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Accessibility
Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece

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\textbf{ABSTRACT}

\textbf{Objectives:} An additive genetic risk score (GRS) for coronary heart disease (CHD) has previously been associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort. In this study, we explore GRS-'environment' joint actions on CHD for several conventional cardiovascular risk factors (ConvRFs), including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet.

\textbf{Design:} A case–control study.

\textbf{Setting:} The general Greek population of the EPIC study.

\textbf{Participants and outcome measures:} 477 patients with medically confirmed incident CHD and 1271 controls participated in this study. We estimated the ORs for CHD by dividing participants at higher or lower GRS and, alternatively, at higher or lower ConvRF, and calculated the relative excess risk due to interaction (RERI) as a measure of deviation from additivity.

\textbf{Results:} The joint presence of higher GRS and higher risk ConvRF was in all instances associated with an increased risk of CHD, compared with the joint presence of lower GRS and lower risk ConvRF. The OR (95\% CI) was 1.7 (1.2 to 2.4) for smoking, 2.7 (1.9 to 3.8) for hypertension, 4.1 (2.8 to 6.1) for T2DM, 1.9 (1.4 to 2.5) for lower physical activity, 2.0 (1.3 to 3.2) for high BMI and 1.5 (1.1 to 2.1) for poor adherence to the Mediterranean diet. In all instances, RERI values were fairly small and not statistically significant, suggesting that the GRS and the ConvRFs do not have effects beyond additivity.

\textbf{Conclusions:} Genetic predisposition to CHD, operationalised through a multilocus GRS, and ConvRFs have essentially additive effects on CHD risk.

\textbf{INTRODUCTION}

Coronary heart disease (CHD) is a leading cause of death and disability worldwide.\textsuperscript{5} Lifestyle and environmental factors, such as cigarette smoking, physical inactivity, chronodisruption and unhealthy diets, play a significant role in its development and are largely responsible for increased risk of this disease.\textsuperscript{2,3} In addition, compelling evidence from the literature suggests a genetic basis for CHD\textsuperscript{4} so that genetic data may identify individuals who have an inherited predisposition to develop CHD.

During the past few years, genome-wide association studies (GWAS) have successfully identified a large number of chromosomal loci and genetic variants that are robustly associated with CHD,\textsuperscript{5–11} although their effects on risk are generally fairly small. To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and CHD, genotypes at multiple genetic variants have previously been combined into scores calculated according to the number of risk alleles carried.\textsuperscript{12–15} To date, several studies have examined the utility of different genetic risk scores (GRSs) to identify participants at increased CHD risk.\textsuperscript{14–18} Ripatti et al\textsuperscript{16}
reported that a GRS based on a series of genetic variants from GWAS for myocardial infarction or CHD was associated with risk of CHD, and that the upper quintile of individuals of European ancestry who carried the most risk alleles had a roughly 1.7 times increased risk of CHD when compared with those in the lowest quintile of GRS. Using a similar approach, we have shown that a GRS based on nine documented genetic variants from GWAS is associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort. Despite the success of GWAS in identifying novel genetic contributors to CHD, the heritability of common disorders cannot be adequately explained by the genes that have been discovered; moreover, for the most part, we do not know how these recently discovered loci interact with the environment and what role such interactions play in the development of the disease. Testing such interactions is thus a new frontier for large-scale GWAS of CHD, and some initial findings support the important role of environmental exposures in influencing the magnitude of the genetic associations with cardiovascular disease or other common diseases and traits.

The aim of the current study was to explore potential GRS-environment interaction effects on CHD for several important conventional cardiovascular risk factors (ConvRFs), including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet (MedDiet). We have used resources generated in the Greek-EPIC cohort in which medically documented incident cases of CHD are recorded during an extended follow-up of this population-based cohort.

