Natural history, quality of life and outcome in cardiac ATTR amyloidosis

Running Title: Outcome in cardiac ATTR amyloidosis

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Background – Cardiac transthyretin amyloidosis (ATTR-CM) is an increasingly recognised cause of heart failure in older individuals. We sought to characterise the natural history of ATTR-CM and compare outcomes and quality of life among patients with acquired (ATTRwt-CM) and hereditary (hATTR-CM) forms of the disease.

Methods – We studied 711 patients with wild-type ATTRwt-CM, 205 with hereditary ATTR-CM associated with the V1221 variant (V122I-hATTR-CM) and 118 with non-V122I-hATTR-CM at the UK National Amyloidosis Centre between 2000 and 2017. Patients underwent prospective protocolized evaluations comprising assessment of cardiac parameters, functional status by 6-minute walk test, quality of life (QoL) according to Kansas City Cardiomyopathy Questionnaire (KCCQ), and survival. Hospital service usage pre- and post-diagnosis was established using English central health records in a subset of patients.

Results - There was substantial diagnostic delay, with patients using hospital services a median (interquartile range) of 17 (9-27) times during the 3 years before diagnosis by which time QoL was poor; diagnosis of ATTRwt-CM was delayed more than 4 years after presentation with cardiac symptoms in 42% of cases. Patients with V122I-hATTR-CM were more impaired functionally ($p<0.001$) and had worse measures of cardiac disease ($p<0.001$) at the time of diagnosis, a greater decline in QoL, and poorer survival ($p<0.001$) compared to the other subgroups.

Conclusions – ATTR-CM is an inexorably progressive and eventually fatal cardiomyopathy associated with poor QoL. Diagnosis is often delayed for many years after symptoms develop. Improved awareness and wider use of recently validated diagnostic imaging methods are urgently required for patients to benefit from recent therapeutic developments.

Keywords: Amyloid, Transthyretin, ATTR, Cardiomyopathy

Clinical Perspective

1) What is new?

- Non-biopsy diagnosis of cardiac ATTR amyloidosis through repurposed bone scintigraphy and cardiac magnetic resonance imaging has lately led to an upsurge in diagnoses, but still at an advanced stage in many patients.
- Patients with the TTR V122I gene variant, who are mostly of Afro-Caribbean ethnicity, typically have worse disease at diagnosis with poorer ejection fraction, renal function and performance status, and poorer survival.
- Both hereditary and acquired cardiac ATTR amyloidosis are associated with markedly poor quality of life at the time of diagnosis, the typical patient having attended hospital a median of 17 times during the prior three years.

2) Clinical Implications

- Frequent hospital attendances with symptoms attributable to cardiac ATTR amyloidosis or evidence of unexplained heart wall thickening provide potential opportunities for earlier diagnosis using bone scintigraphy and cardiac magnetic resonance imaging.
- Index of suspicion of the diagnosis should be especially high among Afro-Caribbean patients, of whom about 4 per cent possess the disease-associated TTR V1221 variant; TTR gene sequencing is a valuable aid to diagnosis.
- New disease-modifying therapies that inhibit production of TTR protein or its conversion to ATTR amyloid show great promise, underscoring the need for improved awareness of the disease.

Introduction

Transthyretin amyloidosis cardiomyopathy (ATTR-CM) has of late been increasingly recognised as a cause of heart failure in older individuals, reflecting advances in imaging technology and greater awareness of the disorder. Whilst it has long been known from autopsy studies that amyloid deposits derived from plasma transthyretin (TTR) are present in the hearts of up to 25% of elderly people,[1, 2] the associated clinical syndrome, predominantly comprising older men with restrictive cardiomyopathy that is often preceded by or associated with carpal tunnel syndrome,[3, 4] lumbar canal stenosis[5] and tendinopathy,[6] is still widely perceived as a rare disease.

In addition there are far less common dominantly inherited forms of ATTR amyloidosis (hATTR), associated with more than 130 different mutations in the TTR gene,[7, 8] which present variously from the third decade of life onwards with permutations of peripheral and autonomic neuropathy, sometimes involvement of other organ systems, as well as causing amyloid cardiomyopathy in most instances.[9] One particular TTR gene variant, V122I, occurs in about 4% of black individuals,[10] and is associated with increased susceptibility to development of late onset predominant cardiac amyloidosis that closely mimics wild-type ATTR-CM.[10, 11] The recent upsurge in diagnosis of ATTR amyloidosis has been driven by repurposed bone scintigraphy and cardiac MRI, both of which yield highly characteristic findings in ATTR-CM,[12, 13] but it nevertheless remains probable that heart failure, conduction disease and arrhythmias due to the disorder are far more prevalent than currently recognised.

ATTR-CM progresses inexorably to death, typically within a few years,[14] and hitherto has not been treatable. However, several very promising new therapies are now in development including tafamidis,[15] a small molecule drug that promotes maintenance of TTR protein in its normal soluble non-amyloid conformation, and two novel inhibitors of TTR production, inotersen,[16] an antisense oligonucleotide, and patisiran,[17] a gene silencing RNA therapy.

In contrast with much progress in diagnostic imaging technology and therapy, there remains a relative paucity of data on the patient pathway and natural history of ATTR-CM, as well as its effects on quality of life (QoL). We aimed to study these themes in our cohort of more than 1000 ATTR-CM patients enrolled into a protocolized observational study at the UK National Amyloidosis Centre (NAC); the study included a specific aim to characterise the pathways and phenotypes of patients with wild-type versus V122I associated ATTR-CM, in light of inconsistent previous data on their features and relative severity.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patients

Patients referred to the NAC between 2000 and 2017 in whom ATTR-CM was confirmed on the basis of validated diagnostic criteria,[18] were invited to participate in a prospective protocolized clinical follow up program comprising systematic evaluation of cardiac parameters, functional status, QoL and survival. Briefly, the diagnosis of ATTR-CM was established on the basis of presence of symptoms of heart failure together with a characteristic amyloid echocardiogram and either direct endomyocardial biopsy (EMB) proof of ATTR amyloid, or presence of ATTR amyloid in an extra-cardiac biopsy along with cardiac uptake on ^{99m}Tc-DPD scintigraphy, or Perugini grade 2 or 3 cardiac uptake on ^{99m}Tc-DPD scintigraphy in the absence of either an abnormal serum free light chain ratio or a monoclonal protein in the serum or urine by immunofixation.[18] All patients who were diagnosed with ATTR-CM underwent sequencing of the TTR gene at the time of diagnosis, as previously described.[8] Patients who did not consent to the protocolized clinical follow up program at NAC and those

who received disease modifying therapy including orthotopic liver transplantation for hATTR amyloidosis, TTR stabilizer therapy for more than 6 months, or a TTR-lowering therapy (within the context of a clinical trial) were excluded from the study.

