GAA-*FGF14* ataxia (SCA27B): phenotypic profile, natural history progression and 4-aminopyridine treatment response

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Abstract

Ataxia due to an autosomal dominant intronic GAA repeat expansion in *FGF14* (GAA-*FGF14* ataxia, Spinocerebellar ataxia 27B [SCA27B]) has recently been identified as one of the most common genetic late-onset ataxias. We here aimed to characterise its phenotypic profile, natural history progression, and 4-aminopyridine (4-AP) treatment response.

We conducted a multi-modal cohort study of 50 GAA-*FGF14* patients, comprising in-depth phenotyping, cross-sectional and longitudinal progression data (up to 7 years), MRI findings, serum neurofilament light (sNfL) levels, neuropathology, and 4-AP treatment response data, including a series of n-of-1 treatment studies.

GAA-*FGF14* ataxia consistently presented as late-onset (60.0 years (53.5-68.5), median (IQR)) pancerebellar syndrome, partly combined with afferent sensory deficits (55%) and dysautonomia (28%). Dysautonomia increased with duration while cognitive impairment remained infrequent, even in advanced stages. Cross-sectional and longitudinal assessments consistently indicated mild progression of ataxia (0.29 SARA points/year), not exceeding a moderate disease severity even in advanced stages (max. SARA score: 18 points). Functional impairment increased relatively slowly (unilateral mobility aids after 8 years in 50% of patients). Corresponding to slow progression and low extra-cerebellar involvement, sNfL was not increased relative to controls. Concurrent second diseases (including progressive supranuclear palsy neuropathology) represented major individual aggravators of disease severity, constituting important caveats for planning future GAA-*FGF14* trials. A treatment response to 4-AP with relevance for everyday living was reported by 86% of treated patients. A series of three prospective n-of-1 treatment experiences with on/off design showed marked reduction in daily symptomatic time and symptom severity on 4-AP.

Our study characterises the phenotypic profile, natural history progression, and 4-AP treatment response of GAA-*FGF14* ataxia. It paves the way towards large-scale natural history studies and 4-AP treatment trials in this newly discovered, possibly most frequent, and treatable late-onset ataxia.

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Abbreviations: FGF14 = fibroblast growth factor 14 gene; INAS = Inventory of Non-Ataxia Signs; MSA-C = multiple system atrophy of cerebellar type; SAOA = sporadic ataxia of adult onset; SARA = Scale for the Assessment and Rating of Ataxia; sNfL = neurofilament light in serum; VOR = vestibulo-ocular reflex; 4-AP = 4-aminopyridine

Introduction

The genetic basis of late-onset ataxia has long remained largely elusive [1] and effective pharmaceutic treatment options have been lacking in this frequent ataxia condition [2]. Autosomal dominant GAA repeat expansions in the first intron of the fibroblast growth factor 14 gene (*FGF14*) have recently been discovered as one of the most common causes of late-onset ataxia (Spinocerebellar ataxia 27B [SCA27B]) with a frequency ranging from 10% to 61% in several late-onset ataxia cohorts of various ethnic backgrounds [3,4]. The high frequency around the world highlights the need to already initiate first steps towards trial-readiness and to identify potential candidate treatments of this novel condition.

We here present a multi-modal study of 50 GAA-*FGF14* ataxia patients, (1) characterising its phenotypic profile and evolution as well as providing first longitudinal natural history progression data, and (2) demonstrating its treatment response to 4-aminopyridine (4-AP), including a series of prospective n-of-1 treatment experiences. These findings indicate the potential of a precision medicine approach to – so far untreatable – late-onset ataxias, with genotypic stratification allowing to identify a subpopulation readily treatable with an existing drug.

Materials and methods

Cohort

We aggregated a consecutive series of 50 GAA-*FGF14* patients from 43 families, recruited at the Department of Neurodegenerative Diseases, Center for Neurology and Hertie Institute for Clinical Brain Research, University of Tübingen (Table 1 for demographic and genetic characteristics). The cohort resulted from screening 231 consecutive degenerative ataxia patients comprising of 69 (= 30%) patients with autosomal-dominant cerebellar ataxia and 162 (= 70%) patients with sporadic adult-onset ataxia (SAOA) [3]. All screened patients were genetically unsolved before *GAA-FGF14* screening and had late-onset ataxia (median age at onset: 54 years, interquartile range: 44-64 years). Index patients identified by this earlier genetic screening were now complemented by additional family members, in-depth clinical and longitudinal data and modelling, treatment data and biomarker measurements. The Institutional Review Board of the University of Tübingen approved the study (AZ 598/2011BO1). All subjects provided written informed consent before participation according to the Declaration of Helsinki.

Genotyping

Genetic analysis of the *FGF14* repeat locus was performed as described previously [3,5]. We amplified the intronic *FGF14* repeat locus by long-range polymerase chain reaction (PCR) and determined the number of repeat units by capillary electrophoresis of fluorescent long-range PCR amplification products [5]. Results of fragment length analysis were confirmed by agarose gel electrophoresis of PCR amplification products. The motif of the repeat expansion was analysed by targeted long-read nanopore sequencing in 43 cases. Repeat-primed PCR was used to ascertain the presence of a GAA repeat expansion in the remaining cases. GAA repeat expansions ≥250 repeat units were considered pathogenic [3].

Deep phenotyping

Longitudinal patient data and records (with follow-up times spanning up to 7 years) were systematically assessed according to a comprehensive data form, including prespecified queries on demographics, genetics, history, neurological phenotype, disease severity, and 4-AP treatment. We assessed disease severity by the Scale for the Assessment and Rating of Ataxia

(SARA) [6] and the burden of non-ataxia features by the Inventory of Non-Ataxia Signs (INAS) [7]. As patients' functional impairment in GAA-*FGF14* ataxia arises mainly from the associated gait disorder, functional impairment was assessed in terms of the required mobility aid (i.e., independent walking, dependence on unilateral or bilateral walking aids, wheelchair dependence) and the Friedreich Ataxia Rating Scale functional disability stage (FARS-FDS) [8]. To determine the specificity of how the individual ataxia domains contribute to overall ataxia severity in GAA-*FGF14* ataxia, the profile of the SARA items in the GAA-*FGF14* cohort was compared to that of another frequent late-onset onset ataxia (*RFC1* disease), with patients matched by their overall SARA score. *RFC1* patients (n=42) were recruited by the *RFC1* Natural History Study [9] (ClinicalTrials.gov: NCT05177809).

MR imaging

Routine MRI scans were aggregated and systematically assessed by two independent raters (CW and MS) where such images were available and digitally transferable for centralised review. To evaluate whether the cerebellar vermis atrophy observed in GAA-*FGF14* patients can be identified reliably on routine clinical imaging without prior knowledge of the genotype, the degree of cerebellar vermis atrophy was additionally assessed in a genotype-blinded manner, using a mixed set of scans of 28 GAA-*FGF14* patients and 27 elderly neurologically healthy controls (selected from the control cohort reported by Lindig et al. 2018) [10]. For this, the degree of cerebellar vermis atrophy was assessed on mid-line sagittal planes as "present" or "absent", with further grading of the atrophy as "mild", "moderate", or "severe".

Blood Neurofilament light levels

We measured the serum levels of neurofilament light (sNfL) in (1) GAA-*FGF14* patients (n=12, age: 66.5 years (59.4-71.8), median and interquartile range), (2) age-matched healthy controls (n=26, age: 63.2 years (58.8-69.4)), and – as age-matched disease controls – (3) patients with sporadic adult-onset ataxia (SAOA, n=34, age: 67.2 years (61.6-72.2)) and (4) patients with multiple system atrophy of cerebellar type (MSA-C) (n=19, age: 66.1 years (64.0-70.1)), with both patient groups also having been recruited at the Department of Neurodegenerative Diseases, University of Tübingen, and tested negative for expansions in *FGF14* and *RFC1*. NfL measurements were performed by single molecule array (Simoa) technique on the Simoa HD-X

analyser (Quanterix), using the NF-light Advantage kit, as described previously [11,12]. Longitudinal patient samples were measured in the same batch. All measurements had a coefficient of variation below 20% and all sNfL levels were in the previously established range of quantification [11,12].

