

Genetic variation and gastric cancer risk: a field synopsis and meta-analysis

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Abbreviations: 95% confidence interval, FPRP: false positive report probability, GWAS: genome wide association study, HP: Helicobacter Pylori, HuGeNet: Human Genome Epidemiology Network, LD: linkage disequilibrium, MAF: minor allele frequency, OR: odds ratio, PAR: population attributable risk, SNP: single nucleotide polymorphism

Key words: gastric cancer, stomach, risk, susceptibility, predisposition, genetic variation, association, polymorphism, variant, SNP, GWAS, meta-analysis, synopsis, systematic review

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Abstract

Background

Data on genetic susceptibility to sporadic gastric carcinoma have been published at a growing pace, but to date no comprehensive overview and quantitative summary has been available.

Methods

We conducted a systematic review and meta-analysis of the evidence on the association between DNA variation and risk of developing stomach cancer. To assess result credibility, summary evidence was graded according to the Venice criteria and false positive report probability (FPRP) was calculated to further validate result noteworthiness. Meta-analysis was also conducted for subgroups, which were defined by ethnicity (Asian vs Caucasian), tumor histology (intestinal vs diffuse), tumor site (cardia vs non-cardia) and *Helicobacter Pylori* infection status (positive vs negative).

Results

Literature search identified 824 eligible studies comprising 2,530,706 subjects (cases: 261,386 [10.3%]) and investigating 2,841 polymorphisms involving 952 distinct genes. Overall, we performed 456 primary and subgroup meta-analyses on 156 variants involving 101 genes. We identified 11 variants significantly associated with disease risk and assessed to have a high level of summary evidence: MUC1 rs2070803 at 1q22 (diffuse carcinoma subgroup), MTX1 rs2075570 at 1q22 (diffuse), PSCA rs2294008 at 8q24.2 (non-cardia), PRKAA1 rs13361707 5p13 (non-cardia), PLCE1 rs2274223 10q23 (cardia), TGFBR2 rs3087465 3p22 (Asian), PKLR rs3762272 1q22 (diffuse), PSCA rs2976392 (intestinal), GSTP1 rs1695 11q13 (Asian), CASP8 rs3834129 2q33 (mixed), and TNF rs1799724 6p21.3 (mixed), with the first nine variants characterized by a low FPRP. We also identified polymorphisms with lower quality significant associations (n=110).

Conclusions

We have identified several high quality biomarkers of gastric cancer susceptibility. These data will form the backbone of an annually-updated online resource which will be integral to the study of gastric carcinoma genetics and may inform future screening programs.

Summary Box

What is already known about this subject ?

- Gastric cancer is a leading cause of death by cancer worldwide
- Germ-line genetic variation is believed to play a key role in cancer predisposition
- Hundreds of studies have linked specific gene polymorphisms to the risk of developing gastric carcinoma

What are the new findings ?

- We built up the first comprehensive database collecting the published evidence on the association between genetic variants and risk of gastric carcinoma
- Data meta-analysis (using dedicated methodology) identified several high quality biomarkers of gastric cancer susceptibility
- Subgroup analysis identified polymorphisms associated with specific subsets of tumor type (intestinal vs diffuse) and site (cardia vs non-cardia)
- Much work is still to be done to identify gene-environment interactions

How might it impact on clinical practice in the foreseeable future?

- These data will form the backbone of an annually-updated online resource which will be integral to the study of gastric carcinoma genetics and may inform future screening programs.

Introduction

Gastric carcinoma is the fifth most common cancer and the third most frequent cause of cancer death worldwide (1), and despite some therapeutic advances its prognosis is often unfavorable (2,3). Although the cascade of molecular events leading to gastric carcinogenesis are still largely unknown (3-5), genetic background (6,7), behavioral factors (e.g., alcohol consumption, smoking habit, diet) (8,9) and infectious agents (i.e., *Helicobacter Pylori* [HP]) (10) have been associated with the risk of developing gastric carcinoma. Of note, its incidence shows remarkable geographic heterogeneity, the highest rates being observed in Asia, which has been at least in part attributed to the differences in genetic polymorphism distribution and prevalence of HP infection (or HP strains) in different populations (11,12).

Germ-line variations of DNA sequence are believed to represent a key aspect of predisposition to most complex traits, such as cancer (13-15). As regards gastric cancer, a small fraction of the familial risk (approximately 3%) can be explained by rare mutations in high-penetrance genes such as *CDH1* (encoding E-cadherin), which is associated with hereditary diffuse gastric cancer (16). Less than 15% of cases are believed to have familial clustering, most of these lacking an association with specific germ-line mutations (3,6), suggesting that most familial clustering of this tumor is due to a combination of multiple alleles with lower penetrance (3,6).

Numerous studies (enrolling tens of thousands of subjects) have been performed to investigate the role of genetic variation in gastric carcinogenesis, and many polymorphisms have been identified as potential risk factors for the development of this disease. Interestingly, different DNA variants appear to be correlated with the risk of specific gastric cancer sites (cardia versus non-cardia) and histological types (intestinal versus diffuse). However, the findings of these studies are not always consistent, and no systematic review covering all tested polymorphisms has been published thus far.

The aim of this work is to fill this gap in the international medical literature by presenting the first systematic synopsis and meta-analysis of the available evidence in the field of DNA variation and the risk of sporadic gastric carcinoma, including the interaction of polymorphisms with both ethnicity, environmental factors (i.e. HP infection) and tumor features (i.e. primary cancer site and histological type).

Materials and methods

Search strategy, eligibility criteria and data extraction

We followed the principles proposed by the Human Genome Epidemiology Network (HuGeNet) for the systematic review of molecular association studies (17-19).

Studies dealing with the association between any genetic variant and predisposition to gastric carcinoma in humans were considered eligible, provided that the raw data (necessary to calculate the risk) or summary data were available. Exclusion criteria were data published in abstract form only, minor allele frequency (MAF) in controls less than 1% (rare variants) and a sample size with fewer than 10 cases or controls.

A two-step search strategy was adopted. First, a systematic review of original articles, reviews and meta-analyses analyzing the association between any genetic variant and gastric cancer risk was performed by searching Medline (via the PubMed gateway). The search included the following three groups of keywords: "cancer", "tumor" and "carcinoma"; "gastric" and "stomach"; "polymorphism", "single nucleotide polymorphism" and its acronym "SNP", "variant", "variation", "risk", and "locus". Searches were conducted using all combinations of at least one keyword from each group. Finally, in the light of the growing use of high-throughput platforms for the investigation of gene variants, the expression "genome wide association study" and its acronym "GWAS" were also utilized as a second version of the third group of keywords.

Once this search was completed, in the second phase: A) each single polymorphism was used as a keyword to further refine the search; B) cited references from eligible articles were also searched; C) publicly-available databases dedicated to genotype/phenotype associations were searched (i.e. Database of Genotypes and Phenotypes [dbGaP], <http://www.ncbi.nlm.nih.gov/gap>; Genome Medicine DataBase of Japan [GeMDBJ], <http://gemdbj.nibio.go.jp>, and HuGeNet Phenopedia, <http://www.hugenavigator.net/HuGENavigator/startPagePhenoPedia.do>); D) authors were contacted whenever unreported data were potentially useful to enable the inclusion of the study into the systematic review or to rule out data published in different articles but regarding overlapping series.

For analysis purposes, the database, which will be updated annually and will be publicly available at <http://www.mmmp.org> (20), was frozen in May 2014.

Additional methodology details are available as online **Supplementary Information**.

Statistical analysis

Odds ratios (OR) and corresponding 95% confidence intervals (CI) were used to assess the strength of association between each genetic variant and cancer risk. Per-allele OR were calculated for each study and each polymorphism, assuming an additive (co-dominant) genetic model. This approach was chosen for the following reasons: A) for some studies (including GWAS), only per-allele OR were available; B) the additive model is widely used as a conservative choice between the recessive and the dominant model; C) one model does not require adjustment for multiple hypotheses (which is necessary when different models are tested); D) methods that let the data dictate the genetic model (e.g. the model-free approach (21)) require raw data on genotype distributions (which were unavailable for some studies); E) when large sample sizes are available (as expected in a systematic review and meta-analysis), non-additive models rarely identify high quality associations unidentified by the additive model: therefore the co-dominant model is largely used in genetic association synopses (22-24).

Summary OR were calculated by performing random-effects meta-analysis (using the inverse variance method), which reduces to a fixed-effect meta-analysis when no between-study heterogeneity exists. The choice of this model was suggested mainly by the heterogeneity typically expected in genetic association studies.

For each variant, a meta-analysis was performed if at least three independent datasets were available. As regards GWAS, if data were available, discovery and replication phases were considered as separate datasets.

Subgroup analysis by ethnicity (Asian vs Caucasian/Other), primary tumor site (cardia vs non-cardia), histological subtype (intestinal vs diffuse) and HP status (positive vs negative) was performed if data permitted.

