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Assessing movement changes in degenerative ataxias: from the pre-ataxic disease stage to the effects of a bio-feedback intervention

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Outline

1 Introduction

1.1 Degenerative ataxias

1.1.1 Overview of degenerative ataxias

Degenerative ataxias are a clinically heterogenous group of movement disorders characterised by progressive ataxia. They are caused by a degeneration of the cerebellum and the spinocerebellar tracts (resulting in cerebellar ataxia) and/or the dorsal columns (leading to afferent ataxia). The age of onset varies across the different types, with clinical manifestations reaching from birth to high ages. Degenerative processes can also affect other regions of the central and peripheral nervous system, such as the basal ganglia, pyramidal tracts or peripheral nerves, underlying additional clinical signs and increasing phenotypic complexity. Causes of cerebellar degeneration can be divided into three main groups: i) acquired causes resulting in secondary ataxias (e.g. paraneoplastic or medication-induced), ii) hereditary or iii) sporadic forms.[1] Recent advances in genetic techniques have expanded the group of genes known to cause ataxias. Increasingly, patients who were initially diagnosed with 'sporadic ataxia', are found to carry mutations in one of these novel genes, showing that the share of genetic causes of ataxia is substantial.[2]

1.1.2 Spinocerebellar ataxias

The genes found to cause hereditary ataxias can follow autosomal-dominant, autosomal-recessive, X-linked and mitochondrial modes of inheritance. Today, genetic ataxias are classified based on the heredity pattern of their underlying genes, and this is also reflected in their clinical nomenclature. Following this genetic classification, the expression 'spinocerebellar ataxias' (SCAs) is used for autosomal-dominant cerebellar ataxias (ADCAs).[3] With a prevalence of about 3/100,000 inhabitants of European populations, SCAs are rare conditions. They are a genetically and clinically heterogenous group, considering the differences in clinical phenotype, underlying genetic mutations and their neuropathological consequences.[4] SCAs can be further subclassified based on the type of their genetic aberration. Within these subgroups, the SCAs with polyglutamine expansions (polyQ SCAs) are the most common ones, and

include SCA1, SCA2, SCA3 and SCA6 as the most frequent subtypes.[5] They are caused by an instable expansion of coding CAG repeats that exceed a specific threshold which is different for each corresponding gene. The clinical presentation of these SCAs varies from a pure cerebellar phenotype (e.g. SCA6) to multisystemic neurodegeneration (e.g. SCA2 and SCA3) that includes non-ataxia signs, such as pyramidal tract signs, extrapyramidal movement disturbances or peripheral neuropathy.[5] Symptoms usually manifest around the third or fourth decade of life, but cases with onset in childhood or higher ages have also been identified. SCA6 typically presents with a prominently later disease onset than the other SCAs. It has been shown that repeat size and age of onset are negatively correlated, meaning that longer repeat sizes correspond to earlier onset, and are associated with faster disease progression.[6, 7] Furthermore, repeat size also affects the clinical phenotype of polyQ SCAs. For example, in the case of SCA3, longer repeat expansions are associated with more prominent features of pyramidal tract involvement.[5]

1.2 Deciphering the preataxic state in spinocerebellar ataxias

1.2.1 The preataxic stage of spinocerebellar ataxia

The early symptomatic and preclinical stages of neurodegenerative disorders, such as Huntington's Disease (HD) and Parkinson's Disease (PD), have recently attracted attention of the scientific community, as they offer fundamental insights into first neurological dysfunctions and their underlying neuropathological processes.[8] This is of particular relevance for future interventional trials, as their efficacy will largely depend on an application in early disease stages, when neurodegeneration is still limited and potentially reversible as possibly the case in the preataxic stage of neurodegenerative diseases. Due to genetic decoding and autosomal-dominant inheritance, the SCAs offer a unique opportunity for predictive genetic analysis and corresponding stratification in individuals at risk who do not yet experience any neurological symptoms and appear normal on clinical examination. A growing number of studies conducted in such preataxic mutation carriers of different SCA types revealed that pathophysiological changes occur already at stages

before clinical ataxia manifestation which is typically defined as a score of at least 3 points on the Scale for the Rating and Assessment of Ataxia (SARA).[9-11] This cut-off was defined in the validation process of the scale, indicating a differentiation between patients with manifest ataxia and controls.[12] Gait disturbances have been described to be the first symptom in two thirds of patients across different SCA types.[13, 14] However, various other non-cerebellar and cerebellar signs or symptoms have been reported to be present in disease stages preceding clinical ataxia onset, suggesting pathophysiological involvement not only of the cerebellum, but of various different regions of the central, peripheral and autonomic nervous system.[10, 11, 15, 16] Such symptoms include cramps, sleeping abnormalities or sensory disturbances, and seem to be rather specific for the different SCA genotypes.[9, 17, 18] This also applies to subclinical signs that were detected by different investigations, e.g. early oculomotor sings in preataxic stages of SCA3 and SCA2[19], or pyramidal tract signs in SCA1, SCA3, and SCA7.[20-23] Some of the detected features even showed a progression with proximity to clinical disease onset, indicating a continuous preataxic neurodegeneration of underlying neurons.[9, 16] Given this evidence that neuropathological processes are already present in SCA stages before clinical onset, the concept of disease evolution has been expanded from clinical to preataxic stages and requires the application of adequate methods for its quantitative characterization.

1.2.2 Non-clinical assessments of the preataxic stage in spinocerebellar ataxias

The assessment of the preataxic stages of spinocerebellar ataxias requires sensitive methods, which are able to detect subclinical neurological changes and ideally quantify their progression. Clinical ataxia rating scales, such as the SARA, are by their nature of limited use in the prodromal phase, as they exhibit floor effects for subtle cerebellar motor dysfunctions due to their design to grade manifest ataxia.[24] Non-clinical assessments can provide objective, quantitative and sensitive measures of subclinical neurological abnormalities, and therefore, present a valuable tool in the assessment of premanifest SCA mutation carriers.

