Clinical features and genetic risk of demyelination following anti-TNF treatment

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Authorship

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted

Contributions

A.S, T.H, N.A.K, J.R.G and T.A participated in the conception and design of the work. S.L, H.D.G, P.H, N.M.H, N.C, B.H, G.J.W, G.A.H, J.H, R.M, A.C, M.S.S, P.M.I, G.C.F, E.S, F.C, E.L, V.A, A.R.W, J.T, R.N.B, M.W, N.A.K, A.S, T.H, J.R.G and T.A were involved in the acquisition, analysis or interpretation of data. The data analysis was performed by S.L and H.D.G. Drafting of the manuscript was conducted by S.L, H.D.G, N.A.K, J.R.G and T.A. All the authors contributed to the critical review and final approval of the manuscript. T.A obtained the funding for the study and is the guarantor of the article.

Abstract

Background

Anti-TNF exposure has been linked to demyelination events. We sought to describe the clinical features of demyelination events following anti-TNF treatment and test whether affected patients were genetically predisposed to multiple sclerosis (MS).

Methods

We conducted a case-control study to describe the clinical features of demyelination events following anti-TNF. We compared genetic risk scores (GRS), calculated using carriage of 43 susceptibility loci for MS, in 48 cases to 1219 patients exposed to anti-TNF who did not develop demyelination.

Results

Overall, 39 (74%) cases were female. The median age (range) of patients at time of demyelination was 41.5 years (20.7 – 63.2). The median duration of anti-TNF treatment was 21.3 months (0.5 - 99.4) and 19 (36%) patients were receiving concomitant immunomodulators. Most patients had central demyelination affecting the brain, spinal cord or both. Complete recovery was reported in 12 (23%) patients after a median time of 6.8 months (0.1 – 28.7). After 33.0 months of follow-up partial recovery was observed in 29 (55%) patients, relapsing and remitting episodes in 9 (17%), progressive symptoms in 3 (6%): 2 (4%) patients were diagnosed with MS. There was no significant difference between MS GRS scores in cases (mean -3.5×10^{-4}, SD 0.0039) and controls (mean -1.1×10^{-3}, SD 0.0042) (p=0.23).

Conclusions

Patients who experienced demyelination events following anti-TNF were more likely female, less frequently treated with an immunomodulator, and had a similar genetic risk to anti-TNF exposed

42 controls who did not. Large prospective studies with pre-treatment neuroimaging are required to
43 identify genetic susceptibility loci.

Introduction

Anti-TNF therapies were licensed for use in 1998 and have revolutionised the management of a range of immune mediated inflammatory disorders. Case reports linking infliximab and etanercept to demyelination events followed and prompted the Food and Drug Administration and the European Medicines Agency to issue safety warnings[1–3]. Contemporaneously, a randomised controlled trial of lenercept (a recombinant TNF receptor p55 immunoglobulin fusion protein) in patients with multiple sclerosis was discontinued early, because of the increased frequency of early and more severe demyelination exacerbations in the treatment compared with placebo arms [4].

Demyelinating events have been reported with all licensed anti-TNF therapies in the treatment of patients with inflammatory bowel disease(IBD)[5], rheumatoid arthritis[6] and psoriasis[7]. Because demyelination was rare in the respective registration trials it is not possible to conclude whether a causal association exists between anti-TNF therapies and demyelination events[7,8]. Data from post-marketing adverse event registries seem to be reassuring, citing similar rates of demyelination to the background risk of multiple sclerosis[9]. However, these data are likely to underestimate rates of anti-TNF related demyelination because of confounding by voluntary reporting. In support of this assertion, data from a Danish population based-cohort study of patients with IBD treated with at least one anti-TNF reported a two-fold relative risk of demyelinating events[10]. Moreover, because demyelination can be clinically silent the actual risk attributable to anti-TNF therapies maybe even higher. Evidence of demyelination was reported in 4% of patients with rheumatoid arthritis or spondyloarthopathies treated with anti-TNF therapies after 18 months in whom pre-treatment MRI imaging was normal [11].

Considerable uncertainty remains, therefore, as to whether anti-TNF exposure induces demyelination in patients genetically pre-disposed to multiple sclerosis or is a chance observation reflecting the evolution of de novo multiple sclerosis, or an idiosyncratic drug reaction. Moreover,

because symptomatic demyelination events following anti-TNF are uncommon their natural history is poorly defined.

Methods

Study design and setting

We conducted a retrospective case-control study to report the clinical features and natural history of demyelination events following anti-TNF therapy. We sought to assess whether demyelination events occurred in patients at increased genetic risk for multiple sclerosis.

Study populations

Potential cases were recruited from 41 UK and six international sites between 2012 and 2018. Patients were identified through: opportunistic clinical encounters, cases reported to the British Neurological Surveillance Unit (BNSU) or to the Medicine and Healthcare Products Regulatory Authority Yellow Card scheme.

