Multitrait genetic association analysis identifies 50 new risk loci for gastro-oesophageal reflux, seven new loci for Barrett’s oesophagus and provides insights into clinical heterogeneity in reflux diagnosis

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ABSTRACT

Objective Gastro-oesophageal reflux disease (GERD) has heterogeneous aetiology primarily attributable to its symptom-based definitions. GERD genome-wide association studies (GWASs) have shown strong genetic overlaps with established risk factors such as obesity and depression. We hypothesised that the shared genetic architecture between GERD and these risk factors can be leveraged to (1) identify new GERD and Barrett’s oesophagus (BE) risk loci and (2) explore potentially heterogeneous pathways leading to GERD and oesophageal complications.

Design We applied multitrait GWAS models combining GERD (78 707 cases; 288 734 controls) and genetically correlated traits including education attainment, depression and body mass index. We also used multitrait analysis to identify BE risk loci. Top hits were replicated in 23andMe (462 753 GERD cases, 24 099 BE cases, 1 484 025 controls). We additionally dissected the GERD loci into obesity-driven and depression-driven subgroups. These subgroups were investigated to determine how they relate to tissue-specific gene expression and to risk of serious oesophageal disease (BE and/or oesophageal adenocarcinoma, EA).

Results We identified 88 loci associated with GERD, with 59 replicating in 23andMe after multiple testing corrections. Our BE analysis identified seven novel loci. Additionally we showed that only the obesity-driven GERD loci (but not the depression-driven loci) were associated with genes enriched in oesophageal tissues and successfully predicted BE/EA.

Conclusion Our multitrait model identified many novel risk loci for GERD and BE. We present strong evidence for a genetic underpinning of disease heterogeneity in GERD and show that GERD loci associated with depressive symptoms are not strong predictors of BE/EA relative to obesity-driven GERD loci.

INTRODUCTION

Gastro-oesophageal reflux disease (GERD) is a complex condition with different consensus statements using a variety of clinical, endoscopic and physiological criteria to define the disease.4,13 Although in a narrow sense, GERD can be considered the result of pathological oesophageal acid exposure5, the heterogeneous definition of GERD may help explain the range of traits that have been
associated with GERD risk including obesity and psychological traits. GERD has risen in prevalence in many western countries in recent years and it now accounts for a substantial proportion of direct healthcare costs for digestive diseases. GERD is the major risk factor for Barrett’s oesophagus (BE), a precancerous condition in which GERD-induced erosions of the distal oesophageal squamous epithelium are replaced by metaplastic columnar epithelium. Most oesophageal adenocarcinomas (EA), a lethal malignancy with poor survival, arise from underlying BE tissue.

Recent studies have sought to better understand GERD and its downstream consequences (BE, EA) by identifying the genes influencing risk of GERD. For most complex conditions, the most successful study design has been the genome-wide association study (GWAS), with large sample size and a key feature in successful studies. Initial GWAS for GERD failed to identify any risk genes, but more recent approaches using a broad phenotype definition to maximise sample size identified 25 risk loci. The recent GWAS also revealed strong genetic relationships between GERD and a variety of traits. For example, there was a strong genetic correlation between depression and GERD, likely reflecting the psychological component previously shown to be associated with GERD. Another genetically correlated trait was obesity, an expected result given being overweight is a major risk factor for GERD.

In this study, we took advantage of the genetic overlap between GERD and other traits to improve our power for gene discovery on GERD and BE. Because some of the traits most strongly correlated to GERD have been subjected to GWAS on a very large scale, there is potential for a large increase in gene mapping power if a multitrait approach is taken. We, hence, employed a multitrait approach using GERD and BE, alongside three traits, which had large sample sizes and high correlation with GERD: obesity, depression and educational attainment (EDU). We then replicated the GERD and BE loci in a large replication cohort and conducted gene enrichment and transcriptome-wide association analyses. Finally, using BE as a clinical endpoint indicative of pathological erosive chronic acid reflux (rather than forms of symptomatic reflux associated with psychological traits such as depression), we then assessed the relevance of subsets of the GERD loci to serious oesophageal disease.

METHODS

Detailed methods are provided in online supplemental materials.