Using observational data from longitudinal natural history studies in SCA1, SCA2, SCA3, and SCA6, mathematical models based on the number of CAG repeats have recently been developed to predict the age of clinical onset.[25] Estimations from these models allow correlation analyses between the number of years to predicted disease onset and outcome variables of quantitative assessments, thus testing for their value to capture preataxic progression of pathological processes. Previous non-clinical assessments of preataxic SCA phases have mainly comprised neuroimaging techniques, different electrophysiological assessments and few quantitative movement analyses. The use of advanced imaging techniques, such as voxel based morphometry (VBM), functional magnetic resonance imaging (fMRI) or single photon emission computed tomography (SPECT) have allowed to capture subtle, structural, functional, biochemical and metabolic alterations of different brain regions in premanifest individuals of different SCA types.[9,10,26-33] Although some results appear promising, the lack of standardized approaches and longitudinal studies, small sample sizes, and selection biases warrant further investigations.[34] Studies using electrophysiological methods have found evidence for an early involvement of the peripheral nervous system, the dorsal root ganglia, and posterior columns of the spinal cord, particularly in preataxic SCA2 mutation carriers by revealing changes in sensory nerve action potentials (SNAP) and somatosensory evoked potentials (SSEP).[16, 35] Corticospinal tract involvement has been found to be present in preataxic SCA1, SCA2 and SCA3 carriers using motor evoked potential (MEP) studies.[20] In SCA2, prolonged central motor conduction times (CMTC) have been shown to correlate inversely with years to estimated disease onset.[36] Oculomotor recordings revealed a progressive reduction of saccadic velocity in preataxic SCA2 carriers and the presence of various eye movement deficits in preataxic SCA3 and SCA6 subjects.[19,37,38] Furthermore, rapid eye movement (REM) sleep disturbances in preataxic SCA2 carriers were quantified by analysing REM density and REM sleep percentage in polysomnography studies.[17] Although these assessments have been shown to detect neurophysiological alterations of preataxic SCA mutation carriers to some extent, their sensitivity to change and thus, their

ability to serve as disease progression markers warrant further investigation. This exercise, however, will be necessary to determine the utility of a promising quantitative assessment as a treatment intervention end point in the preataxic SCA stages and forms the rationale of our work (chapter 2.1).

1.3 Outcome parameters in treatment intervention studies and their limitations

1.3.1 Clinical Scores

In the light of upcoming pharmaceutical options for various types of hereditary ataxias, in particular for repeat SCAs (like e.g. antisense oligonucleotides), there is a clear need for the establishment of outcome parameters, sensitive to capture disease progression and potential treatment benefits.[39] Longitudinal natural history studies of the most common hereditary ataxia types have been dedicated to validate promising clinical scales that allow the rating of ataxia severity over disease course, resulting in a number of clinical, functional and self-reporting ataxia scores that are now frequently used in interventional studies. Both the Scale for Assessment and Rating of Ataxia (SARA) and the International Cooperative Ataxia Rating Scale (ICARS) are the most widely applied rating scales in intervention trials across all SCA types, whereas the Friedreich's Ataxia Rating Scale (FARS) is often used as an end point in trials for Friedreich's Ataxia.[12, 40-42] These semiquantitative scales were developed to grade the major neurological deficits deriving from cerebellar impairment, which are captured in several subitems, such as imbalance during gait and stance, incoordination of upper and lower limbs, and dysarthria. A big advantage of these scores is the fast and easy administration, and their validation in large patient cohorts across different SCAs. However, clinical scores are vulnerable to inter-rater variability, and inherently lack the ability to detect small changes in movement patterns, such as in only mildly affected patients or in short intervention trials. The rarity of the conditions necessitates multi-centre approaches in treatment trials, which makes inter-rater variability a relevant confounder to clinical outcome measures. This demonstrates that there is a

fundamental need to establish objective, quantitative and sensitive measures that can be used as end points in treatment studies.

1.3.2 Non-clinical assessments

Non-clinical assessments, such as neurophysiology studies or brain imaging, are often used in the diagnostic work-up of ataxia patients. Although they have been found to provide potential markers for disease progression in research studies, standardized approaches and clear progression rates validated in big patient cohorts are not available, and therefore their use as trial end points is limited.[42-44] However, single intervention trials have sometimes complemented electrophysiological assessments to address specific research questions.[45-47] Imaging techniques, such as magnetic resonance imaging (MRI), positron emission tomography (PET) or diffusion tensor imaging (DTI), have been experimentally applied in a small number of intervention trials for SCA3, SCA2 and Friedreich's Ataxia (FRDA) to investigate treatment benefits on structural and metabolic properties of different brain regions, but changes were either not evident or of unclear relevance.[48-50] Biochemical blood assessments, particularly in FRDA, have gained more attention in trial settings due to a growing understanding of underlying pathophysiological processes. Based on the mechanisms of investigated pharmaceuticals on cell physiology, desired down-stream effects can be captured by measuring specific protein or enzyme levels, such as the expression of frataxin, the key protein in FRDA pathology, markers of iron metabolism or mitochondrial function.[51-54] Taking into account impaired muscle bioenergetics in FRDA, some interventional studies have investigated changes in exercise capacity, reflected by peak oxygen consumption per unit time (peak VO_2) and peak work rate.[55, 56] Non-clinical assessments have great potential to complement or even replace traditional clinical scores, but need to undergo further validation processes, before they can be systematically applied in intervention trials. Multi-dimensionality in capturing pathophysiological changes beyond clinical observation, objectivity and quantifiability are needed for outcome measures that detect underlying neurological dysfunctions and their potential improvement. Here, we applied

these prerequisites in the establishment of measures based on the core feature of degenerative ataxias – ataxia itself (chapter 2.1).