Patients were managed in accordance with the Declaration of Helsinki and provided written informed consent for retrospective analysis and publication of their data with approval from the Royal Free Hospital ethics committee (ref: 06/Q0501/42).

Protocolized Evaluations

From the time of diagnosis, patients were systematically evaluated at the NAC on a 6-monthly basis. Each study evaluation comprised the following; a full clinical history and examination, routine hematology, serum and urine biochemistry including measurement of N-terminal brain pro-natriuretic peptide (NT-proBNP), troponin T (baseline only), electrocardiography, and detailed echocardiography.

In 2010, functional testing and assessments of quality of life were introduced into the clinic protocol; patients were routinely asked to perform a standardized 6-minute walk test (6MWT) and complete the Kansas City Cardiomyopathy Questionnaire (KCCQ) quality of life assessment tool at each study evaluation. Patients who were unable to complete a 6MWT at follow up were imputed as '0 meters' for analyses of change in 6MWT distance across time.

Hospital Episode Statistics

Data on hospital episode statistics (HES) in England, defined as publicly funded hospital service usage within the NHS in England, were obtained from NHS Digital (NHSD). Study patients were identified on the basis of their unique NHS number. Hospital service usage included inpatient (including surgical procedures), outpatient and A&E (emergency room)

services. All attendances were for medical issues and did not include those for occupational or physical therapy.

In order to ensure robustness of the presented data, only patients in whom there was a complete three year observation window across all three care settings pre-diagnosis of ATTR-CM (n=534, diagnosed April 2010-August 2016) and a complete three year observation window across all three care settings post-diagnosis of ATTR-CM (n=364, diagnosed April 2007-August 2013) were included in analyses of hospital service usage.

Access to HES data was secured via NHS Digital's Data Access Release Service (DARS) (ref: DARS-NIC-60624-B1R2Q) and included relevant permissions and approvals from Research Ethics (ref: 16/LO/1065), and Confidentiality Advisory Group (CAG), for the linkage of datasets under Section 251 of the Health and Social Care act 2014 (ref: 16/CAG/0081). The Independent Group Advising on the Release of Data (IGARD) at NHS Digital also approved the use of HES data for this study.

^{99m}Tc-DPD scintigraphy

Patients were administered intravenously with 700 MBq of ^{99m}Tc-DPD, scanned 3 hours later on either a General Electric (GE) Infinia Hawkeye 4 or Discovery 670 gamma camera and whole body and cardiac a SPECT-CT images were acquired, as previously described.[19] Intensity of myocardial uptake at ^{99m}Tc-DPD scintigraphy was graded 0–3 by two independent observers according to the established Perugini criteria.[20] Rare discrepancies in scoring were resolved by consensus within a multi-disciplinary meeting attended by a panel of experts.

Histology

Biopsies were stained with Congo red by the method of Puchtler *et al*,[21] and immunohistochemistry was performed with a panel of antibodies including an antibody against

TTR, as previously described.[22] After 2010, the presence of ATTR amyloid was further corroborated by proteomic analysis, as previously described.[23]

Echocardiography

Echocardiography was performed at baseline and at every NAC evaluation thereafter by three echocardiographers on GE Vivid 7 machines using EchoPac software. All echocardiograms were reported by two independent observers and discrepancies were reviewed by a group of clinical experts in amyloid echocardiography within a multi-disciplinary meeting.

Quality of life

After 2010, health-related QoL was measured at each evaluation by Kansas City Cardiomyopathy Questionnaire (KCCQ) score.[24] Briefly, the KCCQ is a 23-item patient-reported measure that quantifies physical function, symptoms (frequency, severity and stability), social function, self-efficacy and quality of life. An overall summary score is derived from the physical and social function, symptom and quality of life domains. Scores are represented on a scale from 0 to 100, with higher scores reflecting better health status. A median difference of 5 points on the overall summary score is considered a clinically significant change; a decline of 10 points is considered prognostically significant. It is noteworthy that 88% of patients who performed the KCCQ assessment were diagnosed from 2012 onwards, and that the V122I-hATTR-CM genotypic subgroup were proportionately under-represented (15% vs 20% in the whole cohort) and the ATTRwt-CM over-represented (75% vs 69% in the whole cohort) amongst those completing the KCCQ assessment.

Statistical Analyses

All mortality data were obtained from the UK Office of National Statistics. Date of Censor was 1st October 2017. The three genotypic sub-groups of interest were wild-type ATTR amyloidosis (ATTRwt-CM), V122I-associated hereditary ATTR amyloidosis (V122I-hATTR-CM) and non-V122I-associated hereditary ATTR amyloidosis (non-V122I-hATTR-CM). As a number of the numerical variables had skewed distributions, a Kruskal Wallis test was used to compare the distributions of each of the numerical variables at baseline in the three genotypic subgroups. A significant result was followed by Bonferroni corrected Mann Whitney pairwise comparisons to establish where the differences lay. A chi square test was used to compare the proportion of males in the three groups, and this was followed by Bonferroni corrected pairwise chi square comparisons. A mixed-effect linear regression model with main effects and interactions was used to analyse the longitudinal data to assess the effect of genotype on the outcome measures 6MWT, eGFR and NT-proBNP over time. Baseline variables that were statistically significant at the 0.10 alpha level in univariable Cox proportional hazards models were entered into a multivariable Cox model to investigate the factors independently predictive of survival. Variables that were not significant in the initial multivariable model were removed and an updated multivariable Cox model was re-analysed and presented. The proportional hazards assumption was checked and confirmed where appropriate. Kaplan Meier survival curves were drawn. All data were analysed using Stata software (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC). A significance level of 0.05 was used for all hypothesis tests unless otherwise stated.

Results

Characteristics of the cohort at baseline and comparison of genotypes

There were 1034 patients in the whole study cohort, 711 (69%) with wild-type ATTR-CM (ATTRwt-CM), 205 (20%) with V122I-associated hATTR-CM and 118 (11%) with non-

