Five genes influenced by obesity may contribute to the development of thyroid cancer through the regulation of insulin levels

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Abstract

34   Previous studies indicate that obesity is an important contributor to the proceeding of thyroid

35   cancer (TC) with limited knowledge of the underlying mechanism. Here, we hypothesize that

36   molecules affected by obesity may play roles in the development of TC. To test the hypothesis

37   above, we first conducted a large-scale literature-based data mining to identify genes influenced

38   by obesity and genes related to TC. Then, a mega-analysis was conducted to study the

39   expression changes of the obesity-specific genes in the case of TC, using 16 independent TC

40   array-expression datasets (783 TC cases and 439 healthy controls). After that, pathway analysis

41   was performed to explore the functional profile of the selected target genes and their potential

42   connections with TC. We identified 1,036 genes associated with TC and 534 regulated by

43   obesity, demonstrating a significant overlap (N=176, *p-value*= 4.07e-112). Five out of the 358

44   obesity-specific genes, FABP4, CFD, GHR, TNFRSF11B, and LTF, presented significantly

45   decreased expression in TC patients (LFC<-1.44; and *p-value*<1e^{-7}). Multiple literature-based

46   pathways were identified where obesity could promote the pathologic development of TC

47   through the regulation of these five genes and INS levels. The five obesity genes uncovered

48   could be novel genes that play roles in the etiology of TC through the modulation of INS levels.

49

50   Keywords: thyroid cancer; obesity; data mining; mega-analysis; pathway analysis

51

52   **Introduction**

53   Thyroid cancer (TC) develops from the tissues of the thyroid gland and becomes the fastest-

54   growing cancer of all malignancies (Wolin, Carson, & Colditz, 2010). Approximately 20% of all

55   types of cancers might be caused by excessive weight (overweight or obesity) (Wolin, Carson, &

56   Colditz, 2010). Epidemiologic research suggested that there could be a positive correlation

57   between the increased incidence of both obesity and TC in the past decades (Ogden et al.,

58   2007). The hypothesis has been supported by multiple studies with different methodologies,

59   including cohort study, pooled analysis, and meta-analysis (Engeland et al., 2006; Kitahara et al,

60   2011; Zhao et al, 2012). However, inconsistent results were represented, which could be due to

61   the unbalanced sex in the TC sample (Meinhold et al., 2010), different sample population regions

62   (Oh, Yoon, & Shin, 2005), and the lack of adjustment of other influential factors (Meinhold et al.,

63   2010; Engeland et al., 2006).

64

65   Nevertheless, many studies have been made to explore the mechanisms underlying the obesity-

66 TC relationship, taking the advances made by molecular biologists (Nannipieri et al., 2009; Hard,
67 1998; Liu et al., 2012; Ozgen et al., 2009; Stassi et al., 2003; Iyengar et al., 2017; Park et al,
68 2016). Some studies showed evidence that regional obesity and a tendency to weight gain were
69 associated with the variations in thyroid function. For example, an increase of triiodothyronine (T_3)
70 levels was observed in obese subjects (Nannipieri et al., 2009). TSH is the major stimulator of
71 thyrocyte proliferation; the high level of this hormone could be directly involved in thyroid
72 carcinogenesis in obese subjects (Hard, 1998). In addition, multiple genetic and epigenetic
73 alterations of obesity have been reported as pathophysiological important, with many of them
74 also identified as genetic targets for early diagnosis, prognosis or the therapeutic response to the
75 treatment of TC (Liu et al., 2012; Ozgen et al., 2009; Stassi et al., 2003; Iyengar et al., 2017;
76 Park et al., 2016). For instance, separate sets of studies analyzing the pro- and anti-inflammatory
77 cytokines, TNF-α, IL-6, and IL-10, which are part of the obesity-associated secretory phenotype,
78 showed their roles in the deterioration or treatment of TC (Liu et al., 2012; Ozgen et al.,2009;
79 Stassi et al. 2003; Iyengar et al.2017). However, the mechanism regarding this obesity-
80 promoting-TC relationship remains mostly unclear.

81

82 Taken together, these observations indicated the presence of some not-yet discovered
83 connections between obesity and TC. In this study, we attempted to use a system biology
84 approach to identify the not-yet discovered connections between both diseases, including the
85 data mining of disease-gene relation data, the analysis of molecular pathways, and a mega-
86 analysis of existing expression datasets. The integrated analysis of multiple modalities of data
87 has been proven to be an effective way for disease mechanism study (Liu et al., 2019; Zhang et
88 al., 2019; Lian et al., 2019).

89

Methods and Materials

91 This study was organized as follows. First, the large-scale literature-based TC-gene and obesity-
92 gene relations data were mined, through which obesity- and TC-genes were identified and
93 compared. Then, a mega-analysis was conducted to test genes that were regulated by obesity
94 but not implicated with TC. After that, a literature-based pathway analysis and a gene set
95 enrichment analysis (GSEA) were performed to identify the potential functional network

96   connecting the selected molecules and TC and the biological profile of these molecules.