1.4 Quantitative movement analysis in degenerative ataxias

Ataxic movement disturbance is the defining and unifying feature across all different types of degenerative ataxias and reflects the malfunctioning of the cerebellum. The detailed analysis and characterization of movement disruptions in ataxic patients has therefore yielded significant insights into the functional role of the cerebellum in motor control (as discussed in chapter 1.5). Identifying and quantifying ataxia symptoms is particularly interesting for degenerative ataxias, as it allows to track disease progression. In clinical settings the grading of ataxia severity is traditionally performed by a neurological examination, increasingly with the help of clinical scores. However, due to inherent limitations of clinical ataxia rating scales (as described in chapter 1.3.1) other methods are needed to enable a precise analysis of movements. Quantitative movement analysis methods have the advantage to provide a fine-grained, sophisticated characterization of motion sequences based on spatiotemporal features that are suited to capture the essence of cerebellar motor dysfunction in an objective way. Since postural instability during walking and stance is the main and in the case of spinocerebellar ataxias, often the first feature, many studies have analysed cerebellar-induced posture and gait abnormalities by using motor analysis systems with different incorporated properties.[13] Electromyography (EMG) studies, for example, have detected temporal shifts in leg muscle activation during gait cycle and disrupted agonist/antagonist activation in an upper limb task.[57, 58] The clinical observation of ataxic gait being broad-based and exhibiting irregular foot placement can be quantitatively expressed by spatiotemporal features. They have revealed discrepancies between ataxia patients and healthy controls, specifically reduced gait velocity and cadence, reduced step length and swing phase, increased base width, step times and stance phases, and very characteristically, increased variability in step length and gait cycle time. [59-62] Some of these gait parameters have been shown to correlate with established clinical scores and disease duration in different ataxia

types, thus indicating a sensitivity to disease progression. [63-65] Indeed, rehabilitation studies have even detected improvements at the level of spatiotemporal features after motor training interventions, making them potential candidates as treatment outcome parameters. [66-69] Furthermore, features were able to detect the presence of mild, and even subclinical ataxia.[70-72]

Spatiotemporal analysis of gait can be achieved by different technologies, including pressure sensitive mats or treadmills, tri-axial inertial sensors, pressure sensitive insoles, and 3D motion capture systems.[61, 62, 73-76] The latter presents the most advanced method of capturing gait characteristics, as it not only provides spatiotemporal information on foot placement, but of any joints and anatomical landmarks of interest, including the trunk, and can compute trajectories of the centre of mass (CoM) and centre of pressure (CoP).[77, 78] Therefore, whole-body movement analysis is able to pick up the full picture of ataxic gait that is now increasingly recognized as the result of complex interactions between cerebellar-induced deficits on balance and multi-joint coordination, applied safety strategies and inadequate postural adjustments.[60, 61, 75, 79-81] Despite their reduced informative value, less expensive or space-consuming alternatives to motion capture systems are often needed in clinical settings, such as instrumented mats, or affordable camera-based systems.[70, 82-85] Portable inertial sensors offer the advantage of longer recording times and an application in various different settings. Depending on the underlying technology, they enable the identification of different variables, ranging from basic ambulatory activity to more complex movement patterns. Whereas the former quality has been of interest for daily-life activity recording, more advanced sensors are used for the analysis of standing and walking, and even for kinematic measurements of upper and lower limbs.[86-92] However, the output of these sensors is body-referenced, and provides only indirect measurement of spatiotemporal gait variables. They have partly replaced the use of force plates that are traditionally used in posturography, as they measure only indirect body sway, focus mainly on leg responses and can only be used during standing tasks.[93] However, other groups detected changes in body sway in preclinical mutation carriers of SCA1 and SCA2 using posturography under challenging

conditions.[94, 95] These findings are of high relevance for the conceptualization of the preataxic SCA stages (see chapter 1.2), as they suggest that movement changes may already occur before clinical disease onset. Although these changes are so subtle that traditional clinical scales fail to detect them, sensitive movement analysis methods might be able to capture and quantify them. We explored this notion more systematically in our first study (chapter 2.1). Furthermore, quantitative movement analysis has played a pivotal role in shaping our fundamental understanding of cerebellar motor control, and the consequences of its dysfunction. Such findings are important for the development of novel neurorehabilitation strategies for cerebellar patients. This has, for example, been the case in the promising application of whole-body controlled videogames ('exergames') that aim to address specific cerebellar motor impairments, such as multijoint discoordination and dynamic instability.[96] Quantitative movement analysis has not only shaped the concepts of such rehabilitation approaches through its findings, but has also been successfully applied to measure their effects in intervention trials.[66-69] Following these examples, we analysed the effects of an experimental assistive device on balance in cerebellar patients by using quantitative movement analysis (chapter 2.2).

