*TTC7A* variants previously described to cause enteropathy are observed on a single haplotype and appear non-pathogenic in paediatric inflammatory bowel disease patients

*Reply to* *Lawless et al, Bialellic Mutations in Tetratricopeptide Repeat Domain 7A (TTC7A) Cause Common Variable Immunodeficiency-Like Phenotype with Enteropathy*

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**Conflicts of interests**

The authors declare no conflicts of interest

To the Editor,

Identification of monogenic causes of inflammatory bowel disease (IBD) has increased dramatically over the last five years, largely due to the improved interpretation and value offered through use of whole exome and whole genome sequencing (WES + WGS) techniques (1). There are now over 80 genes associated with Mendelian causes of IBD, with novel associations being described each year (1). Despite this there are significant challenges with interpretation of variants that appear to be deleterious *in silico*, but lack functional validation (1). An additional challenge lies with identification of compound heterozygosity within patients.

We read with interest the report from Lawless *et al* of compound heterozygote variants in the Tetratricopeptide Repeat Domain 7A (*TTC7A*) gene presenting with enteropathy and a common variable immunodeficiency (CVID) phenotype (2). *TTC7A* deficiency has been described as a cause of monogenic IBD in over 50 patients, in an autosomal recessive inheritance pattern (3). Multiple variants have been demonstrated as causal, presenting with a varied phenotype involving inflammatory, gastrointestinal and immunological manifestations (3). Typically nonsense variants are fatal within a year of birth, whereas missense mutations may lead to milder disease and patients surviving into adulthood (2,3). The role of variation within *TTC7A* in non-monogenic forms of IBD is less clear. *TTC7A* is functionally key as a scaffolding and chaperone protein in processes crucial for normal intestinal epithelial cell development (3). It may prove that milder missense mutations are risk variants for polygenic forms of IBD, although this has not been substantiated.

We have previously reported monogenic forms of inflammatory bowel disease for our cohort (4). In this study we describe the two variants observed in *trans* by Lawless *et al* in five of our patients, always observed in *cis*. Additionally, through application of a contemporary *per gene, per individual*, deleteriousness score, GenePy, we assess the evidence for variation in *TTC7A* contributing to polygenic IBD (5).

Patients recruited to the genetics of paediatric IBD study (n=401) at Southampton Children’s Hospital underwent WES. All patients were consented and the study had ethics approval from Southampton and South West Hampshire Research Ethics Committee (09/H0504/125). Data were processed through an in-house pipeline. Parental DNA underwent Sanger sequencing to confirm segregation in potential compound heterozygous patients (5). Variants were annotated using *in silico* deleteriousness metrics including ClinVar (2018 update) and human mutation genetic database (HGMD Pro 2018). Variant call files (VCFs) for *TTC7A* in 401 patients with IBD were assessed for pathogenic mutations.

Five patients were identified harbouring the p.K606R and p.S672P variants (Table 1). Sanger sequencing confirmed all patients had these variants on the same chromosome (inherited maternally or paternally). Neither variant was observed in isolation (without the other) in the remaining 396 patients. In all cases, the parent who transmitted these variants did not have IBD. The brother of patient 1, who also had IBD (Crohn’s disease, presenting in childhood), was found to have both the p.K606R and p.S672P variants. The phenotype of these *TTC7A* patients was varied, 4/5 probands had Crohn’s disease. One had required surgery during follow-up (mean follow-up time 4.0 years) due to stricturing disease but none of the remaining patients had developed complications and disease was controlled with 5-ASA, thiopurine or anti-TNF therapy.

The p.K606R and p.S672P variants are rare, both having a gNomad (all genomes) frequency of 0.0022 and an EXAC (non-Finnish European) frequency of 0.0035. Identical frequency suggests inheritance on a common haplotype. Both are predicted to be highly deleterious, possessing CADD scores of 25.6 and 27.5 respectively. The percentage of our patients, as a proportion of IBD patients sequenced, possessing these alleles is 1.2% (5 patients), contrasting to the 1 patient (0.22-0.35%) expected from curated frequency databases. To determine whether an additional variant could be impacting on the function of the wild-type allele patient VCFs were reviewed. Three patients (patient 1, 3 and 4) harboured the common synonymous p.E232E variant (CADD 9.5) and one (patient 5) harboured the synonymous p.S66S variant (CADD 17.63). Both of these are common, minor allele frequency 0.178 and 0.08 respectively, and have modest deleteriousness scores. Segregation analysis was not performed for these variants. Sanger sequencing to confirm segregation unambiguously confirmed heterozygote status and excluded a deletion in the five patients at the site of the p.K606R and p.S672P variants. To further assess the evidence for large deletions and copy number variants (CNV), WES data from patients harbouring TTC7A variants were examined in the integrative genome viewer (IGV) and compared to patients not harbouring any TTC7A variants, sequencing using the same capture kits. Read depth was calculated across the entire gene and compared to patients without the variants (supplementary figure 1). No deletions or CNVs were identified through either method. We acknowledge the limitations of WES in detection of deletions.

