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ORIGINAL INVESTIGATION

Phenome-wide association study of *TTR* and *RBP4* genes in 361,194 individuals reveals novel insights in the genetics of hereditary and wildtype transthyretin amyloidoses

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Abstract

Transthyretin (*TTR*) gene has a causal role in a hereditary form of amyloidosis (ATTRm) and is potentially involved in the risk of wild-type transthyretin amyloidosis (ATTRwt). To understand the genetics of ATTRm and ATTRwt, we conducted a phenome-wide association study of *TTR* gene in 361,194 participants of European descent testing coding and non-coding variants. Among the 382 clinically relevant phenotypes tested, *TTR* non-coding variants were associated with 26 phenotypic traits after multiple testing correction. These included signs related to both ATTRm and ATTRwt such as chronic ischaemic heart disease (rs140226130, $p = 2.00 \times 10^{-6}$), heart failure (rs73956431, $p = 2.74 \times 10^{-6}$), atrial fibrillation (rs10163755, $p = 4.63 \times 10^{-6}$), dysphagia (rs2949506, $p = 3.95 \times 10^{-6}$), intestine diseases (rs970866, $p = 7.14 \times 10^{-6}$) and anxiety (rs554521234, $p = 8.85 \times 10^{-6}$). Consistent results were observed for *TTR* disease-causing mutation Val122Ile (rs76992529) with respect to carpal tunnel syndrome ($p = 6.41 \times 10^{-6}$) and mononeuropathies of upper limbs ($p = 1.22 \times 10^{-5}$). Sex differences were also observed in line with ATTRm and ATTRwt epidemiology. Additionally, we explored possible modifier genes related to TTR function, observing convergent associations of *RBP4* variants with the clinical phenotypes associated with *TTR* locus. In conclusion, we provide novel insights regarding the molecular basis of ATTRm and ATTRwt based on large-scale cohort, expanding our understanding of the phenotypic spectrum associated with *TTR* gene variation.

Introduction

Transthyretin (TTR) misfolding and the consequent amyloid formation and deposition lead to two form of amyloidoses: a hereditary (or mutant) form (ATTRm) caused by more than 130 *TTR* gene coding mutations (Conceicao et al. 2019; Plante-Bordeneuve and Said 2011), and a senile systemic form that is due to the misfolding of wild type protein (wild-type transthyretin amyloidosis, ATTRwt; i.e. no *TTR* coding mutation present in the individuals affected by ATTRwt; (Westermark et al. 1990). ATTRm is a life-threatening disorder that affects several tissues (e.g. brain, autonomic and peripheral nerves, heart, and gastrointestinal (GI) tract) and is characterized by a complex genotype–phenotype correlation (Conceicao 2012; Hellman et al. 2008; Palaninathan 2012; Parman et al. 2016). Despite *TTR* coding mutations are the cause of the disease, the clinical variability of the ATTRm is likely due to several other factors (Alves-Ferreira et al. 2018; Iorio et al. 2015, 2017a; b; Polimanti et al. 2014). Differently from ATTRm, patients affected by ATTRwt do not present a coding mutation in *TTR* gene and the main symptoms include

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cardiac failure and carpal tunnel syndrome (Connors et al. 2011; Kyle and Gertz 1995; Maceira et al. 2005; Nakagawa et al. 2016; Pitkanen et al. 1984). *TTR* non-coding variation via its regulatory function seems to have a role in the phenotypic heterogeneity of ATTRm and to play a role in the pathogenesis of ATTRwt (Iorio et al. 2017b; Polimanti et al. 2013, 2019; Sikora et al. 2015). Furthermore, variation in other genes encoding for protein products that interact with TTR tetramer seems to modulate the pathogenetic processes involved in the TTR fibrils formation and, consequently, in the course of the disease (Santos et al. 2016; Soares et al. 2005; White and Kelly 2001). Genome-wide datasets generated from cohorts including hundred thousand participants can be leveraged to disentangle complex genotype–phenotype associations. In particular, phenome-wide association studies (PheWAS) can permit us to detect novel associations with respect to known risk loci with respect to a wide range of phenotypes (Bush et al. 2016; Denny et al. 2013; Polimanti et al. 2016). In the present study, we explored the phenotypic spectrum associated with the coding and non-coding variants of *TTR* gene, also investigating potential modifier loci that could modulate TTR amyloidogenic process. To date, the vast majority of the studies that investigated the genetics of ATTRm and ATTRwt have been conducted on cohorts with a limited sample size due to the low disease prevalence (Iorio et al. 2015, 2017b; Sikora et al. 2015). ATTRm and ATTRwt signs are poorly recognized and the correct diagnosis in sporadic cases is usually established several years from the onset of the symptoms (Ando et al. 2013). This consistently limited the ability to collect large informative cohorts needed to investigate the genetics of ATTRm and ATTRwt. To date, it is possible to investigate cohorts including hundred thousand participants to explore the association of genetic variation with clinically relevant phenotypes. Accordingly, we conducted a PheWAS in 361,194 participants of European descent available from the UK Biobank with respect to 382 clinically relevant traits to investigate genetic variation potentially involved in TTR-related pathogenic processes. The results obtained support that non-coding variants located in *TTR* gene and other disease-modifying loci are associated with phenotypic traits related to ATTRm and ATTRwt. On the basis of the current findings, we hypothesize that non-coding variation is involved (1) in the variability of the phenotypic presentation of carriers of *TTR* coding mutations; and (2) in the increased risk of ATTRwt in non-carriers of *TTR* coding mutations.