Overview of methods

Leveraging the strong genetic correlation between GERD and related traits, we applied a multitrait GWAS model combining GWASs for body mass index (BMI), major depressive disorder (MDD), education attainment and GERD (and BE), to identify more susceptibility loci for GERD and BE (figure 1). The brief description for each input GWAS in the multitrait GWAS analysis and the equivalent effect sample size is shown in online supplemental table ST1. Candidate loci for GERD and BE achieving genome-wide significance \( (p < 5 \times 10^{-8}) \) were sent for replication in the independent 23andMe cohort (462,753 cases; 1,127,474 controls). Findings from these GWAS analyses were followed up with transcriptome-wide association studies (TWAS) and tissue enrichment analyses. TWAS analysis allows us to infer if there is a relationship between predicted gene-expression levels and GERD/BE risk. Tissue enrichment analyses allow us to assess whether the relevant GERD/BE-associated genes showed differential enrichment across 44 human tissues including oesophageal-related tissues. We finally applied a simple heuristic to dissect aetiological heterogeneity in GERD by separating GERD risk loci into obesity-driven and depression-driven categories; we then assessed these categories for differences in predicted gene expression in various tissues and for their ability to predict BE/EA susceptibility.

RESULTS

Multitrait analysis reveals 88 risk loci for GERD and 17 for BE with strong evidence of replication

In our multitrait GWAS combining GERD with BMI, MDD and EDU, we identified 88 GERD risk loci, where one or

![Figure 1](https://www.gut.bmj.com/content/first/10.1136/gutjnl-2020-323906)

**Figure 1** Schematic diagram describing overall study approach of the multi-trait GWAS analysis for GERD and BE susceptibility. GWAS data obtained from published studies are shown in bold. BE, Barrett’s oesophagus; EA, oesophageal adenocarcinoma; EDU, Education Attainment; GERD, gastro-oesophageal reflux disease; GWAS, genome-wide association study; MDD, major depressive disorder; MTAG, multitrait analysis of GWAS; PGC, Psychiatric Genomics Consortium; QSKIN, Queensland Sun and Health Study; SSGAC, Social Science Genetics Association Consortium; UKBB, UK Biobank. Traits within the blue/red boxes are traits selected for the multi-trait GWAS analysis for GERD (blue) and BE (red). The trait BE was not modelled in the MTAG model for GERD, to avoid sample overlap bias in the genetic prediction analysis for GERD into the BE/EA datasets. Asterisk (*) highlights genetic correlation estimates for each trait against GERD (shown by the blue arrows) obtained from previous An et al findings.
more single nucleotide polymorphisms (SNPs) exceeded genome-wide significance ($5 \times 10^{-8}$). There was no evidence for inflation of the GWAS test statistic (LD-Score intercept $<1$, QQ-plot in online supplemental figure 1). The association estimates for each of the 88 lead SNPs are presented in online supplemental table ST2. To benchmark the power improvement resulting from our multitrait approach, we compared the average $\chi^2$ test statistic for GERD from the multitrait GWAS (1.72) with that from the standard univariate GWAS (1.44); the increase corresponds to an estimated increase in GERD effective sample size of 64% (estimated via ratio of genomic inflation: $(1.72–1)/(1.44–1))$.

The GWAS Manhattan plot for the multitrait GERD GWAS along with the circular Manhattan plot showing the contribution of the BMI and depression associations to the multitrait GERD GWAS are shown in figures 2A and 3. The associations between independent genome-wide significant GERD loci in the multitrait GWAS model (multitrait analysis of GWAS (MTAG)) against BMI, EDU and MDD are tabulated in online supplemental table ST3. The linkage disequilibrium among SNPs in each of the 88 GERD loci is displayed via locusZoom plots in online supplemental figure 2). The vast majority (78 of 88) of the GERD–MTAG associations replicated at an uncorrected $p<0.05$ level in the independent GERD validation sample from the 23andMe cohort, with 59 significant at $p<0.05$ after Bonferroni correction (ie, $p<8.4 \times 10^{-4}$) for 88 tests (online supplemental table ST2). The effect sizes for GERD in the MTAG and 23andMe replication analyses were highly consistent ($r^2=0.85$) (figure 4A).