V122I-associated hATTR-CM comprising 80% of all referrals who were diagnosed with ATTR-CM at the National Amyloidosis Centre during the study period. The main reason for exclusion from the study was a refusal to consent to the protocolized program of follow up at the NAC. Other reasons, almost exclusively among patients with non-V122I-hATTR-CM, included receipt of disease modifying therapy (liver transplantation, diflunisal, anti-sense oligonucleotide therapy or RNA inhibitor therapy). There was no difference in disease-related parameters or disease severity between patients with ATTR-CM who were included and excluded from the study. Of the study patients with non-V122I-associated hATTR-CM, the majority (n=97, 83%) had the T60A variant, although other pathogenic variants were represented; 96% of non-V122I-associated hATTR-CM patients had coexistent, symptomatic amyloid polyneuropathy at the time of diagnosis as opposed to <5% of patients within the other genotypic subgroups. The number of new diagnoses of ATTR-CM each year stratified by genotype are shown in Figure 1 which highlights, in particular, the exponential recent rise in identification of ATTRwt-CM. Male gender dominated in all three subgroups: 94%, 71% and 69% respectively, although the proportion of males was significantly higher in ATTRwt-CM than hATTR-CM ($p<0.001$). There was no association between gender and survival and nor were there significant differences in baseline disease characteristics between males and females. The characteristics of the cohort at the time of diagnosis of cardiac ATTR amyloidosis are shown in Table 1. At diagnosis, patients with ATTRwt-CM, V122I-associated and non-V122I-associated hATTR-CM differed significantly in terms of age, left ventricular ejection fraction (LVEF), renal function and functional capacity (Table 1). Median age at diagnosis was significantly higher in ATTRwt-CM (79 years) and V122I-hATTR-CM (77 years) than in non-V122I-hATTR-CM (67 years) ($p<0.001$). NT-proBNP concentration was also significantly higher at baseline in patients with ATTRwt-CM and V122I-hATTR-CM compared to those with non-V122I-hATTR-CM ($p<0.001$ for both comparisons, Table 1). At

the time of diagnosis, LVEF and functional status as measured by 6MWT distance were significantly worse among V122I-hATTR-CM patients compared to the two other genotypic subgroups (Table 1).

Survival from baseline

Median patient survival from diagnosis by Kaplan-Meier analysis was 31 months in patients with V122I-hATTR-CM compared to 69 months among patients with ATTRwt-CM ($p<0.0001$) and 57 months in non-V122I hATTR-CM. NAC ATTR Disease Stage, calculated according to previously published cutoffs in NT-proBNP (3000 ng/L) and eGFR (45 ml/min) was, as expected, strongly predictive of survival across all genotypes (as well as within each genotype) with median survival of 68.0, 42.4 and 25.9 months among patients with Stage I, Stage II and Stage III disease respectively (all comparisons $p<0.001$). Survival of patients diagnosed with ATTRwt-CM from 2012, the year that ^{99m}Tc-DPD scintigraphy was routinely introduced into the diagnostic algorithm for ATTR-CM at UK NAC and following which 75% of patients were diagnosed non-invasively, was significantly better (median 60.2 months) than among patients diagnosed with ATTRwt-CM pre-2012 (median 46.3 months) when histology was usually required to establish the diagnosis (63% diagnosed via biopsy, usually EMB) (Figure 2B). Correspondingly, a higher proportion of ATTRwt-CM patients diagnosed pre-2012 had Stage III Disease (20% vs 16%) and a lower proportion had Stage I Disease (41% vs 43%) at the time of diagnosis compared to patients with ATTRwt-CM diagnosed from 2012 onwards.

Univariable analyses showed that factors at diagnosis significantly predictive of survival were age, NT-pro-BNP, left ventricular ejection fraction (LVEF), interventricular septal diameter in diastole (IVSd), eGFR, 6MWT distance, modified BMI (mBMI), 24hr urinary protein excretion, serum albumin, NAC ATTR Disease Stage, genotypic subgroup, and

date of diagnosis (Table 2). A multivariable model combining age, NAC ATTR Disease Stage, LVEF, genotypic subgroup, and 6MWT distance at the time of diagnosis revealed that age (HR, 1.037 per year; 95% CI, 1.008-1.067, p<0.011), NAC ATTR Disease Stage (HR 2.049; CI 1.352-3.104, p=0.001 for Stage II and HR 3.705; CI 2.313-5.933, p<0.001 for Stage III compared to Stage I), LVEF (HR 0.978 per 1% increase; 95% CI 0.963-0.993, p=0.003), genotypic subgroup (HR 2.071; CI 1.415-3.031, p<0.001 for V122I-hATTR-CM and HR 2.727; CI 1.458-5.098, p=0.002 for non-V122I-hATTR-CM compared to ATTRwt-CM) and 6MWT distance (HR 0.881 per 50 meter increase; 95% CI 0.832-0.933, p<0.001) were independently associated with patient survival (Table 2). Interestingly, a comparison of outcomes between genotypic subgroups among patients with each of the three NAC ATTR Disease Stages at the time of diagnosis showed that even within each category of Disease Stage, V122I genotype was an independent predictor of death (HR for V122-hATTR-CM vs ATTRwt-CM between 2 and 3, p<0.002 for all analyses). Survival among patients of African ancestry (n=207) with ATTRwt-CM (n=13) was longer (median 40 months) than among those with V122I-hATTR-CM (n=194) (median 31 months) but this did not reach statistical significance (p=0.85), probably owing to the small number of patients with ATTRwt-CM. It is noteworthy that only 6% (13/207) of the cohort who were of African ancestry had ATTRwt-CM.

Longitudinal analyses of disease parameters and functional status during follow up

Given the prognostic importance of NT-proBNP and eGFR (the variables which determine NAC ATTR Disease Stage) at the time of diagnosis, coupled with that of 6MWT distance, longitudinal analyses of these three variables were undertaken with a comparison between genotypic subgroups. NT-proBNP progressively increased whilst eGFR and 6MWT distance declined during the first 2 years of follow up. There was a more rapid rate of rise in NT-

proBNP concentration among patients with V122I-hATTR-CM than both ATTRwt-CM ($p<0.001$) and non-V122I-hATTR-CM ($p=0.009$) with no significant difference ($p=0.884$) between the latter two genotypic subgroups (Figure 3). Mean rise in absolute NT-proBNP concentration from diagnosis to 12 months was 842 ng/L (CI 551-1134) in patients with ATTRwt-CM, 1043 ng/L (CI 329-1758) in patients with non-V122I-hATTR-CM and 2678 ng/L (CI 658-4698) in patients with V122I-hATTR-CM. The mean (range) rates of decline in eGFR and decline in 6MWT distance from baseline over the course of 2 years were remarkably consistent between different genotypic subgroups; 5 to 10 ml/min (6.5-9.9) and approximately 100 metres (80-111) respectively (p=ns).

Hospitalisation episodes pre- and post-diagnosis of ATTR-CM

The subset of 534 English patients in whom complete data on hospital service usage were available for the full three years prior to diagnosis of ATTR-CM attended hospital a median (IQR) of 17 (9-27) times in the period, including a median (IQR) of 3 (1-5) inpatient hospital admissions. The breakdown of hospital service usage year on year prior to diagnosis is shown in Figure 4A. Median (IQR) diagnostic delay from first presentation with cardiac symptoms was 39 (8-78) months in English patients with ATTRwt-CM with 42% waiting more than 4 years after first presentation with cardiac symptoms and a further 23% waiting between 6 months and 4 years for the diagnosis to be established. Only just over one-third of English patients were diagnosed with ATTRwt-CM within 6 months of first presentation with cardiac symptoms. Median (IQR) time from first presentation with cardiac symptoms to diagnosis of hATTR-CM was 25 (4-60) months.