METHODS
Study population
The EPIC is a longitudinal study aimed at investigating the role of biological, nutritional, lifestyle and environmental factors in the aetiology of cancer and other chronic diseases. The study has been described in detail elsewhere. The recruitment of Greek-EPIC participants was from 1994 to 1999. The active follow-up of study participants is repeated every 2–4 years. In each round, the focus of follow-up is on the update of information related to health status of the participants. For this analysis, exposure data at enrolment and follow-up data until the end of 2009 for outcomes are considered. By December 2009, 788 participants were diagnosed with an incident, medically confirmed, CHD or stroke event and were considered eligible for a study also evaluating genetic predisposition. For each case, an attempt was made to choose two control participants matched for sex, age (±2 years) and date of recruitment (±6 months). Both cases and controls were free of CHD and stroke at baseline; the final study sample consisted of 788 cases (494 CHD, 320 stroke, 26 both diseases) and 1345 controls. For each study participant, a buffy coat sample was drawn from the Greek-EPIC bio-repository and genomic DNA was extracted. CHD events included myocardial infarction, angina and other ischaemic heart disease (cardiac arrest, presence of cardiac and vascular implants and grafts), with several cases following in more than one categories. All procedures were in accordance with the Helsinki Declaration and all participants provided written informed consent.

Selection of genetic variants, genotyping and GRS calculation
We constructed a multilocus GRS by using nine previously reported genetic variants associated with myocardial infarction or CHD from GWAS, with convincing replication evidence in populations with European ancestry. The variants used were: rs11206510 at 1p32 near PCSK9, rs646776 at 1p13 near CELSR2-PSRC1-SORT1, rs17465637 at 1q41 in MIA3, rs6725887 at 2q33 in WDR12, rs9349379 at 6p21 in PHACTRI, rs1746048 at 10q11 near CXCL12, rs1122608 at 19p13 near LDLR, rs9982601 at 21q22 near SLC5A3-MRPS6-KCN2 and the lead variant (rs1333049) at locus 9p21 near CDKN2A/2B identified by the Wellcome Trust Case Control Consortium. Genotyping was performed blindly as to case-control status with the TaqMan allelic discrimination system on the ABI 7900HT platform using custom genotyping assays and probes designed by Applied Biosystems, Inc (Foster City, California, USA). Replicate quality control samples yielded 100% concordance and call rates exceeded 98%. All genotypes were analysed in the Nutrition and Genomics Laboratory, Jean Mayer US Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, USA.

A GRS was computed for each individual as the sum of the number of risk alleles across all nine variants, after weighting each one by its estimated effect size in the discovery samples as generally used and previously described. In this study, the minimum and maximum weighted GRS values were, respectively, 4.6 and 17.7 in control participants and 5.7 and 18.8 in CHD cases.

Conventional risk factors for CHD
We evaluated GRS-environment interaction effects on CHD for several important conventional ConvRFs for which information was collected at enrolment. These factors were: smoking status, hypertension, T2DM, BMI, waist-to-hip ratio, physical activity, energy intake and adherence to the MedDiet. Participants were characterised as current, former or never smokers and were considered as hypertensive if they met one of the following criteria: (1) their measured arterial blood pressure
was 140 mm Hg or higher systolic, or 90 mm Hg or higher diastolic and (2) self-reported intake of an antihypertensive treatment. T2DM was identified through self-reported T2DM-specific medication use or self-reported medical diagnosis of T2DM. Weight, height, waist and hip circumference were measured using standard procedures, and BMI was calculated in kg/m². With respect to physical activity, we used a metabolic equivalent index (MET-value) that expresses the amount of energy per kilogram of body weight expended during an average day. Dietary information of the participants energy per kilogram of body weight expended during respect to physical activity, we used a metabolic equivalent index (MET-value) that expresses the amount of energy per kilogram of body weight expended during an average day. Dietetic information of the participants was measured at baseline using a validated interviewer-administered food frequency questionnaire (FFQ). The frequency of consumption of about 200 foods and recipes that are common in Greece was reflected at the FFQ. The daily energy intake was assessed by recording participants’ energy intake (in kcal). Adherence to the MedDiet was assessed with a MedDiet score that incorporates the salient characteristics of this diet, that is, high intake of plant foods and olive oil, low intake of meat and dairy products and moderate intake of alcohol. This score, with values from 0 to 9 (higher scores indicate greater adherence to the MedDiet), is associated with death from CHD, with lower values predicting higher incidence of death from CHD.