1.5 The role of the cerebellum in processing sensory cues for optimizing motor control

Since movement deficits deriving from cerebellar damage were first described, the relevance of the cerebellum in motor control has been well recognized.[97, 98] The quantitative analysis of movement patterns under different conditions and in various manipulated settings has provided increasing insights into underlying cerebellar control mechanisms. However, the precise function of the cerebellum in the regulation of movements and its underlying computations are still only partially understood, despite the dedication of many studies. Anatomical and functional imaging studies have shown that the cerebellum is interconnected with various other brain regions, not only with the motor system, but also those involved in sensory and higher brain functions.[99, 100] The cerebellum receives

sensory input from a wide range of exteroceptive and proprioceptive channels, such as visual, vestibular and somatosensory information.[101] In accordance with this anatomical evidence, it appears that the cerebellum participates in the processing and integration of sensory feedback signals to control movement and balance.[101-104] By combining information about the current sensory state and ongoing motor commands (via motor efference copies), it is believed that the cerebellum forms feed-forward models that generate a prediction about the expected consequences of an action.[105-107] Such estimations of the future body state function as internal feedback signals to allow rapid adjustments to current motor commands. Internal forward models are subject to constant updating due to changing environments and body dynamics that lead to discrepancies between predicted and actual sensory feedback signals.[108-110] Such sensory prediction error learning is impaired in cerebellar patients and indicated to account for the observed deficits in motor adaptation, e.g. disrupted multi-joint coordination during arm reaching movements or increased postural sway during stance.[111-113] Findings from several studies suggest that patients with cerebellar damage may be forced to increase their reliance on peripheral feedback signals as a compensation strategy for disrupted predictive capabilities.[114-117] When sensory circumstances of the surrounding environment change, a re-weighting process is required to shift dependence on the most reliable sensory channel.[118-120] It is unknown to which extent this process is cerebellum-dependent, but some studies suggest a preserved ability of cerebellar patients to perform sensory re-weighting.[121, 122] Vision in particular seems to present a dominant feedback cue, as indicated by several studies and the clinical observation that patients exhibit larger body sway when having their eyes closed.[123, 124] These findings indicate that augmented sensory feedback signals present a potential assistive strategy that might help cerebellar patients to compensate for deficient processing of internal sensory information, e.g. proprioceptive or vestibular signals. However, this notion has never been systematically tested in cerebellar patients. Given this lack of evidence, we explored the effects of augmented audio-biofeedback of trunk acceleration on postural control (chapter 2.2).

1.6 Research questions

Based on the current scientific state of spinocerebellar ataxias and cerebellar motor control mechanisms, we hypothesized in the present work that (1) quantitative movement analysis allows to detect early movement changes in subjects at the preataxic stage of spinocerebellar ataxia (SCA) when clinical signs have not yet evolved, and to capture motor progression in this stage (chapter 2.1). Furthermore, we hypothesized that (2) quantitative movement analysis enables to identify the effects of a biofeedback intervention on postural sway in patients with degenerative ataxia, where they might be able to exploit real-time acoustic bio-feedback signals (ABF) of trunk acceleration to compensate for impaired balance control (chapter 2.2).

2 Results

2.1 Individual changes in preclinical spinocerebellar ataxia identified via increased motor complexity

This chapter refers to the following publication, including the supplementary information that has been published in the online version:

Ilg, W., Fleszar, Z., Schatton, C., Hengel, H., Harmuth, F., Bauer, P., Timmann, D., Giese, M., Schöls, L., and Synofzik, M. (2016), Individual changes in preclinical spinocerebellar ataxia identified via increased motor complexity. Mov Disord., 31:1891-1900. doi:10.1002/mds.26835

2.1.1 Introduction

The conceptualization of disease progression in spinocerebellar ataxias has recently gained an expansion from clinically manifest to preataxic stages. A detailed understanding of the preataxic phase, including a quantification of pathophysiological processes is crucial in paving the way for future intervention trials that will be applied in earliest stages when neurodegeneration is still limited and potentially reversible. As explicated in more detail in sections 1.2 and 1.4, quantitative analysis has been shown to offer sufficient sensitivity to detect subclinical ataxic movement changes and to reflect effects of treatment interventions. Based on these findings we hypothesized in the following study that quantitative movement analysis allows (i) to identify earliest movement deficits in preataxic SCA mutation carriers in the absence of clinically detectable motor symptoms and (ii) to capture the progression of such movement changes within the preataxic stage.

The experimental procedures of the following study were approved by the ethics committee of the University of Tübingen (Az303/2008BO2).

2.1.2 Original publication

Individual changes in preclinical spinocerebellar ataxia identified via increased motor complexity

Running title: Motor features in preclinical spinocerebellar ataxia

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Abstract

Background: Movement changes in autosomal-dominant spinocerebellar ataxias (SCAs) are suggested to occur many years before clinical manifestation. Detecting and quantifying these changes in the preclinical phase offers a window for future treatment interventions and allows to decipher the earliest dysfunctions starting the evolution of SCA. We hypothesized that quantitative movement analysis of complex stance and gait tasks allows to (i) reveal movement changes already at early stages of the preclinical phase when clinical ataxia signs are still absent, and (ii) to quantify motor progression in this phase.

Methods: 46 subjects (14 preclinical SCA mutation carriers [SCA 1,2,3,6], 9 SCA patients at early stage; 23 healthy controls) were assessed by quantitative movement analyses of increasingly complex stance and walking tasks in a cross-sectional design.

Results: Body sway in stance and spatio-temporal variability in tandem walking differentiated between preclinical SCA subjects and healthy controls ($p<0.01$). Complex movement conditions allowed to discriminate even those mutation carriers without any clinical signs in posture and gait ($SARA_{posture\&gait}=0$; $p<0.04$). Multivariate regression analysis categorized pre-clinical mutation carriers on a single-subject level with 100% accuracy within a range of 10 years to estimated onset. Movement features in stance and gait correlated significantly with genetically estimated time to onset, indicating a gradual increase of motor changes with increasing proximity to disease manifestation.

Conclusion: Our results provide evidence for subclinical motor changes in SCA, which allow to discriminate subjects without clinical signs even on a single-subject basis and may help to capture disease progression in the preclinical phase.

