CHOP 5'UTR-c.279T>C and +nt30C>T variants are not associated with overweight condition or with tumors/cancer in Italians – a case-control study

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Abstract

Background: Type 2 diabetes (T2D) is associated with obesity and has been shown recently to be associated with tumors/cancer. HNF1-beta and JAZF1 genes are associated with T2D and prostate cancer. We have previously shown that CHOP 5'UTR-c.279T>C and +nt30C>T haplotype variants contribute to T2D. CHOP deficiency causes obesity in mice, thus CHOP gene variants may contribute to human obesity. Furthermore, CHOP mediates apoptosis and is implicated in cancer pathogenesis. Hence, we aimed at identifying any potential association of CHOP 5'UTR-c.279T>C and +nt30C>T genotypes and corresponding haplotypes with overweight condition/pre-obesity and tumors/cancer in an Italian dataset.

Methods: We recruited from Italy 45 overweight subjects (body mass index (BMI) ≥ 25) and 44 control subjects (BMI < 25) as well as 54 cases with at least one cancer or at least one tumor and 43 control subjects without tumors/cancer from the general population. We excluded allelic departure from Hardy-Weinberg equilibrium in cases and control subjects, separately.

Results: We assessed the power to detect risk odds ratios by association tests in our datasets. We tested the hypothesis of association of CHOP 5'UTR-c.279T>C and +nt30C>T genotypes and haplotypes with tumors/cancer and, separately, with overweight condition. Both associations were not significant.

Conclusion: From our study, we may conclude that CHOP 5'UTR-c.279T>C and +nt30C>T genotypes and corresponding haplotypes are not associated with tumors/cancer and pre-obesity. However, more studies are warranted to establish the role of CHOP variants in tumor/cancer predisposition and in overweight condition.
Background
Type 2 diabetes (T2D) is associated with obesity. There is increasing evidence that T2D is associated with tumors [1] and cancers of the pancreas [2], prostate, breast, colon, endometrium, and liver [3]. T2D genes, such as HNF-1 beta and JAZFI, have been associated with prostate cancer [4-6]. Thus, T2D candidate genes may not only be obesity predisposing genes, but also tumor/cancer risk genes. CHOP mediates apoptosis and regulates mitochondrial gene expression, thus it may be implicated in beta cell inability to replicate as well as in insulin secretion defects. Following up on a linkage signal in the CHOP region of chromosome 12q13.1 in Italian T2D families, we have previously shown that CHOP 5’UTR-c.279T>C and +nt30C>T haplotype variants are associated with early-onset T2D under a recessive and additive model [7]. In addition, CHOP inhibits adipogenesis [8], thus CHOP gene variants may contribute to insulin resistance [9,10] and/or obesity [11]. Since CHOP is regulating programmed cell death in response to stress stimuli [12], it is implicated in tumor/cancer development.

CHOP is involved in the pathogenesis of myxoid liposarcoma, a rare human tumor in which a reciprocal chromosomal translocation creates a fusion protein consisting of CHOP and TLS, a potent oncoprotein [13]. Other tumor-specific fusion genes, such as EWS-CHOP and TLS/FUS-CHOP, have been detected in solid tumors [14] and liposarcomas [15-17]. Another rearrangement of the CHOP gene has been reported in myxoid liposarcoma [18].

Our aim was to find whether there is any association of the CHOP 5’UTR-c.279T>C and +nt30C>T genotypes and corresponding haplotypes (the latter contributing to T2D) [7] with body mass index (BMI) ≥ 25 (hereby defined as overweight condition/pre-obesity) and/or with tumors/cancer in an Italian dataset.

Methods
We recruited from the general population in Italy 45 subjects with BMI ≥ 25 and 44 control subjects with BMI < 25 and 54 subjects with at least one cancer or at least one tumor and 43 control subjects with no history of tumor or cancer. We obtained the written informed consent from each subject and the approval from the Institutional Review Board accordingly to Helsinki Declaration guidelines. DNA samples were directly sequenced by PCR and an automated fluorescence sequencer with specific primers for the CHOP 5’UTR-c.279T>C and +nt30C>T genotypes.

We calculated via 70% power and type 1 error probability of 0.05, detectable odds ratios for genotype association tests in our two datasets, using the prevalence of 31.3% for overweight condition [19] and the prevalence of 2.7% for tumors/cancer in the Italian population [20].

Via Chi-Square test statistics, we tested the alleles for departure from Hardy-Weinberg equilibrium (HWE) in our two datasets in cases and control subject groups, separately.

Via the Mantel-Haenszel algorithm, we tested the CHOP 5’UTR-c.279T>C and +nt30C>T genotypes for association with BMI ≥ 25 and with tumors/cancer.

In addition, we performed model free and parametric haplotype associations tests (dominant, recessive and additive models) for BMI ≥ 25 and for tumors/cancer, independently (EHPLUS software) [21].

Results
Risk odds ratios of 0.248/2.943 for genotypes association tests were detectable in the pre-obesity dataset. Risk odds ratios of 8.210 for genotype association tests were detectable in the tumors/cancer dataset.

All alleles tested in each group of the two datasets of BMI ≥ 25 and of tumors/cancer were not in departure from HWE.

We did not identify in our dataset any significant and valid association of the CHOP 5’UTR-c.279T>C and +nt30C>T genotype variants with BMI ≥ 25 (Table 1) as well as with tumors/cancer patients (Table 2).

The parametric and non-parametric CHOP 5’UTR-c.279T>C and +nt30C>T haplotype association tests with BMI ≥ 25 as well as with tumors/cancer were also not significant (data not shown).