Statistical analysis

For this study, we have used all incident CHD cases and all available control participants and we have proceeded through unconditional logistic regression.

Mean values of quantitative characteristics, as well as percentages for qualitative ones, by sex and case-control status, were calculated for descriptive purposes. We evaluated whether CHD incidence is related to the aforementioned ConvRFs using logistic regression, adjusting for age, sex and GRS. We evaluated ORs for CHD, as estimates of the incidence rate ratios, in relation to age, sex and higher or lower risk with respect to GRS (above or equal to vs below the sex-specific median score in controls) and, alternatively, on the basis of smoking status (current vs never/former smoker), hypertension (yes vs no), T2DM (yes vs no), physical activity (below vs above or equal to the sex-specific median), energy intake (below vs above or equal to the sex-specific median), MedDiet score (below vs above or equal to the median score of 4.0), BMI (above or equal or below 25 kg/m²) or waist-to-hip ratio (above or equal to vs below the sex-specific median).

In order to access the nature of the joint effects of GRS and ConvRFs, we calculated the relative excess risk due to interaction (RERI), as defined by Rothman. RERI is an estimate of excess or deficit risk that is attributable to the interaction between two exposures, in this case GRS and each one of the ConvRFs; it measures deviation from additivity of effects independently of the risk scale of the outcome. From the ORs of the logistic regression, we computed the RERIs between GRS and ConvRFs as follows: let X+ and Y+ denote the presence of the risk factors X (GRS in our analysis) and Y (conventional factor) and X− and Y− denote the absence of these risk factors. Then, by considering that the OR estimates the relative risk (RR) we have that

\[
RERI(X, Y) = (RR_{X+Y+} - RR_{X-Y-}) - (RR_{X+Y-} - RR_{X-Y+}) - (RR_{X-Y+} - RR_{X-Y-})
\]

that is, RERI(X, Y) = (OR_{X+Y+} - 1) - (OR_{X-Y-} - 1) - (OR_{X-Y+} - 1).

The necessary variance estimators of RERI for the construction of 95% CIs were derived using the standard method. All statistical analyses were conducted using the Stata Statistical Software, release V.11 (StataCorp 2009, StataCorp LP).

RESULTS

Of the 1839 study participants with genotype data (494 patients with incident CHD only and 1345 controls), 91 participants had missing data for one or more of the ConvRFs; thus, our analyses were restricted to 477 CHD cases and 1271 controls with complete datasets. The characteristics of the study participants at enrolment according to sex and case-control status are given in table 1.

The association of ConvRFs with CHD incidence in this prospective cohort study is illustrated intable 2. As expected, smoking, hypertension, T2DM and an increased BMI and waist-to-hip ratio were all associated with a substantial increase in the risk of CHD, whereas higher levels of physical activity and energy expenditure (as reflected in an increased energy intake) were associated with a decrease in risk. Greater adherence to the MedDiet was also associated with an 11% decreased risk of CHD, although this association was not statistically significant.