Our MTAG GWAS analysis focusing on BE discovered 17 BE susceptibility loci achieving genome-wide significance. Of these, 14 replicated at $p<2.9 \times 10^{-3}$ (0.05/17) in the independent 23andMe BE case-control cohort (online supplemental table ST4; QQ-plot in online supplemental figure 1). Relative to Gharakhani et al. [15], seven of the loci were novel (rs2861695, rs10080150, rs10039754, rs622217, rs11792928, rs739414 and rs7187365). The effect sizes for BE in the MTAG and 23andMe replication analyses were highly consistent (figure 4B). Regional locusZoom plots for these novel loci are in online supplemental figure 3.

To assess whether our MTAG results were robust against issues of phenotypic heterogeneity, due to the use of self-report and inference through medication data that are diagnostically less reliable, we repeated our multitrait GWAS analyses using only GERD defined through the International Classification of Disease, tenth version (ICD-10) code (for GERD MTAG) and pathologically confirmed BE diagnosis (for BE MTAG). Differences in GERD and BE definition made no meaningful difference to the results, with strong correlations ($r^2 >0.99$) observed between the SNP effect sizes of the original and revised MTAG models for both the GERD and BE analyses (online supplemental data).
figures 4 and 5). Moreover, results from the MTAG BE model excluding MDD and EDU were not meaningfully different from the BE+GERD+BMI+MDD+EDU model (online supplemental table ST5), suggesting that at least for these BE risk loci, the associated SNPs act on BE risk either via BMI or via pathways to GERD, which are not shown to be robustly related to traits such as MDD.

Transcriptome-wide association analyses reveal more than 200 genes associated with GERD and 49 with BE

Our metaXcan TWAS for GERD identified 37 significant genes when using expression quantitative trait loci (eQTL) information specifically derived from oesophageal muscularis tissue. Similarly, TWAS on gastro-oesophageal junction and oesophageal mucosa tissue identified 19 and 37 genes, respectively (online supplemental table ST6). As well as conducting TWAS on the three oesophageal tissues above, we also employed a TWAS approach using a much broader range of tissues types in multiXcan; this identified 212 significant genes after multiple testing corrections (see online supplemental table ST7).

A multiXcan TWAS for BE identified 49 significant genes using a weighted SNP-eQTL association across multiple tissues (online supplemental table ST8). For a TWAS focusing solely on each of the three oesophageal-related tissues, we identified fewer significant genes (<10 per tissue, online supplemental table ST9). Among the genes identified via TWAS, 31 genes were associated with both GERD and BE (online supplemental figure 6).

Tissue enrichment analysis shows enrichment of GERD-associated genes in brain tissues, but limited findings for BE genes

As expected given our study design, and consistent with previous findings, the majority of GERD loci showed pleiotropic associations with other complex traits

PheWAS analysis on novel hits for BE shows pleiotropic associations with other complex traits

As expected given our study design, and consistent with previous findings, the majority of GERD loci showed pleiotropic associations with other complex traits.
pleiotropic associations with a variety of anthropometric traits, education level, behavioural traits (including smoking). Several GERD genes were also associated with sarcoidosis, sleep duration and proxies of physical activity (online supplemental figure 8). A brief summary of our look-up for GERD loci driven by obesity and depression is in online supplemental table ST10.

For BE, again our study design leverages multiple input traits and, hence, pleiotropic associations are expected. PheWAS plots for the seven novel BE loci against traits available in the Open-Target platform are provided in online supplemental figure 9. The BE-associated SNP rs10080150 is associated with risk of ulcerative colitis, and rs7187365 and rs10039754 are associated with diaphragmatic hernia. Numerous BE SNPs (rs622217, rs10080150, rs11792928) showed pleiotropic associations with cardiovascular traits.

Genetic heterogeneity among the obesity-driven/depression-driven GERD subgroups showing differential patterns of enrichment on oesophageal tissues and genetic prediction onto BE/EA

Of the 88 genome-wide GERD loci, we categorised 27 as depression-driven, 46 as obesity-driven GERD loci and 15 as indeterminate/unclear based on their relative contribution to GERD in the multitrait model. We then sought to validate these categories using two separate approaches.