Discussion
CHOP gene encodes a C/EBP (CCAAT/enhancer binding protein family)-homologous nuclear protein that acts as dominant-negative inhibitor of gene transcription through dimerization with C/EBP [22]. CHOP is implicated in programmed cell death [12]. Several studies reported CHOP gene rearrangement and/or fusion with other genes (such as EWS-CHOP and TLS/FUS-CHOP) in tumors/cancer [13,18].

Cellular and endoplasmic reticulum (ER) stress, occurring in response to toxic and metabolic insult, is a powerful inducer of CHOP [12]. ER stress down-regulates insulin receptor signaling and triggers insulin resistance [9]. Furthermore, insulin increases CHOP expression in adipocytes cells [23], and CHOP inhibits adipocyte differentiation [8]. Thus, CHOP deficiency may contribute to obesity [11].
Glucotoxicity induces cellular stress [24], which activates CHOP [12]. Thus, hyperglycemia may cause CHOP-mediated beta-cell apoptosis and may contribute to T2D. Interestingly, CHOP 5'UTR-c.279T>C and +nt30C>T haplotype variants are significantly associated with early-onset T2D under a recessive and additive model [7]. For all the above reasons, CHOP is not only a T2D gene, but it is also an obesity candidate gene as well as a gene potentially predisposing to tumors and/or cancer. Other T2D genes, such as HNF-1 beta and JAZF1, have already been associated with prostate cancer [4-6]. Of note, while the prostate cancer risk HNF-1 beta variant decreases the risk of T2D [4], variants of JAZF1 gene are associated with both increased risk for T2D and for prostate cancer [5,6]. However, no study has up to date investigated the susceptibility role of CHOP common variants in pre-obese and tumor/cancer patients. This is the first association study focusing on CHOP gene variants in human genomic DNA samples of overweight subjects and tumor/cancer cases.

In our study, we did not identify any association between CHOP 5'UTR-c.279T>C and +nt30C>T genotype and haplotype variants with pre-obesity and tumors/cancer.

Table 1: CHOP 5'UTR-c.279T>C and +nt30C>T genotype association with overweight condition (BMI ≥ 25).

| Genotype      | 45 Cases | 44 Control Subjects | $\chi^2$ | 2-t P | OR  | 95% C.I. |
|---------------|----------|---------------------|---------|-------|-----|----------|
| 5'UTRc.279T>C |          |                     |         |       |     |          |
| TT            | 31       | 14                  | 26      | 18    | 0.92| 0.33     | 1.53     | 0.39–4.02 |
| CT            | 13       | 32                  | 18      | 26    | 1.41| 0.23     | 0.59     | 0.22–1.55 |
| CC            | 1        | 44                  | 0       | 44    | 0.98| 0.32     | 8        | 0.06–8   |
| +nt30C>T      |          |                     |         |       |     |          |
| TT            | 0        | 45                  | 0       | 44    | NA  |          |          |           |
| CT            | 13       | 32                  | 17      | 27    | 0.94| 0.33     | 0.65     | 0.24–1.71 |
| CC            | 32       | 13                  | 27      | 17    | 0.94| 0.33     | 1.55     | 0.58–4.13 |

$X^2$ = Chi-Square, 2-t P = 2-tailed p-value, OR = odds ratio, C.I. = confidence interval

Table 2: CHOP 5'UTR-c.279T>C and +nt30C>T genotype association with tumors/cancer.

| Genotype      | 54 Cases | 43 Control Subjects | $\chi^2$ | 2-t P | OR  | 95% C.I. |
|---------------|----------|---------------------|---------|-------|-----|----------|
| 5'UTRc.279T>C |          |                     |         |       |     |          |
| TT            | 35       | 19                  | 27      | 16    | 0.04| 0.83     | 1.09     | 0.44–2.73 |
| CT            | 17       | 37                  | 14      | 29    | 0.01| 0.91     | 0.95     | 0.37–2.45 |
| CC            | 2        | 52                  | 2       | 41    | 0.05| 0.81     | 0.79     | 0.08–8.28 |
| +nt30C>T      |          |                     |         |       |     |          |
| TT            | 2        | 52                  | 1       | 42    | 0.15| 0.69     | 1.62     | 0.11–46.73 |
| CT            | 17       | 37                  | 16      | 27    | 0.35| 0.55     | 0.78     | 0.31–1.96 |
| CC            | 35       | 19                  | 26      | 17    | 0.19| 0.66     | 1.20     | 0.48–2.99 |

$X^2$ = Chi-Square, 2-t P = 2-tailed p-value, OR = odds ratio, C.I. = confidence interval
If the CHOP gene variants tested were to contribute to overweight condition and/or tumors/cancer with a modest size effect, our datasets are too small to detect such effects. However, we could at least exclude in the current study a CHOP 5' UTR-c.279T>C and +nt30C>T variant risk effect of about 3 for pre-obesity and of about 8 for tumors/cancer.

Conclusion
In summary, we conclude that CHOP 5' UTR-c.279T>C and +nt30C>T variants, both at genotype and at haplotype level, are not contributing to the overweight condition and tumors/cancer in our dataset. However, other studies are warranted to further exclude the role of any other CHOP variants in obesity and in tumor/cancer predisposition. Since obesity is a preventable associated factor in several tumors/cancer [25] and in other comorbidities [26], and, since tumors and cancer may be prevented and/or diagnosed at an earlier stage, genetic studies to identify overweight risk predisposition as well as tumors/cancer risk susceptibility should be further performed to guide disease prediction and prevention.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
CG participated in study design, DNA amplification, sequence reading, project coordination and manuscript drafting and revising. RM carried out the statistical analysis, reference collection, and manuscript drafting. NP and LP executed PCR set up, DNA amplification and sequence analysis, reference collection, and manuscript drafting. All authors have read and approved the manuscript.

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