Inclusion criteria were all of the following: exposure to anti-TNF drug(s) without a preceding history of neurological symptoms suggestive of demyelination; neurological symptoms persisting for at least 24 hours following anti-TNF exposure; MRI brain and/or spinal cord imaging and/or or electrophysiological studies (nerve conduction or evoked potentials) consistent with central nervous system (CNS) or peripheral nervous system (PNS) disease, respectively; and neurological opinion implicating the anti-TNF drug as a cause of demyelination necessitating drug withdrawal if the patient was still receiving the drug.

Investigators at each site completed a custom-designed case report form (Supplemental Appendix 3), that captured the following data: patient demographics (age, weight, height, ethnicity, smoking and inflammatory disease history); drug exposure data (anti-TNF, anti-TNF dose, drug start date, drug stop date) and demyelination history (onset, duration, resolution, investigations and treatment).

Case report forms and supporting imaging and/or electrophysiological tests were reviewed independently by a panel including a neuro-radiologist and at least 2 neurologists. Consistent with our prior pharmacogenetic studies[12–14] we modified the Liverpool Adverse Drug Reaction Causality Assessment Tool to verify cases (Supplemental Figure 1). "Possible" cases were defined as patients who had equivocal investigations or clinical features of demyelination. "Probable" cases demonstrated clinical, radiological and / or electrophysiological features of demyelination with a clear temporal relationship with anti-TNF therapy and no other cause for demyelination. In addition to these criteria, "definite" cases were individuals who had a recurrence of demyelination on anti-TNF therapy rechallenge. Cases assigned as "unlikely" were excluded. Definite, probable and possible cases were included in subsequent analyses. We classified patients according to whether they had central (brain and/or spinal cord) or peripheral nervous system involvement and whether their illness was a clinically isolated syndrome or had a relapsing phenotype. Clinically isolated syndrome was defined as a first episode of neurological symptom lasting for 24 hours and is caused by inflammation or demyelination in the central nervous system. Partial recovery was defined as an episode of demyelination with partial or no resolution of symptoms at the time of follow-up.

Patients recruited to the Personalising Anti-TNF Therapy in Crohn's disease (PANTS) study without a history of demyelination were used as controls. In brief, the PANTS study is a UK-wide, multicenter, prospective observational cohort study of 1610 patients with Crohn's disease treated with infliximab (originator, Remicade [Merck Sharp & Dohme, UK] and biosimilar, CT-P13 [Celltrion, South Korea]), and adalimumab (Humira [Abbvie, USA])[15]. To allow us to identify phenotypic factors associated with demyelination following anti-TNF therapy, each IBD case was matched to five anti-TNF exposed controls from the PANTS cohort by duration of anti-TNF therapy. Genetic risk scores for multiple sclerosis in all cases were compared to scores from control patients without neurological adverse events included in the genetics arm of the PANTS study.

Genetic methods

DNA was extracted from whole blood and genotyped using the Infinium Global Screening (cases) and Illumina CoreExome microarrays (controls). Individuals of non-European ancestry were identified using principal component analyses and excluded. Checks were made for relatedness using KING 1.9[16].

Variants with a genotype call rate of <95%, a minor allele frequency of less than 1% or with significant evidence of deviation from Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$) were excluded. We corrected for batch-effect by removing variants with an uncorrected p-value of < 0.05 for association with batch using Fisher's exact test. Palindromic variants were also removed prior to imputation leaving 130,132 genotyped variants. Single nucleotide polymorphisms were imputed using the Sanger Imputation Service to the Haplotype Reference Consortium (HRC) panel and a post-imputation information score of 0.9 was used as a cut-off. We constructed a multiple sclerosis genetic risk score (GRS) using data from previously identified risk variants[17]. Genetic risk scores were generated by summing the carriage status at each locus multiplied by the log odds ratio of that variant[18,19]. Susceptibility loci included in our GRS were defined as risk variants with a p-value < 5×10^{-6} and no closer in the genome than within 1 mega-base of another risk variant with a lower p-value. Overall, 51 loci were identified, details of their odds ratios and relative weightings are given in Supplemental Table 1.

We validated our GRS using subjects with multiple sclerosis identified in the UK Biobank, a study of over 500,000 individuals aged between 37 and 73 years recruited between 2006 and 2010[20]. Multiple sclerosis cases were defined in the UK Biobank using either the International Classification of Diseases (ICD) 10 code G35, ICD9 code 340, or self-report code 1261. Those with other demyelinating conditions, defined by an ICD10 code of G36 / G37, ICD9 code of 341, or self-report code of 1397, were excluded. We validated the GRS in unrelated Europeans only. European ancestry was determined using principal components analysis and relatedness was determined using KING

Kinship[16]. Imputation was performed by the UK Biobank[21]. The dataset used for validation of the GRS contains 1680 multiple sclerosis cases and 387,932 controls.