Based on the key role *TTC7A* has in regulation of normal intestinal epithelial cell development we hypothesised that high mutation burden in this gene may contribute to IBD through mutations not classified as casual of a monogenic disorder. We utilised a novel *per gene, per individual* deleteriousness score, GenePy, to assess whether variation in *TTC7A* was contributing to the risk of developing inflammatory bowel disease but not in a monogenic inheritance pattern (5). GenePy provides a single score to a gene, for each individual, based on all variants harboured within that gene. Even in genes known to play a role in the development of polygenic disease high scores are uncommon in individual risk genes (5). Therefore, comparison of extreme scores (top 10%, Mann Whitney U-test) in *TTC7A* between all cases and a set of non-IBD controls allows determination of whether variation in *TTC7A* was more deleterious in the IBD cohort. Following application of GenePy, mutation burden was identical in cases and controls, with the median of the top 10% of scores being 0.72 in both, p=>0.05. This provides evidence that *TTC7A* variation observed in our cohort was not contributing to disease development.

The p.K606R and p.S672P variants appear to be most commonly inherited on the same haplotype. The number of patients harbouring these heterozygote variants exceeds that expected from both gNomad and EXAC allele frequency databases. However, there is no evidence a single affected allele would translate into impact on protein expression or function, based on all previous reports of disease associated with *TTC7A* variants it appears to be a Mendelian disorder with autosomal recessive inheritance, although previous data has indicated a role for heterozygote variants in genes associated with autosomal recessive monogenic IBD for other genes (3,4,6). Where these variants have been inherited in *trans,* previous reports have detailed a partial loss-of-function associated with mild CVID and enteropathy phenotype (2). Interestingly, neither of parents of the patient described by Lawless *et al* possessed the p.K606R and p.S672P haplotype, with the mother having the wild-type allele at amino acid position 606 and the father being wild-type at position 672.

In this study we identify five paediatric IBD patients harbouring variants on the same chromosome, previously described as disease-causing when inherited in compound heterozygosity. These variants, p.K606R and p.S672P, appear to exist on a relatively rare ancestral haplotype and are seen in 1.2% of our cohort. *In silico* analysis indicates that exonic variation in *TTC7A* was not contributing to disease in this cohort. We find no evidence of increased *TTC7A* mutation burden in the coding regions for IBD patients compared to controls. These data imply that *TTC7A* is only associated with an autosomal recessive Mendelian disorder, with disease occurring in patients who harbour rare, deleterious variants. We were unable to assess the impact of variation in intronic/promotor regions, and alteration of *TTC7A* expression or alternative splicing could still contribute to disease pathogenesis. Functional analysis is required to assess the role of rare heterozygote variants, not fulfilling the criteria for causing monogenic disease, in the regulation of intestinal development and inflammation.

Ethics Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of Southampton and South West Hampshire Research Ethics Committee (09/H0504/125) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

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**Table 1**- Genotype and phenotype characteristics of five patients identified harbouring the p.K606R and p.S672P *TTC7A* variants

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | ***TTC7A* variants** | **gNomad frequency** | **CADD score** | **Inherited from** | **Second TTC7A variant?** | **Second TTC7A variant CADD score** | **Second TTC7A variant frequency** | **Sex** | **Age at diagnosis** | **Follow-up duration** | **Disease** | **Paris Classification** | **Complications including evidence of immunodeficiency** | **Medications used** | **Additional information** |
| 1 | K606R | 0.0022 | 25.6 | Mother | Synonymous G696A:p.E232E | 9.522 | 0.1778 | Male | 12.9 years | 4.4 years | Ulcerative colitis | E4 | None evident | 5-ASAThiopurine | No FH |
| S672P | 0.0022 | 27.5 |
| 2 | K606R | 0.0022 | 25.6 | Father | None | - | - | Female | 12.6 years | 8.9 years | Crohn’s disease | L3+L4a | None evident | 5-ASAThiopurine | Brother with IBD also heterozygote for variants |
| S672P | 0.0022 | 27.5 |
| 3 | K606R | 0.0022 | 25.6 | Mother | Synonymous G696A:p.E232E | 9.522 | 0.1778 | Male | 15.2 years | 2.7 years | Crohn’s disease | L1 | None evident | Anti-TNF | No FH |
| S672P | 0.0022 | 27.5 |
| 4 | K606R | 0.0022 | 25.6 | Father | Synonymous G696A:p.E232E | 9.522 | 0.1778 | Male | 13.4 years | 2.8 years | Crohn’s disease | L2 | None evident | 5-ASAThiopurine | No FH |
| S672P | 0.0022 | 27.5 |
| 5 | K606R | 0.0022 | 25.6 | Mother | Synonymous C198T:p.S66S | 17.63 | 0.08 | Male | 15.7 years | 1.4 years | Crohn’s disease | L3+L4a | Stricturing disease | Anti-TNF | No FHHas *NOD2* variant |
| S672P | 0.0022 | 27.5 |

**Supplementary figure 1**- Coverage plot for *TTC7A*. Per-base read coverage for 5 patients harbouring *TTC7A* variants. Coverage is shown for each exon with a 500 base pair padding to each side. The bold line represents the mean coverage observed in 15 individuals not harbouring *TTC7A* variants. The dashed line indicates 1 standard deviation below the control mean coverage. Exons from patients PR0096, SOPR0231 and SOPR0409 were captured using Agilent SureSelect V6. Exons from patients PR0101 and PR0156 were captured using Agilent SureSelect V4 and V5 respectively reflecting a chemistry with worse coverage of this gene. All patients lie within 2 standard deviations of the mean.