. of Deaths	MST (months)	$P_{log\text{-}rank}$
Yes	275	179	35.9	
No	431	389	12.0	
Biochemical Index*				.008
0–2	303	230	18.9	
3–6	362	302	16.0	
7–9	41	36	12.9	

MST, median survival time; HR, hazard ratio; CI, confidence interval; CA 19-9, carbohydrate antigen 19-9; PR, partial response; SD, stable disease; PD, progressive disease.

†This information was unavailable for patients without proper histological samples.

‡Tumor response to therapy was evaluated in patients who received preoperative chemoradiotherapy only.

*Biochemical index represents the number of serum markers with abnormal value. The markers include aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase, alanine aminotransferase, amylase, creatinine, hemoglobin, albumin, bilirubin, and fasting glucose.

Table 4

Association of Genotype with Overall Survival

Genotype	Training Set				Validation Set				Combined dataset		
	No. of Patients /No. of Deaths	MST	HR (95% CI) †	*P*	No. of Patients /No. of Deaths	MST	HR (95% CI) †	*P*	MST	HR (95% CI) †	*P*
GCK IVS1+9652C>T											
CC	111/82	26.2	1.0		380/313	16.0	1.0		17.8	1.0	
CT	39/32	19.8	1.56 (1.03–2.38)	.03	149/117	16.0	1.08 (0.87–1.34)	.48	16.9	1.10 (0.90–1.33)	.35
TT	2/2	5.5	18.0 (3.63–89.5)	<.001	20/19	7.1	2.61 (1.61–4.21)	<.001	7.1	2.69 (1.71–4.22)	<.001
CC vs. CT/TT			1.61 (1.06–2.45)	.026*							
GFPT1 IVS14-3094T>C											
TT	113/85	23.9	1.0		367/295	16.9	1.0		18.0	1.0	
TC	39/31	21.5	0.84 (0.54–1.32)	.46	152/129	13.9	1.26 (1.02–1.55)	.03	16.0	1.25 (1.03–1.50)	.022
CC	2/2	4.9	7.64 (1.69–34.6)	.008	23/20	11.9	1.46 (0.91–2.33)	.12	11.9	1.57 (1.01–2.45)	.045
TT vs. TC/CC			0.96 (0.61–1.49)	.84*							
GPI IVS9+2363C>G											
CC	104/86	21.2	1.0		378/309	15.1	1.0		16.5	1.0	
CG	43/29	27.5	0.61 (0.39–0.94)	.027	147/120	16.4	0.88 (0.71–1.09)	.25	17.8	0.85 (0.70–1.03)	.09
GG	6/2	_‡	0.11 (0.03–0.48)	.003	23/18	22.9	0.60 (0.36–0.99)	.049	27.8	0.51 (0.32–0.83)	.006
CC vs. CG/GG			0.53 (0.34–0.84)	.007*							
GPI G163G											
AA	125/98	21.2	1.0		489/403	15.2	1.0		16.4	1.0	
GA	26/19	28.7	0.56 (0.34–0.93)	.02	59/45	20.5	0.80 (0.58–1.09)	.16	22.0	0.77 (0.59–1.02)	.06
GG	3/1	_‡	0.21 (0.03–1.61)	.13	4/2	33.7	0.32 (0.08–1.33)	.12	33.7	0.31 (0.10–0.99)	.048
AA vs. GA/GG			0.52 (0.31–0.86)	.01*			0.75 (0.55–1.03)	.07*		0.73 (0.56–0.96)	.02*
HK2 N692N											
TT	79/49	45.1	1.0		189/143	21.4	1.0		25.1	1.0	
TC	59/53	17.6	1.94 (1.24–3.03)	.004	255/218	15.1	1.26 (1.01–1.57)	.037	15.3	1.39 (1.15–1.69)	.001
CC	16/16	8.3	5.62 (2.67–11.8)	<.001	108/89	12.0	1.80 (1.36–2.38)	<.001	11.6	2.01 (1.55–2.61)	<.001
HK2 L766L											
GG	72/54	25.7	1.0		195/155	16.4	1.0		18.2	1.0	

Genotype	Training Set				Validation Set				Combined dataset		
	No. of Patients /No. of Deaths	MST	HR (95% CI) †	*P*	No. of Patients /No. of Deaths	MST	HR (95% CI) †	*P*	MST	HR (95% CI) †	*P*
GA	60/50	16.9	1.66 (1.10–2.48)	.01	291/242	15.0	1.29 (1.05–1.58)	.01	15.7	1.29 (1.08–1.55)	.006
AA	22/14	38.5	0.71 (0.38–1.30)	.27	66/53	16.9	1.10 (0.80–1.50)	.57	18.7	0.97 (0.73–1.29)	.85
HK2 R844K											
GG	80/67	16.9	1.0		356/297	14.2	1.0		14.6	1.0	
GA	61/45	27.8	0.69 (0.46–1.04)	.07	182/144	18.9	0.77 (0.61–0.96)	.02	20.5	0.76 (0.62–0.93)	.008
AA	13/6	_‡	0.38 (0.15–0.93)	.03	14/9	48.5	0.31 (0.16–0.63)	.001	84.3	0.37 (0.21–0.63)	<.001

MST, median survival time (months); HR: hazard ratio; CI, confidence interval

†HR values were from multivariable Cox regression models including sex, race, tumor resection, CA19-9, performance status, biochemical index and stage when appropriate.