97

Literature-based relation data

99   Literature-based genetic relation data was conducted by using Pathway Studio
100   (www.pathwaystudio.com), and results were organized into a supplementary file
101   (***Obesity_TC.xlsx***). Besides the full lists of genes, we also presented the information of
102   supporting references for each disease-gene relation, including titles of the references and the
103   related sentences where the disease-gene relationships were identified (Obesity_TC→TC genes
104   and Obesity_TC→Obesity_genes). The information could be used to locate a detailed
105   description of how a candidate gene is associated with obesity and/or TC. To increase the
106   reliability of the obesity affected genes, we selected the obesity-gene relationships with at least
107   three supporting references and with a specific polarity (positive or negative regulation).

108

Selection of TC-RNA expression Datasets

110   We search all TC array-expression datasets available at GEO. After the initial search with
111   keyword 'thyroid cancer', we identified 91 expression datasets for TC. Then the following criteria
112   were applied to fulfill the purpose of this study, including 1) The data organism is Homo sapiens;
113   2) The data type is RNA expression; 3) The sample size is no less than 10, and 4) the studies are
114   limited to TC cases vs. healthy controls design.

115

Mega-analysis and target selection

117   A mega-analysis was conducted for each of these genes that were regulated by obesity but not
118   associated with TC, using 16 out of 91 TC array-expression datasets from Gene Expression
119   Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). During this step, both the fixed-effect model
120   and random-effects model were employed to study the effect size of the selected genes in a case
121   *vs.* control expression comparison. The expression log fold change (LFC) was used as the effect
122   size. Results from both models were reported and compared. The heterogeneity of the mega-
123   analysis was analyzed to study the variance within and between different studies. In the case
124   that the total variance Q is equal to or smaller than the expected between-study variance df, the

125 statistic ISq = 100% x (Q-df)/Q will be set as 0, and a fixed-effect model was selected for the
126 mega-analysis. Otherwise, a random-effects model was selected. The Q-p represents the
127 probability that the total variance is coming from within-study only. Significant genes from the
128 mega-analysis were reported, which were identified with the criteria as follows: *p-value*<10^{-7} and
129 abs (effect size (log fold change)) >1. All analysis was conducted by an individually-developed
130 MATLAB (R2017a) mega-analysis package. We used the term 'mega-analysis' rather than
131 'meta-analysis' due to the fact that the log-fold changes of each gene were calculated from the
132 original datasets.

133

134 **Literature-based pathway analysis and GSEA**

135 For the possible risk genes identified through the expression mega-analysis described above, a
136 literature-based pathway was constructed to identify the connection between the target genes
137 and the TC. The analysis was performed using the 'Shortest Path' module of Pathway Studio
138 (www.pathwaystudio.com). Then all the molecules within the identified networks were tested
139 using a GSEA analysis against the Gene Ontology (GO) terms and Pathway Studio pathways.
140 Significantly enriched pathways and corresponding statistics were reported.

141

142 **Multiple linear regression analysis**

143 The MLR model was employed to study the possible influence of three factors on the gene
144 expression change in TC: sample size, population region, and study date. *P-values* and 95%
145 confidence interval (CI) were reported for each of the factors.

146

147 # Results

148 **Common genes for Obesity and TC**

149 As presented in the Obesity_TC database, there were 1,036 genes associated with TC and 534
150 influenced by obesity. A significant overlap of 176 genes was identified for both obesity and TC
151 (Right tail Fisher's Exact test p-value= 4.07e-112), which counts for about one-third of the
152 obesity-regulated genes (32.96%). For detailed information on these genes, please refer to
153 Obesity_TC.

154

155 **The selected gene expression datasets**

156 There were 16 datasets satisfied the selection criteria and were included for the mega-analysis,

157 as shown in Table 1. According to the approach that we acquired the disease-gene relation data

158 (by using Pathway Studio; www. pathwaystudio.com), about 67.04% of the obesity-genes (358

159 out of 534 genes) have not been reported to have an association with TC. Thus, we tested the

160 expression changes of these 358 genes in the case of TC.

161

162 **Put Table 1 above here.**

163

164 **Mega-analysis results**

165 There were five genes (i.g., FABP4, CFD, GHR, TNFRSF11B, and LTF) passed the significance

166 criteria (*p-value*<10^{-7} and abs (LFC)>1), which were provided in Table 2. We presented the

167 mega-analysis results of all obesity-regulated genes in Obesity_TC→Mega_Analysis. There

168 were four other genes (TMEM173, PLA2G7, SOD3, and AGTR1) that showed less significance

169 (p-value< 7.08e-6) but also with a big change in terms of LFC (abs (LFC)>1).However, the

170 discussion and analysis here were focused on the five genes that passed the significance criteria.

171 The LFCs of the genes were estimated from the majority of the studies: 15 out of 16 studies.

172 Notably, the Random-effects model was used for CFD and GHR, and the fixed-effect model was

173 selected for FABP4, TNFRSF11B, and LTF. MLR results showed that the age of studies and the

174 sample sizes presented no significant influence on the effect size (LFC) of all five genes except

175 LTF (*p-value*>0.05), but the sample's population region (country) was a significant factor for all of

176 them (*p-value*<0.033, Table 2).

