The Barrett-associated variants at GDF7 and TBX5 also increase esophageal adenocarcinoma risk

Jessica Becker1,2, Andrea May3, Christian Gerges4, Mario Anders5,6, Claudia Schmidt7, Lothar Veits8, Tania Noder3, Rupert Mayershöfer3, Nicole Kreuser10, Hendrik Manner11, Marino Venerito12, Jan-Hinnerk Hofer13, Orestis Lyros10, Constantin J. Ahlbrand14, Michael Arras14, Sebastian Hofer14, Sophie K. M. Heinrichs1,2, Katharina Weise1,2, Timo Hess1,2, Anne C. Böhmer1,2, Nils Kosiol1,2, Ralf Kiesslich11, Jakob R. Izbicki15, Arnulf H. Hölscher7, Elfriede Bollschweiler7, Peter Malferttheiner12, Hauke Lang14, Markus Moehler16, Dietmar Lorenz17, Katja Ott18,19, Thomas Schmidt19, Markus M. Nöthen1,2, Andreas Hackelsberger20, Brigitte Schumacher4,21, Oliver Pech22, Yogesh Vashist15, Michael Vieth9, Josef Weismüller23, Michael Knapp24, Horst Neuhaus4, Thomas Rösch5, Christian Ell3, Ines Gockel10,a & Johannes Schumacher1,2,a

1Institute of Human Genetics, University of Bonn, Bonn, Germany
2Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany
3Department of Medicine II, Sana Klinikum, Offenbach, Germany
4Department of Internal Medicine II, Evangelisches Krankenhaus, Düsseldorf, Germany
5Department of Interdisciplinary Endoscopy, University Hospital Hamburg-Eppendorf, Hamburg, Germany
6Departments of Gastroenterology and Interdisciplinary Endoscopy, Vivantes Wenczech-Klinikum, Berlin, Germany
7Department of General, Visceral and Cancer Surgery, University of Cologne, Cologne, Germany
8Institute of Pathology, Klinikum Bayreuth, Bayreuth, Germany
9Gastroenterologie am Burgweier, Bonn, Germany
10Department of Visceral Transplant, Thoracic and Vascular Surgery, University Hospital of Leipzig, Leipzig, Germany
11Department of Internal Medicine II, HSK Hospital, Wiesbaden, Germany
12Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Hospital, Magdeburg, Germany
13Magen Darm Zentrum Wiener Platz, Cologne, Germany
14Department of General, Visceral and Transplant Surgery, University Medical Center, University of Mainz, Mainz, Germany
15Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, University of Hamburg, Hamburg, Germany
16First Department of Internal Medicine, University Medical Center, University of Mainz, Mainz, Germany
17Departments of General and Visceral Surgery, Sana Klinikum, Offenbach, Germany
18Department of General, Visceral and Transplant Surgery, University of Heidelberg, Heidelberg, Germany
19Department of General, Visceral and Thorax Surgery, RoMed Klinikum Rosenheim, Rosenheim, Germany
20Gastropraxis, Wiesbaden, Germany
21Departments of Internal Medicine and Gastroenterology, Elisabeth Hospital, Essen, Germany
22Departments of Gastroenterology and Interventional Endoscopy, St. John of God Hospital, Regensburg, Germany
23Gastroenterologische Gemeinschaftspraxis, Koblenz, Germany
24Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany

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Introduction

Barrett’s esophagus (BE) is characterized by replacement of squamous epithelium by metaplastic columnar epithelium and represents a common premalignant condition affecting 1–2% of the adult population in Western developed countries [1]. Individuals with BE have a 2–4% life time risk of esophageal adenocarcinoma (EAC) [2], which presents the end point in the esophagitis-metaplasia-dysplasia-adenocarcinoma sequence. Chronic inflammation due to gastroesophageal reflux disease (GERD) is the predominant etiologic factor for BE [3]. GERD and thereby BE risk is further influenced by hiatal hernia and obesity [3]. In addition, genetic factors play a role in BE and EAC development. So far, two genome-wide association studies (GWAS) for BE have been published. Su et al. used a discovery sample of 1852 BE cases and 5172 controls as well as several replication samples (total of 5986 patients and 12,825 controls) [4]. They identified genome-wide significant BE association at the HLA-region on chromosome 6p21 and near FOXP1 on chromosome 16q24. In a following study using 318 patients and 431 controls it has been shown that both loci confer also EAC risk [5]. The second GWAS by Levine et al. used a combined discovery BE/EAC sample consisting of 2416 BE cases and 5156 EAC cases as well as 3209 controls [6]. Their best findings were followed up in a replication sample (1633 BE/EAC patients and 6911 controls), which lead to genome-wide significant associations near CRTC1 on chromosome 1p13, BARX1 on chromosome 9q22 and FOXP1 on chromosome 3p13. Except for the finding at CRTC1 the associations near BARX1 and FOXP1 have been independently replicated in a sample of 1065 EAC cases and 1019 controls [7].

