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Original Article

The Y-chromosome F haplogroup contributes to the development of Barrett's esophagus-associated esophageal adenocarcinoma in a white male population

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*SUMMARY.***Barrett's esophagus (BE) is a metaplastic condition of the distal esophagus, resulting from longstanding gastroesophageal reflux disease (GERD). BE predisposes for the highly malignant esophageal adenocarcinoma (EAC). Both BE and EAC have the highest frequencies in white males. Only a subset of patients with GERD develop BE, while <0.5% of BE will progress to EAC. Therefore, it is most likely that the development of BE and EAC is associated with underlying genetic factors. We hypothesized that in white males, Y-chromosomal haplogroups are associated with BE and EAC. To investigate this we conducted a multicenter study studying the frequencies of the Y-chromosomal haplogroups in GERD, BE, and EAC patients. We used genomic analysis by polymerase chain reaction and restriction fragment length polymorphism to determine the frequency of six Y-chromosomal haplogroups (DE, F(xJ,xK), K(xP), J, P(xR1a), and R1a) between GERD, BE, and EAC in a cohort of 1,365 white males, including 612 GERD, 753 BE patients, while 178 of the BE patients also had BE-associated EAC. Univariate logistic regression analysis was used to compare the outcomes. In this study, we found the R1a (6% vs. 9%, $P = 0.04$) and K (3% vs. 6%, $P = 0.035$) to be significantly underrepresented in BE patients as compared to GERD patients with an odds ratio (OR) of 0.63 (95% CI 0.42–0.95, $P = 0.03$) and of 0.56 (95% CI 0.33–0.96, $P = 0.03$), respectively, while the K haplogroup was protective against EAC (OR 0.30; 95% CI 0.07–0.86, $P = 0.05$). A significant overrepresentation of the F haplogroup was found in EAC compared to BE and GERD patients (34% vs. 27% and 23%, respectively). The F haplogroup was found to be a risk factor for EAC with an OR of 1.5 (95% CI 1.03–2.19, $P = 0.03$). We identified the R1a and K haplogroups as protective factors against development of BE. These haplogroups have low frequencies in white male populations. Of importance is that we could link the presence of the predominantly occurring F haplogroup in white males to EAC. It is possible that this F haplogroup is associated to genetic variants that predispose for the EAC development. In future, the haplogroups could be applied to improve stratification of BE and GERD patients with increased risk to develop BE and/or EAC.**

KEY WORDS: **Barrett's esophagus, esophageal adenocarcinoma, gastroesophageal reflux disease, genetic polymorphisms, Y-chromosome haplogroup.**

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INTRODUCTION

Barrett's esophagus (BE) is a condition caused by chronic gastroesophageal reflux disease (GERD),[1–4] in which the normal squamous mucosa of the distal esophagus is substituted by a specialized (intestinal) columnar type of epithelium. BE is most prevalent in older white males. BE patients are at an increased risk for developing esophageal adenocarcinoma (EAC).[5]

Besides age, ethnicity, and male gender, risk factors for GERD patients to develop BE are less clear. Apparently, the development of BE and EAC depends on a combination of lifestyle factors[6–12] and a genetic predisposition. The efforts to find specific genetic abnormalities that are associated with development of BE and progression of BE to EAC are ongoing.[13] For example, a study by Sun *et al*. found an increased admixture of European ancestry on chromosome 8 and 11 in African American patients.[14] These chromosomal regions contain several candidate genes that have been suggested to be involved in the development of BE and EAC. A particular other genetic change that has been observed in nondysplastic BE is numerical Y-chromosome abnormalities.[15–17]

Relevant features of the Y chromosome include the haploid status and the inability of the Y chromosome to recombine over most of its length (male-specific region [MSY]). Therefore, the MSY region is transmitted unchanged from father to the male offspring over many generations.