In our first approach, we compared the categories in a tissue enrichment-based analysis. The mapped genes for SNPs from each category using eQTL information and proximity are shown in online supplemental tables ST11 and ST12. While the regulation of gene expression was shown to be predominantly observed in brain tissues for the complete set of 88 genome-wide significant genetic loci, there were clear differences in the trend of gene expression across tissue types in the stratified obesity and depression driven subgroups (figure 5). Results for the directional (upward and downward separately) differential regulation for both subgroups are presented in online supplemental tables ST13 and ST14; directional regulation plots and gene expression heatmaps in online supplemental figures 10-13. Genes mapped in the depression GERD subgroup remain predominantly expressed in brain tissues. In contrast, for genes in the obesity GERD subgroup as well as there being expression in brain tissues, a clear signal can be seen in oesophageal tissues (online supplemental figure 10).

In our second approach, we compared the SNP effect sizes for GERD with those from an independent BE/EA GWAS. Based on the 88 GERD susceptibility loci from the MTAG analysis, each one unit (log(OR)) increase in risk of BE/EA was associated with a 0.6 (p=4.2×10^{-10}) unit (log(OR)) increase in GERD susceptibility (figure 6; summary data for BE/EA for the 88 GERD SNPs available in online supplemental table ST15). However, the Cochran Q statistics for the derived overall estimate suggested substantial evidence for effect size heterogeneity among GERD SNPs (p heterogeneity <0.001), indicating the potential for multiple biological mechanisms in action (online supplemental table ST16). We, hence, followed up with an association analysis for BE/EA based on the two

![Figure 5](https://example.com/figure5.png)

**Figure 5** MAGMA gene-based tissue enrichment analysis for categorised functional and non-functional GERD gene sets on 53 human tissues. The dotted line represents the Bonferroni corrected significance threshold. While both the obesity-driven and neuropsychiatric-driven GERD genes were differentially expressed in brain tissues, only the regulation of gene expression in oesophageal tissues were detected from the obesity GERD gene set. Another observation is that the pattern of regulation is more pleiotropic across tissues for the obesity GERD set, consistent with the complex architecture of adiposity. The results for up-ward and down-ward regulation of gene expression can be viewed in online supplemental materials. GERD, gastro-oesophageal reflux disease
DISCUSSION

Leveraging the known genetic correlation between GERD diagnosis with depression, EDU and anthropometric traits, we identified 88 independent GERD loci, with more than two-thirds showing clear evidence of replication in the independent 23andMe cohort. Applying a similar multitrait model, we also discovered seven additional risk loci for BE that have not been previously reported. To the best of our knowledge, this is also the first study to have presented clear evidence for the existence of genetic subgroups within the complex and heterogeneous GERD (genetic) architecture.

Obesity and depression are known GERD risk factors; here, we first show that they can be combined to identify more loci for a broadly defined GERD phenotype, where we greatly expanded the number of GERD risk loci over and above previous GWAS findings through a multitrait model. More than two-thirds of the MTAG GERD loci replicated in 23andMe suggest that despite heterogeneous phenotyping being used, our findings represent genuine reflux loci. These results are also in good accord with our previous work showing high genetic concordance between these self-reported and clinical phenotypes. Second, we show that among the genome-wide significant GERD loci, a subgroup acts predominantly via effects on obesity while another acts via effects on depression. This is broadly consistent with previous observations that endoscopic findings in many (38%) patients with typical GERD symptoms are normal; in such cases, psychological factors may drive symptom reporting. We further showed that genes involved in the obesity-driven GERD subgroup were differentially expressed in oesophageal and adipose tissues—which was not evident from previous analyses evaluating GERD as a single, homogeneous disease.

It was previously shown that psychosomatic factors are linked with functional oesophageal disorders through the gut-brain axis, which may alter a person’s emotional status, perception of pain and influence the self-reporting of GERD symptoms and severity. Previous studies have shown that among the broad class of individuals affected by GERD, those with functional oesophageal disorders typically have normal oesophageal acid exposure, and this may lead to them having lower risk of downstream BE/EA compared with other patients with GERD. Despite many studies showing strong association between depression with GERD (notably, the nonerosive reflux disease subtype), studies evaluating psychosomatic factors and BE/EA susceptibility had been more limited, likely due to sample size constraints. While there have been no large-scale observational studies to date to clarify any link between depression and BE, the previously documented weak correlation between GERD symptom scores (which correlates strongly with presence of a functional oesophageal disorder) and severity of esophagitis, would imply that psychosomatic factors are unlikely to drive progression towards BE. Here, our results are in keeping with this as they show that the depression-driven GERD loci, which may predispose a person towards a functional oesophageal disorder rather than a disorder with abnormal oesophageal acid exposure, have no effect on BE/EA risk. In contrast, we show that GERD risk loci acting via the obesity-driven pathway do have an effect on BE/EA risk (figure 6).