Materials and methods

This study was conducted leveraging genome-wide association data generated from the UK Biobank (361,194 participants and 382 phenotypes). These datasets was used to explore the phenotypic spectrum associated with common genetic variants located in *TTR* gene and its surrounding regions. Additionally, we tested whether the protein product of *TTR* gene showed strong evidence of interaction with other proteins and verified whether genetic variants located in the encoding gene of these proteins were associated with the same phenotypic traits identified in *TTR* association analysis. A schematic workflow summarizing the analyses conducted is reported in Supplementary File 1.

Genome-wide datasets

The dataset used for the analysis was derived from the UK Biobank. This is an open access resource available to investigate a wide range of serious and life-threatening illnesses (Allen et al. 2014). This project has recruited more than 500,000 people assessed for a wide range of phenotypic information, also including clinically relevant phenotypes. Genetic data are available for the whole cohort and were used to generate genome-wide association datasets that can be used to explore the genetics of human diseases and traits. These genome-wide datasets used in the present study were generated from the analysis of 361,194 unrelated participants of European descent including 194,174 women and 167,020 men. The association analysis for all phenotypes was conducted using appropriate regression models available in Hail (available at https://github.com/hail-is/hail) including the first 20 ancestry principal components, sex, age, age^2, sex × age, and sex × age^2 as covariates. The principal components included in the regression model were generated by the UK Biobank investigators using fastPCA algorithm and considering unrelated subjects and genetic markers pruned for linkage disequilibrium (Bycroft et al. 2018). Details regarding QC criteria, GWAS methods, and the original data are available at https://github.com/Nealelab/UK_Biobank_GWAS/tree/master/imputed-v2-gwas.

Clinically relevant phenotypes

Since our goal was to investigate phenotypes related to the pathogenesis of ATTRm and ATTRwt, we focused on clinically relevant traits available in the UK Biobank. We considered ICD-10 [International Classification of Disease, 10th Revision; (Denny 2012; Wei and Denny 2015)] codes and clinical endpoints derived by the FinnGen project (information available at https://www.finngen.fi/en). ICD-10 codes are a specific terminology that include diseases, signs, symptoms, and procedure codes maintained by the World Health Organization (WHO), and they typically are used to billing data (Denny 2012). The FinnGen project comes from a collaboration among Finnish universities, biobanks, hospital districts, and several international pharmaceutical companies to increase knowledge about human diseases. FinnGen clinical endpoints (available at https://www.finngen.fi/en/

researchers/clinical-endpoints) were developed to conduct genome-wide investigations of phenotypic traits assessed across several national health registers. To remove phenotypes not informative due to a lack of statistical power, we investigated phenotypic traits with a number of cases greater than 1000 individuals. The full list of the phenotypes investigated, and their corresponding sample size is reported in the Supplementary File 2. Additionally, we also conducted sex-stratified analysis to investigate the known differences between sexes in the epidemiology of ATTRm and ATTRwt. A total of 382, 240, and 225 clinically relevant phenotypes were investigated in the total sample and in the sex-stratified analysis (female- and male-specific analyses, respectively).

Data analysis

We considered a total of 382 clinically relevant phenotypes (sex-stratified analysis: 240 for female-specific PheWAS and 225 for male-specific PheWAS) and investigated variants located in *TTR* gene considering a 4-Mb region (NC_000018.9: 27,171,000–31,171,500) including the genic locus (NM_000371; 7257 bp) and the surrounding regions (± ~ 2 Mb from the transcription start/end sites) (Table 1). We initially applied a filter to the variants investigated on the basis of their minor allele frequency (MAF > 1%). Additionally, as recommended in the original UK Biobank analysis (information available at http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas), we considered high-confidence association results generated from variants with at least 25 minor alleles in the smaller group (case or control). A total of 12,719, 12,710, and 12,711 high-confidence *TTR* variants that were investigated in the overall-sample and in the sex-stratified analyses (male- and female-specific, respectively). False Discovery Rate (FDR; (Benjamini and Hochberg 1995) at 10% was applied as significance threshold for multiple testing correction accounting for the number of variants and the number of traits tested. This significance threshold was selected on the basis of the correlation expected among the variants and the phenotypes tested. To estimate the independent association signals within the genomic region tested, PLINK 1.09 (Chang et al. 2015) was used to perform linkage disequilibrium (LD) clumping considering a 0.1 R^2 cut-off within a 500-kb window.