In addition to these loci, three BE associations have been published most recently [8]. The replication of the GWAS in BE by Sue et al. was extended to a total of 8306 BE cases and 15,890 controls, in which additional 65 prioritized SNPs from the discovery phase were genotyped [4]. This lead to genome-wide significant BE associations at rs3072 on chromosome 2p24 and at rs2701108 on chromosome 12q24 [8]. Within the chromosomal 2 region GDF7 represents the most promising risk gene and TBX5 within the chromosomal 12 region. In addition, the authors performed a meta-analysis using both published GWAS datasets and followed up the most significant associations in their replication samples. This lead to an additional genome-wide significant BE association at rs3784262 on chromosome 15q22 with ALDH1A2 being the most promising risk gene at this locus [8].

In this study, we aimed at replicating the observed BE associations at GDF7, TBX5, and ALDH1A2. In addition, we tested whether the implicated loci are also conferring risk to EAC and if so, whether the risk effects differ between BE and EAC.

Material and Methods

Our sample consisted of 542 BE and 1106 EAC cases as well as 1602 controls, all of German descent. In all cases the diagnosis of BE or EAC was histopathologically confirmed. Controls were a population-based sample from the Heinz Nixdorf Recall (HNR) study, a population-based cohort to study risk factors for cardiovascular diseases [9]. All participants signed informed consent and the study was approved by ethics committees from the Universities of Bonn and Leipzig (Germany). Although none of the controls were diagnosed with EAC, they were not screened for Barrett’s esophagus status. The use of unscreened controls may have led to a decrease in statistical power. However, as the prevalence of BE is only 1–2% in the general population [1], the power of the present association study should not have been substantially reduced by the use of unscreened controls [10]. In BE, 171 cases were females and 371 were males, whereas 134 EAC cases were females and 972 EAC cases males. In controls, 802 were females and 800 were males.

In patients, genotyping of all three reported BE risk variants (rs3072, rs2701108, rs3784262) was done using the Sequenom MassARRAY® iPLEX Gold® system (Sequenom, San Diego, USA). For quality control intra- and interplate duplicates were genotyped. In addition, negative controls (H2O) were added on each 384 well plate in order to exclude contamination. Clusterplot of each SNP was visually checked and manually corrected if necessary. In controls, genotypes for all three markers were obtained from Illumina’s HumanOmniExpress BeadArrays (Illumina, San Diego, USA). The genome-wide data of the control sample have been previously used in several GWAS on different traits [11–13].

All genotype data underwent different quality control steps, including Hardy-Weinberg equilibrium P > 0.001 and call rate >99%. Single-marker association analyses including sex as covariate were performed separately for
BE, EAC, and BE/EAC. In addition, for each of the three sample sets the presence of sex-specific association was tested. SAS software (SAS 8.02; SAS Institute Inc, Cary, NC) was used for the quality control as well as the single-marker association analyses.

Results

Table 1 shows the results of the case–control comparison in BE, EAC, and in the combined sample. In BE we could replicate the association at rs3784262 near ALDH1A2 with $P = 9.70 \times 10^{-04}$ (RR = 0.79, Table 1), the same allele was disease-conferring as previously reported [8]. In addition, the same alleles at rs3072 near GDF7 and at rs2701108 near TBX5 that conferred BE risk in the previously published study [8] were more prevalent in patients than in controls (RR = 1.05 and RR = 0.87, respectively), although this was not significant (Table 1). In EAC we found association at rs3072 near GDF7 with $P = 1.48 \times 10^{-03}$ (RR = 1.20) and at rs2701108 near TBX5 with $P = 2.47 \times 10^{-02}$ (RR = 0.88, Table 1). Although the same allele at rs3784262 near ALDH1A2 that confers BE risk was slightly more prevalent in patients than in controls, this association was not significant ($P = 1.30 \times 10^{-01}$, RR = 0.92, Table 1). Given the association findings obtained in the separate analyses, we found significant associations at all three loci in the combined BE/EAC sample. SNP rs3072 at GDF7 was disease associated with $P = 7.53 \times 10^{-03}$ (RR = 1.15), rs2701108 at TBX5 showed disease association with $P = 1.12 \times 10^{-02}$ (RR = 0.88), and rs3784262 at ALDH1A2 with $P = 8.06 \times 10^{-03}$ (RR = 0.88, Table 1). At all three loci, we observed no evidence for sex-specific BE or EAC risk effects (data not shown).