Introduction

It is well-known from various neurodegenerative diseases like Parkinson's disease or Huntington's disease that, at the point of clinical manifestation, large populations of underlying neurons are already lost and most compensatory resources already exhausted[1-3]. The same is likely true also for cerebellar functioning in degenerative spinocerebellar ataxias (SCA)[4-6]. The preclinical phase of SCAs attracts increasing research interest as it could provide a promising window for early therapeutic intervention before substantial irreversible neurodegeneration has occurred[4, 5, 7]. Effectiveness of future interventions studies in SCAs will largely depend on three prerequisites: (1) Detection and quantification of motor control deficits as early as possible; (2) a more detailed understanding of the earliest dysfunctions in cerebellar motor control mechanisms starting the evolution of ataxia; (3) the availability of measures which are able to sensitively quantify progression and intervention benefits in this preclinical stage.

First studies have recently started to investigate pre-symptomatic persons at risk for SCA[4, 6], focussing mainly on clinical ataxia sores like SARA (Scale for the assessment and rating of ataxia)[8] as primary measure. However, by their nature, clinical scores lack the sensitivity to identify subtle movement changes and to quantify the progression in the preclinical phase[9]. Non-clinical measures might thus outperform clinical ataxia scores for identifying and quantifying signs during the preclinical phase of SCA. Quantitative motor measures of posture and gait seem particularly promising for finding a unifying description of preclinical motor symptoms across SCA subtypes, as clinical observation shows that coordinatively demanding gait and stance tasks, like e.g. tandem gait and stance, are abnormal already very early in the clinical disease course. In fact, gait difficulties have been identified as the first symptom in two-thirds of 287 patients across all the main SCA genotypes 1,2,3, and 6[10]. Finding such a SCA-unifying signature of the preclinical phase might complement (or partly

even overtake) early non-ataxia symptoms like muscle cramps, oculomotor signs and sleep disturbances or brain imaging abnormalities [4, 6, 11, 12] which seem largely specific to certain SCA subtypes [5].

The hypothesis of quantitative motor measures as a promising early marker for characterising the preclinical phase of SCA is based on earlier studies on quantification of spatio-temporal movement features in SCAs. It has been shown that movement measures of spatial and temporal variability are distinctively suitable for characterizing ataxic gait[13-17], including mild ataxic and subclinical subjects[13, 18, 19]. In addition, these measures are particularly attractive as they allow also for a fine-grained quantification of treatment effects in degenerative ataxias[20-23].

Here we hypothesized that (i) quantitative movement features might be able to identify movement changes already at early stages of the preclinical phase across SCA subtypes, when clinical signs of ataxia are still absent. Moreover, we speculated that (ii) these movement features might also allow to quantify the progression of motor deficits in the preclinical phase before disease onset.

Methods

Subject populations

We recruited three subject groups: **MC$^{SARA<3}$**: a group of n=14 *preclinical* mutation carriers with a repeat expansion mutation in the genes causing SCA1, SCA2, SCA3 or SCA6 with a SARA score of <3; **SCA$^{SARA3-8}$**: a group of n=9 patients with SCA 1,2,3 or 6 in the early clinical stage (SARA score 3-8); and **HC:** a group of n=23 age- and gender-matched healthy controls (see Table 1). In line with previous studies on preclinical SCA[4, 5] the cutoff-value between preclinical mutation carriers (MC$^{SARA<3}$) versus patients with manifest SCA

($SCA^{SARA3-8}$) was set at a SARA score of 3. HC included 10 members of SCA 1,2,3 or 6 families who were shown to have not inherited the SCA mutation. The other 13 HC subjects had no personal or family history of any neurological or psychiatric disease. All HC subjects did not show any neurological signs upon clinical examination.

To investigate more strictly whether movement analysis allows to identify motor changes in early preclinical stages when any clinical signs in posture and gait control are indeed still completely absent, we formed an additional subgroup out of the $MC^{SARA<3}$ group, selecting only those subjects with $SARA_{posture\&gait}$ subscore=0 ($SARA_{posture\&gait}$ ≡ sum of the three SARA items: gait, stance and sitting)[8, 24] and a SARA total score ≤1 (n=8 subjects total). Since this subgroup $\mathbf{MC^{SARAp\&g=0}}$ differed in age from HC, and since age is known to modulate balance and gait capacities[25, 26], we defined an aged-matched subgroup out of HC, selecting eight age-matched mutation-negative family members of SCA patients (non-mutation carriers; **nMC**), thus allowing to control for unspecific features that might be present in SCA families. Neurological signs other than ataxia were assessed with the Inventory of Non-Ataxia Signs (INAS)[27].

Genetics

All mutation carriers carried repeat expansions in the clearly pathological, fully penetrant range. CAG repeat length was analysed in DNA extracted from EDTA blood samples at the Institute of Medical Genetics and Applied Genomics, University of Tübingen, using well-established methods[4, 28] (for further details see Supplement 6).

Standard protocol approvals and patient consents

All experimental procedures were approved by the local ethics committee (Az303/2008BO2). All subjects gave written informed consent. No descriptive single subject data about

individual age, allele sizes and genotypes are shown in the manuscript, as this might allow participants to re-identify themselves and to recognize her/his genotype status. Means and standard deviation of CAG repeat lengths are provided in Supplement 4.