Genotyping for Y-chromosomal polymorphic markers located on this MSY region of the chromosome, makes it possible to define Y haplogroups, which for example has been used to track human evolution and migration but can also be applied to investigate an association between genes on the Y chromosome and certain diseases as well as potential role of ethnicity on the development of these particular diseases.[18,19]

In this study, we hypothesized that certain Y-chromosomal haplogroups are associated with an increased susceptibility for BE and EAC. To test our hypothesis, we used a set of six Y-chromosome-linked polymorphisms to define the major Y-chromosome haplogroups in white males with BE or BE-associated EAC. Their Y-chromosome haplogroup frequencies were subsequently compared to white males with GERD symptoms that had no BE at endoscopy.

MATERIAL AND METHODS

Study population

This multicenter case control study was approved by all the local ethics committees and all participants agreed to the use of their samples. The total cohort consisted of 1,445 Dutch and US patients (Fig. 1). Of these, 51 were excluded due to insufficient material in the paraffin embedded tissue blocks that were used for DNA extraction. Another 24 patients were excluded because of technical reasons (i.e. the DNA concentration was too low for polymerase chain reaction [PCR] or PCR results were inconsistent). Finally, five patients were excluded because of their Y chromosome falling into the A, B, or C haplogroups which are haplogroups associated with African descent. The Dutch patients underwent upper gastrointestinal (GI) endoscopy between 2002 and 2006 in the Amsterdam University Medical Centers (AUMC, Amsterdam and the Erasmus Medical Center (EMC, Rotterdam). Data on ethnic origin for all individuals were obtained from patient files and/or by questionnaires. The US patients underwent upper GI endoscopy at the Mayo Clinic, Rochester, USA between 1992 and 2010. White ethnic origin of these patients was confirmed using both questionnaires as well as patient charts. GERD was classified according to the Los Angeles (LA) classification. Patients with GERD symptoms (objectified using either a questionnaire or pH-metry) but with no visible reflux esophagitis were classified as nonerosive GERD (grade 0).

For histological confirmation of BE, biopsies were taken below the z-line in the esophagus and at least 1 cm above the gastric folds from the BE mucosa. In case of EAC, biopsies were taken from the tumor mass and adjacent to the mass to confirm BE-associated EAC. Active reflux esophagitis was classified according to the LA classification.

Y-chromosome haplotyping

Genomic DNA of each patient was extracted from normal GI tissue (Qiagen) or from a whole blood sample by standard salt-out procedure. Six Y-linked binary markers, located on the male-specific region of the Y chromosome and known to be polymorphic in the European population, were chosen to genotype all individuals using the following markers: M9, SRY10831, M89, DYS257, Yap, and p12f.[20,21] Genotyping was performed by the PCR and restriction fragment length polymorphism and is further described in the Supplementary Data.

Statistical analysis

Differences in the distribution of the Y-chromosomal haplogroups were determined with Pearson chi-square test (two sided). To assess the predictive power of Y haplogroups on group allocation (odds ratios [ORs]), a univariate logistic regression, using the P haplogroup as a reference group, was performed. Confounding by variables such as familial relationship, ethnicity, or gender was excluded due to the strict patient selection. Multivariate analysis was performed to adjust for age. Statistical significance was set at a P value of <0.05. Data analysis was performed using R statistical software version 3.5.1.