STRING v.11.0 (Szklarczyk et al. 2019) was used to identify protein interaction with *TTR*, considering experiments, co-expression, co-occurrence, gene fusion, and neighbourhood as active sources and a confidence score higher than 0.9. With respect to proteins identified, we investigated variants included in coding, non-coding, and surrounding regions of the encoding gene using the same quality control criteria applied in the analysis of *TTR* variants. Additionally, we investigated the functional enrichments association related to the protein–protein interactions identified considering Gene Ontologies (Ashburner et al. 2000; The Gene Ontology 2019) for biological processes and molecular functions and molecular pathways available in the Reactome Database (Fabregat et al. 2018).

Results

The *TTR* PheWAS identified several phenotypic associations surviving FDR multiple testing correction (Fig. 1). These include associations identified in the overall analysis and in the sex-stratified analyses (Fig. 2, Supplementary File 3). In the analysis of the total cohort (Supplementary File 4), we observed several clinical signs that may be related to ATTRm and ATTRwt: spinal stenosis (FinnGen: M13_SPINSTENOSIS; rs116937108, beta = 0.002, $p = 1.21 \times 10^{-7}$), neuromuscular dysfunction of bladder (ICD-10: N31; rs9953311, beta = − 0.001, $p = 2.30 \times 10^{-6}$; rs575854233, beta = − 0.001, $p = 5.33 \times 10^{-6}$), and anxiety disorders (FINNGEN: KRA_PSY_ANXIETY; rs554521234, beta = 0.003, $p = 8.85 \times 10^{-6}$). Additionally, we also observed significant findings not expected to be directly related to TTR amyloidogenic process: Barret's esophagus (FinnGen: K11_BARRET; rs147570462, beta = 0.003,

Table 1 Details regarding the clinically relevant traits and the genomic regions tested in the overall cohort and in the sex-stratified analyses

Gene	Chromosome Assembly Localization	Sex	Phenotype *N*	Case median *N* (minimum–maximum)	Control median *N* (minimum–maximum)
TTR	NC_000018.9: 27,171,000–31,171,500	Both sexes	382	2534 (1011–208,009)	358,660 (79,185–360,183)
		Female	240	2364 (1008–153,946)	191,810 (40,228–193,166)
		Male	225	2533 (1004–128,063)	164,487 (39,957–166,016)
RBP4	NC_000010.10: 93,353,000–97,353,500	Both sexes	32[a]	2820 (1014–20,857)	358,374 (340,337–360,180)
		Female	32[a]	1095 (331–7355)	193,079 (186,819–193,843)
		Male	31[a]	1581 (441–15,056)	165,439 (151,964–166,579)

We report the number of phenotypes tested and the sample size investigated in the overall-sample and sex-stratified analyses

[a]Significant phenotypic traits identified in the *TTR* PheWAS and tested in the *RBP4* analysis

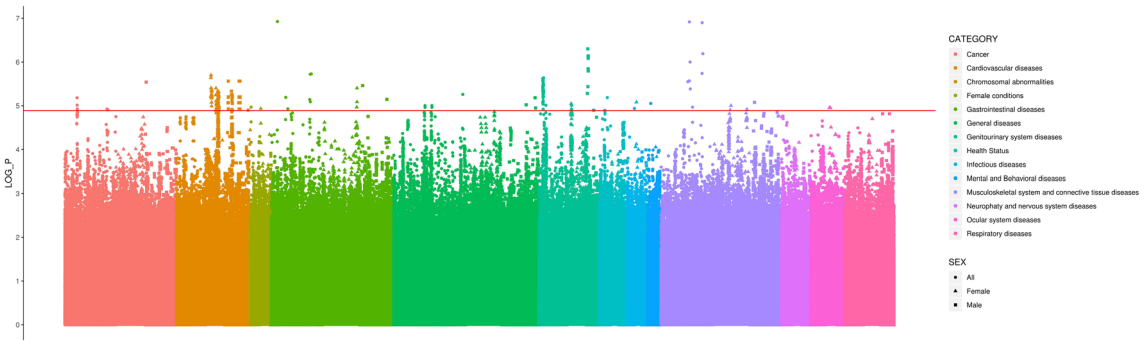

Fig. 1 Manhattan Plot of *TTR* PheWAS in the total sample and in sex-stratified analyses across the different phenotypic categories tested