In the year after diagnosis of ATTR-CM (n=364), the median (IQR) number of hospital inpatient episodes (admissions) per patient was 2 (1-5) with 30% of patients admitted as inpatients to hospital at least 3 times during the period. The median (IQR) number of out-

patient and emergency room attendances per patient in the first year after diagnosis was 8 (5-13) and 1 (1-2) respectively. The median (IQR) number of hospital inpatient admissions per surviving patient was 2 (1-4) in the second year after diagnosis and 3 (1-5) in the third year after diagnosis with no difference between genotypic subgroups (Figure 4B).

Quality of Life (QoL) by KCCQ

Overall KCCQ domain scores within the first 12 months of diagnosis, obtained from 158 patients, showed poor health-related QoL across all 3 genotypic subgroups of ATTR-CM (Figure 5). The lowest scoring domains were physical limitation, social limitation and symptom stability in all three cohorts.

The magnitude and direction of change of QoL scores in each domain were measured in each cohort between 12 and 36 months. The direction of change of QoL scores was overwhelmingly negative in all cohorts and for all domains. V122I-hATTR-CM patients showed clinically significant deterioration in 7 of 10 domains, non-V122I-hATTR-CM patients in 5 of 10 domains and ATTRwt-CM patients in 3 of 10 domains. Median changes in KCCQ summary scores are shown in Table 3. Given that 88% of patients who completed the KCCQ assessment were diagnosed from 2012 onwards, and that patients with ATTRwt-CM were over-represented and those with V122I-hATTR-CM under-represented amongst those completing the KCCQ assessment, it is likely that the overall decline in QoL following diagnosis for the whole cohort would have been even more marked.

Discussion

This prospective observational study of more than 1000 patients with ATTR-CM undergoing comprehensive follow-up at the National Amyloidosis Centre, which is centrally commissioned as the single center in the UK for diagnosis and monitoring amyloidosis, has

yielded many new insights into the diagnosis, natural history, quality of life and outcome of the condition. Firstly, it highlights the exponential increase during recent years in the number of patients diagnosed with ATTR-CM, particularly ATTRwt-CM which is now diagnosed more than twice as frequently than hATTR-CM. This presumably reflects the remarkable sensitivity and increasingly widespread use of cardiac MRI and ^{99m}Tc-DPD (and ^{99m}Tc-PYP) scintigraphy,[12, 13] which in turn have fuelled increased awareness of the condition amongst cardiologists. Despite this however, our data demonstrate huge delays in establishing the diagnosis of ATTR-CM following presentation with cardiac symptoms, this taking more than 4 years in over 40% patients with ATTRwt-CM on a background of a median 17 hospital attendances during the three years prior to diagnosis. This long delay in the face of a progressive disease is likely to have contributed to the identified poor QoL by the time diagnosis was finally established. On a more encouraging note, survival among patients diagnosed with ATTRwt-CM since 2012 has been better than before this time, which in the absence of disease-modifying therapy and coupled with the finding that a higher proportion of such patients had NAC Stage I Disease and fewer had NAC Stage III Disease at the time of diagnosis than patients diagnosed pre-2012, suggests that increased awareness of the disease along with adoption of non-invasive diagnostic imaging techniques,[18] is resulting in patients being diagnosed earlier in the course of their disease. Nonetheless, given the evidence from historical autopsy series that ATTR amyloid deposits are present up to 25% of elderly male hearts, there seems little doubt that many individuals with ATTR-CM are currently not being diagnosed during their lifetime. A challenge for the future will be differentiating clinically significant from incidental myocardial ATTR amyloid deposits given the sensitivity of cardiac scintigraphy and MRI for identifying them. In view of the prolonged survival and reduction of hospitalizations recently reported in ATTR-CM with the TTR-stabilizing therapy, tafamidis, (notwithstanding the comparatively low rate of hospitalizations throughout the ATTR-ACT

trial compared to that reported here),[15] and the improvement in outcomes among patients with hATTR amyloidosis reported with the TTR-lowering RNA inhibitor therapy, patisiran,[17] there is every prospect that awareness and early diagnosis will increase further.

Although access to healthcare in the UK is available free of charge to all residents, this study demonstrated that patients with V122I-hATTR-CM had higher NT-proBNP, lower LVEF, and poorer functional status than those with ATTRwt-CM and non-V122I-hATTR-CM at the time of diagnosis, indicating that they had more advanced cardiac disease. Whilst this may partly explain the reduced survival from the time of diagnosis in V122I-hATTR-CM patients, our finding of a shorter delay from presentation with cardiac symptoms to diagnosis among patients with hATTR-CM compared to ATTRwt-CM, coupled with the finding of poorer outcomes among V122I-hATTR-CM even when stratified by NAC ATTR Disease Stage, suggest that the disease biology may be inherently more aggressive. It is noteworthy that overall QoL scores appeared to worsen more rapidly in hATTR-CM than ATTRwt-CM which is likely to reflect the impact of neuropathy, present in 96% of study patients with non-V122I-hATTR-CM, on physical performance and QoL, coupled with the generally poorer outcomes in V122I-hATTR-CM compared to ATTRwt-CM.

The authors acknowledge a number of limitations to this study. More sensitive echocardiographic parameters of ATTR-CM and of disease progression in the context of ATTR-CM such as longitudinal strain by tissue doppler imaging, myocardial contraction fraction and relative wall thickness were not included due to the fact that measurement of such parameters were only routinely introduced into the UK National Amyloidosis Centre echocardiography protocol within the last five years. Similarly, data on hospital episode statistics were only available from patients living in England (excluding those from Northern Ireland, Scotland and Wales) during the specified time period. Lastly, QoL and functional status were available only in the subset of patients diagnosed with ATTR-CM after 2010,

having been introduced into the clinic protocol at that time. Nonetheless, we believe that the data pertaining to subsets of the ATTR-CM cohort presented in this manuscript are representative of the disease natural history in the population as a whole.

In summary, ATTR-CM is being increasingly recognized although there remains much work to do to establish the diagnosis earlier in the course of the disease, the natural history of which is gradual progression and death some 3-10 years from diagnosis. In this era of promising novel therapies for ATTR amyloidosis, earlier diagnosis assumes greater importance and argues strongly for inclusion of CMR and bone scintigraphy early in the investigative pathway of patients with cardiac failure or cardiomyopathy of uncertain etiology.