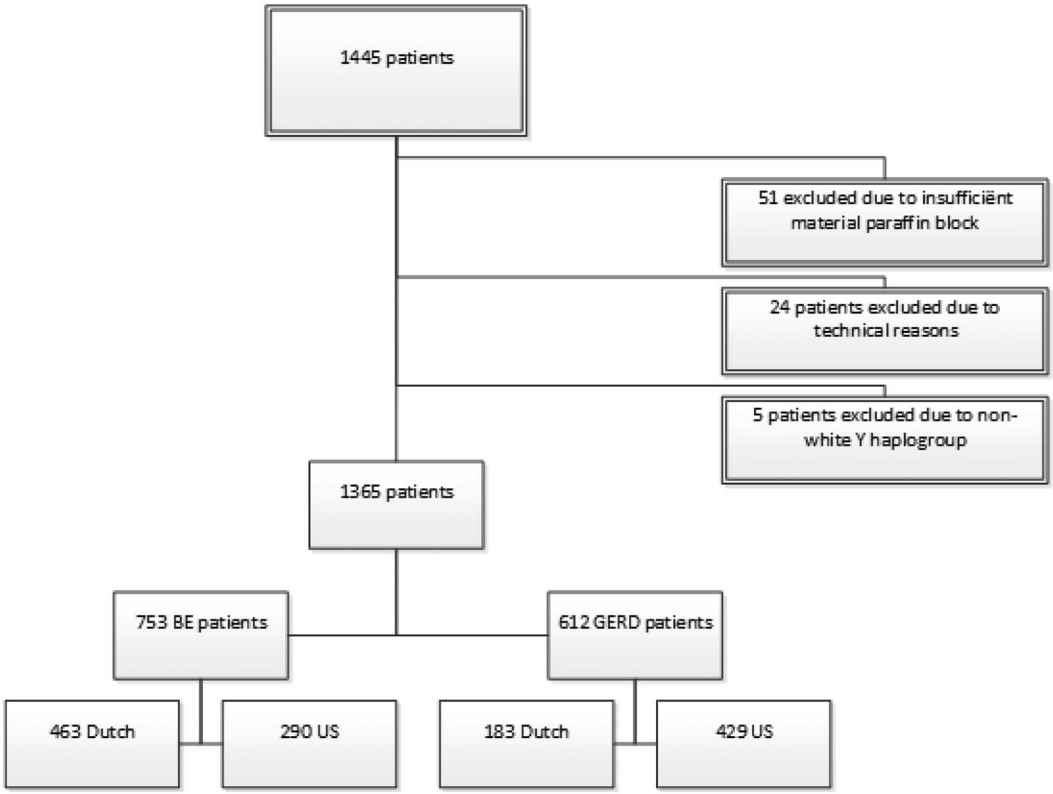

Fig. 1 Flow chart of included patients.

RESULTS

BE and GERD patients

The final cohort consisted of 1,365 patients: 753 BE and 612 GERD. The population consisted of 646 white male patients from the Netherlands (183 GERD, 463 BE) and 719 from the United States (429 GERD, 290 BE) (Fig. 1).

The GERD group consisted of 183 Dutch (all AUMC) and 429 US patients, with a mean age of 55 ± 15 (mean in years ± SD) and served as a white male control group of patients without BE. In the GERD group 58 patients (10%) had grade 0; 463 (76%) patients had grade A/B and 83 (14%) of patients had grade C/D reflux esophagitis.

The BE group consisted of 463 Dutch (235 AUMC and 228 Erasmus MC) and 290 US white males. All patients had histologically proven BE. Mean age was 62 ± 12 (mean in years ± SD). There was a significant difference in age between the GERD and BE group of 55 versus 62 years ($P < 0.001$) (Table 1).

Patients with BE-associated EAC versus BE patients without EAC

One hundred and seventy eight of 753 BE patients (24%) had BE-associated EAC. Mean age for these patients was 65 ± 12 (mean in years ± SD) versus 62 ± 13 in the patients that had BE without EAC, $P = 0.004$.

Overall haplogroup frequencies in the study cohort

The haplogroups most frequently observed were the P and F haplogroups. Approximately 80% of the patients fell within these two haplogroups whereas the DE, J, R1a, and K haplogroups were found less frequently (Table 2).

Differences between the BE (with or without EAC) versus GERD patients

In this study cohort, a significant difference was observed in the overall distribution of Y haplogroups between the BE with EAC (BE/EAC) ($n = 753$) and the GERD ($n = 612$) population ($P = 0.02$; Table 3). This was in part due to the fact that the R1a haplogroup—using the P haplogroup as a reference haplogroup—was significantly underrepresented in the BE/EAC group compared to the GERD group (6% vs. 9%, OR of 0.63 [0.41–0.95 95% CI, $P = 0.03$]). Also, the frequency of the K haplogroup was significantly lower in the BE/EAC group than in the GERD group (3% vs. 6%) corresponding to lower odds to have BE/EAC, with an OR of 0.56 (95% CI 0.33–0.96, $P = 0.03$) compared to the P haplogroup.