Population attributable risk (PAR) was calculated using the Levin's formula: $P (RR - 1) / [1 + P (RR - 1)]$, where P is the proportion of controls exposed to the genotype of interest and the relative risk (RR) was estimated using the summary estimates (OR) calculated by meta-analysis.

The alpha level of significance was set at 0.05, except for the Harbord test and Q-test (0.10), and the Hardy-Weinberg equilibrium test (0.01).

All statistical analyses were performed with STATA 11.0/SE (StataCorp LP, College Station, Texas, USA).

Assessment of cumulative evidence

In order to evaluate the credibility of each nominally statistically significant association identified by meta-analysis, we applied the Venice criteria (18,19). Briefly, credibility level was defined based upon the grade (*A*=strong, *B*=moderate or *C*=weak) of three parameters:

1) Amount of evidence, roughly depending upon the study sample size, was graded by the sum of cases and controls expressing the risk allele: grade *A*, *B* or *C* were assigned for more than 1000, 100-1000 and less than 100, respectively.

2) Replication of the association was graded based on the amount of heterogeneity: grade *A*, *B* or *C* were assigned for I-squared less than 25%, 25-50% and over 50%, respectively.

3) Protection from bias was graded as *A* if there was no observable bias (bias was unlikely to explain the presence of the association), *B* if bias could be present, or *C* if bias was evident. Assessment of protection from bias also considered the magnitude of association: a score of *C* was assigned to an association with a summary OR less than 1.15 (or greater than 0.87 in case of protective effect) (18).

According to these criteria, the credibility level of the cumulative evidence was defined as high (*A* grades only), low (one or more *C* grades) or intermediate (all other combinations).

In addition to the Venice criteria, we assessed the noteworthiness of significant findings by calculating the false positive report probability (FPRP) (25), which is defined as the probability of no true association between a genetic variant and disease (null hypothesis) given a statistically significant finding. The FPRP is based not only on the observed P-value of the association test but also on the statistical power of the test and on the prior probability that the molecular association is real (according to a Bayesian approach). We calculated FPRP values for two levels of prior probabilities: at a low prior ($10E-3$) that would be similar to what is expected for a candidate variant, and at a very low prior ($10E-6$) that would be similar to what would be expected for a random variant. To classify a significant association as "noteworthy", we used a FPRP cut-off value of 0.2 (25).

For each non significant meta-analysis, we assessed the quality of summary evidence for no association (22). To this aim, we calculated the power of detecting a non-negligible association (i.e., $OR \geq 1.15$ or ≤ 0.87) for a hypothetical study with sample size equal to the combined sample sizes of the studies included in the meta-analysis (with an alpha level of significance set at 5%). If the power were $\geq 90\%$, no between-study heterogeneity were found (I-squared $< 25\%$) and no bias were detected, we considered the level of cumulative evidence high (i.e., sufficient to rule out any meaningful relationship between that variant and gastric cancer risk). Either power $< 80\%$, or significant between-study heterogeneity or detection of

bias (or any combination of them) indicated a low level of evidence. In all other cases, the level of evidence for no association was considered intermediate.

Results

Characteristics of eligible studies

The database with the main features and findings of all eligible studies is available online (**Supplementary Table 1**).

We identified 824 eligible articles (**Supplementary Figure 1**), comprising 2,530,706 subjects (cases: 261,386 [10.3%], range: 13-3,632, mean: 316). More than 95% of these studies (788/824) were published after the year 2000 (with a steep positive trend over time, see **Supplementary Figure 2**).

According to the prevalent ancestry (the race of at least 80% of the enrolled subjects), two thirds of the studies were Asian (n=555, 67%).

Based on the design, the majority of the studies were hospital-based case-control studies (n=494, 60%), the remaining being population-based case-control studies, 24 of which were case-control studies within the frame of cohort prospective studies. Data from three GWAS were also available (26-28).

Less than half of the eligible studies specified the histological subtypes (intestinal vs diffuse) of gastric carcinoma (n=329, 40%), the site (cardia vs non-cardia) of the primary tumor (n=338, 41%), and the status (positive vs negative) of gastric *Helicobacter Pylori* infection (n=102, 12%).

Overall, data on 2,841 polymorphisms involving 952 distinct genes were available. Variation consisted mainly of SNP (n=2,784; 97%), followed by insertions/deletions (n=34), variable number of tandem repeats (n=16) and polymorphisms revealed by a specific change in phenotype (n=7, such as presence/absence of enzymatic activity). These genetic variants were located in the DNA upstream the "relevant" (meant as physically closest) gene (including the promoter region) (n=508), downstream the "relevant" gene (n=215), in introns (n=1,461), in exons (n=378), in the 5'-UTR (n=56) and the 3'-UTR (n=182). Among the exonic SNPs, the functional effects were generally missense (n=229) and synonymous coding changes (n=127), with the remaining being stop-gain, frameshift or splicing variations (n=5).

Distribution of variants (and significant associations with gastric cancer risk) across chromosomes are depicted in **Supplementary Figure 3**. Of note, most of the variants tested are located on chromosome 1, no variant on chromosome Y has been ever investigated, and only three variants in mitochondrial DNA have been studied.

Meta-analysis findings

The results of data meta-analysis are comprehensively reported online (**Supplementary Table 2**). At least three independent datasets were available for 156 variants across 101 genes, which allowed us to perform 456 meta-analyses. Of these, 164 were primary meta-analyses and 292 meta-analyses of subgroups as defined by ethnicity (Caucasian [n=44 meta-analyses] versus Asian [n=77]), cancer histological subtype (intestinal [n=41] versus diffuse [n=40]), primary tumor site (cardia [n=34] versus non-cardia [n=36]) and HP infection status (positive, [n=12] versus negative [n=8]).

The number of datasets meta-analyzed ranged from 3 to 68, the mean number being 7. Based on the number of datasets, the ten most studied variants were the following: IL1B rs16944 (data sets, n=68), IL1RN rs2234663 (n=60), GSTM1 wild/null (n=50), IL1B rs1143627 (n=47), GSTT1 wild/null (n=40), TNF rs1800629 (n=38), TP53 rs1042522 (n=33), MTHFR rs1801133 (n=29), IL10 rs1800896 (n=29), and IL10 rs1800872 (n=25).

The number of subjects (cases plus controls) enrolled in the 456 meta-analyses ranged from 477 to 1,496,612 (median: 3,704). Based on the number of subjects, the ten most studied variants were the following: ABO rs8176719/rs8176746/rs8176747 (n=1,496,612 individuals; however, nearly 99% of these subjects were controls), PSCA rs2294008 (n=42,274), PLCE1 rs2274223 (n=33,219), IL1B rs16944 (n=29,789), PRKAA1 rs13361707 (n=25,319), GSTM1 wild/null (n=25,222), IL1RN rs2234663 (n=24,930), MUC1 rs4072037 (n=23,024), GSTT1 wild/null (n=22,008), and IL1B rs1143627 (n=20,922).

Of the 456 meta-analysis performed, 121 (26%) resulted nominally statistically significant (P-value <0.05), the remaining 335 being non-significant. Among the significant associations identified by meta-analysis, the level of summary evidence was high, intermediate or low in 11 (9%), 38 (31%) and 72 (60%) analyses, respectively. Between-study heterogeneity was the most frequent single cause of non-high quality level of evidence (12/38 intermediate level, 56/72 low level). Considering all meta-analyses, the FPRP was optimal (<0.2) at the 10E-3 and 10E-6 level for 28/121 and 14/121 contrasts, respectively. Amongst the high quality associations, the FPRP was optimal at the 10E-3 and 10E-6 level for 9/11 and 5/11 contrasts, respectively.

The details of significant associations characterized by a high or intermediate level of summary evidence are reported in **Table 1**. These polymorphisms, arranged upon their gene main function, are shown in **Figure 1**. Combinations of variants to predict gastric cancer risk are reported in **Table 2**.

High quality significant associations emerged from data meta-analysis are discussed below.

MUC1 rs2070803

Located on chromosome 1q22, rs2070803 is a G>A SNP downstream of both MUC1 and TRIM46. MUC1 codes for mucin-1, a cell surface glycoprotein which has been repeatedly reported to be involved in the carcinogenesis of different tumors (29), including gastric cancer (30,31), and is also widely used in the clinical setting as a tumor marker known as CA15.3. TRIM46 codes for the tripartite motif-containing protein 46, a zinc-finger containing protein of still uncertain function recently associated with serum urate concentrations (32). MUC1 is located downstream of the TRIM46 gene: these two genes are part of a cluster of genes (including also KRTCAP2, THBS3, MTX1, PKLR and HCN3) located in a region of strong linkage disequilibrium (LD), and are transcribed in opposite directions (i.e., convergently). Since TRIM46 is not expressed in gastric mucosa (33), rs2070803 (whose functional effect is unknown) might be a tagging SNP for variants in other genes located in this LD block, such as MUC1, which are associated with gastric carcinogenesis.