We then examined the impact on CHD risk of the joint presence of genetic predisposition and ConvRFs by modelling the data through unconditional logistic regression, adjusting for age and sex. Specifically, we estimated ORs for CHD incidence depending on participants having a higher or lower GRS and simultaneously as being at higher or lower risk on the basis of a conventional risk factor. Table 3 gives the distribution of CHD cases and controls by GRS and each ConvRF (lower vs higher risk for CHD) in men and women. As shown in table 4, in all instances the joint presence of higher GRS and higher-risk ConvRF is associated with a substantial increase in the risk of CHD, compared with the joint presence of lower GRS and lower-risk ConvRF. In addition, participants with higher GRS values (high-risk genetic predisposition) and simultaneously at higher risk because of a ConvRF are characterised by an OR for CHD that is higher than the OR among individuals with high-risk genetic predisposition who belong to the lower risk category of the respective ConvRF (smoking status, OR 1.70 vs 1.49; hypertension, OR 2.72 vs 1.21; T2DM,
OR 4.13 vs 1.34; physical activity, OR 1.86 vs 1.25; energy intake, OR 1.75 vs 1.43; MedDiet score, OR 1.51 vs 1.24; BMI, OR 2.01 vs 1.47; waist-to-hip ratio, OR 1.88 vs 1.25).

Relative excess risks due to interaction (RERIs) between the GRS and each one of the ConvRFs are presented in the last column of table 4. There is some evidence for superadditivity with respect to hypertension, and, on the contrary, some evidence for subadditivity with respect to smoking. Nevertheless, in all instances, RERI values are fairly small and the 95% CIs cover the null values of RERI, suggesting that the GRS and the conventional risk factors do not have effects beyond additivity.

DISCUSSION

In a sizable case–control study nested in the population-based Greek-EPIC cohort, we have found that genetic predisposition to CHD, operationalised through a multilocus GRS (the sum of high-risk alleles in 9 genetic variants) and ConvRFs have essentially additive influence on CHD risk. In other words, people at high risk for CHD because of genetic susceptibility tend to have additively increased RR when also exposed to any of the investigated conventional risk factors. This is highlighted by the fact that, while among people with low genetic risk, only five of the eight investigated ConvRFs were documentable as ‘statistically significant’, all eight were documentable as such among people at high genetic risk.

Evaluation of joint effects in a multiplicative scale through interaction terms in logistic regression and other models that rely on similar principles are very valuable on account of their flexibility and provision of insights on causal pathways. Additive models (and deviations from additivity), however, as evaluated in this paper, convey straightforward answers to questions of preventive and clinical importance by pointing to individual change of risk in relation to values of conventional risk factors and

<table>
<thead>
<tr>
<th>Table 1 Characteristics of conventional cardiovascular risk factors and genetic risk score for incident CHD cases and controls in the Greek-EPIC cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases (n=477)</strong></td>
</tr>
<tr>
<td><strong>Men (n=331)</strong></td>
</tr>
<tr>
<td><strong>Women (n=146)</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong> 60.1 (11.4)</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong> 28.7 (3.8)</td>
</tr>
<tr>
<td><strong>Waist-to-torh ratio</strong> 0.97 (0.06)</td>
</tr>
<tr>
<td><strong>Physical activity (MET-h/day)</strong> 33.8 (5.6)</td>
</tr>
<tr>
<td><strong>Energy intake (kJ)</strong> 9250.8 (3000.8)</td>
</tr>
<tr>
<td>**MedDiet score <strong>4</strong>.4 (1.7)</td>
</tr>
<tr>
<td><strong>Hypertensive, n (%)†</strong> 224 (67.7)</td>
</tr>
<tr>
<td><strong>Type 2 diabetes, n (%)‡</strong> 68 (20.5)</td>
</tr>
<tr>
<td><strong>Current smokers, n (%)</strong> 138 (41.7)</td>
</tr>
<tr>
<td><strong>Weighted GRS§</strong> 12.6 (2.0)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD) unless otherwise indicated.
*The range of the MedDiet score is from 0 to 9, with higher values indicating greater adherence to the MedDiet.
†Defined as a systolic blood pressure of 140 mm Hg or higher or a diastolic blood pressure of 90 mm Hg or higher, or self-reported receipt of an antihypertensive treatment.
‡Identified through self-reported T2DM-specific medication use or self-reported medical diagnosis of T2DM.
§The minimum and maximum weighted GRS values were 4.6 and 18.8.