Neuropathology

Post-mortem examination of the brain of subject ID20559 (79-year-old woman) with a biallelic GAA-*FGF14* repeat expansion (repeat sizes: 276 and 252 units) was performed at the brain bank affiliated with the DZNE/University of Tübingen. Histological analysis of formalin-fixed paraffin embedded tissue included haematoxylin and eosin (H&E) staining and immunohistochemistry with antibodies against ptau (AT8, ThermoFisher, dilution: 1:1000), pTDP-43 (1D3, own production, 1:100) [13], alpha-synuclein (4D6, OriGene, 1:5000), beta amyloid (4G8, Covance, 1:6000), p62 (BD Biosciences, 1:200), and polyQ (1C2, Millipore, 1:5000) using the Ventana BenchMark XT automated staining system with the OptiView DAB detection kit (Ventana).

Treatment response to 4-aminopyridine

A retrospective analysis of the 4-AP treatment response was performed on the group level in all GAA-*FGF14* patients of our cohort who had received 4-AP treatment. In addition, we studied an aggregated series of prospective structured open-label single-subject treatment experiences in three patients. These n-of-1 studies had each been independently performed as single-subject named-patient uses (German: *"individueller Heilversuch"* = "trial of therapy"), structured by a prospective treatment protocol with prespecified documentation, allowing characterisation of patients' 4-AP treatment response with a prospective, intra-individually on/off-controlled treatment protocol design [14] (thus following the guidance of the Declaration of Helsinki [section 37] to systematically collect data also and particularly in such individual "trials of therapy" [15,16]. As treatment endpoints, we defined the patient-recorded total symptomatic time per day and the frequency of days affected by serve symptoms. 4-AP treatments had been initiated in all subjects in a genotype-blind manner, i.e., without knowledge of, and prior to, identification of the underlying *FGF14* GAA repeat expansion.

Statistical analysis

To assess the phenotypic evolution of GAA-*FGF14* disease, we estimated the temporal evolution of phenotypic features from cross-sectional data by logistic regression, modelling the presence of each disease feature as a dichotomous variable (i.e., present/absent) as a function of the disease duration. This provided estimates of the frequency of each phenotypic feature across the observed range of disease durations (ranging from 0 to 25 years). We used the same approach to model patients' functional impairment (in terms of their dependence on several types of mobility aids) over their disease duration. To characterise the longitudinal natural history progression of GAA-*FGF14* disease, we used a linear mixed-effects model of longitudinal SARA scores, thus considering the covariance between repeated scores of each subject. In the model, we included disease duration and age as fixed effects, their interaction, and the random variable subject, modelled by random intercepts (R package: lme4) [17]. We analysed cross-sectional SARA scores and INAS counts by linear regression over disease duration. Between-group comparisons of continuous variables were performed with two-sided Mann-Whitney U-tests. We analysed the data in R (version 4.1).

Data availability

The anonymised data can be accessed on reasonable request addressed to the corresponding author.

Results

Genetic characteristics

50 patients from 43 families with GAA-*FGF14* expansions above the pathogenic threshold (≥250 repeats) were assessed (repeat count of the expanded allele: 349 [316-402] (median [interquartile range]), max. repeat count: 578; note that the repeat count refers to GAA-repeat units, not nucleotide numbers; for descriptive characteristics of the cohort, see Table 1), including two patients (ID20559, ID26913) with biallelic pathogenic expansions (for details on the capillary electrophoresis in biallelic carriers, see Supplementary Figure 1). 51% of index patients had a positive family history for ataxia in the parent or sibling generation (= familial

ataxia), whereas 49% did not have any affected family members (= sporadic ataxia). The repeat count of the trinucleotide expansion was not significantly associated with patients' age at onset (r=-0.02, p=0.874), disease severity in terms of the SARA score (r=-0.07, p=0.660), or cross-sectional disease progression (defined as the quotient of SARA score and disease duration; r=0.06, p=0.729; Pearson's correlations, n=45).

Symptom onset

Symptoms in GAA-*FGF14* disease started on average at 60.0 (53.5-68.5) years of age. The first symptom experienced by patients at clinical onset usually was unsteady gait (79%) but could also – though less frequently – consist of visual disturbances (12%, comprising of oscillopsia, diplopia, blurring), dizziness (as opposed to vertigo), impairment of fine motor skills, and dysarthria (all ≤ 10%) (Fig. 1A). In a subset of patients, clinical disease started with episodic symptoms (13%) (including episodic worsening of gait impairments and dizziness).

Contribution of ataxia domains to GAA-FGF14 ataxia severity

The overall severity of ataxia in GAA-*FGF14* disease, as captured by the SARA score, was primarily driven by gait and stance ataxia and lower-limb ataxia, whereas sitting and speech disturbances contributed only relatively little to the overall ataxia severity, which suggests a caudal-to-rostral gradient of ataxia severity in GAA-*FGF14* disease (Fig. 1B). In comparison to another frequent genetic late-onset ataxia – *RFC1* disease – speech was significantly less affected in GAA-*FGF14* disease (p=0.001, two-sided Mann-Whitney U-test, Bonferroni-corrected for the number of SARA items) (Fig. 1B).

Phenotypic profile

The phenotypic profile of GAA-*FGF14* ataxia consisted of a pancerebellar syndrome, with predominant impairment of balance and gait (95%) and highly prevalent cerebellar oculomotor signs (88%, comprising broken-up smooth pursuit, saccadic dysmetria and downbeat nystagmus) (Fig. 2; Supplementary Table 1 for clinical details). As non-cerebellar systems, GAA-*FGF14* disease compromised mainly the afferent tracts (reflected by hyporeflexia in up to 33% and reduced pallesthesia (≤5/8 on Rydel-Seiffer) in up to 55%) and the autonomous nervous system (reflected by urinary urgency in 28%) (Fig. 2). Bradykinesia and involvement of upper or lower

motor neurons were rare (each < 13%), which suggests that GAA-*FGF14* disease does not frequently affect basal ganglia or motor neurons in a clinically relevant manner. Additional features frequently comprised visual disturbances (48%), dizziness (21%), and clinical evidence of vestibulo-ocular reflex (VOR) impairment (≥23%, corresponding to 11 patients in the total cohort), while – despite patients' advanced age – cognitive impairment (based on clinical judgment) was infrequent (16%) (Fig. 2).

The atrophy pattern of GAA-*FGF14* ataxia was consistently marked by atrophy of the cerebellar vermis (97%) and, though less frequent, of the cerebellar hemispheres (59%), as assessed by two independent reviewers on aggregated routine MRI scans of 29 patients (age: 69.7 years (61.5-74.5)) (Fig. 3A). While atrophy of the cerebral cortex was also commonly observed (31%), this showed a variable regional pattern across patients (e.g., including atrophy of the parietal cortex, hippocampus, or enlarged ventricles) and was thus likely due to other, possibly age-related degenerative processes, rather than GAA-*FGF14* ataxia. We did not observe brain stem atrophy detectable on routine MRI. Longitudinal progression of cerebellar atrophy was mild, as assessed on longitudinal images of 9 patients (follow-up interval: 5.4 years (3.1-5.9)) (exemplified in Fig. 3B and 3C).

The overall key MRI finding – atrophy of the cerebellar vermis – was validated by assessment in a genotype-blinded manner in a mixed set of scans of GAA-*FGF14* patients and elderly neurologically healthy controls, showing that it was significantly more frequent in GAA-*FGF14* patients (20/28 = 71.4%) than in healthy elderly controls (2/27 = 7.4%) (X^2 (1) = 20.88, p < 0.001, Pearson's Chi-square test), with the degree of atrophy in GAA-*FGF14* ataxia ranging from "mild" (14.3%) over "moderate" (50.0%) to "severe" (7.1%).

Phenotypic evolution

The phenotypic evolution of GAA-*FGF14* ataxia was characterised by the presence of balance and gait impairments almost always already at clinical onset, with falls becoming frequent >5 years after onset (i.e., in 50% of subjects at a disease duration of 8 years) (Fig. 4). Dysphagia and particularly dysarthria were frequent features in GAA-*FGF14* ataxia, but their prevalence increased only slightly in the disease course, even in patients with >20 years disease duration. In contrast, the frequency of upper limb ataxia and dysdiadochokinesia increased in later disease stages (Fig. 4). GAA-*FGF14* ataxia frequently comprised damage to the afferent tracts, with high

prevalence of reduced pallesthesia and Romberg test abnormality already at the earliest stages of the disease, and the prevalence of reduced ankle reflexes increasing during the disease course. Autonomic dysfunction was rare at disease onset but became frequent in advanced and late-stage disease. It comprised urinary urgency (28%) and erectile dysfunction (13%), but no positive evidence of postural hypotension. Cognitive impairment remained relatively infrequent – even in advanced disease stages (Fig. 4).