Statistical methods

Pseudonymised data were managed using purpose designed electronic data capture tools at the Royal Devon and Exeter NHS Trust. Statistical analyses were undertaken in R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria), PLINK version 1.90b3.42 and MATLAB version R2017a. All analyses were two tailed and p-values <0.05 were considered significant.

Descriptive statistics were reported, based on normality, as mean (SEM) and median [IQR] for continuous data and as proportions for categorical data unless otherwise stated. We included patients with missing clinical data in analyses for which they had data and specified the denominator for each variable. Propensity matching of IBD cases to PANTS controls on duration of anti-TNF drug exposure was undertaken using the MatchIt package in R[22]. We performed univariable analyses, using Fisher's exact test for categorical data and Mann-Whitney U tests for continuous data, to identify clinical variables associated with demyelination events in cases versus controls.

We tested for differences in multiple sclerosis genetic risk scores between cases and controls both in the UK Biobank and in our case-control study of patients exposed to anti-TNF, using Student's t-tests. Diagnostic performance of these scores was assessed using receiver operating characteristics (ROC) analyses. Fisher's exact test with Bonferroni correction was used to test association at each locus.

Ethical considerations

The protocol was approved by the National Research Ethics Committee (11/SW/0222, Exeter pharmacogenetic PRED4 programme), and international sites sought local ethical approval respectively. All participants involved provided informed written consent. Development and validation of the GRS was conducted using data from the UK Biobank (application 41588).

Results

Study overview

Case disposition through the study is shown in Figure 1. Between 2012 and 2018, 66 patients were recruited from 41 UK and 6 international sites. Following adjudication, we excluded 13 (20%) patients: 7 (11%) in whom review of investigations refuted evidence of demyelination or a temporal relationship with anti-TNF exposure; and 6 (9%) in whom an alternative diagnosis was more likely (mycoplasma infection, hypertension, vitamin B12 deficiency, mononeuritis multiplex, multifocal acquired demyelinating sensory and motor neuropathy and myositis). Only one patient was re-challenged with an anti-TNF drug after a demyelination event.

Control subject disposition through the study is shown in Figure 1. Overall, 2% (34/1610) patients suffered a neurological adverse event during follow-up in the PANTS study and were excluded from this control cohort. The adverse event was attributed to the anti-TNF drug in 24/34 patients, leading to drug withdrawal in half; however, following neurological assessment none were diagnosed with demyelination.

After assessment using genetic quality control methods, we excluded 5 cases: 3 (6%) for non-white European ethnicity and 2 (4%) for failure of genotyping. We did not identify relatives of third degree or closer.

Clinical characteristics

The clinical features of verified cases are summarised in Table 1. Overall, 39 (74%) patients were female and 44 (83%) patients were white European. The median age (range) was 41.5 years (20.7 – 63.2). Thirteen (25%) were current and 13 (25%) were ex-smokers. The indication for anti-TNF therapy was IBD in 32 (60%), rheumatoid arthritis in 12 (23%), psoriasis or psoriatic arthropathy in 7 (13%), and ankylosing spondylitis in 5 (9%) patients, respectively. Three patients received anti-TNF therapy for more than one indication. Demyelination events followed treatment with infliximab in

25 (47%), adalimumab in 19 (36%), etanercept in 7 (13%), golimumab in 1 (2%) and certolizumab in 1 (2%) patient(s), respectively. Concomitant immunomodulator use was observed in 19 (36%) cases, (thiopurine 8 (42%), methotrexate 8 (42%), ciclosporin 2 (11%), leflunomide 1 (5%)). Overall, the median (range) duration of anti-TNF treatment prior to demyelination event was 21.3 [0.5-99.4] months.

Propensity matching in the subset of patients with IBD resulted in a median [IQR] duration of anti-TNF treatment prior to demyelination event of 9.9 [5.1 - 31.9] and 9.9 [5.1 - 25.2] months in cases and controls, respectively (p= 0.44). Cases were more likely to be female (84% [27/32] vs 58% [92/160], respectively, p = 0.008, Table 2) and were less likely to have been treated with a concomitant immunomodulator (immunomodulator 31% [10/32] vs 56% [89/160] respectively, p = 0.02). No differences were seen according to age, ethnicity, body mass index (BMI) or cigarette smoking.