VARIABLE	ATTRwt (Group 1)	V122I-hATTR (Group 2)	Non-V122I hATTR (Group 3)	p*
N (%)	711 (69)	205 (20)	118 (11)	
Male Sex (%)	668 (94)	146 (71)	81 (69)	p < 0.0001 (Gp 1 vs 2) p < 0.0001 (Gp 1 vs 3)
Median (IQR) age (years)	79 (73-83)	77 (72-80)	67 (62–71)	p < 0.0001 (All comparisons)
Median (IQR) LVEF (mm)	58 (45-71)	49 (39-62)	53 (45-60)	p < 0.0001 (Gp 1 vs 2) p < 0.0001 (Gp 1 vs 3)
Median (IQR) IVSd (mm)	17 (15-18)	17 (16-18)	16 (15-18)	p = ns (All comparisons)
Median (IQR) NT-proBNP (ng/L)	3046 (1615-5472)	3337 (1668-6096)	2026 (871-4548)	p < 0.0001 (Gp 1 vs 3) p < 0.0001 (Gp 2 vs 3)
Median (IQR) eGFR (ml/min)	58 (45-71)	60 (47-75)	81 (62-100)	p < 0.0001 (Gp 1 vs 3) p < 0.0001 (Gp 2 vs 3)
Median (IQR) proteinuria (g/24 hr)	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.10 (0.10–0.13)	p = ns (All comparisons)
Median (IQR) albumin (g/L)	44 (42-46)	42 (40-45)	43 (41-45)	p < 0.0001 (Gp 1 vs 2) p = 0.01 (Gp 1 vs 3)
Median (IQR) mBMI	1151 (1044-1282)	1111 (976-1232)	1039 (922-1225)	p < 0.0001 (Gp 1 vs 2) p < 0.0001 (Gp 1 vs 3)
Median (IQR) 6MWT distance (meters)	345 (230-415)	260 (141-364)	374 (276-440)	p < 0.0001 (Gp 1 vs 2) p < 0.0001 (Gp 2 vs 3)
Median (IQR) 6MWT % expected for age	73 (53-88)	57 (34-76)	69 (57-84)	p < 0.0002 (Gp 1 vs 2)

Table 1. Baseline characteristics of 1034 patients with cardiac transthyretin amyloidosis. ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis; LVEF = left ventricular ejection fraction; IVSd = intraventricular septal thickness in diastole; eGFR = estimated glomerular filtration rate (corrected for race); mBMI = modified Basal Metabolic Index;

MWT = 6-minute walk test. IQR = interquartile range. Gp = Group. * Comparison of numerical variables was by Kruskal Wallis test followed, where relevant, by Bonferroni-corrected Mann Whitney pairwise comparisons.

UNIVARIABLE ANALYSIS			
Variable	**HR for death**	**95% CI**	**p**
Age (per year increase)	1.04	1.025–1.054	<0.001
NT-proBNP (per log unit increase)	3.77	2.915–4.862	<0.001
LVEF (per 1% increase)	0.96	0.954–0.972	<0.001
IVSd (per mm increase)	1.11	1.065–1.149	<0.001
eGFR (per 10 ml/min increase)	0.82	0.776–0.863	<0.001
6MWT (per 50 meter increase)	0.81	0.767–0.0.847	<0.001
mBMI (per unit increase)	0.99	0.998-0.999	<0.001
24hr protein excretion (per 0.1 g increase)	1.07	1.039-1.095	<0.001
Serum albumin (per 1 g/L increase)	0.91	0.882-0.934	<0.001
Gender			
Female	1		
Male	0.91	0.692-1.204	0.519
NAC ATTR Disease Stage			
Stage I	1		
Stage II	2.05	1.631-2.583	<0.001
Stage III	7.05	4.876-10.182	<0.001
Genotype			
ATTRwt	1		
V122I-hATTR	1.91	1.526-2.385	<0.001
Non-V122I-hATTR	0.80	0.582-1.100	0.168
Date of Diagnosis			
Pre-2012	1		
2012 onwards	0.78	0.627-0.961	0.020
MULTIVARIABLE ANALYSIS			
Variable	**HR for death**	**95% CI**	**p**
Age (per year increase)	1.04	1.008–1.067	0.011
NAC ATTR Disease Stage			
Stage I	1		
Stage II	2.05	1.352-3.104	0.001
Stage III	3.71	2.313-5.933	<0.001
LVEF (per 1% increase)	0.98	0.963–0.993	0.003
Genotype			
ATTRwt	1		
V122I-hATTR	2.07	1.415-3.031	<0.001
Non-V122I-hATTR	2.73	1.458-5.098	0.002
6MWT (per 50 meter increase in distance)	0.88	0.832–0.933	<0.001

Table 2. Univariable/Multivariable proportional hazards model of factors at the time of diagnosis of cardiac ATTR amyloidosis that were predictive of survival. NAC ATTR

Disease Stage based on eGFR (< or ≥ 45 ml/min) and NT-proBNP (> or ≤ 3000 ng/L).
LVEF = left ventricular ejection fraction; IVSd = intraventricular septal thickness in diastole; eGFR = estimated glomerular filtration rate; 6MWT = six-minute walk test; mBMI = modified body mass index; ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

	Clinical Summary	Quality of Life	Social Limitation	Overall Summary
ATTRwt 12m				
Median (IQR)	64 (42-79)	58 (38-75)	50 (25-81)	57 (39-79)
Mean (CI)	61 (56-65)	57 (53-62)	51 (45-57)	58 (53-62)
ATTRwt 36m				
Median (IQR)	55 (40-73)	63 (39-81)	38 (19-70)	52 (37-71)
Mean (CI)	56 (50-62)	59 (53-66)	43 (34-52)	54 (48-60)
Change ATTRwt 12m to 36m				
Median (IQR)	**-9 (-2 to -6)**	**4 (1 to 6)**	**-13 (-6 to -11)**	**-5 (-2 to -8)**
Mean (CI)	**-5 (-6 to -3)**	**2 (0 to 4)**	**-8 (-11 to -6)**	**-3 (-5 to -2)**
V122I-hATTR 12m				
Median (IQR)	62 (36-79)	75 (33-83)	42 (13-75)	67 (31-77)
Mean (CI)	57 (47-66)	61 (51-71)	45 (32-58)	55 (45-55)
V122I-hATTR 36m				
Median (IQR)	51 (25-80)	42 (29-67)	25 (3-63)	44 (21-70)
Mean (CI)	52 (28-76)	50 (31-69)	35 (7-63)	47 (24-70)
Change V122I-hATTR 12m to 36m				
Median (IQR)	**-11 (-11 to 1)**	**-33 (-4 to -17)**	**-17 (-9 to -13)**	**-23 (-10 to -7)**
Mean (CI)	**-4 (-19 to 10)**	**-11 (-20 to -3)**	**-10 (-25 to 5)**	**-8 (-21 to 5)**
Non-V122I-hATTR 12m				
Median (IQR)	70 (39-84)	46 (23-71)	38 (25-66)	63 (31-71)
Mean (CI)	64 (48 to 80)	48 (30 to 67)	45 (27 to 63)	56 (40 to 72)
Non-V122I-hATTR 36m				
Median (IQR)	42 (26-90)	46 (27-94)	31 (19-94)	41 (25-92)
Mean (CI)	54 (28-80)	56 (30-83)	45 (10-79)	54 (26-81)
Change Non-V122I-hATTR 12 to 36m				
Median (IQR)	**-28 (-13 to 5)**	**0 (4 to 23)**	**-6 (-6 to 28)**	**-22 (-6 to 21)**
Mean (CI)	**-10 (-21 to 0)**	**8 (0 to 16)**	**-1 (-17 to 16)**	**-3 (-14 to 9)**