Natural history progression of ataxia, non-ataxia features and functional impairment

Disease progression of GAA-*FGF14* ataxia was slow, as suggested by the slow increase of cross-sectional SARA scores with disease duration (≈ 0.29 SARA points/year at age 70 years) (F (3, 36) = 6.04, p=0.002, adjusted R^2=0.28, linear regression with the factors age [b=0.43, p=0.003], duration [b=2.73, p=0.001], and their interaction [b-0.03, p=0.003]) (Fig. 5A). This was confirmed by the slow intra-individual longitudinal increase of SARA scores in subjects with longitudinal SARA scores (≈ 0.23 SARA points/year) (linear mixed-effects model analysing 73 observations of 17 subjects with the fixed effect duration [b=0.23, p=0.007] and the random variable subject, modelled as random intercepts) (Fig. 5B). Overall, SARA scores remained within moderate levels (maximum SARA score: 18 points), even in subjects with >20 years disease duration, indicating an only moderate level of maximal disease severity in GAA-*FGF14* disease. The SARA items gait and stance each correlated significantly with disease duration (gait: rho = 0.35, p = 0.029; stance: rho = 0.44, p = 0.004; n = 40; Spearman's correlation) (Supplementary Figure 2), which suggests that – if validated longitudinally – these items may be particularly relevant in future natural history studies. One patient (ID26913) with a biallelic expansion (and no evidence of any concomitant second brain disease) did not differ from the heterozygous expansion carriers in terms of longitudinal disease progression.

The overall burden of non-ataxia features was relatively low at early disease stages and remained low even throughout the disease, as indicated by the slow increase of cross-sectional INAS counts with disease duration (linear regression: F (1, 39) = 5.89, p= 0.020, adjusted R^2= 0.11, effect of duration: b=0.07, p=0.020), which was non-significant if correcting for age (linear regression including age, duration, and their interaction: F (3, 37) = 2.35, p=0.088, adjusted R^2=0.09) (Fig. 5C). In line with the slow progression of ataxia and non-ataxia severity scores,

functional impairment – in terms of mobility impairment – increased only relatively slowly with disease duration, with 50% of subjects requiring unilateral mobility aids after 8 years and bilateral mobility aids after 15 years, respectively (Fig. 5D). Wheel-chair dependence was rare and, if at all, only occurred in advanced disease (>10 years, two patients). The slow increase of functional impairment was reflected by a statistical trend towards a positive association of the FARS-FDS disease stage with disease duration (Spearman's rho = 0.29, p = 0.058, n = 43) (Fig. 5E).

Second independent brain diseases in GAA-*FGF14* ataxia

Two GAA-*FGF14* patients showed abnormally high baseline values and rapid intra-individual increases of the SARA score (Fig. 5B). Closer analysis revealed evidence of a second, independent brain disease ("double hit") in both subjects: severe cerebral small vessel disease (CSVD) in subject ID19467, and progressive supranuclear palsy (PSP) neuropathology in subject ID20559 (additionally carrying a biallelic expansion; Supplementary Text 1 for case vignettes of the two subjects). In the latter, post-mortem analysis revealed two distinct patterns: pathological changes characteristic of PSP with typical tau-pathology, and additional cerebellar atrophy with severe loss of Purkinje cells in the absence of tau aggregation, unusual for PSP, but well compatible with GAA-*FGF14* ataxia (Fig. 6) [3]. The absence of polyQ immunoreactivity suggests that GAA-*FGF14* ataxia is not associated with (CAG-)repeat inclusion pathology (Fig. 6), also p62 staining was negative. These findings exemplify that second brain diseases might be a recurrent phenomenon which needs to be considered when preparing and analysing single subject, natural history, and trial data in genetic late-onset ataxias like GAA-*FGF14* ataxia, where age-related second neurological conditions are more likely to occur.

Neurofilament light levels in blood

While sNfL levels in GAA-*FGF14* subjects (16.1 pg/ml (11.7-19.7)) significantly increased with age ($F(1, 12) = 13.8$, $p=0.003$, adjusted $R^2=0.50$) (Fig. 3E) and disease duration ($F(1, 12) = 5.84$, $p=0.033$, adjusted $R^2=0.27$) (Fig. 3F), they were not significantly higher than in age-matched healthy controls (14.0 pg/ml (11.0-19.3)) ($U=251$, $z=0.53$, $p=0.609$, two-sided Mann-Whitney U-test) (Fig. 3E) and similar to those in SAOA (15.7 pg/ml (11.1-21.7)) ($U=289$, $z=0.18$, $p=0.873$), but significantly lower than in MSA-C (30.3 pg/ml (20.3-36.6)) ($U=116$,

z=3.08, p=0.001, two-sided Mann-Whitney U-tests) (Fig. 3G). This indicates that GAA-*FGF14* ataxia is not associated with rapid widespread axonal degeneration, as commonly observed in MSA-C (but not in healthy controls or SAOA). This finding thus complements and adds molecular support to the clinical findings of slow disease progression and low amount of extra-cerebellar damage.

4-aminopyridine treatment effect

In our cohort, 7 of 50 GAA-*FGF14* patients had received treatment with 4-aminopyridine (4-AP). 6 of them (86%) reported a treatment response with relevance for everyday living (Fig. 7A, Supplementary Table 2). In addition to this retrospective cohort analysis, also a series of prospective structured n-of-1 treatment open-label experiences had been studied in three patients, allowing us to characterise this treatment response in more detail and with a prospective, on/off-controlled design (Fig. 7B-I, Supplementary Table 3). During on-periods with 4-AP treatment, patients documented a reduction in the symptomatic time per day (Fig. 7C, 8G) and the frequency of days affected by severe symptoms (Fig. 7D, 7H), accompanied by a reduction in objective scores of ataxia severity (i.e., SARA score, Fig. 7I). The beneficial effect of 4-AP vanished in each of the three patients during the off-period without 4-AP treatment (Fig. 7B, 7F, 7I), thus demonstrating a tight on/off-association between ataxia improvement and medication intake in all three patients. The short time until onset of symptom improvement as well as the absence of prolonged wash-out times suggests a symptomatic – rather than disease-modifying – drug effect as the mechanism of action of 4-AP.

Discussion

With GAA-*FGF14* expansions recently identified as one of the most frequent causes of late-onset ataxia [3,4], our study aimed to take the next step in preparing large-scale natural history studies and trial-readiness in this novel and – as shown here – potentially treatable disease.

Characterising its phenotypic profile, our study demonstrates that GAA-*FGF14* ataxia consistently presented as a late-onset pancerebellar syndrome with predominant impairment of balance and gait and frequent cerebellar oculomotor signs. As this syndrome is highly consistent across cohorts, confirming the initial descriptions [3,4], it seems to present the core syndrome of

GAA-*FGF14* ataxia. This descripted syndrome corresponds well to the pattern of brain atrophy on MRI, which by visual analysis mainly affects the cerebellar vermis and, less frequently, hemispheres. GAA-*FGF14* ataxia thus clearly differs from other genetic forms of late-onset ataxia, particularly the multisystemic polyglutamine spinocerebellar ataxias (e.g., SCA1, SCA2, SCA3) [18], and multiple system atrophy of cerebellar type (MSA-C) [19], which usually show clinical and MRI signs of widespread degeneration, involving the pyramidal tract and/or the basal ganglia. Given its late-onset, predominantly cerebellar syndrome, GAA-*FGF14* ataxia constitutes a genetic differential diagnosis to SCA6 [18], and, as presenting frequently in a sporadic manner (as in 49% of our patients), to sporadic ataxia of adult onset (SAOA) [1,20].

Our study is the first to delineate the temporal phenotypic evolution of GAA-*FGF14* ataxia, covering disease durations of up to 25 years. Disease started on average at 60.0 (53.5-68.5) years (median and interquartile range), with age at onset not being associated with repeat length unlike previously reported [3,4]. This might be possibly due to the smaller sample size, the more heterogenous genetic background, and the absence of large families with several affected members in our cohort as compared to the previously reported French-Canadian cohort [3]. Impairments of balance and gait almost always mark the clinical onset, while upper limb incoordination and afferent deficits increase in frequency during the disease course. Autonomic dysfunction, mostly in the form of urinary urgency, is rare at onset but becomes frequent in advanced and late-stage disease, yet – unlike in MSA-C – does not reach a high degree of severity and usually is not accompanied by other dysautonomic features. Cognitive impairment, as judged clinically, remains relatively infrequent even in advanced disease. The overall burden of non-ataxia signs as captured by the INAS count remained low throughout the disease course. Taken together, given the predominant cerebellar core phenotype, with only mild and variable non-cerebellar dysfunction, future natural history studies and treatment trials in GAA-*FGF14* ataxia should primarily focus on endpoints in cerebellar, rather than non-cerebellar, domains.