177

178 **Put Table 2 above here.**

179

180 **Literature-based pathway analysis**

181 To explore the functional association between the five obesity-regulated molecules and TC, we

182 conducted a literature-based functional network analysis and presented in Figure 1 the identified

183 pathways. Results showed that genes GHP, TNFRSF11B, and LTF could be inhibitors of TC,

184 through the stimulation of TC inhibitors or deactivation of TC promoters. In the case of obesity,

185 the activity of these molecules was suppressed. On the contrary, CFD and FABP4 were

186 suggested as two facilitators of the pathological development of TC. CFD stimulates INS, which

187 is a promoter of TC. FABP4 inhibits three TC suppressors, including BCL2, PTEN, and PPARG.

188 Notably, obesity activates these two molecules. The pathways revealed in Figure 1 suggested

189 possible mechanisms of the TC-promoting effect of obesity. For the supporting references of the

190 relationships presented in Figure 1, please refer to TC_Obsesity→ShortestPath.

191

192 **Put Figure 1 above here.**

193

194 **GSEA results**

195 To understand the functional profile of the 14 genes involved in the pathways presented in Figure

196 1, we conducted a GSEA against GO terms and Pathway Studio Pathways

197 (www.pathwaystudio.com) and presented the top 10 results in Table 3. The full list of 31

198 pathways/GO terms enriched with p-value<0.005 (q=0.005 for FDR correction) has been listed in

199 TC_Obsesity→GSEA. Notably, all the 14 genes were involved in the 31 pathways, and 12 out of

200 14 were included in the top 10 pathways.

201

202 **Put Table 3 above here.**

203

204 Based on the GSEA results, we analyzed the connection of the five potential TC-genes and nine

205 of their targets presented in Figure 1, in terms of their shared pathways, as shown in Figure 2.

206 The number in a cell represents the number of shared pathways/GO terms by the two

207 corresponding genes, and a number on the diagonal represents the number of pathways

208 enriched by the specific gene. As shown in Figure 2, most of these molecules play roles together

209 with other molecules in multiple pathways, indicating they were functionally connected.

210

211 **Put Figure 2 above here.**

212

213 **Discussion**

214 In this study, we attempted to explore the mechanism underlying the TC-promoting effect of

215 obesity at the genetic level. Towards this purpose, we first used the knowledge-based algorithms

216 to analyze disease-gene relation data and reveal 176 obesity-regulated genes. Identified genes
217 were also related to TC and utilized to build a common background at the genetic level for the
218 etiology of both obesity and TC. We also uncovered 358 obesity-regulated genes that have not
219 been implicated with TC. To test the potential connection between each of the 358 obesity-
220 regulated genes and TC, we queried and selected the qualified TC-RNA expression datasets
221 from GEO (https://www.ncbi.nlm.nih.gov/geo/), then we conducted a mega-analysis. Five genes
222 were suggested as novel targets for the development of TC, including FABP4, CFD, GHR,
223 TNFRSF11B, and LTF (see Table 2; *p-value*$<10^{-7}$ and LFC<-1.44).

224

225 Notably, we used log fold change (LFC) instead of original expression levels for the mega-
226 analysis, which was calculated as the expression levels of the expression level of TC patients
227 over the mean of the expression level of healthy controls, followed by log2 transformation. We
228 assume that, within the same dataset, patients and controls shared a similar background. Thus,
229 by using the LFC, the influence of the background noise was minimized. In addition, we
230 conducted a heterogeneity test for each gene, and a random-effects model was used in the case
231 there was a significant between-study variance such that the study-specific expression variances
232 were taken into account.

233 FABP4 encodes the fatty acid binding proteins that bind long-chain fatty acids and other
234 hydrophobic ligands. The roles of FABPs include fatty acid uptake, transport, and metabolism
235 (Furuhashi et al., 2015). FABP4 has been showed to induce proteasome degradation of PPARγ
236 (Nishina et al., 2017), and decrease the expression of PTEN (Jin et al., 2018) and BCL2 (Yao et
237 al., 2015). Reduced expression of PPARγ, PTEN, and BCL2 family proteins have been shown to
238 play critical roles in the pathologic development TC (Copland et al., 2006; Leonardi et al., 2012;
239 Gunda et al., 2017). These pathways in Figure 1 suggested FABP4 as a facilitator of the
240 development of TC through the down-regulation of its inhibitors.

241

242 CFD encoded protein adipsin that stimulates the secretion of insulin (Lo et al., 2014), which has
243 been suggested to play roles in the proliferation of TC cells by promoting insulin-like growth
244 factor (Oberman et al., 2015). Therefore, CFD could be a direct promoter of TC. Add together the
245 fact of increased protein levels of FABP4 and CFD in obese patients (Cabré et al., 2012; Kwon et
246 al., 2012) could partially explain the contribution of obesity to TC.