The primary meta-analysis (including 8,789 subjects) showed a statistically highly significant association between this SNP and gastric cancer risk (summary OR: 0.66, 95%CI: 0.59-0.74, P-value=2.55E-13); however, the level of evidence was intermediate due to between-study heterogeneity. When subgroups were considered, the meta-analysis of six datasets (five deriving from two GWAS) including 7,279 subjects (all of Asian ancestry) demonstrated that the rare A allele was associated with a reduced risk of developing the diffuse subtype of gastric carcinoma (summary OR: 0.585, 95%CI: 0.526-0.650, P-value=2.24E-23), with a high level of cumulative evidence as per the Venice criteria and a low false positive report probability (FPRP) at both prior probability levels (10E-3 and 10E-6) (see **Table 1**). Noticeably, this SNP did not result to be associated with intestinal subtype gastric carcinoma (summary OR: 0.759, 95%CI: 0.568-1.012, P-value= 0.061), suggesting that rs2070803 might be specifically linked to diffuse gastric cancer.

In our synopsis, data on a second MUC1 variant (rs4072037) were meta-analyzed, but the association resulted significant only with a low level of evidence.

MTX1 rs2075570

This intronic A>G SNP in the MTX1 gene (on chromosome 1q22) is in strong linkage disequilibrium with the previously described MUC1 rs2070803. MTX1 encodes metaxin-1, a mitochondrial outer membrane protein involved in apoptosis (34,35).

According to the primary meta-analysis (including 8,189 subjects), there was a highly significant association, although the level of evidence was intermediate due to between-study heterogeneity. Upon subgroup meta-analysis, data from five datasets (all deriving from two GWAS) including 6,902 subjects (all of Asian ancestry) showed that the G allele was associated (with a high level of evidence) with a reduced risk of developing the diffuse subtype of gastric carcinoma (summary OR: 0.587, 95%CI: 0.525-0.655, P-value=3.00E-21), with a low FPRP at both prior probability levels. This SNP was not significantly associated with the intestinal subtype of gastric carcinoma (summary OR: 0.848, 95%CI: 0.643-1.117, P-value= 0.241), suggesting that rs2075570 might be specifically linked to diffuse gastric cancer.

PKLR rs3762272

This intronic A>G SNP of the PKLR gene (chromosome 1q22) codes for pyruvate kinase (liver and red blood cell isoenzyme). This enzyme catalyzes the transphosphorylation of phosphoenolpyruvate into pyruvate and ATP, the rate-limiting step of glycolysis (36) that is the key metabolic event underlying the Warburg effect in malignant cells (37).

Upon primary meta-analysis (including 5,750 subjects) the association with this SNP was statistically marginal with a high degree of heterogeneity which made the level of evidence low. Instead, the meta-analysis of three datasets (all deriving from one GWAS) including 4,463 subjects (all of Asian ancestry) showed that the G allele is associated (with a high level of evidence) with a reduced risk of developing the diffuse subtype of gastric carcinoma (summary OR: 0.710, 95%CI: 0.631-0.799, P-value=1.42E-08), with a low FPRP at the higher prior probability level (10-3). Noticeably, this SNP did not result to be associated with intestinal subtype gastric carcinoma (summary OR: 0.927, 95%CI: 0.717-1.197, P-value= 0.559), suggesting that rs3762272 might be specifically linked to diffuse gastric cancer.

PSCA rs2294008 and rs2976392

The C>T SNP rs2294008 is located in exon 1 of PSCA (chromosome 8q24.2) and leads to a frameshift variation that causes a change in the starting codon and is associated with reduced gene transcription (26). PSCA was first identified as a prostate-specific antigen overexpressed in prostate cancer (38), but was then found to also be expressed by other tumor types as well as some normal tissues (including stomach and bladder epithelial cells) (39). PSCA protein product can inhibit proliferation of gastric cancer cells (26), but its role in carcinogenesis appears complex and is still debated (40).

The primary meta-analysis of 15 datasets (42,274 subjects) revealed a highly significant association with disease susceptibility; however, the level of summary evidence was low due to between-study heterogeneity. When subgroup analysis was performed, a similar situation occurred with ancestry and histotype subgroups. In contrast, seven datasets (none from GWAS) including 12,665 subjects (both Asian and Caucasian ancestry) showed that the T allele is associated with a high level of evidence with an increased risk of developing the non-cardia subtype of gastric carcinoma (summary OR: 1.329, 95%CI: 1.254-1.408, P-value<1.00E-20), with a low FPRP at both prior probability levels. SNP rs2294008 was not associated with cardia subtype gastric carcinoma (summary OR: 1.119, 95%CI: 0.933-1.342, P-value= 0.225), suggesting that this SNP might be specifically linked to non-cardia gastric cancer.

Other meta-analyses have recently confirmed the association between gastric cancer and rs2294008 (41,42) (43,44), a variant also associated with the risk of bladder cancer (41,45).

The intronic A>G polymorphism rs2976392 (whose functional effect is unknown) is in strong linkage disequilibrium with the previously discussed PSCA rs2294008.

Primary meta-analysis (16,338 subjects) revealed a highly significant association, but as with SNP rs2294008, the resultant level of evidence was low due to between-study heterogeneity. Upon subgroup meta-analysis, data from four datasets (two deriving from a GWAS) including 7,045 subjects (only Asian ancestry) showed that the G allele is associated (with a high level of evidence) with a decreased risk of developing the intestinal subtype of gastric carcinoma (summary OR: 0.808, 95%CI: 0.751-0.870, P-value=1.42E-08), with a low FPRP at the higher prior probability levels. This variant was also associated with disease risk in patients with diffuse subtype stomach carcinoma (summary OR: 0.619, 95%CI: 0.549-0.697, P-value=4.22E-15), although for this subgroup the level of evidence was low due to between-study heterogeneity.

We could meta-analyze the data regarding a third PSCA polymorphism (rs2976391) that resulted significantly associated with stomach cancer risk with an intermediate level of summary evidence.

PRKAA1 rs13361707

This T>C SNP (whose functional effect is unknown) is located in the first intron of PRKAA1 (chromosome 5p13), a gene encoding the catalytic alpha subunit of AMP-activated protein kinase (AMPK). This enzyme is a cellular energy sensor that maintains energy homeostasis (46) and might be involved in cancer development since its activation is associated with

decreased phosphorylation of mammalian target of rapamycin (mTOR) and S6 kinase, which in turn causes a general reduction in mRNA translation and protein synthesis (47,48).

The primary meta-analysis (25,319 subjects) demonstrated a highly significant association between this variant and gastric cancer, although the level of evidence was low due to between-study heterogeneity. Nevertheless, restricting the meta-analysis to five datasets (three from a GWAS, all of Asian ancestry) comprising 15,478 subjects supported with a high level of evidence the link between the C allele of rs13361707 and an increased risk of non-cardia subtype gastric cancer (summary OR: 1.406, 95%CI: 1.334-1.482, P-value<1.00E-20) with a low FPRP at both prior probability levels. rs13361707 resulted associated also to cardia subtype gastric cancer, although this evidence was of intermediate level due to potential leading study bias (linked to a study (49) identified by the leave-one out sensitivity analysis).

PLCE1 rs2274223

This A>G SNP located in exon 26 of the PLCE1 gene (chromosome 10q23) causes a missense variation (His>Arg) in the protein product named phospholipase-C epsilon-1. This enzyme catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate to generate two second messengers (i.e. inositol 1,4,5-triphosphate [IP3] and diacylglycerol [DAG]) that subsequently regulate various processes affecting cell growth, differentiation, and gene expression (50). Notably, recent investigations have demonstrated that PLCE1 might have a role in gastric carcinogenesis as it is overexpressed in precancerous chronic atrophic gastritis tissues and stomach carcinoma (as compared with normal gastric tissues) (51), and its inhibition has therapeutic potential in a xenograft model (52). Nevertheless, the ultimate function of PLCE1 in cancer development is still debated, since this molecule is downregulated in other tumors and shows a tumor suppressor activity in some animal models (53).

When all available datasets were included in the meta-analysis (n=11, subjects: 33,219; both Asian and Caucasian ancestry), the G allele was significantly associated with an increased risk of gastric cancer (summary OR: 1.219, 95%CI: 1.081-1.373, P-value= 0.001). However, the level of evidence was low (due to a high degree of between-study heterogeneity) and the FPRP was high at both prior probability levels.

In contrast, the meta-analysis of five datasets (two from a GWAS) composed of 21,366 subjects (Asian ancestry only) showed the G allele of rs2274223 to be associated (with a strong level of evidence) with an increased risk of cardia subtype gastric cancer (summary OR: 1.565, 95%CI: 1.486-1.648, P-value<1.00E-20) with a low FPRP at both prior

probability level. When the only Caucasian study is included (54), this association is still highly significant (summary OR: 1.539, 95%CI: 1.386-1.709, P-value: 8.88E-16) but the level of evidence becomes low due to between-study heterogeneity. Interestingly, rs2274223 was not associated with non-cardia gastric cancer with a high level of evidence, which suggests that this SNP is specifically linked to the carcinomas originating in the cardia region of the stomach.