Natural history of demyelination

Five (9%) patients had a family history of multiple sclerosis, although none were first degree relatives of a patient with multiple sclerosis. Four (8%) patients had MRI of the brain or spinal cord before the index demyelinating event. Three MRI scans were conducted prior to drug commencement and between 12.0 – 108.0 months before the demyelinating event. The indications were paraesthesia, seizures and an independent research study. Excluding the research MRI scan, none showed evidence of demyelination. The most common presentation was of central demyelination, observed in 44/53 (83%) patients. 31/44 (70%) patients with central demyelination had features in keeping with a clinically isolated syndrome (CIS). Of these 13/31 (42%) patients were noted to have a single lesion on MRI, and the remaining 18 (58%) multifocal lesions. Both cerebral and spinal lesions were noted (Figure 2).

The anti-TNF drug was withdrawn in all patients. In 24 (45%) patients no additional treatment was used, 21 (40%) patients received corticosteroids, 8 (15%) were treated with intravenous immunoglobulin and 4 (8%) patient received plasma exchange (Table 3). One patient who was re-treated with an anti-TNF developed symptoms of demyelination after each of two re-challenges. The median (range) duration of follow-up after the index demyelination event was 31.0 months (2.0 – 171.0); only 5/53 (9%) patients had less than six months of follow-up. Complete recovery was reported in 12 (23%) patients after a median (range) time of 6.8 months (0.1 – 28.7), partial recovery in 29 (55%) patients after a median (range) time of 33.0 months (2.0 – 118.0) of follow up. Relapsing and remitting episodes were observed in 9 (17%) patients, and 3 (6%) patients experienced progressive symptoms; all patients with relapsing and remitting episodes or progressive symptoms had central demyelination. Overall, 2 (4%) patients were subsequently diagnosed with multiple sclerosis.

Genetic Analysis

After genetic imputation, we excluded 8 of the 51 target loci from the genetic risk score analyses: 6 loci because of poor imputation (INFO score <0.9) and 2 loci because they were not included in the HRC reference panel. The 43 loci that were used to construct our multiple sclerosis GRS are shown in Supplementary Table 1. We used this multiple sclerosis GRS in the UK Biobank and observed a significant difference between multiple sclerosis cases and controls ($p = 3.2 \times 10^{-116}$) (Figure 3) with an area under the curve (95% CI) of 0.65 (0.64 – 0.66) (Figure 4).

There was no significant difference in multiple sclerosis GRS scores between cases and controls (cases [mean -3.5×10^{-4}, SD 0.0039] vs. controls [mean -1.1×10^{-3}, SD 0.0042], p=0.23) (Figure 5). Moreover, no significant associations with demyelination were seen at any individual locus (Supplementary Table 2). We did not observe genomic inflation for the SNPs used in our GRS (Supplementary Figure 2). The AUC (95% CI) for predicting anti-TNF related demyelination in our cases compared with PANTS control subjects was 0.55 (0.46 – 0.64) (Figure 4).

Discussion

Key results

Anti-TNF exposed patients who suffered demyelination events were more likely to be female and less frequently treated with an immunomodulator. Patients who developed demyelination events had similar genetic risk scores for multiple sclerosis to control patients who did not develop demyelination events after anti-TNF therapy. Following almost three years of follow-up, about half of our demyelination cases had received one or more treatments for demyelination and three-quarters had ongoing neurological symptoms.

Interpretation

Shared genetic susceptibility between autoimmune and inflammatory conditions may account for the increased risk of multiple sclerosis reported in patients with rheumatoid arthritis and IBD[23,24]. Previous genetic studies of anti-TNF induced demyelination are limited to a negative candidate gene study of *TNFRSF1A* in patients with rheumatoid arthritis[25]. Here, we have shown that anti-TNF treated patients who developed demyelination events had overlapping genetic risk scores for multiple sclerosis with anti-TNF exposed controls who did not develop demyelination. It is unlikely, then, that anti-TNF therapies lead to demyelination only in individuals genetically pre-disposed to multiple sclerosis. In support of this assertion only two cases in our study were subsequently diagnosed with multiple sclerosis.

There was a female predominance amongst patients with demyelination following treatment with anti-TNF therapies. We did not observe any other classical risk factors for multiple sclerosis arguing against the hypothesis that these events represent the chance development of de novo multiple sclerosis. For example, compared to previously reported case series of patients with multiple sclerosis our cases were older [26], less likely to be cigarette smokers [27] and no one reported a first

degree relative with multiple sclerosis [28] . In support of anti-TNF related demyelination being an adverse drug reaction, we observed rapid recurrence of neurological symptoms in the one individual who was re-challenged with an anti-TNF drug after a demyelination event.