247

248 On the contrary, pathways analysis suggested GHP, TNFRSF11B, and LTF as potential TC
249 inhibitors (Figure 1). It has been shown that GHP inhibits the expression of PD-1 (Zhou et al.,

250 2017), which is a TC treatment target (Bi et al., 2019). TNFRSF11B stimulates the secretion of

251 CXCL12 (Benslimane-Ahmim et al., 2011), which was suggested to contribute to TC

252 development by regulating cancer cell migration and invasion (Zhang et al., 2017). Finally, the

253 reduced TGF-beta Type-II receptor (TGFBR2) mRNA was shown to play a role in the

254 pathogenesis of papillary TC (Matoba et al., 1998), while LTF interacts with TGFBR2 to activate

255 TGF-β signaling and initiates the formation of TbRIII:TbRII:TbRI complex (Jang et al., 2015).

256 Thereby, decreased levels of GHP, TNFRSF11B, and LTF in obesity could facilitate the

257 pathologic development of TC.

258

259 GSEA analysis showed that the five genes (FABP4, CFD, GHR, TNFRSF11B, and LTF) and

260 nine of the downstream target genes mainly played roles in the cell growth related signaling

261 pathways (Table 3). Notably, all these five genes regulate the insulin (INS) levels positively or

262 negatively, while INS level was related to proliferation and carcinogenesis of TC cells (Oberman

263 et al., 2015; Malaguarnera et al., 2017). Our results suggested that obesity may partially affect

264 the pathologic development of TC through its influence on the INS levels.

265

266 Moreover, these genes demonstrated a robust functional connection in terms of shared common

267 pathways (Figure 2). The relationship between TC and the nine target genes (INS, BCL2, PTEN,

268 PPARG, PDCD1, CXCL12, EP300, TGFBR2, and CHI3L1) were supported by previous studies

269 (see TC_Obesity→ShortestPath), which supports the potential association between the five

270 obesity-regulated genes (FABP4, CFD, GHR, TNFRSF11B, and LTF) and TC.

271

272 Nevertheless, this study has several limitations that can be addressed in the future work. First,

273 the connections between the TC and the five obesity genes were suggested by mega-analysis

274 and explored by literature-based pathways analysis. Biologic experiments are needed to test

275 these relationships. Second, due to the lack of space, the discussion was focused on the five

276 genes that passed the significance criteria in the mega-analysis. However, more genes with less

277 significance may also be worthy of inspection (e.g., TMEM173, PLA2G7, SOD3, and AGTR1).

278 Third, further validation of the relationships between the five target genes, insulin, TC, and

279 obesity, can be done using other tools and data sources (e.g., Hetionet v1.0;

280 https://neo4j.het.io/browser/).

281

Conclusions

283 Using the system biology approach, we mined a set of genes influenced by obesity to uncover

284 five genes (FABP4, CFD, GHR, TNFRSF11B, and LTF) as previously unrecognized contributors

285 to the development of TC. An analysis of functional network built upon these genes points

286 towards INS as a remarkable bridging factor connecting obesity and TC.

287

288 **Additional Information and Declarations**

289 **Competing interests**

290 The authors declare that they have no competing interests.

291

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294

295 **Author Contributions**

296 Jiaming Chen collected the original data, analyzed the data, wrote the original paper, and

297 approved the final manuscript.

298

299 Hongbao Cao developed the study design, revised the original paper, and approved the final

300 manuscript.

301

302 Meng Lian developed the study design, revised the original paper, and approved the final

303 manuscript.

304

305 Jugao Fang developed the study design, wrote the original paper, and approved the final

306 manuscript.

307

308 **Data Availability**

309 All data generated during this study are included in this article and the supplemental files.

310

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314 # Reference

315 Bi Y, Ren X, Bai X, Meng Y, Luo Y, Cao J, Zhang Y, Liang Z. 2019. PD-1/PD-L1 expressions in

316 medullary thyroid carcinoma: Clinicopathologic and prognostic analysis of Chinese population.

317   Eur J Surg Oncol. 45(3):353-358.

318   Benslimane-Ahmim Z, Heymann D, Dizier B, Lokajczyk A, Brion R, Laurendeau I, Bièche I,
319   Smadja DM, Galy-Fauroux I, Colliec-Jouault S, Fischer AM, Boisson-Vidal C. 2011.
320   Osteoprotegerin, a new actor in vasculogenesis, stimulates endothelial colony-forming cells
321   properties. J Thromb Haemost. 9(4):834-843.

322   Cabré A, Babio N, Lázaro I, Bulló M, Garcia-Arellano A, Masana L, Salas-Salvadó J. 2012.
323   FABP4 predicts atherogenic dyslipidemia development. The PREDIMED study. Atherosclerosis.
324   222(1):229-234.

325   Copland JA, Marlow LA, Kurakata S, Fujiwara K, Wong AK, Kreinest PA, Williams SF, Haugen
326   BR, Klopper JP, Smallridge RC. 2006. Novel high-affinity PPARgamma agonist alone and in
327   combination with paclitaxel inhibits human anaplastic thyroid carcinoma tumor growth via
328   p21WAF1/CIP1. Oncogene. 25(16):2304-2317.

329   Engeland A, Tretli S, Akslen LA, Bjørge T. 2006. Body size and thyroid cancer in two million
330   Norwegian men and women. Br J Cancer. 95(3):366-70.

331   Furuhashi M, Saitoh S, Shimamoto K, Miura T. 2015. Fatty Acid-Binding Protein 4 (FABP4):
332   Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and Cardiovascular
333   Diseases. Clin Med Insights Cardiol. 8(Suppl 3):23-33.