Estimation of disease onset

Movement features of $MC^{SARA<3}$ subjects were related to the genetically estimated disease onset, which was calculated according to the previously established model[28] (=unadjusted model). This estimate is based on the genotype, number of CAG repeats, and age of the subject. However, it is known that the mutant CAG repeat allele explains only 60% of the age of onset variance[29]. About 55% of the remaining age of onset variance is due to familial factors[29, 30]. These factors probably consist of a number of cis- and trans-acting genetic modifiers and shared familial environmental factors that influence age of onset in addition to the expanded CAG repeat allele itself[29, 31]. To account for these intra-familial effects, we adjusted the CAG-based age of onset estimation of the index subject by the difference between the actual disease onset and the CAG-based age of onset estimation in the affected parent (equation 1; adjusted model).

$$\begin{aligned} estimated\ disease\ onset_{par}^{CAG} \\ &= estimated\ disease\ onset^{CAG} \\ &+ \left[actual\ disease\ onset(parent) \right. \\ &\left. - estimated\ disease\ onset^{CAG}\ (parent) \right] \qquad (equation\ 1) \end{aligned}$$

For example, the estimated time to onset in a given index subject is increased, if his/her parents' actual disease onset was later than estimated from the CAG-repeat number. The differences of both prediction models in relationship to the SARA score are shown in Figure

1. The “actual” disease of onset in the parents was determined by subjects’ self-report of first onset of gait difficulty during their examination by a neurologist, as done previously[28].

Analysing posture and gait tasks with increasing complexity

To unravel first preclinical changes in SCAs, we employed a battery of coordinatively demanding stance and gait tasks, predicting that motor abnormalities might unravel in particular with increasing motor demands. According to this strategy, we started with movement tasks identical to those used in clinical tests, followed by tasks with gradually increasing balance requirements and motor complexity.

We examined three different stance conditions of increasing motor demand: standing still for 30 seconds with feet closed in Romberg position and (i) eyes open: RB , (ii) eyes closed: $RB^{\text{closed eyes}}$, (iii) eyes closed on a mattress: $RB^{\text{closed eyes}}_{\text{mattress}}$ (Figure 2A). Additionally, we examined different walking conditions of increasing motor demand: straight walking, tandem walking, and tandem walking on a mattress (Figure 2B). Subjects walked on a 10 meters long walkway, whereby the capture volume contained only the 7 meters in the centre of the walkway, to exclude step cycles during acceleration and deceleration phases. From each subject we analysed 15-20 step cycles within five trials at a self-determined pace. In tandem walking, subjects were instructed to walk on an imagined straight line placing one foot directly after the other (heel-to-toe). From each subject we recorded 20-25 step cycles within three trials.

Motor performance was quantitatively assessed using a VICON motion capture system with 10 cameras. The three-dimensional movement trajectories were recorded at a sampling rate of 120 Hz, using 41 reflecting markers. The trajectories were pre-processed using commercial software provided by VICON, which fits a kinematic model to the marker trajectories and

extracts velocities, joint angles, and the course of the centre of mass (CoM). For stance analyses, body sway was determined by measuring the path length of the centre of gravity (projection of the CoM on the floor) (Figure 2C). For gait analyses, we focused on spatio-temporal variability measures of step length and step cycle time which have been shown to be most sensitive to characterize ataxic gait[14-16] and to detect subclinical gait changes[13, 19]. Variability measures were calculated using the coefficient of variation $CV=\sigma/\mu$, normalizing the standard deviation with the mean value[32]. In addition, we analysed gait speed and gait asymmetry to show that they had no influence on gait variability (Supplement 1).

Statistics

Group differences (HC, nMC, $MC^{SARAp\&g=0}$, $MC^{SARA<3}$ and $SCA^{SARA3-8}$) on movement features were determined by the non-parametric Kruskal-Wallis–test. When the Kruskal-Wallis–test yielded a significant effect ($p<0.05$), post-hoc analysis was performed using a Mann-Whitney U-test for comparisons between groups. We report two significance levels: uncorrected ($p<0.05$*) and Bonferroni-corrected for multiple comparisons (**: Romberg conditions: $p<0.05/3$; Tandem: $p<0.05/4$). Differences in multi-variate analysis combining different movement features and age were determined using multi-variate logistic regression models[33] for nMC and $MC^{SARAp\&g=0}$. To generate and validate the logistic regression models, we used a three-step procedure. 1.) Models were established to discriminate between non-mutation carriers and mutation carriers using feature sets from groups $MC^{SARA<3}$ (here excluding subgroup $MC^{SARAp\&g=0}$) and $SCA^{SARA3-8}$ as prototypes for mutation carriers, and from healthy controls (HC) (here excluding subgroup nMC) as prototypes for non-mutation carriers. 2.) In the test step we determined model outputs as the degree of ataxic movement characteristics for the two critical groups, namely $MC^{SARAp\&g=0}$ and nMC (for graphical overview, see Supplement 5). 3. We analysed the generated outputs of groups nMC and $MC^{SARAp\&g=0}$ in respect to (a) group differences and (b) categorization capabilities by using

them as input into a ROC (receiver operating characteristic) analysis[34] in order to determine the accuracy of the identification of preclinical mutation carriers $MC^{SARAp\&g=0}$ on a single subject level.

For this multi-variate analysis, we selected those features as candidates for logistic regression, which showed a significant group difference in the Romberg test and tandem walking. The factor "age" was included into the logistic regression analysis to control for a possible influence of age. Using these features we examined all 10 permutations of features sets comprising three features. The Bonferroni-corrected significance level for the multi-variate analysis is set to $p<0.05/10$ (n=10: number of analyzed feature sets).

Spearman's rho was used to examine the correlation between movement features and SARA scores as well as estimates of time to disease onset. Statistical analysis was performed using MATLAB and SPSS.

Results

Changes in posture and gait control in the pre-clinical stage

Differences in body sway were identified in all three stance conditions (Kruskal-Wallis tests: $\chi^2>30.1$, $p<0.0013$), post-hoc analysis showed an increased body sway in both $MC^{SARA<3}$ and $SCA^{SARA3-8}$ versus HC ($p<0.004$**) (Figure 3A).