‡MST can not be calculated.

*When the number of sample is less than 10, homozygous and heterozygous mutants were combined.

Table 5

Association of Genotype with Overall Survival by Clinical Stage

Stage	Genotype	No. of Patients /No. of Deaths	MST	HR (95% CI)†	*P*
Localized	*GFPT1* IVS14-3094T>C				
	TT	239/169	28.7	1.0	
	TC	81/58	29.8	1.12 (0.82–1.53)	.497
	CC	9/8	14.2	3.69 (1.69–8.09)	.001
	HK2 N692N				
	TT	156/93	45.1	1.0	
	TC	135/113	20.3	2.02 (1.51–2.72)	<.001
	CC	42/31	14.7	2.53 (1.61–3.97)	<.001
	HK2 R844K				
	GG	176/133	21.2	1.0	
	GA	137/94	31.2	0.60 (0.45–0.81)	.001
	AA	20/10	90.5	0.32 (0.16–0.64)	.001
Locally Advanced	*GCK* IVS1+9652C>T				
	CC	151/137	15.0	1.0	
	CT	55/46	16.8	0.97 (0.68–1.38)	.89
	TT	5/5	7.1	3.40 (1.15–10.0)	.027
	CC/CT vs. TT			1.01 (0.71–1.43)	.958
	HK2 N692N				
	TT	64/56	20.3	1.0	
	TC	107/95	14.2	1.36 (0.96–1.91)	.087
	CC	40/37	11.7	1.84 (1.16–2.91)	.013
Metastatic	*GFP1**4058A>G				
	GG	70/63	8.37	1.0	
	GA	62/54	9.70	0.65 (0.44–0.98)	.038
	AA	21/18	10.3	0.52 (0.30–0.92)	.025
	GCK IVS1+9652C>T				
	CC	104/94	9.3	1.0	

Cancer. Author manuscript; available in PMC 2014 March 21.

Stage	Genotype	No. of Patients /No. of Deaths	MST	HR (95% CI)†	*P*
	CT	46/38	9.4	1.19 (0.80–1.77)	.40
	TT	10/10	4.4	4.13 (2.00–8.52)	<.001
	HK2 Ex18+407T>G				
	GG	46/37	12.2	1.0	
	GT	84/76	9.2	1.47 (0.96–2.23)	.075
	TT	30/28	8.5	1.89 (1.11–3.22)	.019
Locally Advanced + Metastatic	*GCK* IVS1+9652C>T				
	CC	255/231	12.3	1.0	
	CT	101/84	12.5	1.09 (0.85–1.41)	.499
	TT	15/15	6.1	3.89 (2.20–6.89)	<.001
	HK2 N692N				
	TT	112/99	13.2	1.0	
	TC	179/158	12.1	1.04 (0.80–1.35)	.768
	CC	82/74	10.3	1.61 (1.16–2.23)	.004

MST, median survival time (months); HR: hazard ratio; CI, confidence interval.

†HR was from multivariable Cox regression model with adjustment of sex, race, tumor resection, CA19-9, performance status, and biochemical index.

‡MST can not be calculated.