334   Gunda V, Sarosiek KA, Brauner E, Kim YS, Amin S, Zhou Z, Letai A, Parangi S. 2017. Inhibition
335   of MAPKinase pathway sensitizes thyroid cancer cells to ABT-737 induced apoptosis. Cancer
336   Lett. 395:1-10.

337   Hard GC. 1998. Recent developments in the investigation of thyroid regulation and thyroid
338   carcinogenesis. Environ Health Perspect. 106(8):427-36.

339   Iyengar NM, Brown KA, Zhou XK, Gucalp A, Subbaramaiah K, Giri DD, Zahid H, Bhardwaj P,
340   Wendel NK, Falcone DJ, Wang H, Williams S, Pollak M, Morrow M, Hudis CA, Dannenberg AJ.
341   2017. Metabolic Obesity, Adipose Inflammation and Elevated Breast Aromatase in Women with
342   Normal Body Mass Index. Cancer Prev Res (Phila). 10(4):235-243.

343   Jang YS, Seo GY, Lee JM, Seo HY, Han HJ, Kim SJ, Jin BR, Kim HJ, Park SR, Rhee KJ3, Kim
344   WS4, Kim PH1. Lactoferrin causes IgA and IgG2b isotype switching through betaglycan binding

345   and activation of canonical TGF-β signaling. 2015. Mucosal Immunol. 8(4):906-17.

346   Jin J, Zhang Z, Zhang S, Chen X, Chen Z, Hu P, Wang J, Xie C. 2018. Fatty acid binding protein
347   4 promotes epithelial-mesenchymal transition in cervical squamous cell carcinoma through
348   AKT/GSK3β/Snail signaling pathway. Mol Cell Endocrinol. 461:155-164.

349   Kitahara CM, Platz EA, Freeman LE, Hsing AW, Linet MS, Park Y, Schairer C, Schatzkin A,
350   Shikany JM, Berrington de González A. 2011. Obesity and thyroid cancer risk among U.S. men
351   and women: a pooled analysis of five prospective studies. Cancer Epidemiol Biomarkers Prev.
352   20(3):464-72.

353   Kwon EY, Shin SK, Cho YY, Jung UJ, Kim E, Park T, Park JH, Yun JW, McGregor RA, Park YB,
354   Choi MS. 2012. Time-course microarrays reveal early activation of the immune transcriptome
355   and adipokine dysregulation leads to fibrosis in visceral adipose depots during diet-induced
356   obesity. BMC Genomics.13:450.

357   Leonardi GC, Candido S, Carbone M, Colaianni V, Garozzo SF, Cinà D, Libra M. 2012.
358   microRNAs and thyroid cancer: biological and clinical significance (Review). Int J Mol Med.
359   30(5):991-999.

360   Lian X, Baranova A, Ngo J, Yu G, Cao H. 2019. UGT2B17 and miR-224 contribute to hormone
361   dependency trends in adenocarcinoma and squamous cell carcinoma of esophagus. Biosci Rep.
362   39(7). pii: BSR20190472.

363   Liu D, Cao H, Kural KC, Fang, Q, Zhang F. 2019. Integrative Analysis of shared Genetic
364   Pathogenesis by Autism Spectrum Disorder and Obsessive-Compulsive Disorder, Biosci Rep.
365   39(12). pii: BSR20191942.

366   Liu Z, Brooks RS, Ciappio ED, Kim SJ, Crott JW, Bennett G, Greenberg AS, Mason JB. 2012.
367   Diet-induced obesity elevates colonic TNF-α in mice and is accompanied by an activation of Wnt
368   signaling: a mechanism for obesity-associated colorectal cancer. J Nutr Biochem. 23(10):1207-
369   13.

370   Lo JC, Ljubicic S, Leibiger B, Kern M, Leibiger IB, Moede T, Kelly ME, Chatterjee Bhowmick D,
371   Murano I, Cohen P, Banks AS, Khandekar MJ, Dietrich A, Flier JS, Cinti S, Blüher M, Danial NN,
372   Berggren PO, Spiegelman BM. 2014. Adipsin is an adipokine that improves β cell function in
373   diabetes. Cell. 158(1):41-53.

374 Malaguarnera R, Vella V, Nicolosi ML, Belfiore A. 2017. Insulin Resistance: Any Role in the
375 Changing Epidemiology of Thyroid Cancer? Front Endocrinol (Lausanne). 8:314.

376 Matoba H, Sugano S, Yamaguchi N, Miyachi Y. Expression of transforming growth factor-beta1 and
377 transforming growth factor-beta Type-II receptor mRNA in papillary thyroid carcinoma. 1998.
378 Horm Metab Res. 30(10):624-8.

379 Meinhold CL, Ron E, Schonfeld SJ, Alexander BH, Freedman DM, Linet MS, Berrington de
380 González A. 2010. Nonradiation risk factors for thyroid cancer in the U.S. Radiologic
381 Technologists Study. Am J Epidemiol. 171(2):242–252.
382 Nannipieri M, Cecchetti F, Anselmino M, Camastra S, Niccolini P, Lamacchia M, Rossi M, Iervasi
383 G, Ferrannini E. 2009. Expression of thyrotropin and thyroid hormone receptors in adipose tissue
384 of patients with morbid obesity and/or type 2 diabetes: effects of weight loss. Int J Obes (Lond).
385 33(9):1001-6.