For straight walking (Kruskal-Wallis tests: $\chi^2>5.195$, $p<0.07446$), $SCA^{SARA3-8}$ showed an increased step length variability compared to $MC^{SARA<3}$ ($p=0.02$) and HC ($p=0.01$), but no significant differences were observed between $MC^{SARA<3}$ and HC ($p=0.25$). In contrast, for Tandem and $Tandem_{mattress}$ (Kruskal-Wallis tests: $\chi^2>22.1$, $p<0.01$), $MC^{SARA<3}$ showed significant increased variability in step length and in step cycle time compared to HC ($p<0.006$**, Figure 3B).

Motor changes are already present in preclinical subjects without clinical signs of gait and posture disturbances

To investigate subtle movement changes in preclinical subjects still completely without any clinical signs of gait and posture disturbances ($SARA_{posture\&gait}=0$), we compared motor performance between $MC^{SARAp\&g=0}$ and nMC. There was a difference between $MC^{SARAp\&g=0}$ and nMC for the most challenging stance condition $RB^{closed\ eyes}_{mattress}$ ($p<0.001$**, see Figure 3A). For gait, differences were observed in Tandem (step length variability, p=0.01; step cycle time variability, p=0.02) and $Tandem_{mattress}$ (step cycle time variability, p=0.02).

To further explore the discrimination between $MC^{SARAp\&g=0}$ and nMC, we performed a multivariate analysis (see methods and supplement 5). We selected those features as candidates for logistic regression, which showed a significant difference in stance and gait tasks. The factor "age" was included to control for its possible influence.

Logistic regression analysis revealed differences in model output (interpreted as degree of ataxic movement characteristics) for a set of three features: [age, body sway in $RB^{closed\ eyes}_{mattress}$, variability in step cycle time for Tandem]. Degrees of ataxic movement characteristics differed between HC and $MC^{SARA<3}$ (p=0.0001) and between nMC und $MC^{SARAp\&g=0}$ (p=0.0006) (Figure 3 C).

Classification of pre-clinical subjects

In order to examine whether the identified feature set is capable to discriminate $MC^{SARAp\&g=0}$ subjects from nMC subjects *on a single-subject level*, we computed a ROC analysis based on the outputs of the regression model (model output ≡ "degree of ataxic movement characteristics"). This ROC analysis revealed that for a threshold of 0.127 our model allowed a classification of individual $MC^{SARAp\&g=0}$ subjects as mutation carriers with 100% sensitivity and 87.5% specificity (accuracy 93.8%) (Figure 3 C). A single-subject analysis shows that

only the two $MC^{SARAp\&g=0}$ subjects with an estimated disease onset of more than 10 years showed a smaller model output than one of the nMC subjects.

Motor features reflecting progression within the pre-clinical phase

We finally aimed to determine whether motor features allow to capture progression within the preclinical phase. To this end, we examined the correlations between movement features and estimated time to disease onset in $MC^{SARA<3}$. Step length variability in $Tandem_{mattress}$ was associated with estimated disease onset for both models (unadjusted model: p=0.036; adjusted model: p=0.0068) (Figure **4**). Also body sway in $RB^{closed\ eyes}_{mattress}$ correlated with estimated disease onset (p=0.026), observed only with the adjusted model. Overall, increases in step length variability and in body sway, respectively, with proximity to estimated disease onset were steeper in complex motor tasks (e.g. mattress) than in simple motor tasks (Figure 4). For diagrams of step cycle timing variability see Supplement 2.

Discussion

Effectiveness of future interventions in the earliest stages of SCA will depend on the identification of biomarkers measuring preclinical disease progression in mutation carriers. As motor symptoms are the key feature across SCAs, quantification of motor deficits as early as possible is crucial. In addition, such assessments will lead to a more detailed understanding of the earliest dysfunctions starting the evolution of ataxia. While earlier studies have been restricted mostly to clinical scores like SARA[8] and ICARS[35] or on qualitative descriptions of motor tasks[6, 36], quantitative studies are scarce[18, 37, 38]. Here we show that movement features allow to identify changes in preclinical SCAs when clinical signs are still completely absent, and even on a single-subject level.

Preclinical SCA affects both posture and gait control, particularly in complex motor tasks

Body sway in Romberg conditions and spatio-temporal variability in Tandem differentiated between preclinical subjects ($MC^{SARA<3}$) and healthy controls. In contrast, no increased gait variability was observed in preclinical subjects for straight walking. These results contrast a finding of an earlier study on preclinical SCA 6 subjects[18] which used, however, a different ataxia score ICARS [35] and cutoff value (ICARS cutoff value=7 vs. SARA: cutoff value=3). In fact, several of the preclinical subjects in this previous study already showed first signs on ICARS gait items[18].

Our finding of increased gait variability in preclinical subjects not in normal walking, but in more complex gait conditions is in line with previous studies in other neurodegenerative diseases. Studies in preclinical Parkinson's Disease[39] and Fragile X-associated tremor-ataxia syndrome[40, 41] observed increased gait variability only for more complex walking conditions.

Subclinical motor changes are detectable before their clinical manifestation

Although SARA<3 is commonly defined as preclinical phase[4, 5, 8], mutation carriers presenting with a SARA score of 2 or 2.5 can already show first distinct clinical signs of ataxia. Correspondingly, $MC^{SARA<3}$ revealed a difference in the SARA score compared to healthy controls (p=0.002), confirming the results of a larger multi-centre SCA study[4]. Thus, the advantage of motor measures would be particularly convincing if they allow to identify ataxia-related movement changes even in those subjects where no clinical signs of ataxia are seen, and where clinical scores do not show differences to healthy controls. This is the case for the $MC^{SARAp\&g=0}$ group, who showed no difference in SARA (p=0.97) and INAS (p=0.5) compared to nMC. In contrast to these clinical assessments, movement analysis indeed allowed to unravel changes for complex gait and posture conditions $MC^{SARAp\&g=0}$ compared to nMC.