Moreover, our study provides the first data on disease progression in GAA-*FGF14* ataxia, building on both cross-sectional and longitudinal objective assessments. Our study shows that progression of ataxia was slow (≈ 0.29 SARA points/year), with scores not exceeding a moderate severity level even in advanced stages (max. SARA score: 18 points). Disease progression was not associated with repeat length. Overall, disease progression in GAA-*FGF14* ataxia was considerably slower than in multisystemic repeat-expansion SCAs (e.g., SCA3: 1.56 points/year)

[18], and slower than in other frequent genetic late-onset ataxias, e.g. SCA6 (0.80 points/year) [18] or *RFC1* ataxia (1.3 points/year) [9]. Correspondingly, also functional impairment – namely impaired mobility, the domain most prevalently affected in GAA-*FGF14* ataxia – increased relatively slowly with disease duration, with subjects on average requiring unilateral mobility aids after 8 years and wheelchair dependence remaining rare even in advanced stages. By delineating the phenotypic evolution of GAA-*FGF14* ataxia, its longitudinal progression, and the associated functional impairment relevant for everyday living, our study makes significant headway towards preparing future multicentric natural history studies and treatment trials.

While blood levels of NfL are frequently increased in genetic ataxias [11,12,21,22], our study demonstrates that blood NfL levels in GAA-*FGF14* ataxia remained within the range of age-matched healthy controls and SAOA, but below the range of MSA-C, indicating that GAA-*FGF14* ataxia does not involve rapid widespread axonal decay. This complements and adds molecular support to the clinical and imaging findings of slow disease progression and low amount of extra-cerebellar damage. This finding also indicates the need to develop further objective biomarkers to quantify neurodegeneration in GAA-*FGF14* ataxia.

Our study identified two GAA-*FGF14* patients with evidence of a second, independent brain disease ("double hit") who stood out from the cohort in terms of abnormally high baseline values and rapid intra-individual increases of the SARA score. Such co-pathologies can be confirmed by imaging or neuropathology, as shown here. These findings exemplify that in late-onset genetic ataxias – like GAA-*FGF14* ataxia – atypically high disease severity or progression should prompt work-up for concomitant, but independent second diseases, rather than assuming them to be part of a supposedly broad phenotypic and progression spectrum of the genetic disease itself. This has also important implications for future trial designs in GAA-*FGF14* ataxia, as – in contrast to many other genetic ataxias, mostly of earlier onset – second hit age-related brain diseases aggravating disease progression and phenotype will be a recurrent finding, as shown here. Trial inclusion and exclusion criteria will need to be particularly strict to exclude such concomitant second diseases, and trial stratification and analysis will need to incorporate the additional data heterogeneity and variability which arises from such – possibly initially masked or only evolving – independent second diseases.

Cerebellar neuropathology of GAA-*FGF14* disease shows cerebellar cortical atrophy, predominantly of the vermis, less of the hemispheres (with marked loss of Purkinje cells in both cerebellar regions), thus supporting our MRI findings. Specific neuropathological signatures (e.g., p62 or polyQ immunoreactive inclusions), however, were absent. This finding, which is in line with neuropathological findings recently reported on two other cases [3], highlights the need to identify specific neuropathological signatures of GAA-*FGF14* disease which – if no specific staining signatures will be found – might need to comprise specific proteomic and/or transcriptomic signatures.

GAA-*FGF14* ataxia might be amenable to treatment with a drug readily available on the market: 4-Aminopyridin (4-AP). Our retrospective cohort analysis suggests a treatment response to 4-AP with relevance for everyday living in 86% of treated patients. These retrospective findings are confirmed by a prospective series of three structured n-of-1 treatment experiences, showing a tight on/off-association between symptom improvements and drug intake in all three patients. Thus, our study provides first prospective, on/off-controlled evidence for the efficacy of 4-AP in GAA-*FGF14* ataxia. A symptomatic – rather than disease-modifying – drug effect hereby appears likely given the short time until the onset of symptom improvement and the absence of prolonged wash-out times. The outcome variables used in our n-of-1 treatment protocols – daily symptomatic time and symptom severity – do not constitute standard outcome measures for clinical trials, thus limiting the robustness of this preliminary evidence of efficacy. However, changes in these outcomes are nevertheless valuable as they reflect patients' impression in a systematic, predefined manner and thus indicate high patient meaningfulness of the treatment response. In fact, the FDA recently emphasised the need to monitor change in patient-centred outcomes over standard clinical outcomes (like e.g., the SARA scale) which might fail to or only indirectly capture patient meaningfulness [23]. Our findings might pave the way towards a randomised placebo-controlled trial or aggregated n-of-1 trials [14] providing robust evidence for the efficacy of 4-AP in GAA-*FGF14* ataxia.

These findings also allow further mechanistic insights into the so far elusive molecular mechanisms underlying GAA-*FGF14* ataxia. *FGF14* has been shown to exert effects on the gating properties of voltage-gated sodium channels when expressed in heterologous systems [24,25]. Furthermore, overexpression of a mutant FGF14 protein, which is associated with spinocerebellar ataxia in humans, in cultured hippocampal mouse neurons decreases Nav

expression at axon initial segments and impairs neuronal firing [25]. Similarly, knocking out *FGF14* in mice leads to ataxia and reduces both expression of Nav1.6 and neuronal firing in Purkinje neurons [26]. 4-AP, a known blocker of mainly Kv1 (A-type) potassium channels, has been shown to restore neuronal firing precision of Purkinje neurons in another ataxia mouse model mimicking SCA6 [27]. We thus hypothesise that 4-AP may exert its positive effect on ataxia, which we observed in our patients, by compensating a firing deficit of Purkinje neurons that is induced by *FGF14* loss-of-function via its impaired ability to interact with Nav channels. This hypothesis sets the stage for unravelling the link between GAA-*FGF14* pathways and K^+ channel modulation using targeted ion channel electrophysiology experiments with 4-AP in transfected neuronal cell or animal models.

Our study has several limitations. First, larger multicentric natural history studies are warranted to confirm our longitudinal findings on the phenotypic evolution and disease progression of GAA-*FGF14* ataxia, and to further explore the relation between GAA-FGF14 repeat size and clinical features, ideally also including patients of other ethnic backgrounds. Second, further research is needed to determine the degree to which the afferent deficits and dysautonomic features observed in our study are inherent to GAA-*FGF14* ataxia and not the reflection of independent, possibly age-related processes, which may well concur given the age of our cohort. However, the similarity in the temporal evolution between afferent deficits and some cerebellar features (dysdiadochokinesia and upper limb ataxia) support the notion that at least some degree of the afferent deficits is due to GAA-FGF14 ataxia. Third, more in-depth and standardised cognitive and autonomic profiling is needed, using more fine-grained quantitative tests and rating scales. Fourth, while our study provides first MRI data on the pattern and temporal evolution of brain atrophy in GAA-*FGF14* ataxia, based on both cross-sectional and longitudinal observations from systematically aggregated and centrally reviewed routine scans, longitudinal high-resolution volumetric MRI scans by standardised protocols are needed to capture the full spectrum of atrophy and develop MRI-based progression biomarkers for GAA-*FGF14* ataxia. While cerebellar vermis atrophy in GAA-*FGF14* ataxia can be identified reliably by inspection when already moderate or severe, it can be challenging to assess when still mild. Fifth, our findings on the 4-AP treatment response require further validation and specification in randomised, placebo-controlled, blinded trials with objective outcome variables.

In conclusion, our study provides comprehensive insight into the phenotypic profile, the natural history progression, and the 4-AP treatment response of GAA-*FGF14* ataxia, opening an avenue for future large-scale natural history studies and 4-AP treatment trials in this novel, frequent and likely treatable late-onset ataxia. Clinical observations had long included the experience that some ataxia patients do show a beneficial 4-AP response, but without the knowledge and tools to identify GAA-*FGF14* mutations as one of the main drivers underlying this response, cohort studies of 4-AP in non-stratified ataxia cohorts failed [28] (for reviews see: Feil et al. 2016, Kalla and Strupp, 2019) [29,30]. Our findings now indicate the potential of a precision medicine approach to late-onset ataxias, whereby genotypic stratification allows to identify a subpopulation that appears treatable with an existing drug.

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Competing interests

CW has nothing to disclose.

DP has nothing to disclose.

DM has served as consultant for Biogen, unrelated to the manuscript.

MCD has nothing to disclose.

MJD has nothing to disclose.

MN has nothing to disclose.