Discussion

BE and EAC represent two stages within the esophagitis-metaplasia-dysplasia-adenocarcinoma sequence. It has been shown that genetic risk factors are relevant in the etiology of BE and EAC. However, this does not necessarily mean that genetic variants confer equal risk to all different stages of the disease sequence. In this study, we analyzed three previously published BE variants [8] in BE and EAC samples from Germany in order to determine their risk effects during BE/EAC sequence. Our data show that rs3072 at GDF7 and rs2701108 at TBX5 are also conferring risk to EAC. Although the genetic risk effect of rs2701108 was similar in BE and EAC (RR = 0.87 and RR = 0.88, respectively), the risk effect of rs3072 was even higher in EAC compared to BE (RR = 1.20 and RR = 1.05, respectively). We therefore conclude that both loci are conferring disease risk also at later stages of the BE/EAC sequence. In contrast, rs3784262 at ALDH1A2 was highly significantly BE associated ($P = 9.70 \times 10^{-04}$), but showed no association with EAC ($P = 1.30 \times 10^{-01}$), although the size of the latter sample was substantially larger (542 BE vs. 1106 EAC cases). Although many reasons may have led to an overestimated risk effect of rs3784262 in BE and an underestimated risk effect of this variant in EAC, our data do not provide evidence that this locus confer equal risk in early and late stages of the BE/EAC sequence.

Based on their genomic location and biological function, GDF7 near rs3072, TBX5 at rs2701108 and ALDH1A2 near rs3784262 are all promising risk-conferring genes for BE and EAC (summarized in Palles et al. [8]). GDF7 encodes the BMP12 protein and thereby functions in the PMP pathway that has been already implicated in BE development [14]. Among various functions TBX5 plays

| Phenotype | SNP | Chromosome | Position (bp) | Allele  | MAF (%) in cases | MAF (%) in controls | RR (95% CI) | P value | Nearby gene |
|-----------|-----|------------|--------------|--------|-----------------|---------------------|-------------|---------|-------------|
| BE        | rs3072 | 2p24 | 20,741,887 | G/A | 38.1 | 37.0 | 1.05 (0.91–1.21) | 5.32 × 10^{-01} | GDF7 |
| BE        | rs2701108 | 12q24 | 113,158,644 | G/A | 35.5 | 38.6 | 0.87 (0.75–1.01) | 6.38 × 10^{-02} | TBX5 |
| BE        | rs3784262 | 15q22 | 56,040,398 | G/A | 40.7 | 46.4 | 0.79 (0.68–0.91) | 9.70 × 10^{-04} | ALDH1A2 |
| EAC       | rs3072 | 2p24 | 20,741,887 | G/A | 41.3 | 37.0 | 1.20 (1.07–1.34) | 1.48 × 10^{-03} | GDF7 |
| EAC       | rs2701108 | 12q24 | 113,158,644 | G/A | 35.6 | 38.6 | 0.88 (0.78–0.98) | 2.47 × 10^{-02} | TBX5 |
| EAC       | rs3784262 | 15q22 | 56,040,398 | G/A | 44.3 | 46.4 | 0.92 (0.82–1.03) | 1.30 × 10^{-01} | ALDH1A2 |
| BE/EAC    | rs3072 | 2p24 | 20,741,887 | G/A | 40.3 | 37.0 | 1.15 (1.04–1.27) | 7.53 × 10^{-03} | GDF7 |
| BE/EAC    | rs2701108 | 12q24 | 113,158,644 | G/A | 35.6 | 38.6 | 0.88 (0.79–0.97) | 1.12 × 10^{-02} | TBX5 |
| BE/EAC    | rs3784262 | 15q22 | 56,040,398 | G/A | 43.1 | 46.4 | 0.88 (0.79–0.97) | 8.06 × 10^{-03} | ALDH1A2 |

1Chromosomal position according to hg18.
2First allele represents the minor allele.
3Minor allele frequency (MAF) is given for cases and controls.
4Relative Risk (RR) with 95% Confidence Interval (CI) indicating the genetic effect size is given for the minor allele.
5Nearest gene to the associated SNPs is shown.

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a role in the development of diaphragmatic musculature [15] and genetic variation influencing TBX5 may predispose to hiatus hernia and thereby GERD. ALDH1A2 encodes for an enzyme that catalyzes the synthesis of retinoic acid and may also be involved in alcohol metabolism [16] and hence be relevant for inflammation. Of note, alcohol consumption has been discussed as a risk factor for BE/EAC [17, 18]. However, future studies (including animal models) have to show whether GDF7, TBX5 and ALDH1A2 represent the true risk-conferring genes at the disease associated loci. Furthermore, GWAS have led to the identification of more than 7 BE and EAC risk variants within the past 3 years [4–6, 8]. Aside from functional analyses in order to elucidate the pathophysiological mechanism at each implicated locus and to identify pathways in which risk genes are enriched, further GWAS and GWAS meta-analyses on larger and detailed phenotyped sample sizes with BE and EAC are needed. This also will allow for mapping of all risk variants and genes in the BE/EAC sequence in order to identify biomarkers that predict EAC conversion in future.

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