In the present work, data on other four PLCE1 variants were meta-analyzed (rs11187842, rs3765524, rs3781264 and rs753724), with only one of them significantly associated with disease risk (rs3765524), albeit with a low level of summary evidence.

TGFB2 rs3087465

This G>A SNP is located in the promoter region of TGFB2 (chromosome 3p22), which encodes the transforming growth factor beta receptor 2, a transmembrane Ser/Thr protein kinase protein that forms a heterodimeric complex with TGFBR1 and binds TGFB ligands. In turn, this mediates a variety of physiological and pathological processes including cell cycle arrest in epithelial and hematopoietic cells, control of mesenchymal cell proliferation and differentiation, wound healing, extracellular matrix production, immunosuppression, epithelial-mesenchymal transition, as well as carcinogenesis in different tumor models (55,56), including gastric cancer (57,58).

The meta-analysis of the four available datasets (subjects: 4,670; Asian ancestry only) showed a highly significant association between the minor allele (A) of rs3087465 and a decreased risk of gastric cancer (summary OR: 0.691, 95%CI: 0.620-0.769, P-value= 1.54E-11). This evidence was of a high level and the FPRP was low at the higher prior probability level. No subgroup analysis could be performed due to the lack of permissive data.

GSTP1 rs1695

This A>G SNP is located in exon 5 of GSTP1 (chromosome 11q13) and causes a missense variation (Ile>Val) in the encoded protein glutathione S-transferase pi-1, which leads to a lower activity of the enzyme. GSTP1 belongs to a family of enzymes that play a key role in detoxification of a variety of endogenous and exogenous compounds by catalyzing their conjugation with reduced glutathione (59). Given its importance in the maintenance of integrity of different cell components (e.g. membranes and DNA), GSTP1 has been extensively investigated in the field of carcinogenesis (60).

The primary meta-analysis of all 20 datasets (subjects: 11,258; both Asian and Caucasian ancestry) showed no association between the minor allele (G) of rs1695 and gastric cancer risk, with low level evidence (due to between-study heterogeneity). However, when the meta-analysis was performed separately in Asian and Caucasian subjects, a significant association of high quality was observed only in the former subgroup (summary OR: 1.191, 95%CI: 1.092-1.299, P-value: 7.88E-05), with a low FPRP at the higher prior probability level, with no association being demonstrated in the other subgroups.

CASP8 rs3834129

This variant consists of a 6-nucleotide deletion in the promoter region of CASP8 (chromosome 2q33), which leads to a reduced gene transcription. The gene product, named caspase-8, is a cysteine-aspartic acid protease and is a pivotal mediator of programmed cell death. In brief, once activated by death receptors, it activates both caspase-3 (which in turn activates enzymes with DNase activity) and Bid (which initiates the mitochondrial apoptotic pathway) (61). Given the importance of malignant cell escape from apoptosis, the role of CASP8 in carcinogenesis has been extensively investigated in different tumor models (62-65). The meta-analysis of the three datasets (n=1,701; both Asian and Caucasian ancestry) revealed that the minor allele (deletion) of rs3834129 is associated, with a high level of evidence, with a decreased risk of gastric cancer (summary OR: 0.732, 95%CI: 0.617-0.869, P-value: 3.48E-04), although the FPRP was high at both prior probability levels. Available data did not enable us to perform subgroup analysis.

TNF rs1799724

This C>T SNP is located in the promoter region of the TNF gene (chromosome 6p21.3), which encodes a TNF superfamily member known as tumor necrosis factor. This is a pro-inflammatory cytokine that plays an important role in the immune response to infectious agents but is also involved in the pathogenesis of autoimmune diseases (66) and presents pleiotropic functions in cancer development and progression (67,68).

Primary meta-analysis of the ten available datasets (n=5,953; both Asian and Caucasian ancestry) showed a significant association between the minor allele (T) of rs1799724 and an increased risk of gastric cancer (summary OR: 1.173, 95%CI:1.047-1.314, P-value: 0.006), characterized by a high level of evidence but also by a high FPRP at both prior probability levels. This association remained significant when the meta-analysis was restricted to Asian subjects (intermediate level of evidence), but not when it was restricted to

the other two subgroups (Caucasian subjects and non-cardia subtype) for which data were available (low level of evidence).

Another four TNF polymorphisms (rs1800629, rs1799964, rs1800630 and rs361525) have been meta-analyzed in this synopsis, but only one (rs1800629) was significantly associated to gastric cancer risk with an intermediate level of evidence.

Non-significant associations

Among 335 non-significant primary or subgroup meta-analyses, the level of evidence for lack of association was high, intermediate or low for 5 (1.5%), 21 (6.3%) and 309 (92.2%) analyses, respectively (**Supplementary Table 2**). The most frequent single cause of non-high level of evidence was insufficient statistical power (130/330), followed by between-study heterogeneity (71/330).

The details of non-significant associations characterized by a high or intermediate level of evidence are reported in **Table 3**.

Discussion

In this article, we describe the results of the first systematic synopsis and meta-analysis (with evaluation of the quality of the cumulative evidence) in the field of genetic predisposition to sporadic gastric carcinoma. We found that out of 2,841 variants investigated, 11 polymorphisms in ten genes were associated with susceptibility to gastric cancer in general or to the susceptibility of specific subgroups identified by ancestry (Asian vs Caucasian), primary tumor site (cardia vs non-cardia) and histological subtype (intestinal vs diffuse), with a high level of evidence. Of note, for seven of these 11 variants (MUC1 rs2070803, MTX1 rs2075570, PKLR rs3762272, PRKAA1 rs13361707, TGFBR2 rs3087465, CASP8 rs3834129, TNF rs1799724) this is the first time that a meta-analysis confirms their association with risk of developing gastric carcinoma.

These findings provide investigators with a robust platform of genetic information useful to elucidate the molecular mechanisms underlying the pathogenesis of gastric carcinoma. For instance, our data support the hypothesis that the genetic information contained in chromosome 1 might play a predominant role in the susceptibility of this disease: in fact, the highest number of statistically significant contrasts with a high or intermediate level of evidence are located in this chromosome (16/49, 32.6%). Interestingly, these twelve variants are located in the long chromosomal arm and nine of them belong to a region (chromosomal band 1q21-22) repeatedly reported to be affected by chromosomal aberrations (e.g. copy number variations) in a range of tumors (such as breast (69,70), ovarian (70), hepatocellular (71,72), lung (73), esophageal (74), cervical (75) and oral (76) carcinomas, neuroblastoma (77), and liposarcoma (78)), including stomach sporadic carcinoma (79). Moreover, GWAS have recently identified this region as a susceptibility locus for cutaneous melanoma (80) and testicular germ cell tumor (81). Therefore, this region of the genome represents a promising target for more extensive investigations in the field of gastric cancer research. On the other side, this synopsis also points out DNA regions scarcely investigated in the literature and thus worth new efforts.

By arranging our data upon (main) gene function, another interesting observation is that the majority of statistically significant results with high or intermediate level of evidence regard variants of genes involved in immunity/inflammation or adhesion/invasiveness (24/49, 48.9%). This finding is in line with the pivotal role of HP infection in the development of gastric cancer (10). In particular, our data draw the attention to polymorphisms that might

explain the still enigmatic relationship between the high prevalence of HP infection in the general population (up to 70% (82)) and the relatively low gastric cancer incidence (up to 50 cases/100,000/year (1)) (11). Unfortunately, the scarcity of published data on the interaction between HP status and DNA variants only allowed us to perform 20 HP specific subgroup meta-analyses (out of 456 meta-analyses) and did not enable us to yield any meaningful results.

The 11 common low-penetrance variants identified by our meta-analysis for their strong evidence of association with gastric cancer could also be used to set up prediction algorithms for stratifying disease risk within the frame of targeted screening programs (83-87).

Since it is well recognized that each genetic locus confers a small contribution to the overall risk of a polygenic disease such as cancer, it is daunting to consider any single polymorphism as a test for routine clinical use (83). In our synopsis, the mean PAR of the 11 high-quality biomarkers was 18%. Nevertheless, their combination can raise the fraction of the disease burden attributable to genetic variation and thus improve the predictive power of tests based on genetic profiles, as suggested for other tumors such as breast (84), colorectal (85) and prostate cancer (86).

In this regard, haplotype studies have already shown the potential of polymorphism combination in the identification of subjects at high risk of gastric carcinoma (88-90) by showing odds ratios higher than 2 (or lower than 0.5) and PAR up to 60%. However, the published data did not enable us to perform any meta-analysis since a minimum of three independent datasets was not available for any of the haplotypes studied so far.

To provide readers with an idea of the predictive potential of combining multiple polymorphisms, we calculated four joint-PAR (using seven variants unrelated to one other as defined by a pairwise correlation coefficient $r^2 < 0.1$), whose values ranged from 21.3% through 51.2%. Of note, these joint-PAR would lead to a number needed to screen (number of people who must be tested to prevent one case of gastric carcinoma (91)) that varies from 22 through 990, depending also on the lifetime risk of disease (range: 1% to 5%) (**Table 2**).