AT has nothing to disclose.

HL has nothing to disclose.

SZ has nothing to disclose.

LS has nothing to disclose.

BBe has nothing to disclose.

BBr has nothing to disclose.

HH has nothing to disclose.

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Supplementary material

Supplementary material is available at *Brain* online.

Appendix 1

Members of the RFC1 study group who contributed data

Jennifer Faber, Richard Roxburgh, José Luiz Pedroso, Paula Camila Alvez, Orlando Barsottini, Chiara Pane, Francesco Saccà, Alessandro Filla, Filippo M. Santorelli, Ivana Ricca.

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Figure legends

Figure 1 Initial symptoms and contribution of ataxia domains to GAA-*FGF14* ataxia. (A) Patients' initial symptom mostly consisted in gait unsteadiness but could also comprise visual disturbances (particularly: oscillopsia, diplopia, visual blurring), dizziness (as opposed to vertigo), impaired fine motor skills and dysarthria. **(B)** The contribution of each SARA item to overall ataxia severity was compared between GAA-*FGF14* patients (blue, n=40) and *RFC1* patients (light grey, n=42), using Mann-Whitney U-tests (Bonferroni-corrected for multiple comparisons, i.e., the number of SARA items). Boxes visualise medians with lower and upper quartiles, whiskers extend across the entire range of data. ns: $p>0.05$.

Figure 2 Phenotypic profile of GAA-*FGF14* ataxia. Frequency of symptoms and signs in GAA-*FGF14* ataxia (n=48). Numerator and denominator in brackets indicate the number of patients with positive evidence for the feature and the number of patients assessed for it, respectively. As an exception, the denominator for "impaired vestibulo-ocular reflex" was set to the total cohort size because only its presence, but not its absence could reliably be extracted from the patient data, which may result in under-estimation of the frequency of this feature. The features were assessed as by either history (indicated by h in the second pair of brackets) or examination (indicated by e).

Figure 3 MRI findings and serum levels of neurofilament light in GAA-*FGF14* ataxia. (A) The pattern of atrophy was assessed by two independent reviewers on centrally aggregated routine MRI scans (n=29). The atrophy of the cerebral cortex showed a variable regional pattern across patients (including atrophy of the parietal cortex, hippocampus, and enlarged ventricles). **(B)** Brain atrophy in GAA-*FGF14* ataxia was consistently marked by atrophy of the cerebellar vermis, as exemplified the MRI of subject ID29526. **(C)** Longitudinal progression of cerebellar atrophy was typically mild, as exemplified by the follow-up MRI of the same subject. **(D)** Age-matched elderly control without cerebellar vermis atrophy. **(E)** Serum levels of neurofilament light (NfL) were measured in GAA-*FGF14* patients (n=12, age: 66.5 years (59.4-71.8)) and age-matched healthy controls (n=26, age: 63.2 years (58.8-69.4)). Two additional GAA-*FGF14* subjects aged >80 years were visualised in the plot (as rhombus) but excluded from the analysis due to unavailability of controls for this age range. **(F)** Cross-sectional and longitudinal NfL levels relative to disease duration. Lines connect data of the same individual. **(G)** NfL levels in *GAA-FGF14* ataxia were similar to those in sporadic adult-onset ataxia (SAOA, n=34, age: 67.2 years (61.6-72.2)), but lower than in multiple-system atrophy of cerebellar type (MSA-C, n=19, age: 66.1 years (64.0-70.1)). Boxes visualise medians with lower and upper quartiles, whiskers extend to data within 1.5·IQR of the median.

Figure 4 Phenotypic evolution of GAA-*FGF14* ataxia. The frequency of each phenotypic feature for a given disease duration was estimated by logistic regression modelling the presence of the feature as a dichotomous variable (i.e., present/absent) as a function of the disease duration (blue: ataxia-related features, green: afferent features, orange: additional features, including autonomic features). Shaded areas represent the 95% confidence interval.

Figure 5 Natural history progression and functional impairment in GAA-*FGF14* ataxia. (A) Cross-sectional progression of ataxia severity as indicated by the Scale for the Assessment and Rating of Ataxia (SARA) score relative to disease duration (n=40). Two subjects with biallelic repeat expansions are marked by grey dots (ID20559, ID26913). Two subjects with evidence of a second, independent brain disease (ID20559, ID19467) are marked by black circles (marked by #, excluded from group analysis), one of them also carrying a biallelic expansion.

(B) Longitudinal intra-individual progression of ataxia severity (n=17). Observations of the same individual are connected by dotted lines. Intra-individual regressions of the SARA score over disease duration are visualised by solid lines. Two subjects (grey lines, marked by #) with evidence of a second, independent brain disease ("double hit") had high baseline scores and/or rapid disease progression (excluded from group analysis, as in panel A). **(C)** Cross-sectional progression of the overall burden of non-ataxia features as indicated by the Inventory of Non-Ataxia Signs (INAS) count relative to disease duration (n=41). **(D)** Functional impairment was assessed in terms of the required mobility aid (i.e., the share of subjects requiring at least the indicated type of aid), modelled by logistic regression over disease duration (n=43). **(E)** Disease stage according to the Friedreich Ataxia Rating Scale functional disability stage (FARS-FDS) relative to disease duration.

Figure 6 Neuropathology of a GAA-*FGF14* patient. Post-mortem brain examination of subject ID20559 (biallelic GAA-FGF14 repeat expansions) revealed two neuropathological patterns. (1.) Typical neuropathology of PSP including degeneration of the substantia nigra with presence of globoid neurofibrillary tangles (A) and characteristic tau-immunoreactive pathology with neurofibrillary tangles, tufted astrocytes and oligodendroglial coiled bodies as shown for substantia nigra (B), pallidum (C), and frontal cortex (D). (2.) In addition, and unusual for PSP, cerebellar atrophy with severe loss of Purkinje cells was present (E). No tau pathology was detectable in the cerebellar cortex (F), and no polyQ immunoreactive signal (G). Insert in G shows intranuclear inclusion in Huntington's disease as positive control for polyQ. Scale bar: 50 µm (A-D); 100 µm (E-G). Staining: H&E stain (A, E), immunohistochemistry anti-ptau (B-D, F), immunohistochemistry anti-polyQ (G).

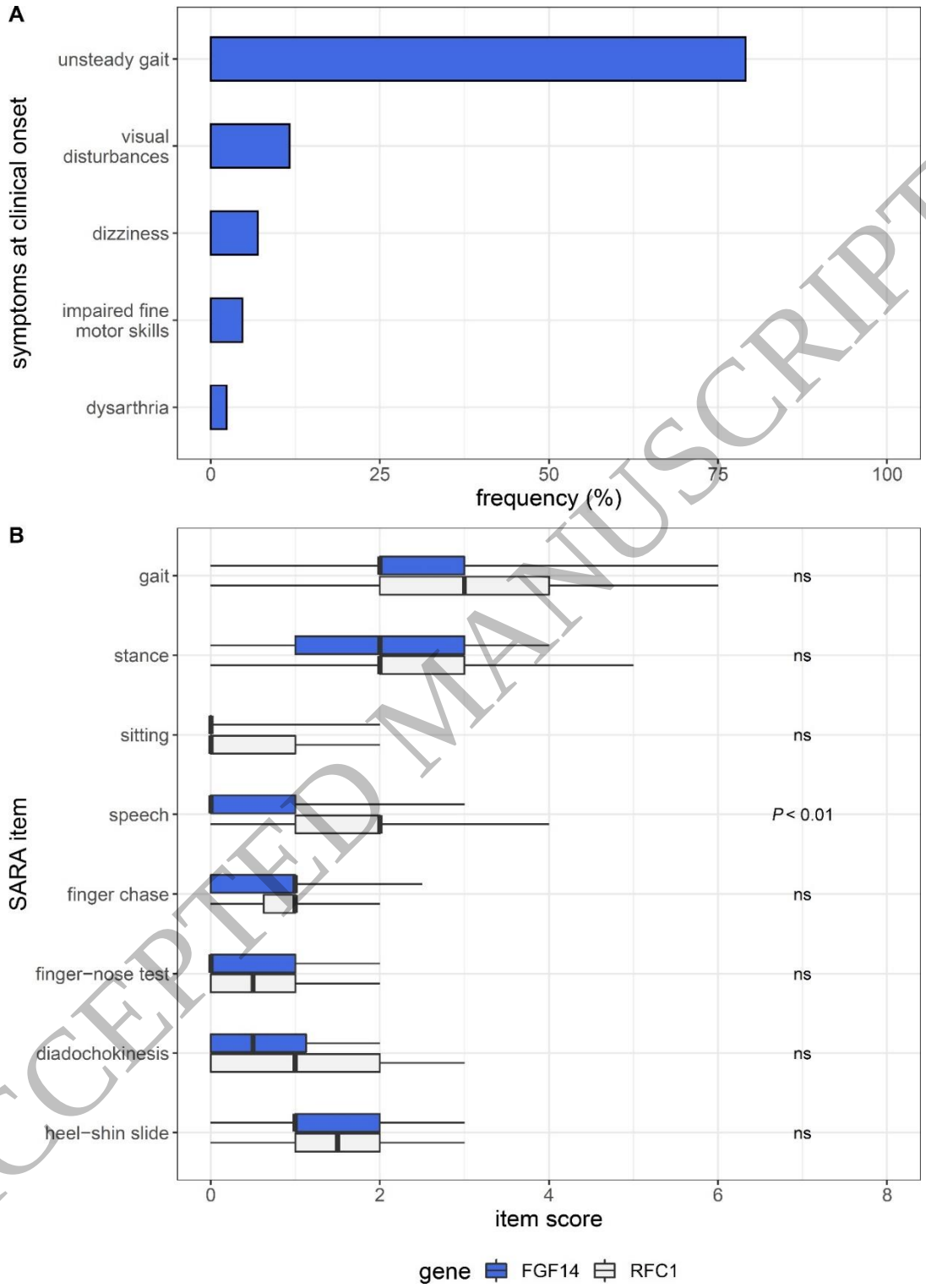
Figure 7 Treatment response of GAA-*FGF14* patients to 4-aminopyridine. (A) Among the 7 patients who had received 4-AP treatment, 6 patients (86%) reported a treatment response with relevance for everyday living. **(B)-(I)** The response to 4-aminopyridine (4-AP) treatment was assessed in a series of structured prospective n-of-1 treatment experiences in three subjects, using an intra-individually controlled on/off treatment protocol, and by additional retrospective analysis of our cohort. **(B)-(E) Subject #1** reported reduction of the symptomatic time per day