Table 1 Patient characteristics

Patient characteristics	GERD	BE/all	BE/EAC
Age (mean ± SD)	55 ± 15	62 ± 12	65 ± 12
Weight (BMI)			
Normal (<25)	79 (17%)	113 (25%)	24 (28%)
Overweight (25–30)	216 (45%)	202 (44%)	34 (40%)
Obese (>30)	183 (38%)	147 (32%)	27 (32%)
Total	478	462	85
Missing data	134 (22%)	291 (39%)	93 (52%)
LA grade reflux esophagitis			
Grade 0	58 (10%)		
Grade A/B	463 (77%)		
Grade C	54 (9%)		
Grade D	29 (5%)		
Total	604		
Missing data	8 (1%)		

BE, Barrett's esophagus; BMI, body mass index; EAC, esophageal adenocarcinoma; GERD, gastroesophageal reflux disease; LA, Los Angeles.

Table 2 Haplogroup distribution

	BE/all	GERD	BE/EAC
DE	27 (3.6%)	27 (4.4%)	5 (2.8%)
F (xJ, xK)	200 (26.6%)*	139 (22.7%)*	**61 (34.3%)***
J	23 (3.1%)	26 (4.3%)	4 (2.3%)
K (xP)	25 (3.3%)	34 (5.6%)#	**3 (1.7%)#**
R1a	47 (6.2%)	57 (9.3%)	9 (5.1%)
Px (R1a)	431 (57.2%)	329 (53.8%)	96 (53.9%)
Total no.	753	612	178

*,#$p < .05$

Both of these findings remained significant after adjusting for age.

Differences between BE-associated EAC versus BE (without EAC) and GERD patients

To investigate if particular Y haplogroups were associated with EAC, we compared the haplogroup frequencies between BE with EAC (BE/EAC) versus BE without EAC (BE) and the GERD patients. We observed a significant overrepresentation of the F haplogroup in patients with EAC as compared to the BE and the GERD patients (35% vs. 27% and 23%, respectively). Univariate logistic regression analysis showed that the F haplogroup was associated with an increased risk for BE-associated EAC as compared to BE and GERD patients as demonstrated by an OR of 1.50 (95% CI 1.03–2.19, $P = 0.03$) and 1.53 (95% CI 1.05–2.23, $P = 0.03$).

Since GERD is a risk factor for BE and BE may develop in GERD patients at later age, and because age by itself is a known risk factor for EAC,[22,23] we adjusted results for age by using multivariate analysis. After adjusting for age, the differences became less significant, but there was still a clear trend (OR 1.42; 95% CI 0.94–2.13, $P = 0.09$ and OR 1.42; 95% CI 0.96–2.09; $P = 0.07$, respectively).

We also found that the K haplogroup was present in lower frequencies in the BE/EAC as compared to the GERD group (2% vs. 6%, respectively) (Table 3). This difference was borderline significant in the univariate analysis (OR 0.30; 95% CI 0.07–0.86; $P = 0.05$) but became highly significant after correcting for age. Thus, the K haplogroup also seems to protect against development of BE-associated EAC as indicated by an OR of 0.23 (95% CI 0.05–0.68, $P = 0.047$).

DISCUSSION

Although GERD is the major risk factor for BE and EAC, there is also a clear genetic predisposition, which for instance, accounts for the high incidence in males compared to females and higher frequencies in white population.

Here, we performed a genetic association study to investigate the impact of Y-chromosomal haplogroups on the susceptibility to BE and EAC. We determined the Y-chromosomal haplogroup distribution in BE patients with and without EAC as well as in a control group of GERD patients while controlling for confounding factors such as ethnicity, familial disposition, and age. The most frequently observed Y haplogroup (56–60%) in the cohort was haplogroup P(xR1a). Indeed, this haplogroup is the most common in Europeans and populations of European ancestry.[24] Haplogroup F(xK, J) was the second most frequently observed Y haplogroup (23–31%), which is also in agreement with literature.[25,26] Haplogroups DE, J, K(xP), and R1a were observed to have the lowest frequencies (2–8).