In this synopsis, other interesting results came from subgroup meta-analysis. First, we confirmed the difference between the results obtained in populations of different ancestry (see **Supplementary Information** and **Supplementary Figures 4** and **5**), which support the hypothesis that the molecular pathway to gastric cancer susceptibility is not necessarily the same across different ethnicities and underscore the need for race specific risk prediction

tools. Moreover, our results also show some evidence as regards the genetic pathways specific for some gastric cancer subtypes (see **Supplementary Information** and **Supplementary Figures 6** and **7**). Considering the different epidemiology and aggressiveness of different subsets of the disease (8,92), these subgroup specific findings might help elucidate the molecular mechanisms underlying not only the development but also the progression of gastric cancer subtypes.

As regards the already existing literature, the past decade has witnessed the publication of a growing number of systematic reviews and meta-analyses addressing genetic predisposition to a variety of tumor types (93,94). In the field of stomach cancer, we found 243 such type of publications, which almost always take into consideration single polymorphisms, polymorphisms of single genes or variants of genes belonging to the same cell pathway. In a single article, published in 2009, the authors extend their analysis to 225 variants across 95 genes by collecting the data from 203 primary studies, and report the findings of 61 primary or subgroup meta-analyses (the only subgrouping variable being ethnicity) (95). A previous similar work, published in 2002, gathered a much lower number of primary studies (n=31) (96). Finally, other investigators have reviewed the meta-analyses regarding the association between genetic variations and gastric cancer (97) or different tumor types including gastric cancer (14).

The present work - which outnumbers the previously published literature by number of articles retrieved, variants investigated, meta-analyses performed, subgroups considered and subjects enrolled - is characterized not only by a truly comprehensive search of the literature in this field, but also by the effort to grade the quality of the cumulative evidence based on criteria specifically set up by a panel of international experts (i.e. Venice criteria). Moreover, for each statistically significant meta-analysis, we calculated the FPRP in order to further increase the information provided to readers on the reliability of the findings generated by our analysis. This way, we provide an updated and more critical summary of the available evidence compared to the already existing literature in this field.

We must also recognize the limits of this synopsis. Firstly, despite our efforts to be comprehensive and accurate, we might have missed some relevant articles and may have included partially overlapping series.

Secondly, some series have been utilized for more than one meta-analysis, which potentially raises the statistical issue of type I error inflation. However, following the implementation of

the FPRP method, this occurrence should be minimized; moreover, even after Bonferroni correction for multiple testing, all 11 high quality biomarkers identified by our meta-analysis would remain statistically significant.

Thirdly, we considered only one genetic model (i.e. additive, which generates per allele odds ratios), as explained in the Methods section. The aim of this work was not to define the best genetic model for some specific well established biomarkers but rather to quantitatively summarize the evidence regarding hundreds of variants, a job that is best done using a conservative approach such as the additive model. In addition, no genotype data are available for a large number of variants. Also, the use of different models in primary and subgroup meta-analyses would have approximately tripled the number of meta-analyses, which would have in turn amplified the issue of type I error inflation.

Fourthly, as previously mentioned, the paucity of data on gene-environment interactions did not allow us to thoroughly investigate potential sources of between-study heterogeneity, with special regard to HP infection status. For other potential effect modifiers, such as smoking, alcohol and dietary habits (though they are less important than HP infection (8,9)), available data were so scarce that we could not even take them into consideration. Although some articles reported odds ratio values adjusted by one or more confounding factors, this was not always the case. The lack of data, coupled with the fact that adjusting covariates of multivariable logistic regression analysis differed in most circumstances, prevented us from implementing this information in our analyses, which are exclusively based on raw (unadjusted) odds ratios.

Finally, we must remind readers that some polymorphisms (e.g. those located on chromosome 1q22) we found associated with gastric cancer risk are in strong linkage disequilibrium with each other, which might imply they actually represent a single signal; moreover, some of our result interpretation - such as the clues on genes involved in the molecular pathways leading to gastric carcinogenesis - rely on the a priori assumption that the relevant gene of extra-genic variants is the nearest gene. Clearly, fine mapping and functional studies are required to identify the most likely causal variants and the genes they control before any predictive genetic tool is set up and any therapeutic target is considered, which highlights once again the burden of work still to be done in this field.

In conclusion, we performed the first systematic review and meta-analysis of the evidence linking DNA variation to the development of gastric carcinoma. We hope that this unprecedented collection of data might represent a useful platform for investigators involved

in this research field not only for designing future studies but also to create a continuously updated databank dedicated to the genetic basis of this disease, a long standing gap in the initiatives of the international scientific community.

Figure 1 legend

Genetic variants statistically significantly associated with risk of gastric carcinoma (with a high or intermediate level of evidence according to the Venice criteria) arranged by (main) gene function.

References

- 1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2014.
- 2 Hartgrink HH, Jansen EP, van Grieken NC, van de Velde CJ. Gastric cancer. *Lancet* 2009;374:477-90.
- 3 Wadhwa R, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol* 2013;10:643-55.
- 4 Wang K, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet* 2014;46:573-82.
- 5 Figueiredo C, Garcia-Gonzalez MA, Machado JC. Molecular pathogenesis of gastric cancer. *Helicobacter* 2013;18 Suppl 1:28-33.
- 6 McLean MH, El-Omar EM. Genetics of gastric cancer. *Nat Rev Gastroenterol Hepatol* 2014;11:664-74.
- 7 Tan IB, Ng I, Tai WM, Tan P. Understanding the genetic basis of gastric cancer: recent advances. *Expert Rev Gastroenterol Hepatol* 2012;6:335-41.
- 8 de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. *Gastroenterol Clin North Am* 2013;42:219-40.
- 9 Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* 2003;56:1-9.
- 10 Polk DB, Peek RM, Jr. Helicobacter pylori: gastric cancer and beyond. *Nat Rev Cancer* 2010;10:403-14.
- 11 Miwa H, Go MF, Sato N. H. pylori and gastric cancer: the Asian enigma. *Am J Gastroenterol* 2002;97:1106-12.
- 12 Zabaleta J. Multifactorial etiology of gastric cancer. *Methods Mol Biol* 2012;863:411-35.
- 13 Frazer KA, Murray SS, Schork NJ, Topol EJ. Human genetic variation and its contribution to complex traits. *Nat Rev Genet* 2009;10:241-51.
- 14 Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *Jama* 2008;299:2423-36.

- 15 Bernig T, Chanock SJ. Challenges of SNP genotyping and genetic variation: its future role in diagnosis and treatment of cancer. *Expert Rev Mol Diagn* 2006;6:319-31.
- 16 Corso G, Figueiredo J, Biffi R, Trentin C, Bonanni B, Feroce I, et al. E-cadherin germline mutation carriers: clinical management and genetic implications. *Cancer Metastasis Rev* 2014.
- 17 Little J, Higgins J. The HuGENet™ HuGE Review Handbook, version 1.0; 2006.
- 18 Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, Vineis P, et al. Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol* 2008;37:120-32.
- 19 Sagoo GS, Little J, Higgins JP. Systematic reviews of genetic association studies. Human Genome Epidemiology Network. *PLoS Med* 2009;6:e28.
- 20 Mocellin S, Rossi CR. The melanoma molecular map project. *Melanoma Res* 2008;18:163-5.
- 21 Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. *Stat Med* 2005;24:1291-306.
- 22 Zhang B, Beeghly-Fadiel A, Long J, Zheng W. Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. *Lancet Oncol* 2011;12:477-88.
- 23 Chatzinasiou F, Lill CM, Kypreou K, Stefanaki I, Nicolaou V, Spyrou G, et al. Comprehensive field synopsis and systematic meta-analyses of genetic association studies in cutaneous melanoma. *J Natl Cancer Inst* 2011;103:1227-35.
- 24 Theodoratou E, Montazeri Z, Hawken S, Allum GC, Gong J, Tait V, et al. Systematic meta-analyses and field synopsis of genetic association studies in colorectal cancer. *J Natl Cancer Inst* 2012;104:1433-57.
- 25 Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 2004;96:434-42.
- 26 Sakamoto H, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, et al. Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet* 2008;40:730-40.
- 27 Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, et al. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet* 2010;42:764-7.