PhENWWAS results reveal that these novel loci are enriched with anthropometric traits and cardiovascular-related risk factors (online supplemental figure 9), perhaps as a result of a pleiotropic effect on obesity and cardiovascular disease. Given our results showing very weak association between depression-driven GERD and BE/EA, we did not expect to see pleiotropy between the BE-associated SNPs and depression; while this was broadly true, one of the novel BE loci (rs28861693) showed moderate evidence of association with depression (p < 6.7e-4). Moreover, dropping proxies of socioeconomic status (EDU) and depression (MDD) from the model did not change our GWAS findings for BE (online supplemental table ST5). While our analyses showed a potential shared pathway between the obesity-driven GERD loci and BE, due to limited power, very few loci achieved genome-wide significance (ie, p < 5e-8) across both traits. However, most of the BE loci did show suggestive evidence (ie, p < 1e-5) of an association with GERD (online supplemental table ST18).

Two of the novel MTAG GERD loci, rs96366202 (nearest gene PGPPE1) and rs7206608 (nearest gene CDH113), were previously shown to be associated with periodontitis. Apart from actual pH-monitoring, periodontitis is also reported as one of the risk factors that are more commonly observed among cases with erosive GERD. However, whether the potential role of the MTAG GERD loci replicated in 23andMe suggest that despite heterogeneous phenotyping being used, our findings represent genuine reflux loci. These results are also in good accord with our previous work showing high genetic concordance between these self-reported and clinical phenotypes. Second, we show that among the genome-wide significant GERD loci, a subgroup acts predominantly via effects on obesity while another acts via effects on depression. This is broadly consistent with previous observations that endoscopic findings in many (38%) patients with typical GERD symptoms are normal; in such cases, psychological factors may drive symptom reporting. We further showed that genes involved in the obesity-driven GERD subgroup were differentially expressed in oesophageal and adipose tissues—which was not evident from previous analyses evaluating GERD as a single, homogeneous disease.

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PGPEP1 and CDH13 on reflux esophagitis and poor oral health represents a common effect on (1) dietary behaviour or (2) changes in adiposity (resulting in genetic associations with BMI) remains unclear. Our gene-based PheWAS identified substantial overlap of GERD-associated genes with those involved in sarcoidosis, consistent with previous findings estimating one-third of patients with sarcoidosis being diagnosed with GERD-related symptoms and irritable bowel syndrome.18 Although fine-mapping is required to unambiguously link SNPs to genes, genes associated with several of the loci identified here (CRTC1, CDK2, PDE4B, DPDY, PDE1C) are targeted by drugs that are currently on trial for oesophageal and digestive system cancers.18 Despite the fact that we have not undertaken the substantial task of unambiguously fine-mapping each associated GERD SNP to the relevant gene(s), many of the genes we identify here may be promising drug targets for further research.

Our observation of a greater magnitude of association between obesity-driven GERD loci and BE/A is not surprising, given prior evidence showing that genetically predicted obesity was associated with increased risk of BE and EA. To evaluate whether obesity is a complete mediator between GERD and BE, we examined the genetic effect of obesity-driven GERD on BE adjusting for BMI and observed only partial attenuation (IVW-logOR) from 0.726 (p=2.23e-8) to 0.513 (p=0.001) of the GERD effect on BE (online supplemental table ST17). This implies that the relationship between GERD and BE cannot be solely explained by a common direct effect of obesity. Formal Mendelian randomisation (MR) analyses have been attempted previously;10 however, interpreting the results from such studies is difficult given we have shown that there are at least two pathways leading to GERD (one acting via obesity associated SNPs, another acting via depression SNPs).