Limitations and generalisability

Our study has several strengths including rigorous cross-disciplinary independent case verification, and for the first time we explored the value of a multiple sclerosis GRS in a study of anti-TNF related demyelination. We acknowledge, however, the following important limitations: first, in keeping with all case-control studies our data are susceptible to recall bias, with greater recruitment of more severe cases. Second, because this was a convenience sample, we were unable to report the incidence of demyelination events. However, in our prospectively collected control cohort of 1610 patients, 2% reported neurological symptoms during follow-up although none were confirmed as being due to demyelination. Third, our retrospective data collection from medical records is subject to missingness and interpretation bias. In particular, we have no data relating to other important environmental risk factors for multiple sclerosis including vitamin D deficiency and previous Epstein-Barr virus infection. Fourth, our genetic analyses were limited to patients of white European ancestry and only patients with Crohn's disease made up the control cohort, which limits the generalisability of our findings. Finally, despite the study being open for six years we accept that our sample size was too small to permit a pharmacogenetic genome wide association study to identify novel variants associated with demyelination following treatment with anti-TNF and we were also underpowered to detect a difference in our cases and multiple sclerosis cases from the UK Biobank.

Conclusion

This large case-control study adds comprehensive clinical information to the existing reports of demyelinating events associated with anti-TNF therapy for inflammatory disorders. Demyelination events were no more common in patients at genetic risk for multiple sclerosis. Further pharmacogenetic studies with prospective neuroimaging are required to define the risk of demyelination following anti-TNF therapy and to identify genetic susceptibility loci.

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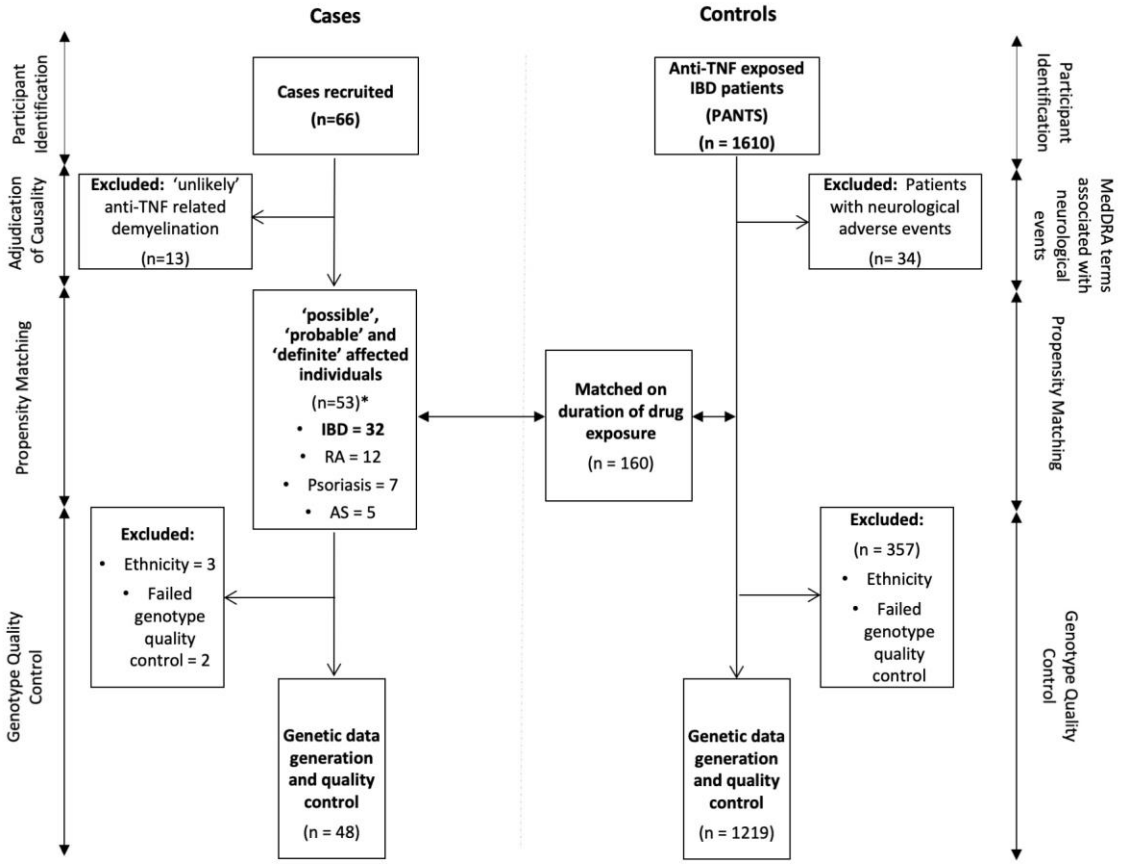
Figures and Tables

Figure 1. Flow diagram and Study Overview of Case and Control Cohorts



* Three patients received anti-TNF therapy for more than one indication

Abbreviations: IBD = Inflammatory Bowel Disease, PANTS = Personalised Anti-TNF Therapy in Crohn's disease, MedDRA = Medical Dictionary for Regulatory Activities, RA = Rheumatoid Arthritis, AS = Ankylosing Spondylitis