$p = 1.19 \times 10^{-7}$), abscess of anal and rectal regions (ICD-10: K61; rs72948149, beta = 0.002, $p = 1.87 \times 10^{-6}$); fissure and fistula of anal and rectal regions (ICD-10: K60; rs72927361, beta = − 0.003, $p = 1.90 \times 10^{-6}$); complications of other internal prosthetic devices, implants and grafts (ICD-10: T85; rs34444556, beta = 0.003, $p = 5.5 \times 10^{-6}$), ulcer of esophagus (FinnGen: K11_OESULC; rs74481699, beta = 0.004,$p = 6.43 \times 10^{-6}$), adjustment and management of implanted device (ICD-10: Z45; rs117672477, beta = 0.002, $p = 6.51 \times 10^{-6}$); primary lymphoid and hematopoietic malignant neoplasms (FinnGen: C3_PRIMARY_LYMPHOID_HEMATOPOIETIC; rs17718949, beta = 0.01, $p = 6.57 \times 10^{-6}$). Among the 240 clinically relevant phenotypes tested in the female-specific analysis ($N = 194{,}174$), we identified 8 LD-independent variants associated with specific pathological conditions (Supplementary File 5): chronic ischaemic heart disease (ICD-10: I25; rs140226130, beta = 0.003, $p = 2.00 \times 10^{-6}$), dysphagia (ICD-10: R13; rs2949506, beta = 0.002, $p = 3.95 \times 10^{-6}$), other disorders of urinary system (ICD-10: N39; rs71173870, beta = 0.003, $p = 9.81 \times 10^{-6}$), other specified/unspecified soft tissue disorders (ICD-10: M13_SOFTTISSUENAS; rs558461933, beta = 0.007, $p = 9.92 \times 10^{-6}$), other cataract (ICD-10: H26; rs12966815, beta = 0.003, $p = 1.10 \times 10^{-5}$), retinal detachments and breaks (ICD-10: H33; rs117637258, beta = 0.003, $p = 1.12 \times 10^{-5}$), other abnormal products of conception (ICD-10: O02; rs139327590, beta = 0.004, $p = 1.16 \times 10^{-5}$), and shoulder lesions (ICD-10: M75; rs77728273, beta = − 0.004, $p = 1.21 \times 10^{-5}$). Several of these clinical manifestations (e.g., I25 ~ Chronic ischaemic heart disease, R13 ~ Dysphagia, N39 ~ Other disorders of urinary system, H26 ~ Other cataract and H33 ~ Retinal detachments and breaks) seem to be closely related to the expected symptoms of ATTRm and ATTRwt. The male-specific PheWAS (225 phenotypic traits tested) revealed several significant associations that could be related to cardiac, gastrointestinal, and urinary symptoms of ATTRm and ATTRwt (Supplementary File 6): other disorders of bladder (ICD-10: N32; rs138038371, beta = 0.009, $p = 5.01 \times 10^{-7}$), heart failure (FinnGen: I9_HEARTFAIL; rs73956431, beta = 0.005, $p = 2.74 \times 10^{-6}$), malignant neoplasm of colon (ICD-10: C18; rs78431500, beta = 0.006, $p = 2.89 \times 10^{-6}$), Barret's esophagus (ICD-10: K11_BARRET; rs147570462, beta = 0.005, $p = 3.45 \times 10^{-6}$), atrial fibrillation and flutter (ICD-10: I48; rs10163755, beta = 0.003, $p = 4.63 \times 10^{-6}$), calculus of kidney and ureter (ICD-10: N20; rs8090264, beta = 0.002, $p = 5.23 \times 10^{-6}$), complications of procedures not elsewhere classified (ICD-10: T81; rs182526571, beta = 0.008, $p = 6.57 \times 10^{-6}$), other diseases of intestine (ICD-10: K63; rs970866, beta = − 0.005, $p = 7.14 \times 10^{-6}$) and fibroblastic disorders (FinnGen: M13_FIBROBLASTIC; rs60487427, beta = − 0.004, $p = 8.38 \times 10^{-6}$).

Amyloidogenic coding variants in *TTR* gene are the established cause of ATTRm (Conceicao 2012; Parman et al. 2016; Plante-Bordeneuve and Said 2011). The large sample size of the UK Biobank cohort includes carriers of *TTR* amyloidogenic mutations with high-quality genotype information (imputation info score > 0.8; Supplementary File 7). However, due to the very low MAF of these coding mutations, the phenotypic associations of these variants should be considered only a "low-confidence" result as described in the methods. Being aware of this limitation, we explored their phenotypic spectrum in the UK Biobank (Table 2). The most relevant results were observed for the *TTR* Val122Ile mutation (rs76992529) in the female participants with respect to two well-known symptoms of the amyloidogenic process: carpal tunnel syndrome (FinnGen: G6_CARPTU; beta = 0.307, $p = 6.41 \times 10^{-6}$) and mononeuropathies of upper limb (ICD-10: G56; beta = 0.306, $p = 1.22 \times 10^{-5}$).