<table>
<thead>
<tr>
<th>Table 2 ORs for CHD incidence by conventional risk factors in the Greek-EPIC cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR (95% CI)</strong> <strong>p Value</strong></td>
</tr>
<tr>
<td>Smoking status (current vs never/former smokers) 1.39 (1.08 to 1.80)</td>
</tr>
<tr>
<td>Hypertension (yes vs no) 2.16 (1.68 to 2.78)</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus (yes vs no) 3.36 (2.52 to 4.47)</td>
</tr>
<tr>
<td>Physical activity (≥sex-specific median vs &lt;sex-specific median) 0.70 (0.56 to 0.87)</td>
</tr>
<tr>
<td>Energy intake (≥sex-specific median vs &lt;sex-specific median) 0.75 (0.60 to 0.93)</td>
</tr>
<tr>
<td>MedDiet score (≥sex-specific median vs &lt;sex-specific median) 0.89 (0.71 to 1.11)</td>
</tr>
<tr>
<td>Body mass index (≥25 vs &lt;25 kg/m²) 1.45 (1.08 to 1.96)</td>
</tr>
<tr>
<td>Waist-to-torh ratio (≥sex-specific median vs &lt;sex-specific median) 1.46 (1.17 to 1.81)</td>
</tr>
</tbody>
</table>

*Association tested with unconditional logistic regression adjusted for age, sex and genetic risk score; median values according to the overall sample (cases and controls combined).

CHD, coronary heart disease; EPIC, European prospective investigation into cancer and nutrition; GRS, genetic risk score; MedDiet, Mediterranean diet; MET, metabolic equivalent.
Table 3  Distribution of CHD cases and controls by genetic risk score and conventional cardiovascular risk factors (lower/higher risk for CHD), in men and women

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Men (n=1115)</th>
<th>Women (n=663)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=331)</td>
<td>Controls (n=784)</td>
</tr>
<tr>
<td><strong>GRS (lower risk: &lt;sex-specific median of controls; higher risk: ≥sex-specific median of controls)</strong></td>
<td>150/181 (45/55)</td>
<td>400/384 (51/49)</td>
</tr>
<tr>
<td>Smoking status (lower risk: never/former smokers; higher risk: current smokers)</td>
<td>193/138 (58/42)</td>
<td>515/269 (66/34)</td>
</tr>
<tr>
<td>Hypertension (lower risk: no; higher risk: yes)</td>
<td>107/224 (32/68)</td>
<td>332/452 (42/58)</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus (lower risk: no; higher risk: yes)</td>
<td>263/68 (79/21)</td>
<td>410/374 (52/48)</td>
</tr>
<tr>
<td>Physical activity (lower risk: ≥sex-specific median; higher risk: &lt;sex-specific median)</td>
<td>148/133 (45/55)</td>
<td>410/374 (52/48)</td>
</tr>
<tr>
<td>Energy intake (lower risk: ≥sex-specific median; higher risk: &lt;sex-specific median)</td>
<td>153/178 (46/54)</td>
<td>405/379 (52/48)</td>
</tr>
<tr>
<td>MedDiet score (lower risk: ≥4; higher risk: &lt;4)</td>
<td>224/107 (68/32)</td>
<td>545/239 (69/31)</td>
</tr>
<tr>
<td>Body mass index (lower risk: &lt;25 kg/m²; higher risk: ≥25 kg/m²)</td>
<td>51/260 (15/85)</td>
<td>160/624 (20/80)</td>
</tr>
<tr>
<td>Waist-to-hip ratio (lower risk: &lt;sex-specific median; higher risk: ≥sex-specific median)</td>
<td>147/184 (44/56)</td>
<td>410/374 (52/48)</td>
</tr>
</tbody>
</table>