386 Nishina A, Itagaki M, Sato D, Kimura H, Hirai Y, Phay N, Makishima M. 2017. The Rosiglitazone-
387 Like Effects of Vitexilactone, a Constituent from Vitex trifolia L. in 3T3-L1 Preadipocytes.
388 Molecules. 22(11). pii: E2030

389 Oberman B, Khaku A, Camacho F, Goldenberg D. 2015. Relationship between obesity, diabetes
390 and the risk of thyroid cancer. Am J Otolaryngol. 36(4):535-541.
391 Ogden CL, Yanovski SZ, Carroll MD, Flegal KM. 2007. The epidemiology of obesity.
392 Gastroenterology. 132(6):2087–102.

393 Oh SW, Yoon YS, Shin SA. 2005. Effects of excess weight on cancer incidences depending on
394 cancer sites and histologic findings among men: Korea National Health Insurance Corporation
395 Study. J Clin Oncol. 23(21):4742–54.

396 Ozgen AG, Karadeniz M, Erdogan M, Berdeli A, Saygili F, Yilmaz C. 2009. The (-174) G/C
397 polymorphism in the interleukin-6 gene is associated with risk of papillary thyroid carcinoma in
398 Turkish patients. J Endocrinol Invest. 32(6):491-494.

399 Park JW, Han CR, Zhao L, Willingham MC, Cheng SY. 2016. Inhibition of STAT3 activity delays
400 obesity-induced thyroid carcinogenesis in a mouse model. Endocr Relat Cancer. 23(1):53-63.

401 Stassi G, Todaro M, Zerilli M, Ricci-Vitiani L, Di Liberto D, Patti M, Florena A, Di Gaudio F, Di
402 Gesù G, De Maria R. 2003. Thyroid cancer resistance to chemotherapeutic drugs via autocrine

403   production of interleukin-4 and interleukin-10. Cancer Res. 63(20):6784-6790.

404   Wolin KY, Carson K, Colditz GA. 2010. Obesity and cancer. Oncologist. 15(6):556-65.

405   Yao F, Li Z, Ehara T, Yang L, Wang D, Feng L, Zhang Y, Wang K, Shi Y, Duan H, Zhang L. 2015.
406   Fatty Acid-Binding Protein 4 mediates apoptosis via endoplasmic reticulum stress in mesangial
407   cells of diabetic nephropathy. Mol Cell Endocrinol. 411:232-242.

408   Zhang G, Wang W, Huang W, Xie X, Liang Z, Cao H. 2019. Cross-disease analysis identified
409   novel common genes for both lung adenocarcinoma and lung squamous cell carcinoma. Oncol
410   Lett. 18(4):3463-3470.

411   Zhang S, Wang Y, Chen M, Sun L, Han J, Elena VK, Qiao H. 2017. CXCL12 methylation-
412   mediated epigenetic regulation of gene expression in papillary thyroid carcinoma. Sci Rep.
413   7:44033.

414   Zhao ZG, Guo XG, Ba CX, Wang W, Yang YY, Wang J, Cao HY. 2012. Overweight, obesity and
415   thyroid cancer risk: a meta-analysis of cohort studies. J Int Med Res. 40(6):2041-50.

416   Zhou M, Yang WL, Aziz M, Ma G, Wang P. Therapeutic effect of human ghrelin and growth
417   hormone: Attenuation of immunosuppression in septic aged rats. 2017. Biochim Biophys Acta
418   Mol Basis Dis. 1863(10 Pt B):2584-2593.

419   **Figure Legends**

420

421   **Figure 1 The potential pathways connecting the five obesity-regulated genes and thyroid**
422   **cancer.**

423

424   **Figure 2 Heat map of the shared pathways by the five potential TC-genes and nine of their**
425   **targets.** The number in a cell represents the number of shared pathways/GO terms by the two
426   corresponding genes.