One major finding of our study was that the F haplogroup, which can be found in approximately one third of the general white population in Northern Europe showed a correlation with the development of EAC suggesting that this, for white male common, haplogroup might predispose for cancer development

Table 3 Odds ratios for BE/EAC development associated with different Y haplotypes—univariate analysis

Haplotype	BE all vs. GERD	BE/EAC vs. GERD	BE/EAC vs. BE without EAC
OR (95% CI); *P*			
DE	0.76 (0.44–1.33); 0.34	0.63 (0.21–1.56); 0.36	0.79 (0.26–1.99); 0.65
F (xJ, xK)	1.10 (0.85–1.43); 0.48	**1.50 (1.03–2.19); 0.03***	**1.53 (1.05–2.23); 0.03***
J	0.68 (.38–1.21); 0.18	0.53 (0.15–1.39); 0.24	0.73 (.21–2.01); 0.58
K (xP)	**0.56 (0.33–0.96); 0.03***	**0.30 (0.07–0.86); 0.05***	0.48 (0.11–1.41); 0.24
R1a	**0.63 (0.42–0.95); 0.03***	0.54 (0.24–1.08); 0.10	0.83 (0.36–1.70); 0.62
Px(R1a)	1 (reference)	1 (reference)	1 (reference)
Total no.	753 vs. 612	178 vs. 612	178 vs. 575

*$p < .05$

in BE patients. Although, this correlation was less strong after adjusting for age, there was still a clear trend after entering yet another degree of freedom into the model in a subgroup that is already relatively small.

Other interesting findings were the protective effect that the K and R1a haplotype seemed to confer for the development of BE. The difference in susceptibility for BE and EAC development between oriental countries, Africa, and Europe is thought to be at least partly due to a distinct genetic background and it is possible that a higher prevalence of the K and R1a in these populations has a protective effect for development of BE or BE-associated EAC.[27–30] It would be highly interesting to study the frequency of the Y haplogroups with respect to BE and EAC in different ethnic groups.

The limitations of this study are that this is a retrospective study. The possibility of false-positive results because of the geographical stratification and ethnical distribution of Y haplogroups must also be considered.[31] To avoid these confounding factors, the appropriate choice of the control population (nonblood relatives from the same geographical area as the disease group) is crucial. This was the case for both our patient cohorts. The white descent of the Dutch patients was verified to the third or at least to the second generation and confirmed by the absence of A, B, or C haplogroups. Therefore, the haplogroups associations found in the Dutch cohort are unlikely to be due to bias caused by different ethnical background. The US patients were thoroughly checked by both questionnaires and medical charts. Nevertheless, in the US subgroup, we found that <1% of patients were possibly of African descent, still the overall haplogroup distribution was very similar between the Dutch and US cohort, suggesting similar ethnic backgrounds and decreasing the likelihood of stratification bias. Since we excluded patients that were genetically related, a bias due to familial predisposition is highly unlikely.

Another important limitation is the fact that in recent years, with the development of next generation sequencing (NSG), the Y-haplotree has undergone significant revisions. Therefore, the application of NGS in future studies will allow to further subdivide the F haplogroup into the G, H, and I haplogroups which will lead to more specific conclusions.