Figure 2. Pattern of anti-TNF related demyelination in 53 cases

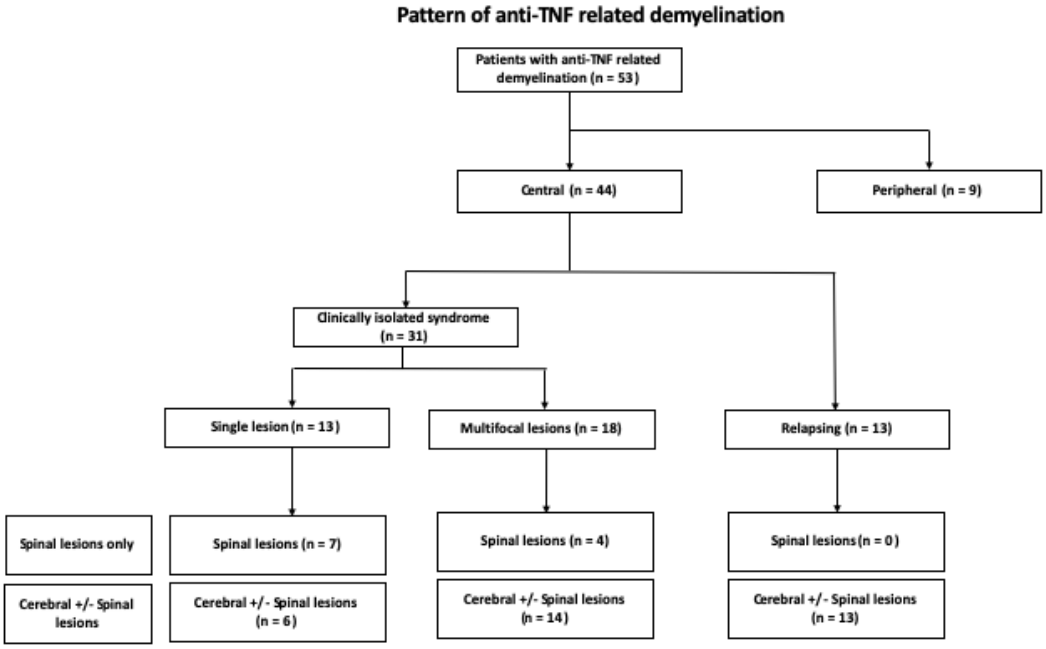
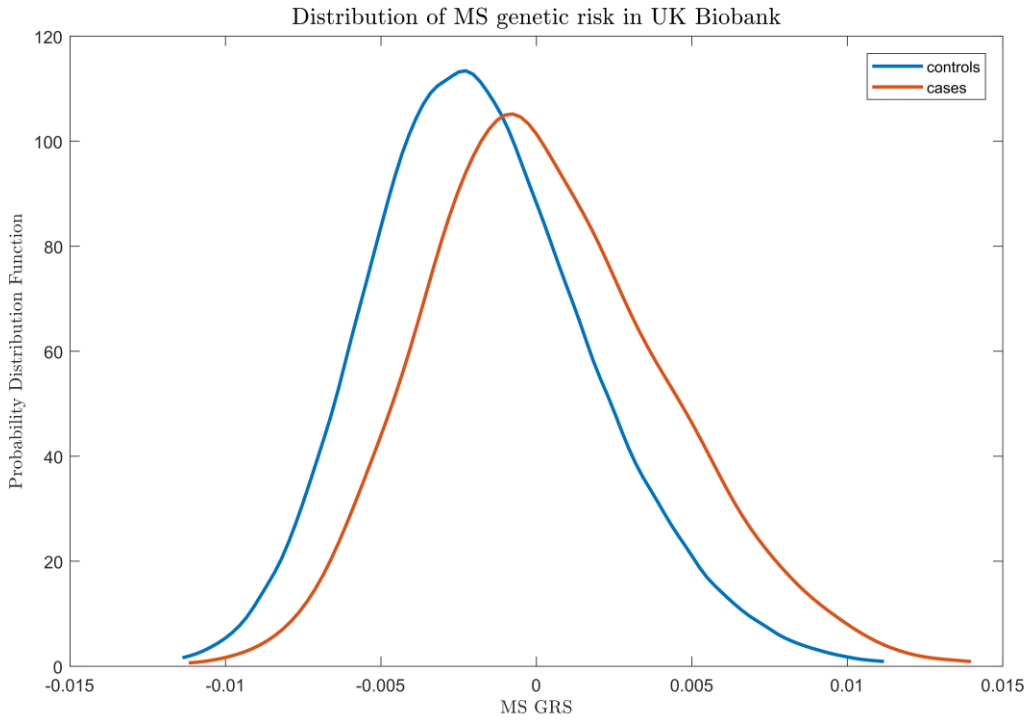