Figure 1

The potential pathways connecting the five obesity-regulated genes and thyroid cancer.
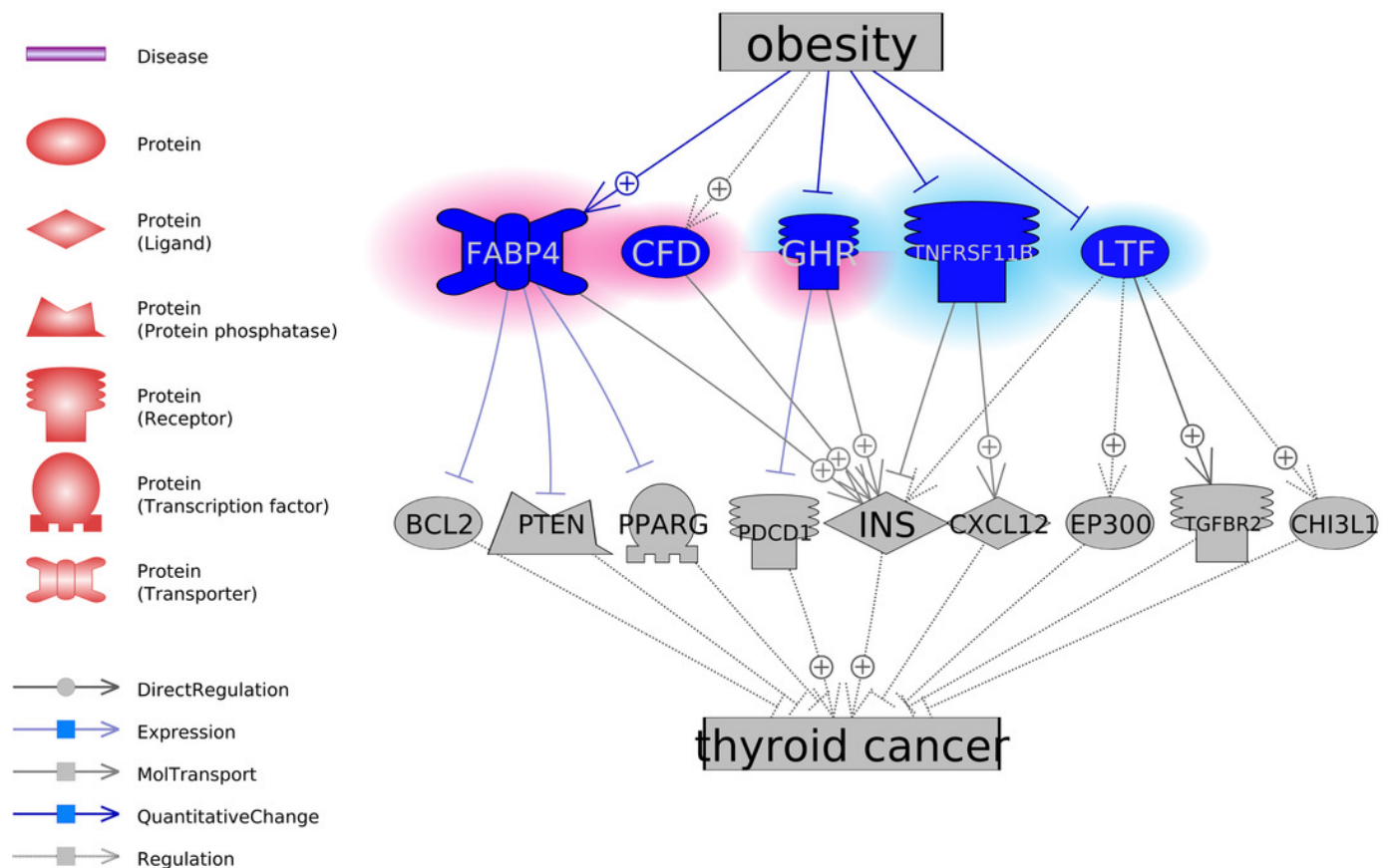
Figure 2

Heat map of the shared pathways by the five potential TC-genes and nine of their targets.

The number in a cell represents the number of shared pathways/GO terms by the two corresponding genes.
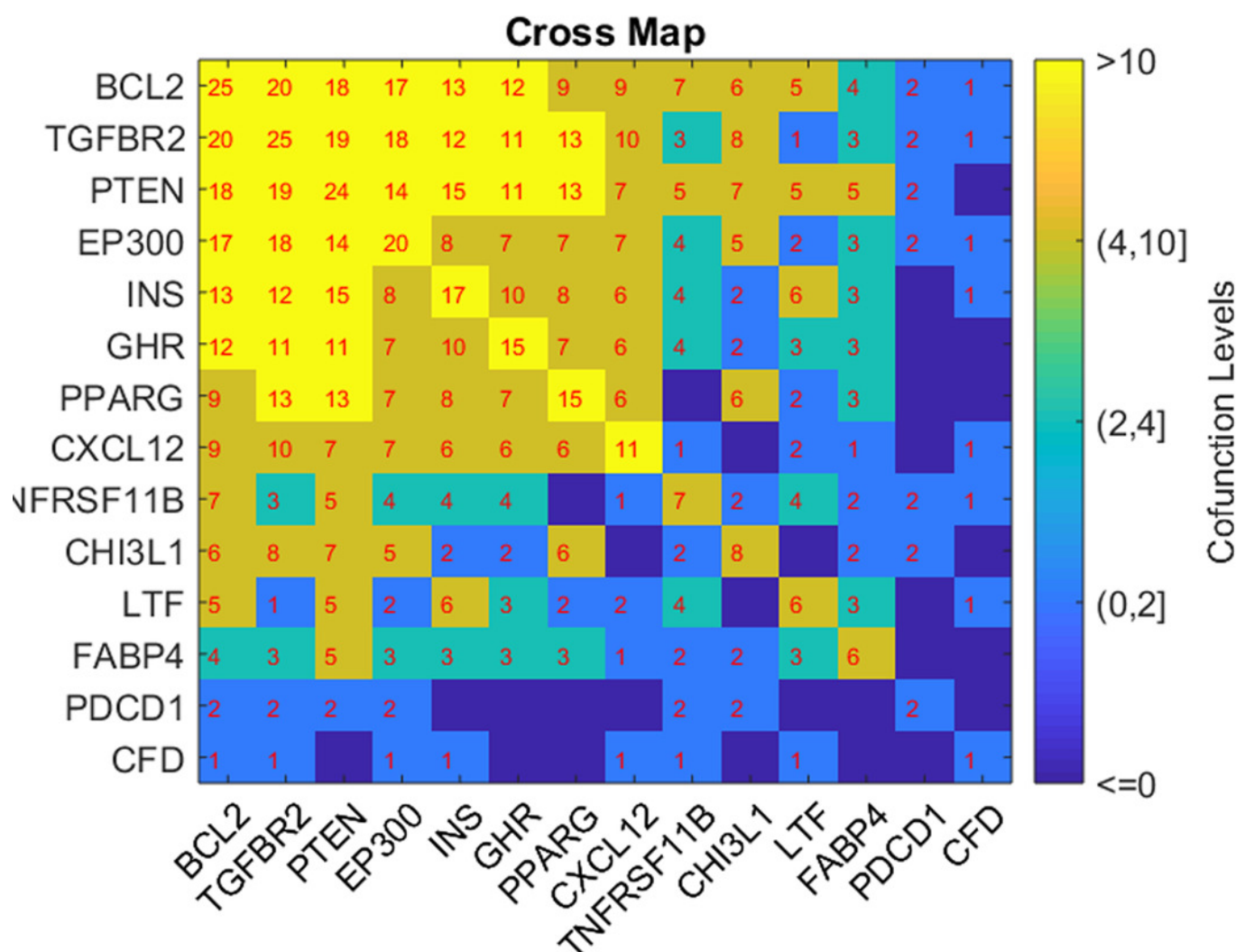
Table 1 The datasets used for gene-TC relation mega-analysis