Table 3. Kansas City Cardiomyopathy Questionnaire summary scores at 12 and 36 months from diagnosis of cardiac ATTR amyloidosis in the three genotypic sub-groups. Clinical summary score = physical limitation score + total symptom score; Overall summary score = clinical summary score + Quality of Life score + Social Limitation score). A 5-point change in the overall summary score reflects a clinically significant change in heart failure status; a 10-point change is considered prognostically significant. Changes in score between 12 months post-diagnosis and 36 months post-diagnosis are shown. ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

Figure Legends

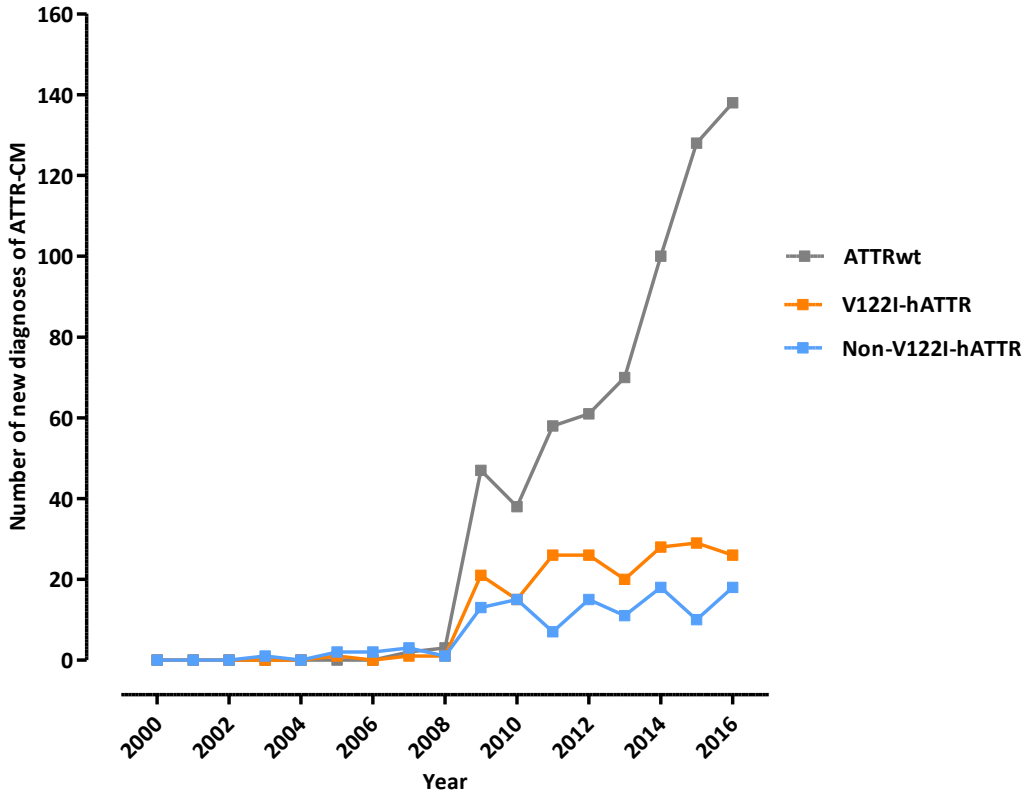
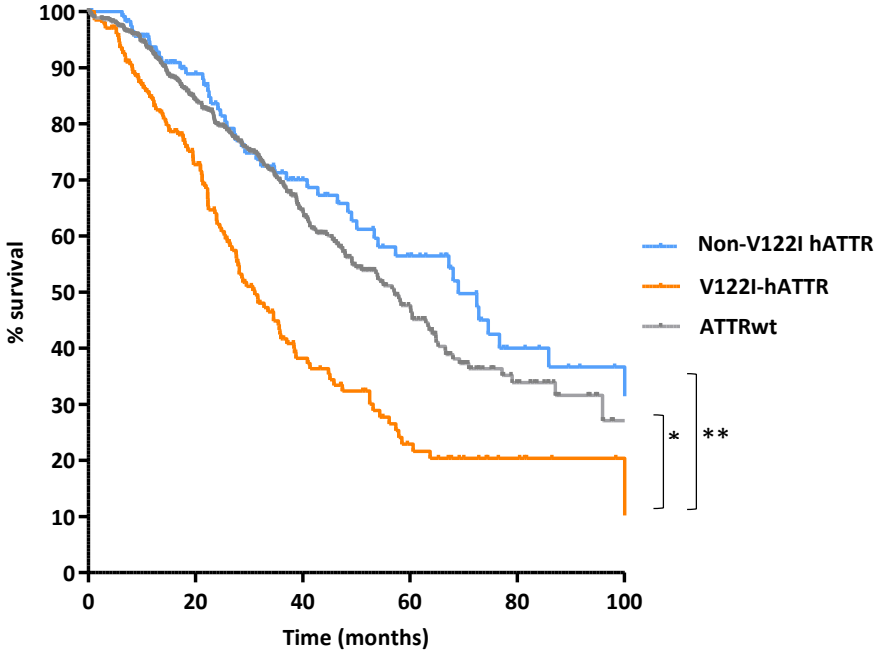


Figure 1. Number of new diagnoses of cardiac transthyretin amyloidosis by year according to genotypic subgroup. ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

A)