The mechanism behind the Y-chromosomal haplogroups influence on the susceptibility to BE and EAC is not clear. We know, however, that Y chromosomes from distinct Y haplogroups show considerable structural variation.[32] One hypothesis might be that Y chromosomes of specific haplogroups are associated with protection against occurrence of certain Y-chromosomal rearrangements (deletions or amplifications) influencing expression of genes important for BE and EAC development. The candidate genes on the Y chromosome involved in this process may be those that are expressed ubiquitously in the body, including, for instance, SRY, ZFY, and SMCY transcription factors, PRKY protein kinase or EIF1AY translational inaction factor. It is however also possible that Y-chromosomal haplogroups are associated with functional variants of genes on autosomal chromosomes.[33] Such an association, for instance, was found between a Y-chromosome variant and the polymorphism on the autosomal gene of aldosterone synthase in European men with high blood pressure. In the future, linkage disequilibrium studies may reveal genetic variants on Y chromosome itself, for example, the tumor suppressor gene TMSB4Y, or on autosomal chromosomes.

In summary, this study is the first to report an association of K and R1a Y-chromosomal haplogroups with a lower susceptibility to BE/EAC in a GERD population of white males. Furthermore, we found that in the white males relatively frequently occurring F haplotype was associated with an increased risk to develop EAC. For a better understanding of the exact mechanisms underlying these associations further linkage analysis as well as detailed molecular studies are needed. Our results, however, indicate that specific haplogroups might be linked to genetic variants that protect of, or predispose to BE/EAC development. The identification of such genetic variants in the future may improve our understanding of the pathogenesis of BE and EAC.

AUTHOR CONTRIBUTIONS

WMW, AMR, KKK, KKW, and SR designed the study. WMW and NSM performed the statistical analyses. WMW, AMR, and ALR performed the data collection. WMW, AMR, ALR, SO, LMGM, MPP, SAM, MJ, and PDS performed the sample collection. SR was responsible for the initial experimental setup and provided the primers. WMW, AMR, GMJW, and ALR performed the sample processing and laboratory analysis. WMW, AMR, and KKK performed the statistical data analysis and interpretation of statistical data. WMW, AMR, KKW, and KKK wrote the paper and created the tables and figures. Critical revision of the manuscript was performed by KKK, KKW, SR, PDS, MPP, and LMGM. All authors reviewed the drafts of the article and gave final approval of the version to be published.

SUPPLEMENTARY DATA

Supplementary data mentioned in the text are available to subscribers in *DOTESO* online.