Table 6

Association of Haplotype Diversity with OS in All Patients

Haplotype*	Frequency	MST	HR (95% CI)‡	*P*
GCK				
GGCCGCA	0.2337	18.4	1.0	
GGTCGCA	0.1248	16.5	0.96 (0.79–1.18)	.73
GGCTATC	0.1218	16.2	1.21 (0.98–1.48)	.078
GGCCACC	0.098	18.7	0.93 (0.74–1.17)	.52
AACCACC	0.0925	17.6	0.90 (0.72–1.14)	.38
GACCACC	0.0572	17.9	1.22 (0.91–1.63)	.18
Others†	0.272	17.3	1.05 (0.88–1.25)	.57
GFPT1				
TTTG	0.4165	17.6	1.0	
TTGA	0.2162	17.6	0.97 (0.82–1.13)	.66
TCTG	0.1468	16.0	1.16 (0.97–1.38)	.10
CTGA	0.1288	18.1	0.93 (0.76–1.13)	.46
TTGG	0.0358	15.3	1.20 (0.87–1.66)	.26
Others†	0.0559	18.2	0.96 (0.71–1.29)	.77
GPI				
TCA	0.808	16.6	1.0	
TGA	0.1154	16.9	0.87 (0.72–1.06)	.17
CGG	0.0555	29.8	0.66 (0.49–0.88)	.004
CCG	0.01	13.0	2.17 (1.16–4.06)	.01
Others†	0.0111	37.1	0.64 (0.33–1.25)	.19
HK2				
GCCGTGG	0.0817	16.2	1.0	
ATTGTGG	0.0553	16.3	1.01 (0.76–1.34)	.93
GCTATGG	0.0391	25.8	0.62 (0.44–0.88)	.007
ACCATGG	0.0353	23.9	0.85 (0.62–1.17)	.33
ATTACAT	0.0308	13.6	1.42 (1.02–1.96)	.03
ACTATGG	0.0299	27.5	0.95 (0.62–1.47)	.83
GCCGCAT	0.028	11.7	1.46 (0.99–2.14)	.055
ACTGTGG	0.0247	24.6	0.92 (0.58–1.44)	.71
GCCATGG	0.0219	21.5	1.12 (0.64–1.95)	.70
ATTGCAT	0.0214	14.2	1.47 (0.98–2.21)	.06
Others†	0.6319	17.3	1.05 (0.87–1.27)	.60
OGT				
GA	0.5394	17.8	1.0	
GG	0.3098	15.4	1.09 (0.95–1.24)	.23
AG	0.1201	16.3	1.12 (0.92–1.35)	.26
AA	0.0307	18.2	0.81 (0.54–1.22)	.31

MST, median survival time (months); HR: hazard ratio; CI, confidence interval.

[*] Haplotypes of *GCK* −515G>A, IVS1-11823G>A, IVS1+6037T>C, IVS1+9652C>T, IVS1+11382G>A, IVS3-1489C>T, IVS6+87A>C; *GFPT1* IVS12-1764C>T, IVS14-3094T>C, Ex19-115G>T, 4058A>G; *GPI* IVS6-378T>C, IVS9+2363C>G, G163G; *HK2* IVS1-6165G>A, IVS1+7072C>T, IVS2+3581C>T, Ex1+318A>G, Ex15+41C>T (N692N), Ex16-78A>G (L766L), Ex18+407T>G; and *OGT* IVS8-72G>A and IVS18-424A>G. Three *HK2* genotypes were not included in the haplotype analysis because the major allele was present in each of the haplotype group presented.

[‡] HR values were from multivariable Cox regression models including sex, race, clinical stage, tumor resection, CA19-9, performance status and biochemical index.

[†] Others include all the haplotypes with an extremely low frequency.

Table 7

Association of Genotypes with Other Clinical Parameters

Variable	Genotype	No. (%)	No. (%)	*P* (χ^2)	OR† (95%CI)	*P*†
Response to Therapy		**PR/SD**	**PD**			
	HK2 N692N			<.001		
	TT	126 (93.3)	9 (6.7)		1.0	
	TC	90 (77.6)	26 (22.4)		4.31 (1.84–10.1)	.001
	CC	22 (62.9)	13 (37.1)		5.96 (2.09–17.0)	.001
	HK2 R844K			.003		
	GG	114 (47.9)	35 (72.9)		1.0	
	GA	105 (44.1)	13 (27.1)		0.36 (0.16–0.82)	.015
	AA	19 (8.0)	0		-	
Tumor Stage		**Localized**	**Advanced**			
	HK2 N692N			<.001		
	TT	156 (46.8)	112 (30.0)		1.0	
	TC	135 (40.5)	179 (48.0)		1.57 (1.10–2.25)	.013
	CC	42 (12.6)	82 (22.0)		2.38 (1.46–3.86)	<.001
	HK2 R844K			<.001		
	GG	176 (52.9)	260 (69.7)		1.0	
	GA	137 (41.1)	106 (28.4)		0.43 (0.30–0.64)	<.001
	AA	20 (6.0)	7 (1.9)		0.29 (0.11–0.78)	.014
Tumor Resection		**Resected**	**Not resected**			
	HK2 N692N			<.001		
	TT	146 (53.1)	122 (28.3)		1.0	
	TC	101 (36.7)	213 (49.4)		2.29 (1.58–3.31)	<.001
	CC	28 (10.2)	96 (22.3)		3.62 (2.14–6.14)	<.001
	HK2 R844K			<.001		
	GG	136 (49.5)	300 (69.6)		1.0	
	GA	118 (42.9)	125 (29.0)		0.43 (0.29–0.64)	<.001
	AA	21 (7.6)	6 (1.4)		0.15 (0.05–0.42)	<.001

OR, odds ratio; CI, confidence interval; PR, partial response; SD, stable disease; PD, progressive disease.

[†] OR and

[†] P value were calculated from logistic regression adjusted for sex, race, CA19-9, performance status, and biochemical index.