1 **Table 1 The datasets used for gene-TC relation mega-analysis**

Dataset GEOID	nControl	nCase	Study Region	Study Age
GSE35570	51	65	Poland	4
GSE58545	18	27	Poland	4
GSE58689	18	27	Poland	4
GSE60542	34	33	Belgium	4
GSE65144	13	12	USA	4
GSE39156	16	48	Belgium	6
GSE53157	3	24	Portugal	6
GSE29265	20	29	Belgium	7
GSE33630	45	60	Belgium	7
GSE27155	4	95	USA	8
GSE5364	58	270	Singapore	11
GSE6339	135	48	France	12
GSE9115	4	15	USA	12
GSE3678	7	7	USA	13
GSE6004	4	14	USA	13
GSE3467	9	9	USA	14

Table 2 Significant Obesity-genes from mega-analysis for TC

1 **Table 2 Significant Obesity-genes from mega-analysis for TC**

	Gene Name	FABP4	CFD	GHR	TNFRSF11B	LTF
Mega-analysis Results	Using Random Effects Model	0	1	1	0	0
	#Study	15	15	15	15	15
	Effect size (LFC)	-1.83	-1.79	-1.70	-1.50	-1.44
	p-value	6.4E-09	2.03E-08	3.61E-09	3.24E-09	4.17E-09
MLR Results	nSample	0.22	0.078	0.53	0.11	0.0012
	Country	0.0019	1.01E-05	2.24E-05	0.033	0.029
	studyAge	0.46	0.94	0.98	0.37	0.14

Table 3 The top 10 enriched pathways/GO terms by the 14 genes in regulating pathway identified in Figure 1

1 **Table 3 The top 10 enriched pathways/GO terms by the 14 genes in regulating pathway**
2 **identified in Figure 1**

Name	GO ID	# of Entities	Overlap	Overlapping Entities	FDR corrected p-value
regulation of growth	0040008	887	8	PPARG;BCL2;PTEN;GHR; INS;CXCL12;TGFBR2; EP300	0.00028
response to estrogen	0043627	155	5	PPARG;TGFBR2;EP300; TNFRSF11B;BCL2	0.00028
leukocyte activation	0045321	991	8	BCL2;INS;CHI3L1;CFD; CXCL12;LTF;TGFBR2; EP300	0.00028
positive regulation of growth	0045927	354	6	INS;BCL2;CXCL12;TGFBR2;EP300;GHR	0.00028
response to nutrient levels	0031667	730	7	PPARG;BCL2;PTEN;GHR; TNFRSF11B;INS;TGFBR2	0.00052
response to extracellular stimulus	0009991	761	7	PPARG;BCL2;PTEN;GHR; TNFRSF11B;INS;TGFBR2	0.00052
regulation of developmental growth	0048638	445	6	BCL2;PTEN;CXCL12;GHR; TGFBR2;EP300	0.00052
response to glucose	0009749	219	5	INS;BCL2;PTEN;TGFBR2; EP300	0.00052
response to alcohol	0097305	460	6	PPARG;BCL2;PTEN;GHR; TGFBR2;EP300	0.00052
response to hexose	0009746	227	5	INS;BCL2;PTEN;TGFBR2; EP300	0.00052