- 28 Shi Y, Hu Z, Wu C, Dai J, Li H, Dong J, et al. A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. *Nat Genet* 2011;43:1215-8.
- 29 Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends Mol Med* 2014;20:332-42.
- 30 Saeki N, Sakamoto H, Yoshida T. Mucin 1 gene (MUC1) and gastric-cancer susceptibility. *Int J Mol Sci* 2014;15:7958-73.
- 31 Silva F, Carvalho F, Peixoto A, Seixas M, Almeida R, Carneiro F, et al. MUC1 gene polymorphism in the gastric carcinogenesis pathway. *Eur J Hum Genet* 2001;9:548-52.
- 32 Kottgen A, Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet* 2013;45:145-54.
- 33 Saeki N, Saito A, Choi IJ, Matsuo K, Ohnami S, Totsuka H, et al. A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. *Gastroenterology* 2011;140:892-902.
- 34 Wang X, Ono K, Kim SO, Kravchenko V, Lin SC, Han J. Metaxin is required for tumor necrosis factor-induced cell death. *EMBO Rep* 2001;2:628-33.
- 35 Cartron PF, Petit E, Bellot G, Oliver L, Vallette FM. Metaxins 1 and 2, two proteins of the mitochondrial protein sorting and assembly machinery, are essential for Bak activation during TNF alpha triggered apoptosis. *Cell Signal* 2014;26:1928-34.
- 36 van Wijk R, van Solinge WW, Nerlov C, Beutler E, Gelbart T, Rijksen G, et al. Disruption of a novel regulatory element in the erythroid-specific promoter of the human PKLR gene causes severe pyruvate kinase deficiency. *Blood* 2003;101:1596-602.
- 37 Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009;324:1029-33.
- 38 Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, et al. Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci U S A* 1998;95:1735-40.
- 39 Yang X, Guo Z, Liu Y, Si T, Yu H, Li B, et al. Prostate stem cell antigen and cancer risk, mechanisms and therapeutic implications. *Expert Rev Anticancer Ther* 2014;14:31-7.

- 40 Saeki N, Gu J, Yoshida T, Wu X. Prostate stem cell antigen: a Jekyll and Hyde molecule? *Clin Cancer Res* 2010;16:3533-8.
- 41 Zuo L, Zhang LF, Wu XP, Zhou ZX, Zou JG, He J, et al. Association of a common genetic variant in prostate stem cell antigen with cancer risk. *Arch Med Sci* 2014;10:425-33.
- 42 Zhang QH, Yao YL, Gu T, Gu JH, Chen L, Liu Y. Association of the PSCA rs2294008 C>T polymorphism with gastric cancer risk: evidence from a meta-analysis. *Asian Pac J Cancer Prev* 2012;13:2867-71.
- 43 Wang T, Zhang L, Li H, Wang B, Chen K. Prostate stem cell antigen polymorphisms and susceptibility to gastric cancer: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2012;21:843-50.
- 44 Gu X, Zhang W, Xu L, Cai D. Quantitative assessment of the influence of prostate stem cell antigen polymorphisms on gastric cancer risk. *Tumour Biol* 2014;35:2167-74.
- 45 Zhao Y, Gui ZL, Liao S, Gao F, Ge YZ, Jia RP. Prostate stem cell antigen rs2294008 (C>T) polymorphism and bladder cancer risk: a meta-analysis based on cases and controls. *Genet Mol Res* 2014;13:5534-40.
- 46 Krishan S, Richardson DR, Sahni S. AMP kinase (PRKAA1). *J Clin Pathol* 2014.
- 47 Chen S, Zhu X, Lai X, Xiao T, Wen A, Zhang J. Combined Cancer Therapy with Non-Conventional Drugs: All Roads Lead to AMPK. *Mini Rev Med Chem* 2014;14:642-54.
- 48 van Veelen W, Korsse SE, van de Laar L, Peppelenbosch MP. The long and winding road to rational treatment of cancer associated with LKB1/AMPK/TSC/mTORC1 signaling. *Oncogene* 2011;30:2289-303.
- 49 Song HR, Kim HN, Kweon SS, Choi JS, Shim HJ, Cho SH, et al. Genetic variations in the PRKAA1 and ZBTB20 genes and gastric cancer susceptibility in a Korean population. *Mol Carcinog* 2013;52 Suppl 1:E155-60.
- 50 Bunney TD, Baxendale RW, Katan M. Regulatory links between PLC enzymes and Ras superfamily GTPases: signalling via PLCepsilon. *Adv Enzyme Regul* 2009;49:54-8.
- 51 Chen J, Wang W, Zhang T, Ji J, Qian Q, Lu L, et al. Differential expression of phospholipase C epsilon 1 is associated with chronic atrophic gastritis and gastric cancer. *PLoS One* 2012;7:e47563.

- 52 Yan F, Fu Q. PLCepsilon1: a potential target of RNA interference therapy for gastric cancer. *Biochem Biophys Res Commun* 2014;448:409-13.
- 53 Martins M, McCarthy A, Baxendale R, Guichard S, Magno L, Kessaris N, et al. Tumor suppressor role of phospholipase C epsilon in Ras-triggered cancers. *Proc Natl Acad Sci U S A* 2014;111:4239-44.
- 54 Palmer AJ, Lochhead P, Hold GL, Rabkin CS, Chow WH, Lissowska J, et al. Genetic variation in C20orf54, PLCE1 and MUC1 and the risk of upper gastrointestinal cancers in Caucasian populations. *Eur J Cancer Prev* 2012;21:541-4.
- 55 Kaklamani VG, Pasche B. Role of TGF-beta in cancer and the potential for therapy and prevention. *Expert Rev Anticancer Ther* 2004;4:649-61.
- 56 Principe DR, Doll JA, Bauer J, Jung B, Munshi HG, Bartholin L, et al. TGF-beta: duality of function between tumor prevention and carcinogenesis. *J Natl Cancer Inst* 2014;106:djt369.
- 57 Achyut BR, Yang L. Transforming growth factor-beta in the gastrointestinal and hepatic tumor microenvironment. *Gastroenterology* 2011;141:1167-78.
- 58 Mishra L, Shetty K, Tang Y, Stuart A, Byers SW. The role of TGF-beta and Wnt signaling in gastrointestinal stem cells and cancer. *Oncogene* 2005;24:5775-89.
- 59 Vasieva O. The many faces of glutathione transferase pi. *Curr Mol Med* 2011;11:129-39.
- 60 Tew KD, Manevich Y, Grek C, Xiong Y, Uys J, Townsend DM. The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer. *Free Radic Biol Med* 2011;51:299-313.
- 61 Kantari C, Walczak H. Caspase-8 and bid: caught in the act between death receptors and mitochondria. *Biochim Biophys Acta* 2011;1813:558-63.
- 62 Fulda S. Caspase-8 in cancer biology and therapy. *Cancer Lett* 2009;281:128-33.
- 63 Stupack DG. Caspase-8 as a therapeutic target in cancer. *Cancer Lett* 2013;332:133-40.
- 64 Chen D, Ma T, Liu XW, Liu Z. CASP-8 -652 6N ins/del polymorphism and cancer risk: A literature-based systematic HuGE review and meta-analysis. *Exp Ther Med* 2012;4:762-70.
- 65 Yin M, Yan J, Wei S, Wei Q. CASP8 polymorphisms contribute to cancer susceptibility: evidence from a meta-analysis of 23 publications with 55 individual studies. *Carcinogenesis* 2010;31:850-7.

- 66 Mackay F, Kalled SL. TNF ligands and receptors in autoimmunity: an update. *Curr Opin Immunol* 2002;14:783-90.
- 67 Balkwill F. TNF-alpha in promotion and progression of cancer. *Cancer Metastasis Rev* 2006;25:409-16.
- 68 Mocellin S, Rossi CR, Pilati P, Nitti D. Tumor necrosis factor, cancer and anticancer therapy. *Cytokine Growth Factor Rev* 2005;16:35-53.
- 69 Parris TZ, Danielsson A, Nemes S, Kovacs A, Delle U, Fallenius G, et al. Clinical implications of gene dosage and gene expression patterns in diploid breast carcinoma. *Clin Cancer Res* 2010;16:3860-74.
- 70 Cheng KW, Lahad JP, Kuo WL, Lapuk A, Yamada K, Auersperg N, et al. The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers. *Nat Med* 2004;10:1251-6.
- 71 Wong N, Chan A, Lee SW, Lam E, To KF, Lai PB, et al. Positional mapping for amplified DNA sequences on 1q21-q22 in hepatocellular carcinoma indicates candidate genes over-expression. *J Hepatol* 2003;38:298-306.
- 72 Skawran B, Steinemann D, Weigmann A, Flemming P, Becker T, Flik J, et al. Gene expression profiling in hepatocellular carcinoma: upregulation of genes in amplified chromosome regions. *Mod Pathol* 2008;21:505-16.
- 73 Goeze A, Schluns K, Wolf G, Thasler Z, Petersen S, Petersen I. Chromosomal imbalances of primary and metastatic lung adenocarcinomas. *J Pathol* 2002;196:8-16.
- 74 Chattopadhyay I, Singh A, Phukan R, Purkayastha J, Kataki A, Mahanta J, et al. Genome-wide analysis of chromosomal alterations in patients with esophageal squamous cell carcinoma exposed to tobacco and betel quid from high-risk area in India. *Mutat Res* 2010;696:130-8.
- 75 Narayan G, Bourdon V, Chaganti S, Arias-Pulido H, Nandula SV, Rao PH, et al. Gene dosage alterations revealed by cDNA microarray analysis in cervical cancer: identification of candidate amplified and overexpressed genes. *Genes Chromosomes Cancer* 2007;46:373-84.
- 76 Vincent-Chong VK, Anwar A, Karen-Ng LP, Cheong SC, Yang YH, Pradeep PJ, et al. Genome wide analysis of chromosomal alterations in oral squamous cell carcinomas revealed over expression of MGAM and ADAM9. *PLoS One* 2013;8:e54705.
- 77 Diskin SJ, Hou C, Glessner JT, Attiyeh EF, Laudenslager M, Bosse K, et al. Copy number variation at 1q21.1 associated with neuroblastoma. *Nature* 2009;459:987-91.