References

1 Rex D K, Cummings O W, Shaw M *et al.* Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. Gastroenterology 2003; 125: 1670–7.
2 Ronkainen J, Talley N J, Storskrubb T *et al.* Erosive esophagitis is a risk factor for Barrett's esophagus: a community-based endoscopic follow-up study. Am J Gastroenterol 2011; 106: 1946–52.
3 Westhoff B, Brotze S, Weston A *et al.* The frequency of Barrett's esophagus in high-risk patients with chronic GERD. Gastrointest Endosc 2005; 61: 226–31.
4 Zagari R M, Fuccio L, Wallander M A *et al.* Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. Gut 2008; 57: 1354–9.
5 Peters Y, Al-Kaabi A, Shaheen N J *et al.* Barrett oesophagus. Nat Rev Dis Primers 2019; 5: 35.
6 Anderson L A, Cantwell M M, Watson R G P *et al.* The association between alcohol and reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma. Gastroenterology 2009; 136: 799–805.
7 Cook M B, Shaheen N J, Anderson L A *et al.* Cigarette smoking increases risk of Barrett's esophagus: an analysis of the Barrett's and esophageal adenocarcinoma consortium. Gastroenterology 2012; 142: 744–53.
8 Corley D A, Kubo A, Levin T R *et al.* Iron intake and body iron stores as risk factors for Barrett's esophagus: a community-based study. Am J Gastroenterol 2008; 103: 2997–3004.
9 Edelstein Z R, Bronner M P, Rosen S N *et al.* Risk factors for Barrett's esophagus among patients with gastroesophageal reflux disease: a community clinic-based case-control study. Am J Gastroenterol 2009; 104: 834–42.
10 Kramer J R, Fischbach L A, Richardson P *et al.* Waist-to-hip ratio, but not body mass index, is associated with an increased risk of Barrett's esophagus in white men. Clin Gastroenterol Hepatol 2013; 11: 373–381.e1.
11 Kubo A, Levin T R, Block G *et al.* Cigarette smoking and the risk of Barrett's esophagus. Cancer Causes Control 2009; 20: 303–11.
12 Kubo A, Levin T R, Block G *et al.* Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. Gastroenterology 2009; 136: 806–15.
13 Reid B J, Paulson T G, Li X. Genetic insights in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology 2015; 149: 1142–1152 e3.
14 Sun X, Chandar A K, Canto M I *et al.* Genomic regions associated with susceptibility to Barrett's esophagus and esophageal adenocarcinoma in African Americans: the cross BETRNet admixture study. PLoS One 2017; 12: e0184962.
15 Garewal H S, Sampliner R, Liu Y *et al.* Chromosomal rearrangements in Barrett's esophagus. A premalignant lesion of esophageal adenocarcinoma. Cancer Genet Cytogenet 1989; 42: 281–6.
16 Krishnadath K K, Tilanus H W, van Blankenstein M *et al.* Accumulation of genetic abnormalities during neoplastic progression in Barrett's esophagus. Cancer Res 1995; 55: 1971–6.
17 Walch A K, Zitzelsberger H F, Bruch J *et al.* Chromosomal imbalances in Barrett's adenocarcinoma and the metaplasia-dysplasia-carcinoma sequence. Am J Pathol 2000; 156: 555–66.
18 Kim W, Yoo T K, Kim S J *et al.* Lack of association between Y-chromosomal haplogroups and prostate cancer in the Korean population. PLoS One 2007; 2: e172.
19 Nathanson K L, Kanetsky P A, Hawes R *et al.* The Y deletion gr/gr and susceptibility to testicular germ cell tumor. Am J Hum Genet 2005; 77: 1034–43.
20 Underhill PA, Jin L, Lin AA *et al.* Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography. Genome Res. 1997;7:996–1005.
21 Underhill PA, Passarino G, Lin AA, *et al.* The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. Ann Hum Genet. 2001;65:43–62.
22 Martinez P, Timmer M R, Lau C T *et al.* Dynamic clonal equilibrium and predetermined cancer risk in Barrett's oesophagus. Nat Commun 2016; 7: 12158.
23 Timmer M R, Martinez P, Lau C T *et al.* Derivation of genetic biomarkers for cancer risk stratification in Barrett's oesophagus: a prospective cohort study. Gut 2016; 65: 1602–10.
24 Jobling M A, Tyler-Smith C. The human Y chromosome: an evolutionary marker comes of age. Nat Rev Genet 2003; 4: 598–612.
25 Repping S, Skaletsky H, Brown L *et al.* Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 2003; 35: 247–51.
26 Underhill P A, Shen P, Lin A A *et al.* Y chromosome sequence variation and the history of human populations. Nat Genet 2000; 26: 358–61.
27 Ford A C, Forman D, Reynolds P D *et al.* Ethnicity, gender, and socioeconomic status as risk factors for esophagitis and Barrett's esophagus. Am J Epidemiol 2005; 162: 454–60.
28 Hongo M. Review article: Barrett's oesophagus and carcinoma in Japan. Aliment Pharmacol Ther 2004; 20(Suppl 8): 50–4.
29 Mason R J, Bremner C G. The columnar-lined (Barrett's) oesophagus in black patients. S Afr J Surg 1998; 36: 61–2.
30 Pera M, Manterola C, Vidal O *et al.* Epidemiology of esophageal adenocarcinoma. J Surg Oncol 2005; 92: 151–9.
31 Cox N J, Bell G I. Disease associations. Chance, artifact, or susceptibility genes? Diabetes 1989; 38: 947–50.
32 Repping S, van Daalen S K, Brown L G *et al.* High mutation rates have driven extensive structural polymorphism among human Y chromosomes. Nat Genet 2006; 38: 463–7.
33 Charchar F J, Tomaszewski M, Padmanabhan S *et al.* The Y chromosome effect on blood pressure in two European populations. Hypertension 2002; 39: 353–6.