To test the presence of modifier genes involved in TTR-related pathogenic processes, we investigated potential protein interaction and observed the highest-confidence interaction with respect to RBP4 protein, which is supported by experiments and co-expression interaction sources (STRING interaction score = 0.914). The TTR-RBP4 protein interaction is associated with functional enrichments for GO terms, including retinol metabolic process (GO:0042572, FDR $q = 6.3 \times 10^{-4}$) and protein

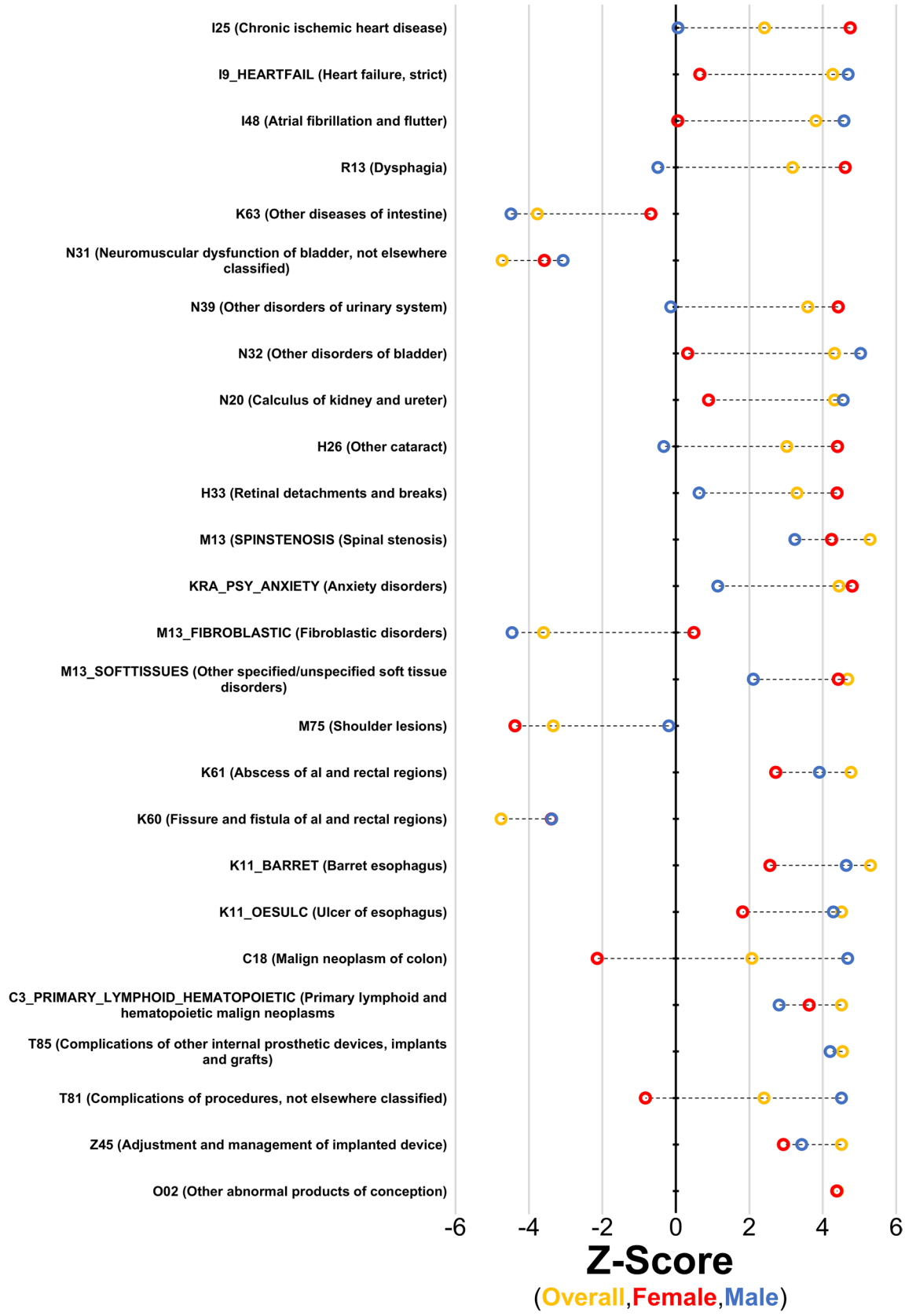

Fig. 2 Associations (Z-Scores) of non-coding variants surviving multiple testing correction in the *TTR* PheWAS in the overall-sample and sex-stratified analyses. Details of the associations are reported in Supplementary File 3

Table 2 Significant associations identified with respect to *TTR* coding mutations

Phenotypic trait	rsid	Substitution	Beta	SE	*P* value	FDR
Both sexes						
FinnGen: XVII_MALFORMAT_ABNORMAL ~ Congenital malformations, deformations and chromosomal abnormalities	rs138657343	Arg5His	0.0264	0.00550	1.64×10^{-6}	0.029
Female						
ICD10: R87 ~ Abnormal findings in specimens from female genital organs	rs138657343	Arg5His	0.0379	0.00724	1.65×10^{-7}	0.004
FinnGen: XVII_MALFORMAT_ABNORMAL ~ Congenital malformations, deformations and chromosomal abnormalities			0.0368	0.00756	1.12×10^{-6}	0.019
ICD10: N94 ~ Pain and other conditions associated with female genital organs and menstrual cycle	rs76992529	**Val122Ile**	0.158	0.0334	2.31×10^{-6}	0.032
FinnGen: G6_CARPTU ~ Carpal tunnel syndrome			0.307	0.0681	6.41×10^{-6}	0.063
ICD10: G56 ~ Mononeuropathies of upper limb			0.306	0.0699	1.22×10^{-5}	0.079
FinnGen: K11_HERNIA ~ Hernia	rs121918074	**His90Asn**	0.210	0.0485	1.53×10^{-5}	0.090