- 78 Forus A, Larramendy ML, Meza-Zepeda LA, Bjerkehagen B, Godager LH, Dahlberg AB, et al. Dedifferentiation of a well-differentiated liposarcoma to a highly malignant metastatic osteosarcoma: amplification of 12q14 at all stages and gain of 1q22-q24 associated with metastases. *Cancer Genet Cytogenet* 2001;125:100-11.
- 79 Larramendy ML, el-Rifai W, Kokkola A, Puolakkainen P, Monni O, Salovaara R, et al. Comparative genomic hybridization reveals differences in DNA copy number changes between sporadic gastric carcinomas and gastric carcinomas from patients with hereditary nonpolyposis colorectal cancer. *Cancer Genet Cytogenet* 1998;106:62-5.
- 80 Macgregor S, Montgomery GW, Liu JZ, Zhao ZZ, Henders AK, Stark M, et al. Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. *Nat Genet* 2011;43:1114-8.
- 81 Ruark E, Seal S, McDonald H, Zhang F, Elliot A, Lau K, et al. Identification of nine new susceptibility loci for testicular cancer, including variants near DAZL and PRDM14. *Nat Genet* 2013;45:686-9.
- 82 Peleteiro B, Bastos A, Ferro A, Lunet N. Prevalence of Helicobacter pylori infection worldwide: a systematic review of studies with national coverage. *Dig Dis Sci* 2014;59:1698-709.
- 83 Khoury MJ, Yang Q, Gwinn M, Little J, Dana Flanders W. An epidemiologic assessment of genomic profiling for measuring susceptibility to common diseases and targeting interventions. *Genet Med* 2004;6:38-47.
- 84 Pharoah PD, Antoniou AC, Easton DF, Ponder BA. Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med* 2008;358:2796-803.
- 85 Hawken SJ, Greenwood CM, Hudson TJ, Kustra R, McLaughlin J, Yang Q, et al. The utility and predictive value of combinations of low penetrance genes for screening and risk prediction of colorectal cancer. *Hum Genet* 2010;128:89-101.
- 86 Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, et al. Cumulative association of five genetic variants with prostate cancer. *N Engl J Med* 2008;358:910-9.
- 87 Furberg AH, Ambrosone CB. Molecular epidemiology, biomarkers and cancer prevention. *Trends Mol Med* 2001;7:517-21.
- 88 Duraes C, Munoz X, Bonet C, Garcia N, Vencesla A, Carneiro F, et al. Genetic variants in the IL1A gene region contribute to intestinal-type gastric carcinoma susceptibility in European populations. *Int J Cancer* 2014;135:1343-55.

- 89 Li B, Liu HY, Guo SH, Sun P, Gong FM, Jia BQ. M113 genetic variants affect risk of gastric cancer in the chinese han population. *Asian Pac J Cancer Prev* 2013;14:4239-42.
- 90 Companioni O, Bonet C, Munoz X, Weiderpass E, Panico S, Tumino R, et al. Polymorphisms of Helicobacter pylori signaling pathway genes and gastric cancer risk in the European Prospective Investigation into Cancer-Eurgast cohort. *Int J Cancer* 2014;134:92-101.
- 91 Vineis P, Schulte P, McMichael AJ. Misconceptions about the use of genetic tests in populations. *Lancet* 2001;357:709-12.
- 92 Crew KD, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006;12:354-62.
- 93 Boccia S, De Feo E, Galli P, Gianfagna F, Amore R, Ricciardi G. A systematic review evaluating the methodological aspects of meta-analyses of genetic association studies in cancer research. *Eur J Epidemiol* 2010;25:765-75.
- 94 Ntzani EE, Khoury MJ, Ioannidis JP. Combining molecular and genetic data from different sources. *IARC Sci Publ* 2011:323-36.
- 95 Loh M, Koh KX, Yeo BH, Song CM, Chia KS, Zhu F, et al. Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. *Eur J Cancer* 2009;45:2562-8.
- 96 Gonzalez CA, Sala N, Capella G. Genetic susceptibility and gastric cancer risk. *Int J Cancer* 2002;100:249-60.
- 97 Gianfagna F, De Feo E, van Duijn CM, Ricciardi G, Boccia S. A systematic review of meta-analyses on gene polymorphisms and gastric cancer risk. *Curr Genomics* 2008;9:361-74.

Table 1. Meta-analysis results: genetic variants significantly associated with gastric cancer risk with a high or intermediate level of summary evidence

Gene	Variant ID	Chr	Risk Allele	Datasets	Cases	Controls	Subgroup	sOR	LL	UL	P-value	Venice criteria	Level of Evidence	FPRP (10E-3)	FPRP (10E-6)
MUC1	rs2070803	1	A	6	2376	4903	Diffuse	0.585	0.526	0.650	<1.00E-20	AAA	High	0.00	0.00
MTX1	rs2075570	1	G	5	2299	4603	Diffuse	0.587	0.525	0.655	<1.00E-20	AAA	High	0.00	0.00
PRKAA1	rs13361707	5	C	5	7587	7891	Non-cardia	1.406	1.334	1.482	<1.00E-20	AAA	High	0.00	0.00
PLCE1	rs2274223	10	G	5	4869	16497	Cardia	1.565	1.486	1.648	<1.00E-20	AAA	High	0.00	0.00
PSCA	rs2294008	8	T	7	5690	6975	Non-cardia	1.329	1.254	1.408	<1.00E-20	AAA	High	0.00	0.00
TGFB2	rs3087465	3	A	4	2323	2347	Asian	0.691	0.620	0.769	1.54E-11	AAA	High	0.00	0.53
PKLR	rs3762272	1	G	3	1362	3101	Diffuse	0.710	0.631	0.799	1.42E-08	AAA	High	0.03	0.97
PSCA	rs2976392	8	G	4	2602	4443	Intestinal	0.808	0.751	0.870	1.42E-08	AAA	High	0.00	0.36
GSTP1	rs1695	11	G	11	2425	4731	Asian	1.191	1.092	1.299	7.88E-05	AAA	High	0.12	0.99
CASP8	rs3834129	2	del	3	616	1085	Mixed	0.732	0.617	0.869	3.48E-04	AAA	High	0.93	1.00
TNF	rs1799724	6	T	10	2136	3817	Mixed	1.173	1.047	1.314	0.006	AAA	High	0.89	1.00
PSCA	rs2294008	8	T	13	7380	26388	Intestinal	1.284	1.206	1.367	5.33E-15	ABA	Intermediate	0.00	0.00
TRIM46	rs3814316	1	C	3	1362	3101	Diffuse	0.582	0.506	0.670	3.71E-14	AAB	Intermediate	0.00	0.79
THBS3	rs2066981	1	C	3	1362	3101	Diffuse	0.585	0.507	0.674	1.57E-13	AAB	Intermediate	0.00	0.22
ABO	rs8176719	9	A (vs O)	15	8737	1069256	Caucasian	1.225	1.161	1.293	1.92E-13	ABA	Intermediate	0.00	0.00
MUC1	rs2070803	1	A	6	3886	4903	Asian	0.660	0.590	0.738	2.55E-13	ABA	Intermediate	0.00	0.01
KRTCAP2	rs4971088	1	T	3	1362	3101	Diffuse	0.608	0.526	0.703	1.60E-11	AAB	Intermediate	0.02	0.96
MTX1	rs2075570	1	G	5	3586	4603	Asian	0.672	0.596	0.759	1.44E-10	ABA	Intermediate	0.01	0.90
PPARG	rs1805192	3	G	5	546	903	Mixed	2.265	1.695	3.027	3.20E-08	BAA	Intermediate	0.78	1.00
PPARG	rs1805192	3	G	3	227	598	Caucasian	2.149	1.536	3.007	8.03E-06	BAA	Intermediate	0.96	1.00
PSCA	rs2294008	8	T	4	1187	3162	Caucasian	1.337	1.177	1.519	8.51E-06	ABA	Intermediate	0.45	0.99
PSCA	rs2976391	8	A	4	1921	2504	Asian	0.725	0.625	0.840	2.00E-05	ABA	Intermediate	0.71	1.00
FAM189B	rs2072647	1	G	3	1362	3101	Diffuse	0.727	0.626	0.844	2.82E-05	ABA	Intermediate	0.44	0.99
PTGS2	rs20417	1	C	6	1464	3000	Asian	1.773	1.356	2.318	2.85E-05	BBB	Intermediate	0.92	1.00
THBS3	rs2066981	1	C	3	2649	3101	Asian	0.721	0.615	0.845	5.63E-05	ABA	Intermediate	0.60	0.99
IL1B	rs1143634	2	T	4	434	450	HP positive	1.721	1.321	2.243	5.84E-05	BAB	Intermediate	0.93	1.00
IL17F	rs763780	6	G	4	1760	2362	Asian	1.288	1.138	1.458	6.28E-05	AAB	Intermediate	0.63	0.99
YY1AP1	rs3738590	1	T	3	1362	3101	Diffuse	0.750	0.651	0.864	6.78E-05	ABA	Intermediate	0.77	1.00
KRTCAP2	rs4971088	1	T	3	2649	3101	Asian	0.725	0.618	0.852	9.04E-05	ABA	Intermediate	0.87	1.00