and the days with severe symptoms in the periods on 4-AP treatment. The symptom improvement on 4-AP treatment comprised reduction of days affected by diplopia, dizziness, and dysarthria. **(F)-(H) Subject #2** also reported reduction of the symptomatic time per day and the days with severe symptoms while on 4-AP. **(I) Subject #3** reported an increase in the severity of both gait and speech impairment after discontinuation of 4-AP, associated with an increase of the SARA score which had been stable at lower levels under 4-AP treatment.

Table 1 Demographic, clinical, and genetic characteristics of the GAA-*FGF14* cohort

cohort	**GAA-*FGF14* subjects**
n	50
women	50%
age at examination (years)	73.4 (66.5–78.0)
age at clinical onset (years)	60.0 (53.5–68.5)
disease duration (years)	9.7 (5.5–15.2)
repeat count of longer allele	349 (316–402)
repeat count of shorter allele	16 (8–52)

Data are reported as median and interquartile range. The repeat count of the shorter allele excludes the values of two subjects with biallelic expansions.



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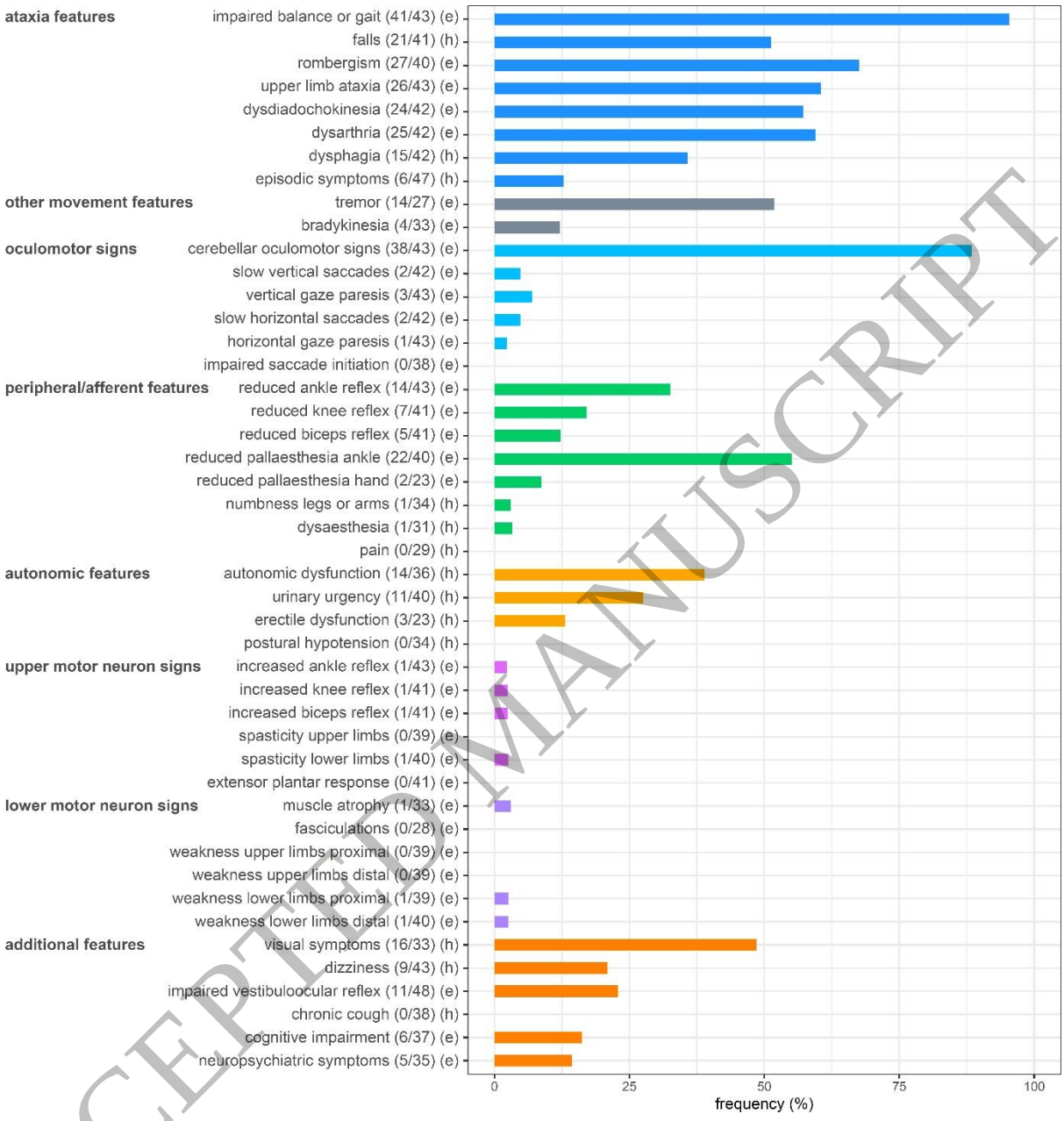