Information about beta value, standard error (SE), *p* value and false discovery rate (FDR) *q* value are reported. Bold text: TTR protein substitution. Underline text: TTR precursor substitution. Information about allele frequency and minor allele frequency are reported in Supplementary File 7

heterodimerization activity (GO:0046982, FDR $q=0.02$), and for Reactome molecular pathways, including retinol cycle disease events (HSA-2453864, FDR $q=9.98\times10^{-6}$), canonical retinoid cycle in rods (HSA-2453902, FDR $q=1.1\times10^{-5}$), and retinoid metabolism and transport (HSA-975634, FDR $q=2.84\times10^{-5}$). On the basis of these data, we hypothesize a convergence where variants located in *TTR* and *RBP4* genes are associated with clinically relevant phenotypes related to ATTRm and ATTRwt pathogenesis. Accordingly, we investigated 13,226, 13,231, and 13217 high-confidence variants (overall-sample, female-specific, and male-specific analyses, respectively) located in *RBP4* gene and its surrounding regions (NC_000010.10: 93,353,000–97,353,500) with respect to the significant phenotypic traits observed in the *TTR* PheWAS (Table 1). We identified significant associations in the overall cohort and in the sex-stratified analyses (Supplementary File 3). Considering the traits tested across different phenotypic categories, we observed a concordant trend between *TTR* and *RBP4* associations observed in the overall sample and in the sex-stratified analyses (Fig. 3). In the *RBP4* analysis conducted in the overall sample, we confirmed some of the *TTR* associations previously observed with respect to *TTR* gene that are related to known clinical signs of ATTRm and ATTRwt (Supplementary File 8): heart failure (FinnGen: I9_HEARTFAIL; rs1326222, beta = 0.001, $p=5.25\times10^{-7}$) and anxiety disorders (FinnGen: KRA_PSY_ANXIETY; rs112059561, beta = 0.002, $p=6.44\times10^{-6}$). Among the phenotypes that do not seem to be related to TTR amyloidogenic processes, Barret's esophagus was also significantly associated with a *RBP4* variant (FinnGen: K11_BARRET; rs12573026, beta = 0.001, $p=9.96\times10^{-8}$). In the female-specific analysis, we observed significant associations with respect to cardiac symptoms which are one of the leading signs of ATTRm and ATTRwt: atrial fibrillation and flutter (ICD-10: I48; rs4917692, beta = 0.002, $p=1.15\times10^{-5}$) and heart

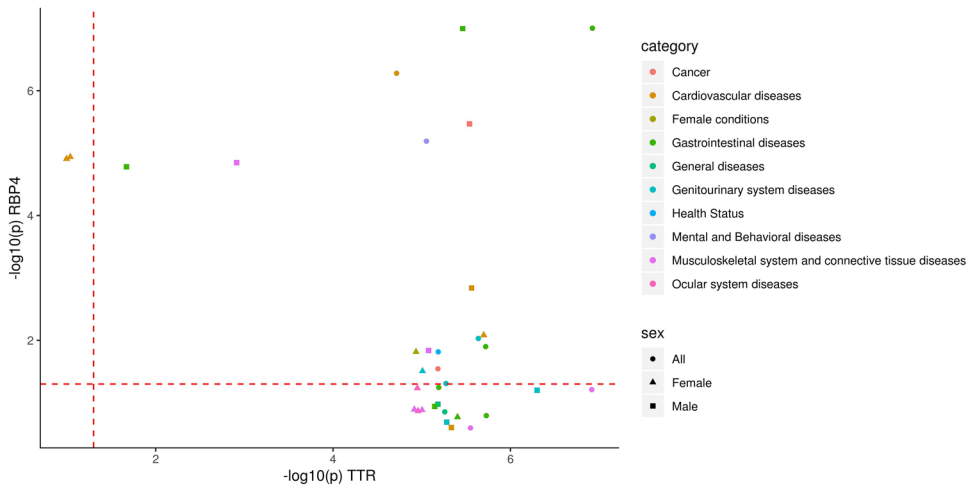

Fig. 3 Association of *RBP4* common genetic variants with traits surviving multiple testing correction in the *TTR* PheWAS in the overall-sample and sex-stratified analyses. Red dotted lines represent nominal significance ($p<0.05$)