Figure 3. Probability distribution of genetic risk scores (GRS) in patients with multiple sclerosis in the UK Biobank



413 **Figure 4. Receiver operating characteristic (ROC) curves of multiple sclerosis (MS) genetic risk**
414 **scores (GRS) in MS patients in the UK Biobank and anti-TNF related demyelination cases**

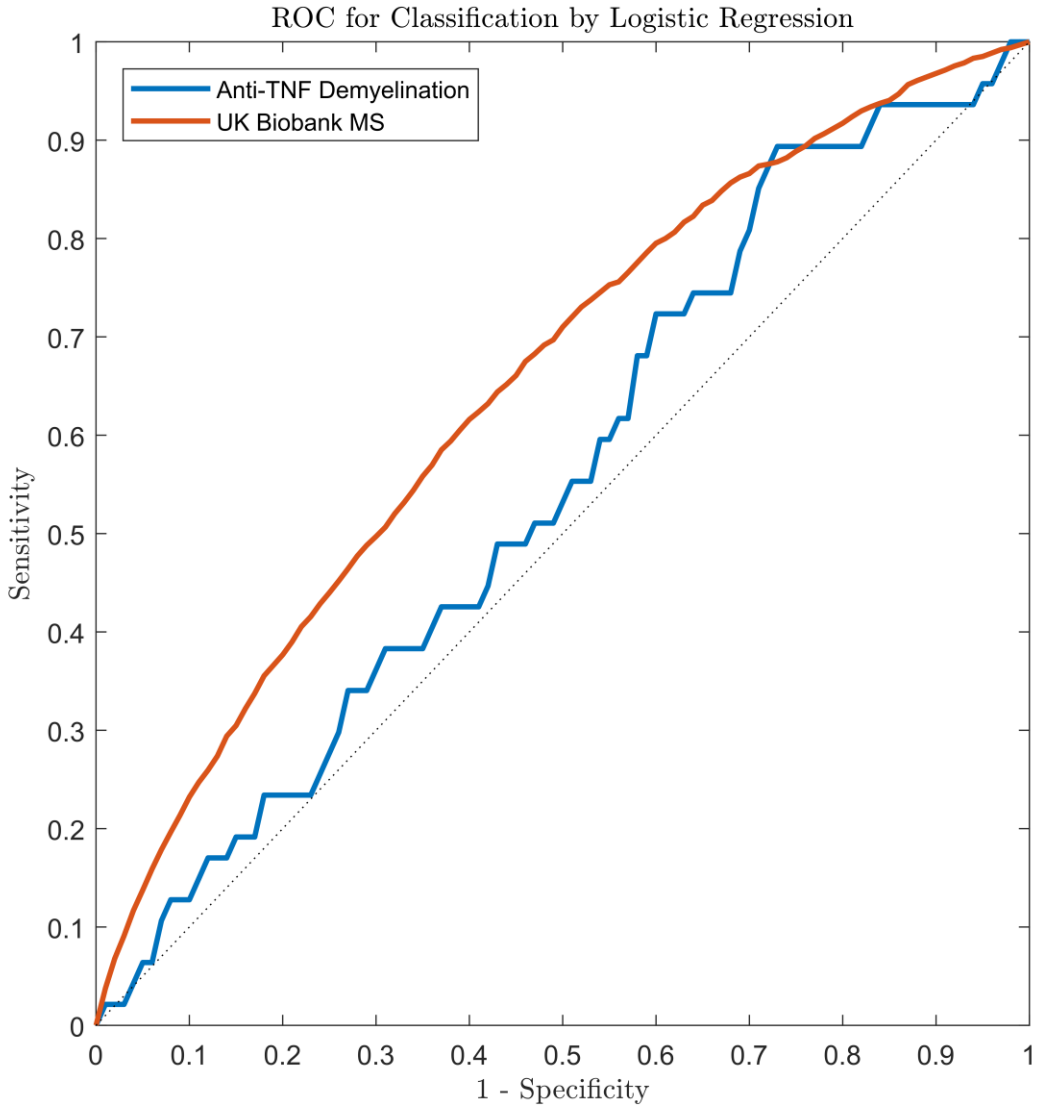


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Figure 5. Probability distribution of genetic risk scores (GRS) in cases and controls