This study has several limitations. First, a proportion of UKB GERD cases was determined through use of heartburn-related medication;17 however, as noted in our previous study, the genetic correlation between GERD inferred through medication, self-report and clinical diagnosis was very high. Similarly, our validation cohort (23andMe) adopted a broad GERD definition and diagnosis of BE were self-reported as opposed to being histologically verified. Overall, our approach of using a large GWAS sample size with a broad phenotype was much more effective in identifying novel loci than previous smaller studies using more well-defined phenotypes.10,13 Our heuristic for making the GERD obesity versus depression-driven subgroup classifications is limited by the accuracy of GWAS data, focused on only 88 SNPs, and, for ease of implementation, we used the BMI and depression GWAS results to group SNPs. Furthermore, while the combined effect of the obesity-driven GERD genome-wide significant loci (assembled in a genetic risk score) was robustly associated with BE/EA risk, the predictions from this genetic risk score lack precision to reliably predict BE/EA in clinical settings. Finally, some SNPs may have pleiotropic effects on both obesity and on psychiatric traits or could simply reflect other pleiotropic pathways not mediated by these factors (eg, mucosal tight junctions, inflammation and repair, oesophageal and gastric motility). Modelling these risk factors which are independent of the obesity and depression axis in our multimtrait model could conceivably increase power to detect additional loci for both GERD and BE. GWAS studies on these additional traits are currently limited in size but investigating this will be worthwhile in future work.

In conclusion, using a multimtrait framework, we greatly expanded the number of genome-wide significant genetic loci for GERD and also increased the number of BE loci by seven. Here, we present strong evidence for a genetic underpinning of disease heterogeneity in GERD, where we showed that GERD loci associated with depressive symptoms are not strong predictors of BE/EA relative to obesity-driven GERD loci.

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**Correction notice**

This article has been corrected since it published Online First. An author’s name has been updated and figure 4 replaced.

**Collaborators**

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SM has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: J-ES, JA, PG, BJK, SM. Data collection: J-ES, JA, IG, AB, JJ, CP, CMO, REN, RF, APT, TLV, MB, DAH, SM. Data preparation: JSO, JA, XH, PG, SM. Drafting of the manuscript: J-ES, JA, PG, BJK, SM. Statistical analysis and interpretation of data: J-ES, JA, XH, MH, PN, PG, BJK, SM. Acquisition of data and obtained funding: J-ES, IG, AB, JJ, CP, CMO, REN, RF, APT, TLV, MB, DAH, SM. Data management: JSO, JA, XH, PG, SM. Drafting of the manuscript: J-ES, JA, XH, MH, PG, BJK, SM. Critical review of the manuscript for important intellectual content: J-ES, JA, JJ, CP, CMO, REN, RF, APT, MB, DAH, PG, BJK, SM. Study supervision: SM. All authors read and approved the final version for submission.

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Competing interests Authors listed in the 23andMe Research Team are employees for the company 23andMe Co.

Patient consent for publication Not required.

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Data availability statement Data are available in a public, open access repository. Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. The MTAG GWAS summary statistics on GERD (study accession GCTST90000154) and BE (GCTST90000151) can be obtained from the GWAS catalog (ebi.ac.uk). Sources for the individual GWAS summary statistics used in the multi-trait model are listed in Supplementary Information. GWAS summary statistics for the 23andMe samples are available via direct request to 23andMe (dataset-request@23andMe.com; a data transfer agreement is required). The raw genomic and phenotypic UK Biobank data can be obtained via direct application to the UK Biobank (http://www.ukbiobank.ac.uk/).

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Editor's quiz: GI snapshot

Polyps and skin lesions: what could it be?

**CLINICAL PRESENTATION**

A 51-year-old man presented to our gastroenterology clinic complaining of bloating and belching. His medical history was significant for diabetes mellitus and thyroidectomy for papillary thyroid cancer. He reported occasional heartburn but denied any abdominal pain, change in bowel habits, weight loss or family history of malignancy. One of his children had autism. Physical examination was noted for asymptomatic skin-coloured lesions over the feet (figure 1A) but was otherwise negative. Upper gastrointestinal endoscopy showed numerous whitish plaques in the oesophagus (figure 1B), along with multiple small polyps in the stomach (figure 1C) and duodenum. Colonoscopy revealed numerous polyps scattered throughout the colon (figure 1D) and multiple polyps in the terminal ileum. Biopsies from the stomach, duodenum and colon were all consistent with hyperplastic polyps (figure 1E).

**QUESTION**

What is your diagnosis?

See page 1116 for answer

![Figure 1](http://gut.bmj.com/).