failure (FinnGen: I9_HEARTFAIL; rs1326222, beta = 0.001, $p = 1.23 \times 10^{-5}$) (Supplementary File 9). Finally, in the male participants, we observed a convergence between *RBP4* and *TTR* findings with respect to two additional phenotypes expected to be related to ATTRm and ATTRwt (FinnGen: M13_SPINSTENOSIS ~ spinal stenosis, rs112288944, beta = 0.005, $p = 1.42 \times 10^{-5}$; ICD-10: R13 ~ Dysphagia, rs61886346, beta = 0.003, $p = 1.66 \times 10^{-5}$). Some conditions that are not expected to be linked to amyloidogenic processes such as Barret's esophagus (FinnGen: K11_BARRET; rs12573026, beta = 0.002, $p = 1.01 \times 10^{-7}$) and malignant neoplasm of colon (ICD-10: C18; rs142083973, beta = 0.005, $p = 3.40 \times 10^{-6}$) were also associated with both *RBP4* and *TTR* gene variants in the male sample (Supplementary File 10). Supplementary File 11 summarizes all significant associations, providing the allele frequencies of *TTR* and *RBP4* variants identified.

Discussion

TTR misfolding and the following amyloid formation and deposition are the cause of ATTRm and ATTRwt due to *TTR* coding variants and the misfolding of the wild type protein, respectively (Plante-Bordeneuve and Said 2011; Westermark et al. 1990). With respect to ATTRm, several studies investigated the role of *TTR* coding and non-coding variation to dissect the molecular machineries at the basis of its complex phenotype-genotype correlation (Iorio et al. 2015, 2017a, b; Polimanti et al. 2013, 2014, 2019; Sikora et al. 2015). Differently, limited information is available regarding the genetic basis of ATTRwt (Sikora et al. 2015). PheWAS design is a powerful tool to broaden the knowledge about the phenotypic spectrum associated with disease-causing genetic variations (Bush et al. 2016; Denny et al. 2013; Polimanti et al. 2016) and its performance is enhanced by the availability of genomic data of large cohorts. Accordingly, we conducted a phenome-wide investigation of *TTR* coding and non-coding variants in more than 300,000 participants of European descent, also evaluating the *RBP4* gene as a possible modifier of *TTR* pathogenetic mechanisms.

Our results pointed out novel associations of *TTR* non-coding variants with phenotypic conditions potentially related to ATTRm and ATTRwt pathogenesis. In the sex stratified analysis, we observed strong evidence of the effect of *TTR* non-coding variations on cardiac involvement (ICD-10: I25 ~ Chronic ischaemic heart disease; I48 ~ Atrial fibrillation and flutter; FinnGen: I9_HEARTFAIL ~ Heart failure, strict), one of the leading clinical signs of ATTRm and ATTRwt. With respect to ATTRwt, heart is the most affected organ in elderly patients, and, the main manifestations are cardiomyopathy (resulting in increase of biventricular wall thickness and ventricular stiffness) and atrial fibrillation (Ando et al. 2013; Siddiqi and Ruberg 2018). The symptoms associated with ATTRm include a restrictive amyloid cardiomyopathy along with a combination of several other signs, including gait, gastrointestinal, neurological, urinary/renal, and ocular involvement frequently reported among the affected carriers (Ando et al. 2013; Parman et al. 2016; Plante-Bordeneuve and Said 2011; Siddiqi and Ruberg 2018).

GI symptoms were identified in our phenome-wide investigation (ICD-10: R13 ~ Dysphagia and K63 ~ Other diseases of intestine). The occurrence of GI manifestations has been reported in both ATTRm and ATTRwt and these include nausea, vomiting, constipation, faecal incontinence, and weight loss (Ando et al. 2013; Collins et al. 2018; Wixner et al. 2014). Although GI involvement is reported in ATTRwt patients, these symptoms are less common than the ones reported in ATTRm (Wixner et al. 2014), where GI manifestations are more frequent in early-onset patients (Wixner et al. 2014).

Significant associations of *TTR* variants with respect to bladder, urinary tract and kidney were identified in the overall sample and in the sex-stratified analyses (ICD-10: N31 ~ Neuromuscular dysfunction of bladder, not elsewhere classified; N39 ~ Other disorders of urinary system; N32 ~ Other disorders of bladder; N20 ~ Calculus of kidney and ureter). It is well known the evolution of neurogenic bladder in ATTRm that is linked to autonomic nerve dysfunction, and, renal involvement due to the amyloid deposition in the glomeruli arterioles and medium vessels (Ando et al. 2013; Lobato and Rocha 2012).

The female sample showed that *TTR* non-coding variants are associated with ocular involvement (ICD-10: H26 ~ Other cataract; H33 ~ Retinal detachments and breaks). These findings are consistent with the known ocular manifestations described in ATTRm, which, in agreement with our sex-stratified result, occur more frequently in women (Reynolds et al. 2017). The ocular symptoms reported in ATTRm patients include vitreous opacities, retinal vein occlusion and direct optic nerve infiltration (Reynolds et al. 2017).