Data are numbers (% in parenthesis). Median values for GRS are based on controls only, whereas for conventional risk factors median values are based on cases and controls combined. CHD, coronary heart disease; GRS, genetic risk score; MedDiet, Mediterranean diet.
<table>
<thead>
<tr>
<th>Smoking status (lower risk: never/former smokers higher risk: current smokers)</th>
<th>1st (reference)</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>Relative Excess Risk due to Interaction (RERI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRS: lower risk</td>
<td>ConvRF: lower risk</td>
<td>OR (95% CI)</td>
<td>N</td>
<td>OR (95% CI)</td>
<td>N</td>
</tr>
<tr>
<td>Smoking status (lower risk: never/former smokers higher risk: current smokers)</td>
<td>630</td>
<td>1.75 (1.22 to 2.49)</td>
<td>223</td>
<td>1.49 (1.15 to 1.92)</td>
<td>664</td>
</tr>
<tr>
<td>Hypertension (lower risk: no; higher risk: yes)</td>
<td>318</td>
<td>2.07 (1.45 to 2.94)</td>
<td>535</td>
<td>1.21 (0.81 to 1.80)</td>
<td>305</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus (lower risk: no; higher risk: yes)</td>
<td>740</td>
<td>3.72 (2.45 to 5.63)</td>
<td>113</td>
<td>1.34 (1.05 to 1.71)</td>
<td>765</td>
</tr>
<tr>
<td>Physical activity (lower risk: ≥sex-specific median; higher risk: &lt;sex-specific median)</td>
<td>425</td>
<td>1.36 (0.99 to 1.88)</td>
<td>428</td>
<td>1.25 (0.92 to 1.71)</td>
<td>451</td>
</tr>
<tr>
<td>Energy intake (lower risk: ≥sex-specific median; higher risk: &lt;sex-specific median)</td>
<td>439</td>
<td>1.47 (1.07 to 2.03)</td>
<td>414</td>
<td>1.43 (1.05 to 1.94)</td>
<td>436</td>
</tr>
<tr>
<td>MedDiet score (lower risk: ≥4.0; higher risk: &lt;4.0)</td>
<td>574</td>
<td>1.03 (0.73 to 1.43)</td>
<td>279</td>
<td>1.24 (0.95 to 1.60)</td>
<td>616</td>
</tr>
<tr>
<td>Body mass index (lower risk: &lt;25 kg/m²; higher risk: ≥25 kg/m²)</td>
<td>143</td>
<td>1.56 (0.99 to 2.46)</td>
<td>710</td>
<td>1.47 (0.84 to 2.56)</td>
<td>152</td>
</tr>
<tr>
<td>Waist-to-hip ratio (lower risk: &lt;sex-specific median; higher risk: ≥sex-specific median)</td>
<td>433</td>
<td>1.40 (1.02 to 1.93)</td>
<td>420</td>
<td>1.25 (0.92 to 1.71)</td>
<td>439</td>
</tr>
</tbody>
</table>

Association tested with unconditional logistic regression adjusted for age and sex. Statistically significant results ($p \leq 0.05$) are in italic fonts. CHD, coronary heart disease; ConvRF, conventional cardiovascular risk factor; EPIC, European prospective investigation into cancer and nutrition; GRS, genetic risk score; MedDiet, Mediterranean diet.
In conclusion, this study provides evidence that genetic and conventional cardiovascular risk factors tend to have additive consequences on CHD, an issue that may be of preventive importance when genetic predisposition could not be assessed through an ad hoc genetic risk score but simply through a positive family history.

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**Contributors**

NY, AT, JMO and DT participated in design and development of the study. NY, AT, JMO and DT participated in acquisition of data. NY, MK, AT, JMO and DT participated in drafting and critical revision of the manuscript for important intellectual content. NY, MK, AT, JMO and DT participated in statistical analysis. NY, MK, AT, JMO and DT provided administrative, technical and material support. NY, AT and NY participated in study supervision.

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**Competing interests**

None.

**Patient consent**

Obtained.

**Ethics approval**

All procedures were in accordance with the Helsinki Declaration. The study protocol was approved by the ethics committees of the International Agency for Research on Cancer and the Medical School of the University of Athens.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**Data sharing statement**

No additional data are available.

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