TNF	rs1800629	6	A	38	7896	12982	Mixed	1.166	1.074	1.267	2.67E-04	ABB	Intermediate	0.26	0.99
TLR4	rs4986790	9	G	3	774	1129	Non-cardia	2.022	1.351	3.026	6.17E-04	BBA	Intermediate	0.99	1.00
MDM2	rs2279744	12	G	4	778	2272	Cardia	1.384	1.133	1.690	0.001	ABB	Intermediate	0.97	1.00
TLR4	rs4986790	9	G	8	1859	3276	Caucasian	1.475	1.159	1.878	0.002	BBB	Intermediate	0.98	1.00
TNF	rs1800629	6	A	19	3516	6604	Caucasian	1.187	1.065	1.322	0.002	ABA	Intermediate	0.76	1.00
IL1B	rs16944	2	T	9	644	4867	Cardia	1.199	1.066	1.349	0.003	AAB	Intermediate	0.83	1.00
IL8	rs4073	4	A	8	1058	2620	Intestinal	1.240	1.077	1.428	0.003	ABA	Intermediate	0.95	1.00
ABO	rs8176719	9	A (vs AB)	13	4696	620332	Caucasian	1.234	1.068	1.427	0.004	ABB	Intermediate	0.96	1.00
TNF	rs1799724	6	T	7	1503	2517	Asian	1.189	1.043	1.355	0.010	BAA	Intermediate	0.94	1.00
TLR4	rs4986790	9	G	9	1919	3438	Mixed	1.433	1.089	1.886	0.010	BBB	Intermediate	0.99	1.00
IL10	rs1800871	1	T	3	78	399	Diffuse	0.567	0.367	0.875	0.010	BAB	Intermediate	0.99	1.00
EPHX1	rs1051740	1	C	3	427	1401	Caucasian	1.236	1.049	1.456	0.011	BAB	Intermediate	0.98	1.00
IGFBP3	rs2854744	7	C	3	1100	1338	Mixed	1.181	1.026	1.360	0.020	BAB	Intermediate	0.98	1.00
TP53	rs17878362	17	ins	3	342	635	Mixed	1.373	1.051	1.793	0.020	BAB	Intermediate	0.99	1.00
PRKAA1	rs13361707	5	C	3	1113	5261	Cardia	1.177	1.025	1.350	0.020	AAB	Intermediate	0.97	1.00
TIMP2	rs2277698	17	T	3	692	685	Mixed	0.807	0.669	0.975	0.026	BAB	Intermediate	0.99	1.00
IL8	rs2227306	4	T	4	348	1040	Asian	1.225	1.017	1.476	0.032	BAB	Intermediate	0.99	1.00
TNF	rs1800629	6	A	7	1239	4520	Cardia	1.180	1.010	1.379	0.037	BAB	Intermediate	0.98	1.00
TNF	rs1800629	6	A	8	1338	3836	Diffuse	1.262	1.010	1.578	0.041	BBB	Intermediate	0.99	1.00
KIAA0907	rs625658	1	C	3	1362	3101	Diffuse	0.842	0.713	0.993	0.042	ABB	Intermediate	0.99	1.00

Chr: chromosome; sOR: summary odds ratio; LL: sOR 95% lower level; UL: sOR 95% upper level; Venice criteria: A (high), B (moderate), C (weak) credibility for 3 parameters (amount of evidence, heterogeneity and bias; see text for more details); level of evidence: overall level of summary evidence according to the Venice criteria; FPRP: false positive report probability (at two levels of prior probability, see text for more details)

Table 2: Combination of independent polymorphisms (pairwise $r^2 < 0.1$) to predict risk of gastric carcinoma.

Gene	Variant ID	Target	PAR (%)	Joint PAR (%)	Joint MAF	NNS (ltr: 1%)	NNS (ltr:5%)
PSCA	rs2294008	Non-cardia	15.3	28.8	0.24	666	133
PRKAA1	rs13361707	Non-cardia	16.0				
PLCE1	rs2274223	Asian	11.1	37.1	0.12	990	198
TGFBR2	rs3087465	Asian	26.4				
GSTP1	rs1695	Asian	3.9				
CASP8	rs3834129	Mixed	19.0	21.3	0.30	707	141
TNF	rs1799724	Mixed	2.9				
MUC1	rs2070803	Diffuse	36.5	51.2	0.05	424	22
PKLR	rs3762272	Diffuse	23.2				

PAR: population attributable risk; MAF: minor allele frequency (in controls); NNS: number needed to screen; ltr: life-time risk

Table 3. Meta-analysis results: variants with *non* significant association to gastric cancer risk with a high or intermediate level of summary evidence

Gene	Variant ID	Chr	Risk Allele	Datasets	Cases	Controls	Ethnicity	sOR	LL	UL	P-value	Power	Heterogeneity	Bias	Evidence
TGFB1	rs1800470	19	C	4	1011	1522	Caucasian	0.917	0.817	1.030	0.143	A	A	A	High
PLCE1	rs2274223	10	G	5	2078	5694	Non-cardia	1.067	0.977	1.165	0.151	A	A	A	High
IL4	rs2243250	5	T	8	1878	3098	Mixed	1.063	0.953	1.187	0.271	A	A	A	High
IL1B	rs16944	2	T	22	1725	7989	Diffuse	1.024	0.944	1.111	0.568	A	A	A	High
IL1B	rs1143627	2	C	14	1199	3429	Diffuse	1.103	1.000	1.217	0.050	B	A	B	Intermediate
GSTM1	wild/null	1	null	5	3081	3322	Non-cardia	1.090	0.998	1.190	0.056	A	A	B	Intermediate
IL10	rs1800871	1	T	15	2756	5075	Mixed	0.903	0.810	1.006	0.063	A	B	B	Intermediate
CD14	rs2569190	5	C	6	1231	2408	Non-cardia	1.119	0.977	1.281	0.105	B	B	B	Intermediate
GSTT1	wild/null	22	null	6	1736	3280	Intestinal	1.168	0.965	1.413	0.111	B	B	A	Intermediate
LTA	rs909253	6	G	16	3197	6459	Mixed	1.072	0.981	1.172	0.125	A	B	B	Intermediate
MIR196a2	rs11614913	12	C	4	1343	3440	Diffuse	0.937	0.854	1.029	0.174	B	A	A	Intermediate
FAS	rs2234767	10	A	7	1748	2695	Mixed	1.062	0.960	1.175	0.242	B	A	A	Intermediate
FAS	rs2234767	10	A	6	1634	2235	Asian	1.067	0.957	1.190	0.242	B	A	B	Intermediate
IL4	rs2070874	5	C	4	1423	2741	Mixed	0.908	0.742	1.110	0.346	B	B	B	Intermediate
PTPN11	rs12229892	12	A	3	1361	2740	Asian	0.960	0.875	1.054	0.394	B	A	A	Intermediate
CDH1	rs16260	16	A	8	1585	3701	Intestinal	0.951	0.841	1.075	0.424	B	A	A	Intermediate
KIAA0907	rs625658	1	C	3	2649	3101	Asian	0.945	0.816	1.094	0.448	B	B	A	Intermediate
IL10	rs1800896	1	G	9	1880	3614	Caucasian	0.964	0.854	1.088	0.555	A	B	B	Intermediate
MTHFR	rs1801131	1	C	13	3784	5029	Mixed	0.982	0.874	1.102	0.751	A	B	B	Intermediate
XRCC1	rs25487	19	A	16	3628	7866	Mixed	0.990	0.904	1.084	0.827	A	B	A	Intermediate
RUNX3	rs6672420	1	A	3	1322	2394	Asian	0.984	0.853	1.136	0.830	B	B	A	Intermediate
OGG1	rs1052133	3	G	14	2107	4223	Mixed	0.988	0.867	1.126	0.853	A	B	A	Intermediate
ERCC2	rs13181	19	C	8	1483	4251	Caucasian	1.007	0.922	1.101	0.876	B	A	B	Intermediate

Chr: chromosome; sOR: summary odds ratio; LL: sOR 95% lower level; UL: sOR 95% upper level; power, heterogeneity, bias: quality of evidence (A: high, B: moderate, C: weak) for 3 parameters (amount of evidence, heterogeneity and bias; see text for more details); evidence: overall level of summary evidence