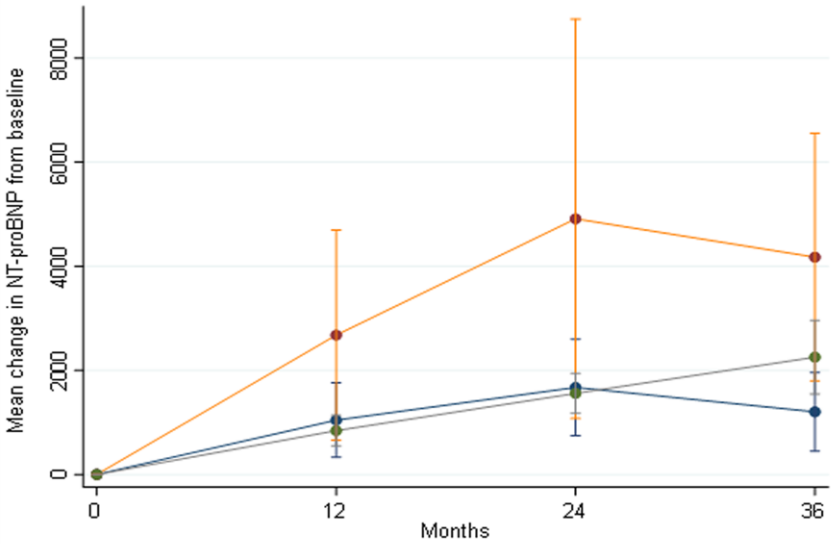


Numbers at risk

Non-V122I-hATTR	118	87	52	34	14	7
V122I-hATTR	205	122	42	18	7	3
ATTRwt	711	415	188	76	24	2

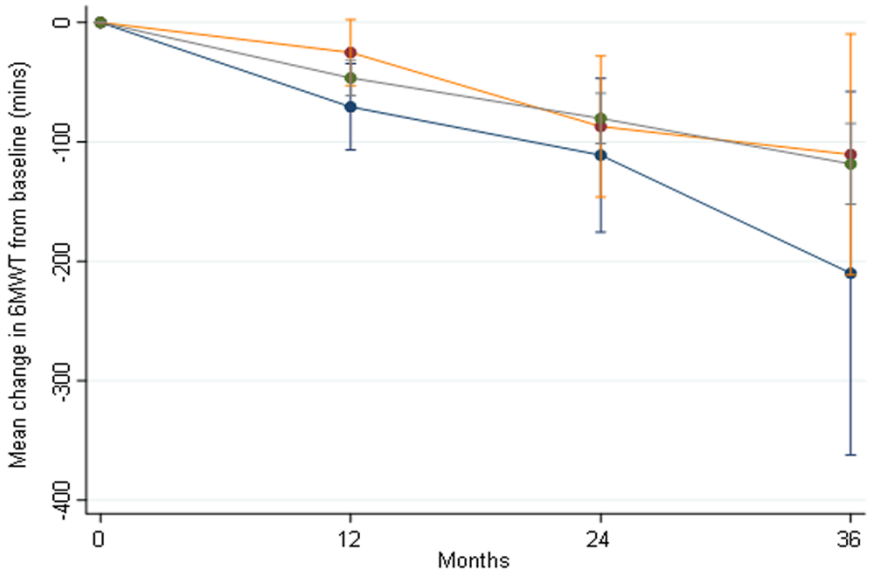
Figure 2. A) Kaplan-Meier survival stratified by genotype for the whole cohort (*p<0.0001; **p<0.0001). **B) Survival of patients with ATTRwt-CM stratified by year of diagnosis** (*p=0.009). ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

A



Number			
V122I-hATTR	124	68	27
ATTRwt	428	282	128
Non-V122I-hATTR	75	51	33

B



Number			
V122I-hATTR	61	31	11
ATTRwt	289	175	66
Non-V122I-hATTR	32	17	9

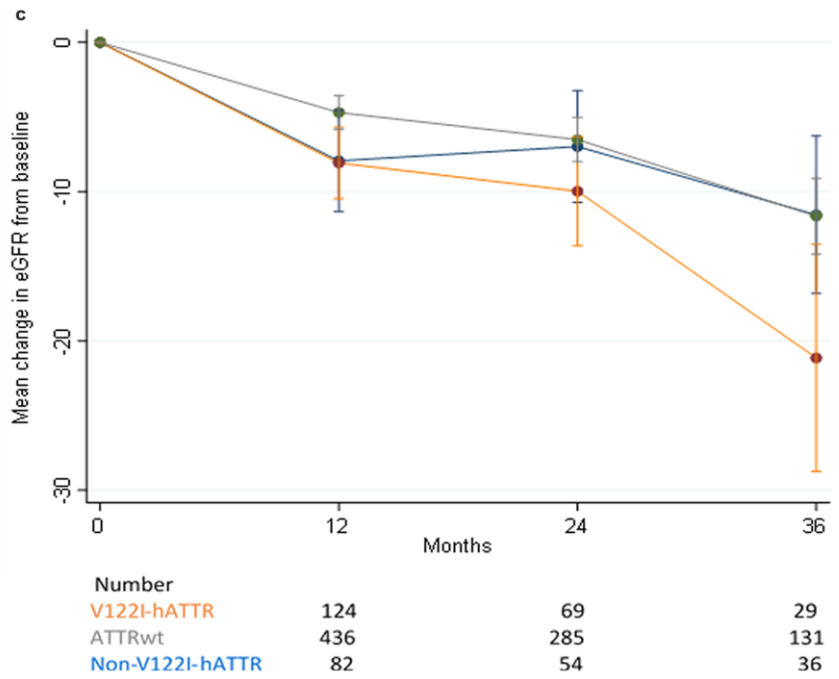


Figure 3. Change in NT-ProBNP, six-minute walk test distance and estimated glomerular filtration rate from baseline to 12, 24 and 36 months stratified by genotypic subgroup. A) Mean change (with 95% CI) in NT-proBNP concentration from baseline to 12, 24 and 36 months. The mean rate of rise in NT-proBNP concentration was significantly greater in V122I-hATTR-CM than in both ATTRwt-CM ($p<0.001$) and non-V122I-hATTR-CM ($p<0.009$) but was not significantly different between the latter genotypic sub-groups ($p=0.884$). B) Mean change in six minute walk test (6MWT) distance from baseline to 12, 24 and 36 months ($p>0.05$). C) Mean change in estimated glomerular filtration rate (eGFR) from baseline to 12, 24 and 36 months ($p>0.05$). ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