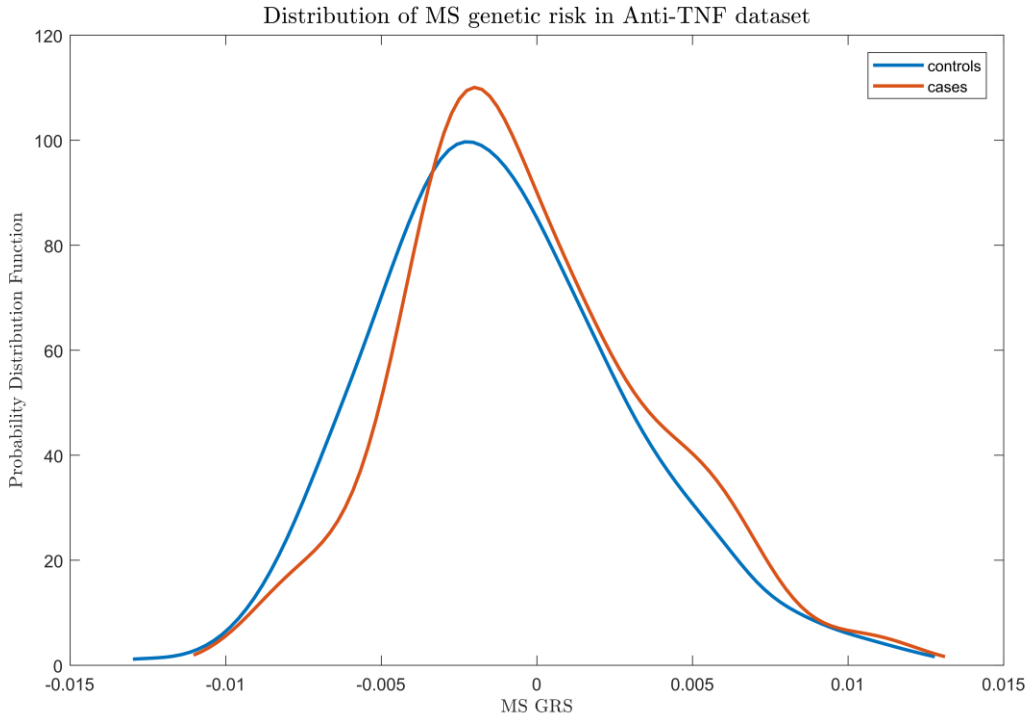

Table 1. **Baseline demographic of cases with demyelination related to anti-TNF therapy**

Characteristic	**Cases**
Patients, n	**53**
Gender	
Female	39 (74%)
Male	14 (26%)
Age	
Mean (SD)	40.6 (10.5)
Median [Min, Max]	41.5 [20.7, 63.2]
Ethnicity	
White European	44 (83%)
Other white background	4 (8%)
Mixed white and asian	2 (4%)
Any other Asian	2 (4%)
Carribean	1 (2%)
BMI	
Median [Min, Max]	24.9 [18.0, 43.2]
Missing	5 (9%)
Condition	
IBD	32 (60%)
RA	12 (23%)
Psoriasis	7 (13%)
AS	5 (9%)
Drug	
Infliximab	25 (47%)
Adalimumab	19 (36%)
Etanercept	7 (13%)
Certrolizumab	1 (2%)
Golimumab	1 (2%)
Family History	
Yes	5 (9%)
No	42 (79%)
Smoking	
Current	13 (25%)
Ex	13 (25%)
Never	21 (40%)
Immunomodulator	
Yes	19 (36%)
No	34 (64%)
Duration on anti-TNF (months)	
Median [Min, Max]	21.3 [0.460, 99.4]

420 BMI, body mass index; IBD, inflammatory bowel disease; RA, rheumatoid arthritis; AS, ankylosing
421 spondylitis

Table 2. Characteristics of anti-TNF exposed inflammatory bowel disease cases and controls

Characteristic (IBD patients)	**Case** **n = 32**	**Control** **n = 160**	***p* value**
Sex			
Female	27 (85%)	92 (58%)	0.008
Male	5 (16%)	68 (43%)	
Age (median [IQR])	34.1 [29.5 - 46.5]	33.9 [25.0 - 48.0]	0.542
BMI (median [IQR])	23.6 [20.6 - 27.1]	24.1 [20.3 - 28.9]	0.539
Smoking			
Current	6 (22%)	27 (17%)	0.75
Ex	9 (33%)	50 (32%)	
Never	12 (44%)	81 (51%)	
Concurrent immunomodulator	10 (31%)	89 (56%)	0.02

BMI, body mass index.

Table 3. Clinical characteristics of demyelination events in anti-TNF exposed cases

Characteristic of demyelination events	**Cases (n = 53)**
Investigations	
Lumbar puncture	32 (60%)
Nerve conduction studies	8 (15%)
Electrophysiology	19 (36%)
Treatment	
Steroids	21 (40%)
IVIG	8 (15%)
Plasma exchange	4 (8%)
None	24 (45%)
Other	1 (2%)
Time to recovery (Months)	
Median [Min, Max]	6.75 [0.10, 28.7]
Duration of follow-up (Months)	
Median [Min, Max]	31.0 [2.00, 171]

IVIG, intravenous immunoglobulin.