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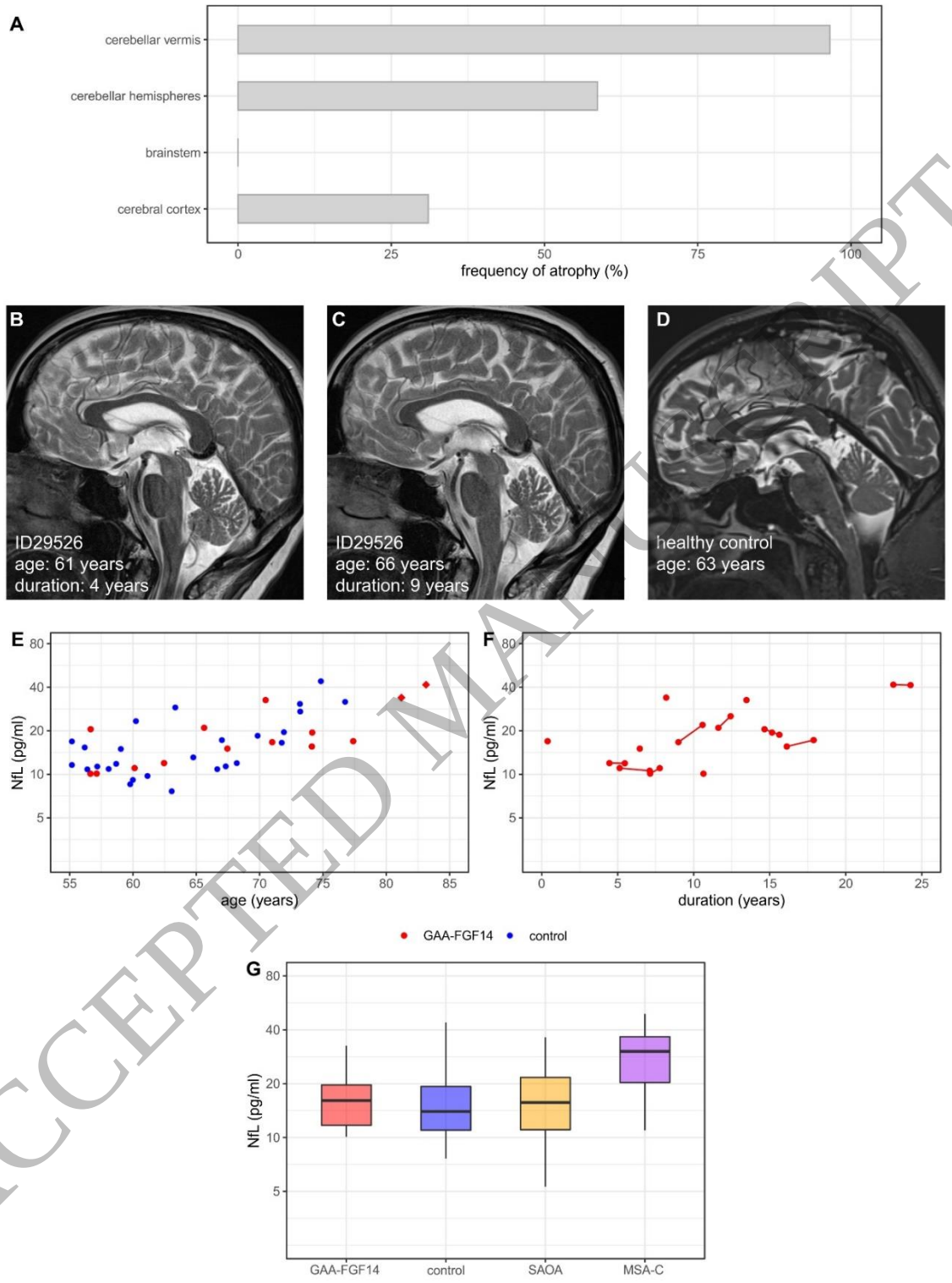

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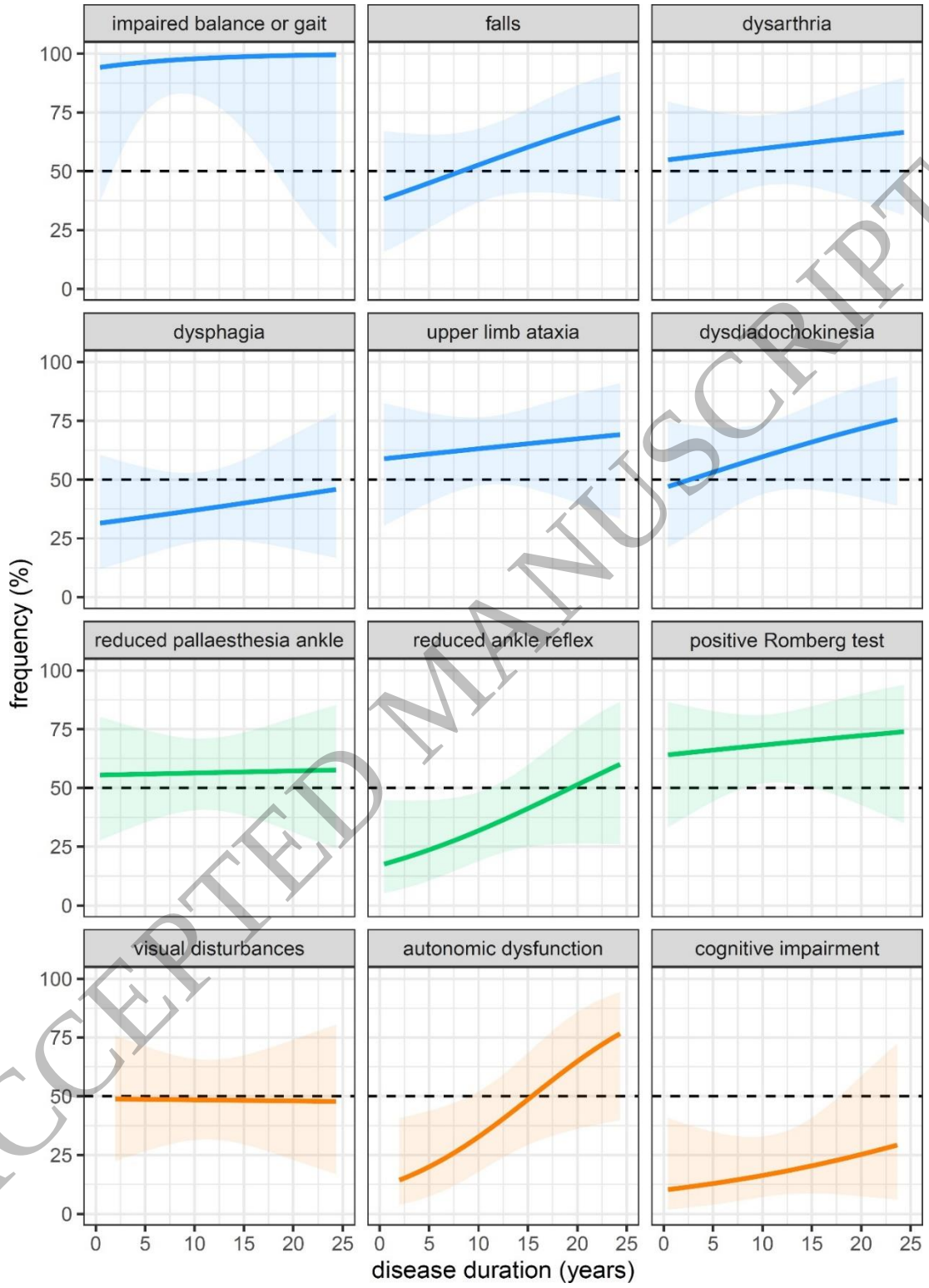

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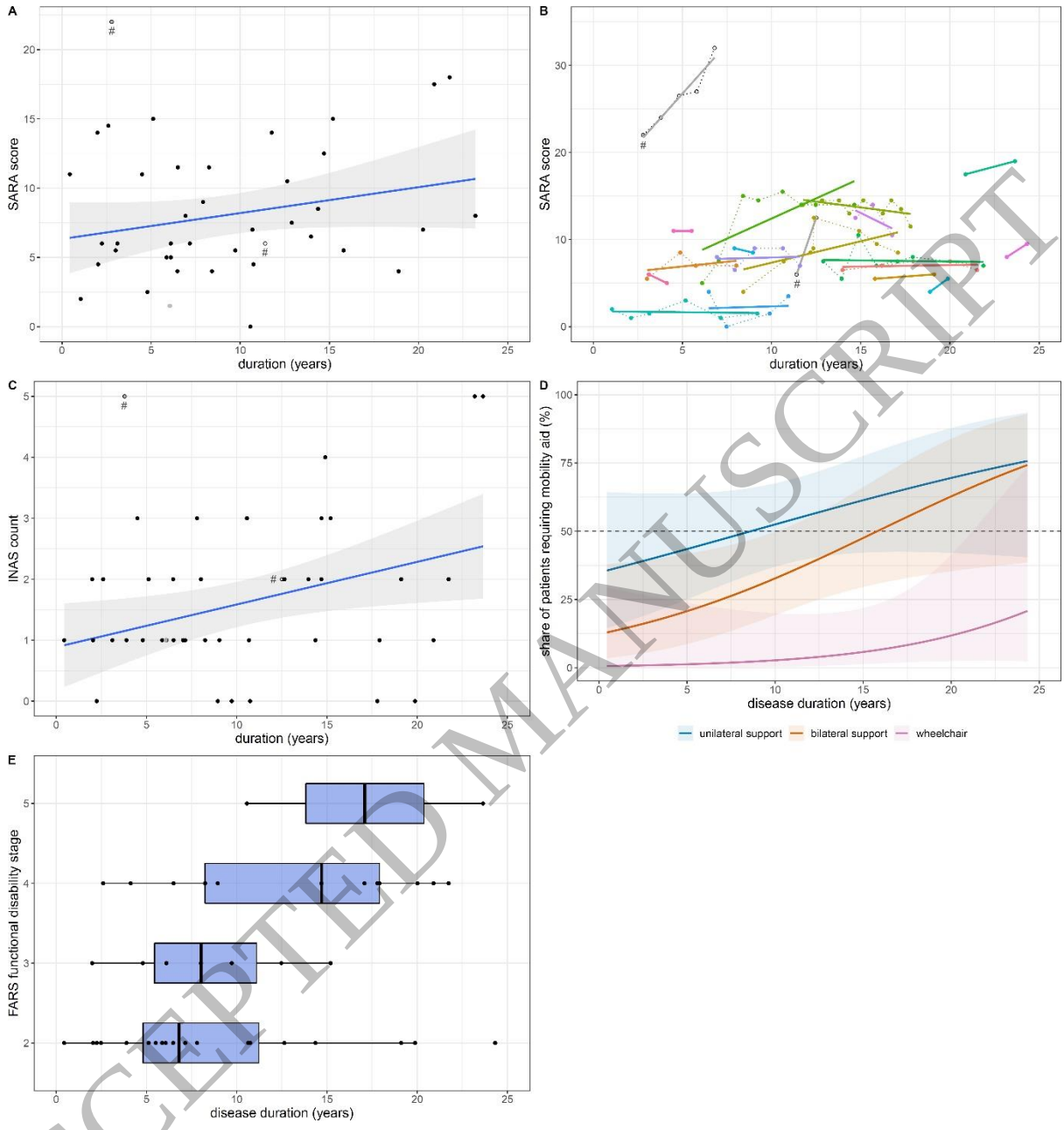