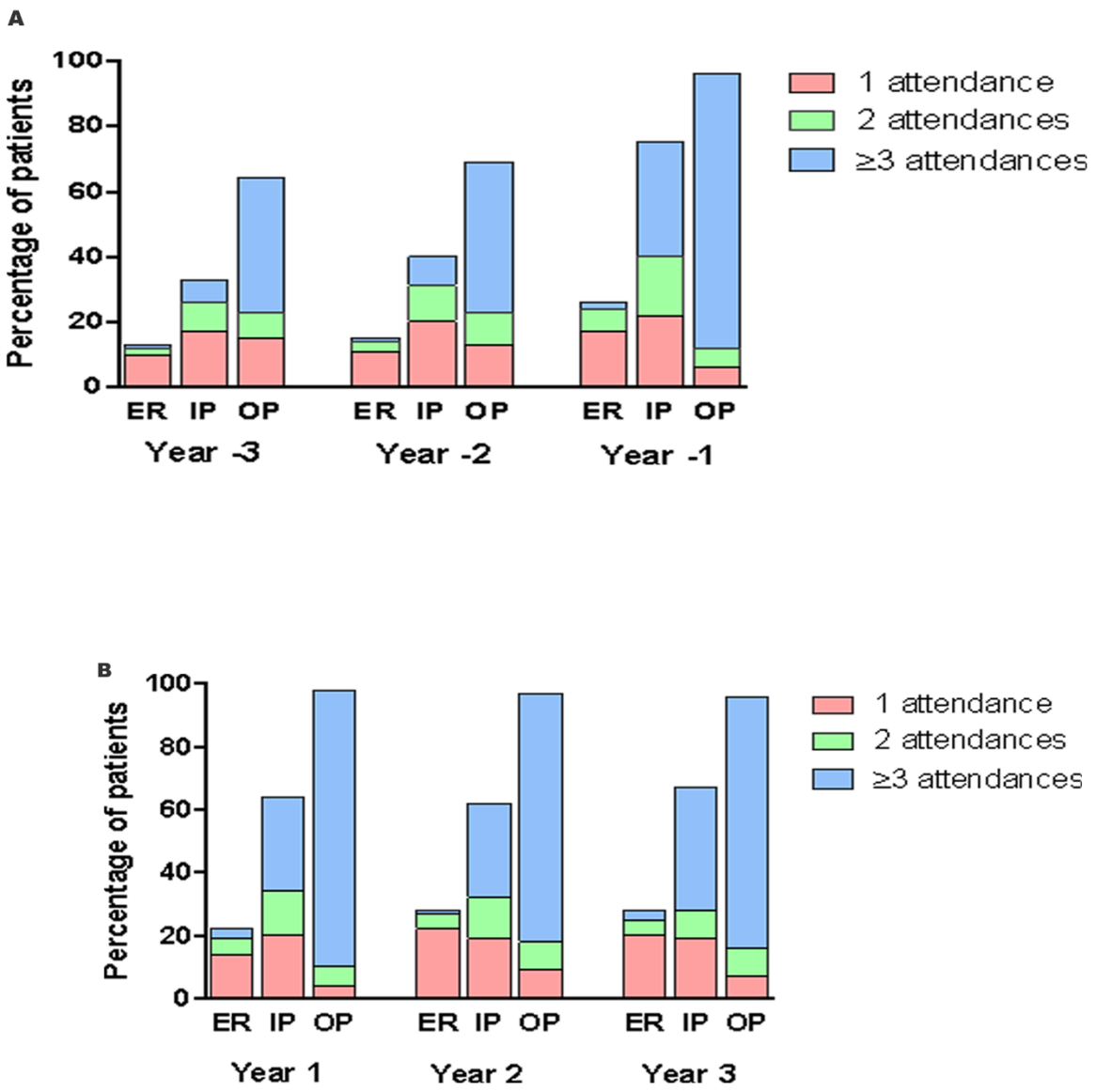


Figure 4. English National Health Service (NHS) hospital services usage. A) English NHS hospital services usage covering emergency room (ER), inpatient admissions (IP) and outpatient services (OP) in the three years prior to diagnosis of ATTR-CM. B) English NHS hospital services usage, covering emergency room (ER), inpatient admissions (IP) and outpatient services (OP) during the first 3 years after diagnosis of ATTR-CM (percentages adjusted for surviving patients at each timepoint).

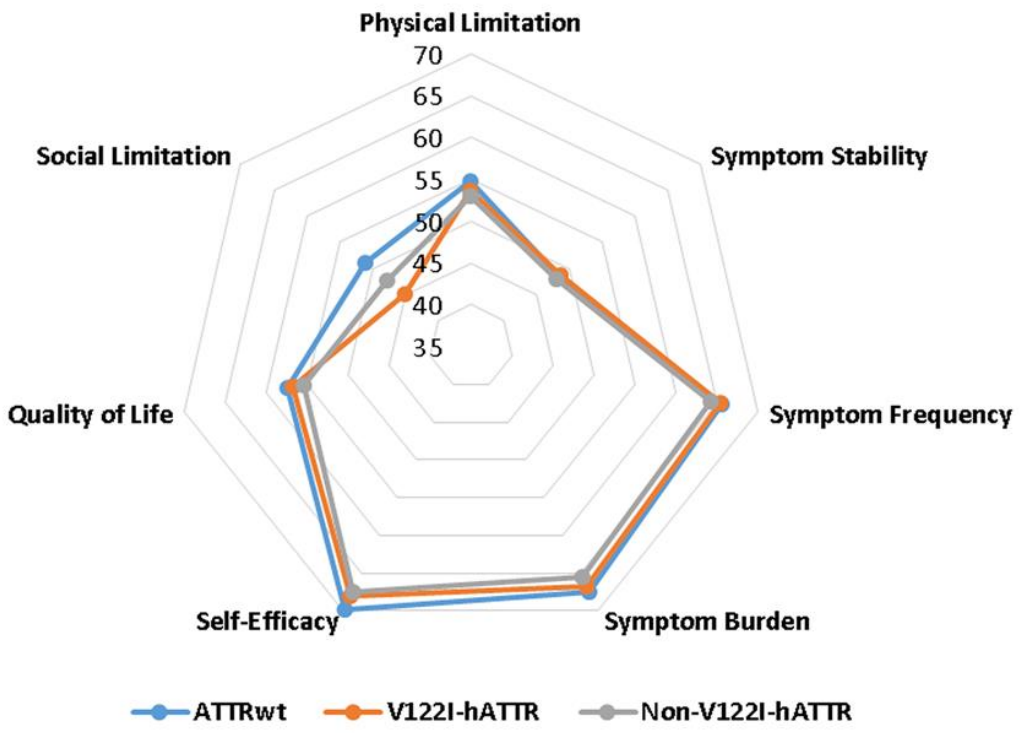


Figure 5. Health-related quality of life as measured by the Kansas City Cardiomyopathy Questionnaire in 158 patients within the first 12 months of diagnosis stratified by genotype. A score of 100 indicates perfect health. ATTRwt = wild-type ATTR amyloidosis; V122I-hATTR = V122I-associated hereditary ATTR amyloidosis; Non-V122I-hATTR = Non-V122I-associated hereditary ATTR amyloidosis.

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Dr Lane has received consultancy fees from Alnylam Pharmaceuticals and Eidos Therapeutics Inc. Dr Whelan serves on advisory boards for Alnylam Pharmaceuticals and Akcea Therapeutics. Professor Gillmore serves on advisory boards for Alnylam Pharmaceuticals, Akcea Therapeutics, Pfizer Inc. and GlaxoSmithKline. The other authors report no conflicts.

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