Remarkably, a significant genetic association between *TTR* gene and anxiety disorders (FinnGen: KRA_PSY_ANXIETY) was observed. There is a growing literature regarding the effect of rare life-threatening diseases on the mental health of the patients and their families. With respect to ATTRm, several studies reported considerable psychological consequences in carriers of *TTR* mutations after the onset of the symptoms and the diagnosis of the disease (Graceffa et al. 2009; Lopes et al. 2018). These behavioural changes seem to be related to the stressful scenarios related to amyloidosis diagnostic path, including the pre-symptomatic genetic testing confirming the presence of an amyloidogenic mutation in family members and the time (usually

years) intervening between the onset of the symptoms and the diagnosis of the diseases in novel ATTRm cases.

Spinal stenosis (FinnGen: M13_SPINSTENOSIS) was another phenotype identified in our *TTR* Phewas. This symptom has been reported in both ATTRm and ATTRwt patients (Ando et al. 2013; Siddiqi and Ruberg 2018), and it usually co-occurs with other known signs of *TTR* amyloidogenic process such as carpal tunnel syndrome and neuropathies (Carr et al. 2019). These phenotypic traits were identified as associated to *TTR* Val122Ile mutation (Table 2). This is also consistent with phenotypic presentation of Val122Ile carriers affected by ATTRm where the carpal tunnel syndrome represents an early sign of the disease whereas upper limb involvement is a clinical manifestation observed in a later stage (Ando et al. 2013). Although our findings are related to carriers of European descent and Val122Ile mutation is mainly identified in individuals of African descent, a similar clinical phenotype has been reported in Val122Ile carriers of both ancestry groups (Cappelli et al. 2016).

Our findings support a phenotypic convergence between *TTR* and *RBP4* genetic associations, also including traits expected to be associated with ATTRm and ATTRwt clinical spectrum (i.e., heart failure and atrial fibrillation). This evidence supports the putative role of *RBP4* as modifier gene with respect to TTR amyloidogenic process. RBP4 interacts with TTR via formation of TTR-RBP4 protein complex that stabilizes the TTR tetramer, inhibiting monomer dissociation and fibril formation (Palaninathan 2012). In carriers of *TTR* amyloidogenic mutations, there is an increase of the TTR tetramer dissociation with a consequent decrease of the TTR-RBP4 complex and an increase of the RBP4 urinary excretion (Palaninathan 2012; Arvanitis et al. 2017). Accordingly, *RBP4* genetic variation could affect the functionality of the TTR-RBP4 complex, contributing to the complex phenotype-genotype correlation in carriers of *TTR* amyloidogenic mutations and increasing the risk of ATTRwt in non-carriers.

Although the findings presented provided an unprecedented amount of information regarding the phenotypic spectrum associated with common genetic variants located in *TTR* gene and its surrounding region, our study present several limitations. UK biobank is an amazing resource to investigate the molecular bases of human diseases. However, the depth of the phenotypic characterization available in the UK Biobank at this time does not permit to fully characterize certain aspects related to ATTR pathogenesis. For instance, we observed significant association of *TTR* variants with heart failure. However, ATTR is associated with heart failure with preserved ejection fraction. Further studies will be needed to verify common variants in *TTR* gene are associated with the same cardiac phenotype observed in ATTR patients. Another limitation is that the UK Biobank cohort includes subjects representative of the general population that were not recruited specifically for ATTR research. Accordingly, several of the phenotypic association observed appear to be unrelated to ATTR pathogenesis. These traits can potentially be related to the inter-individual variability of the main physiological function of *TTR* and *RBP4* protein products: the transport of retinol (vitamin A) (Noy 2016). Altered homeostasis of vitamin is associated with several disorders (Mason et al. 2015) and some of them could be associated with the effect of *TTR* and *RBP4* variants on retinol metabolism, explaining some of the phenotypic traits identified in the present study.

In conclusion, the present study provides novel insights about *TTR* gene variation, confirming its role in phenotypic variability observed in ATTRm patients and supporting its potential involvement in the predisposition to ATTRwt. These data support the relevance of large biobanks to investigate complex genotype–phenotype associations. Additionally, the insights provided support the necessity of further investigations of *TTR* non-coding variation together with putative modifier loci to develop tools able to anticipate the course of the disease to improve the diagnosis and management of patients affected by ATTRm and ATTRwt.

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Data availability Data supporting the findings of this study are available within this article and its additional files. UK Biobank GWAS summary association data are available at https://github.com/Nealelab/UK_Biobank_GWAS/tree/master/imputed-v2-gwas.

Compliance with ethical standards

Conflict of interest Drs. Fuciarelli and Polimanti are both receiving research grants from Pfizer Inc. to conduct epigenetic studies of TTR amyloidosis. The other authors reported no biomedical financial interests or potential conflicts of interest.

Ethical approval This study was conducted using summary association data generated by previous studies. Owing to the use of previously collected, deidentified, aggregated data, this study did not require institutional review board approval.

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