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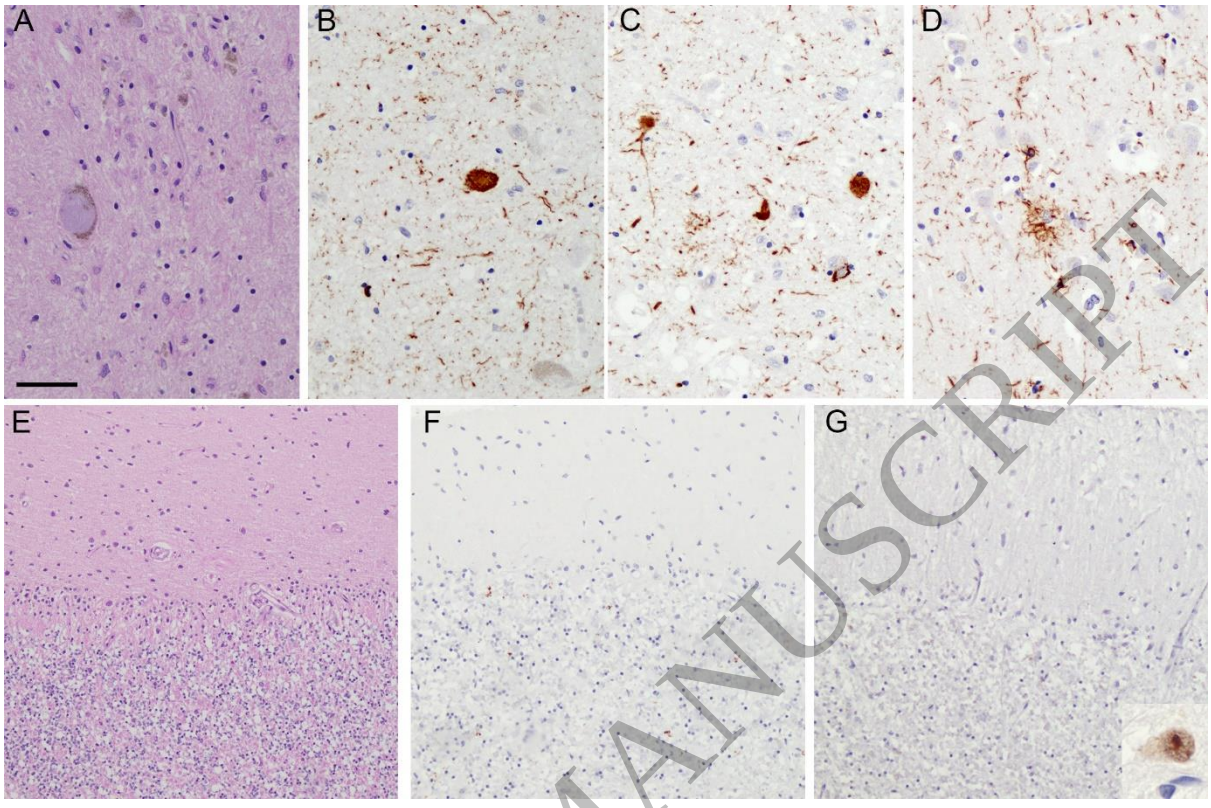

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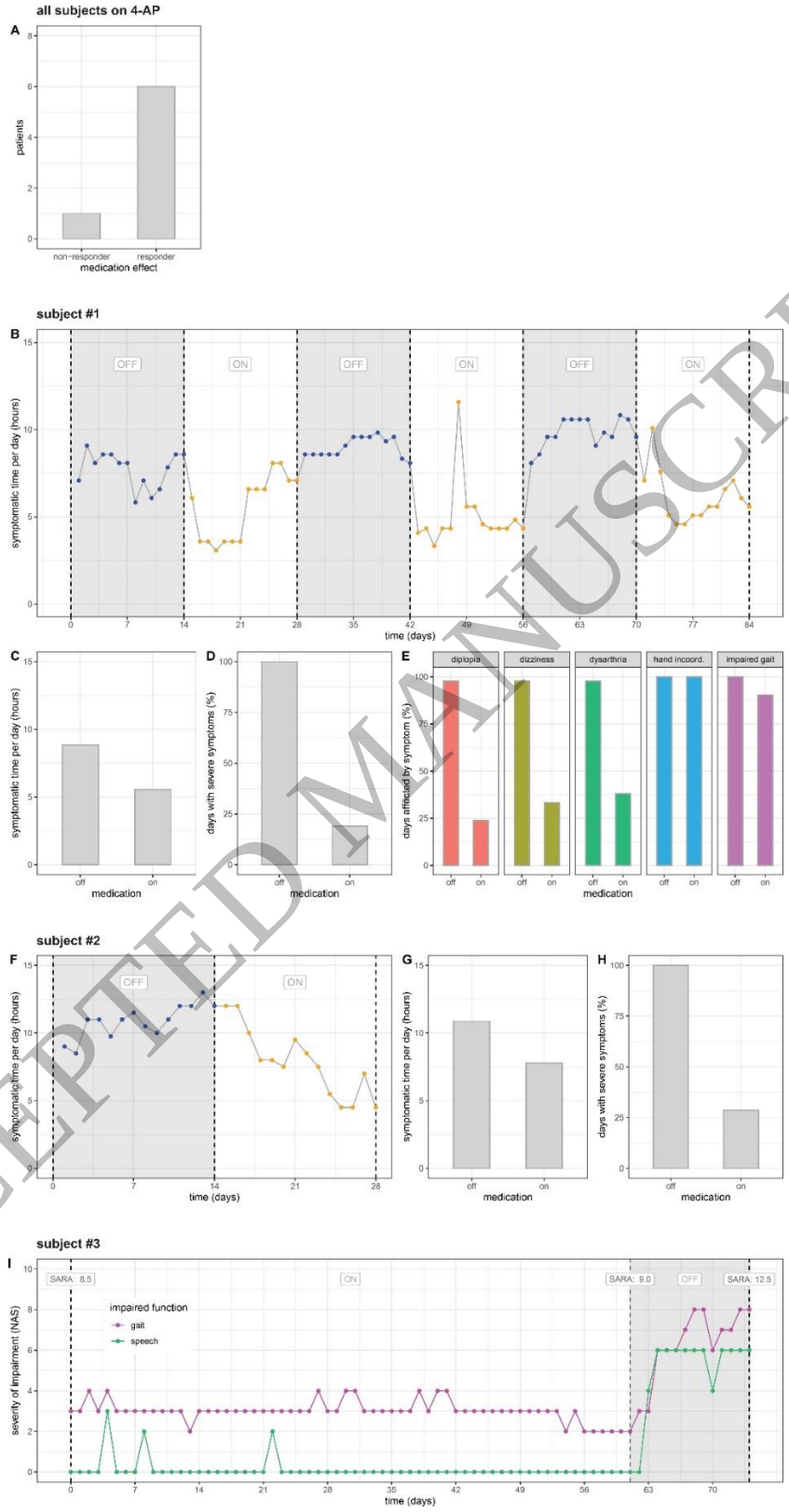

Figure 7
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