This difference is remarkable as nMC consisted exclusively of blood-related non-mutation carriers who are not aware of their carrier status, thus serving as an ideal blinded control group controlling for unspecific factors that might be present in members of SCA families (e.g. subjective uncertainty under close motor assessment scrutiny). Interestingly, the SARA score of nMC was on average as high as of $MC^{SARAp\&g=0}$ (Table 1). This observation confirms the low specificity at the lower end of the SARA score[9] . Indeed, it was shown that about 20% of healthy controls have positive ratings in at least one SARA item, predominantly related to kinetic functions of the non-dominant hand[8]. Thus, movement analysis can refine the clinical assessments performed in earlier studies on preclinical SCA[4, 6]. Specifically, the higher sensitivity might allow to detect preclinical motor changes much earlier than clinical measures. The estimated time to onset for the $MC^{SARAp\&g=0}$ is 7.6 years on average. In contrast, clinical assessments by visual detection of missteps identified abnormalities in tandem gait about 1.2 years before disease onset, as shown in a longitudinal study of SCA2[6].

Movement-based classification of mutation carriers

While discriminations mutation carriers and non-mutation carriers on a group level are informative, even more meaningful would be measures which allow to identify an affected subject on a *single-subject* level. Indeed, our feature set including features from two complex motor tasks and age allows to discriminate $MC^{SARAp\&g=0}$ subjects from nMC subjects on a single-subject level with 100% accuracy for a range up to 10 years before estimated disease onset. Such a measure will be particularly valuable to classify preclinical SCA subjects in the future, given that in particular intervention studies in preclinical subjects of this rare disease will likely depend of small subject series.

The selection of the factor ‘age’ in the feature set with the highest discrimination capabilities indicates the importance to control for this factor in preclinical SCA studies. Subclinical movement changes are susceptible to aging, as shown for tasks like Romberg test and tandem

walking[25, 26]. Such an age-effect was confirmed by our observation of a correlation between age and body sway in the Romberg test for healthy controls (p=0.0031). This emphasizes the need to carefully select age-matched control groups, and for an age-dependent interpretation of subtle movement changes in challenging motor tasks.

Quantifying the preclinical course of SCA

Our study provides a fine-grained analysis on quantifying the *progression* of preclinical motor changes. Body sway in different Romberg conditions and spatio-temporal variability in tandem correlated with estimated time to onset, indicating a gradual increase of motor changes with increasing proximity to disease onset. $Tandem_{mattress}$ showed the fastest progression during the pre-clinical phase (Figure 4), indicating that in particular complex motor tasks might be suitable to capture early motor changes.

An adjusted estimate of the predicted time to onset

In general, correlations between movement changes and estimated time to onsets have to be interpreted with caution since estimations of time to onset are based on prediction models, which explain only 60% of the age of onset variance[29]. Specifically, the current unadjusted SCA prediction model does not take into account familial factors contributing to the individual's age of onset. Our adjusted model includes these factors. We observed the largest adjustment effects for subjects with a SARA score of 2-2.5. Although already close to the threshold of SARA=3, these subjects would still be up to 10 years *before* their estimated onset according to the unadjusted model[28] (Figure 1). This estimate thus seems implausible given the natural progression of 0.8-2.1 SARA points per year in the SCAs studied here[42]. In summary, our adjusted model leads to a closer capture of the SARA scores to estimated time to disease onset, and to more plausible averages of time to onsets for $MC^{SARAp\&g=0}$ and $MC^{SARA<3}$ (Table 1). Prospective cohort studies of larger pre-clinical SCA populations are certainly warranted to further validate this adjusted formula.

Limitations

This study has some limitations. The small sample size does not allow to exclude the possibility that non-significant findings might be just due to a lack of power. For example, step length variability in tandem walking on a mattress is marginally not significant (p=0.09) in the distinction between $MC^{SARAp\&g=0}$ and nMC, although the same parameter is significant for tandem on the ground (Figure 3B). This seems to be in particular relevant for more complex motor tasks, which can show also increased variability in healthy controls. On the other hand, if significant results are observed even in such small groups, this indicates that these effects are robustly present in the cohort. Another limitation of our study is its cross-sectional nature. However, the current data serve as baseline for an ongoing prospective longitudinal study. Here we will also investigate the preclinical course for specific SCA subtypes. In the current study, subjects shared the same mutational SCA mechanism (CAG repeat expansion), but differed in their genotype (SCA 1,2,3,6), thus representing clinically and biologically distinct SCA types, which are characterized by heterogeneous patterns of disease progression and distinct determinants on motor deterioration[42]. Our current study, however, was primarily designed to investigate early changes in posture and gait *across* specific SCA types, allowing to find SCA-general markers of early motor changes in the preclinical phase across SCAs.

Conclusions

The results of this study provide evidence for (1) quantitative measures of preclinical motor changes in SCA across specific types, which allow (2) to discriminate subjects even on a single-subject basis in complex motor tasks and (3) which enable the quantification of disease progression in the preclinical phase. Thus, this study provides the basis for future observational studies investigating the characteristics and evolution of the preclinical phase of SCAs and also for both pharmaceutical and rehabilitative intervention trials.

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Table 1 Description of subject populations.

Groups	# subjects	Age (years)	Gender (f/m)	SARA	$SARA_{p\&g}$	INAS	Estimated disease onset (years)		SCA type # (1,2,3,6)
							$eT2DO^{CAG}$	$eT2DO^{CAG}_{par}$	
$SCA^{SARA3-8}$	9	40.5±13.3 [20-68]	4/5	5.1±1.3 [3-8]	2.1±1.6 [0-4]	3.3±2 [1-8]	n.a.	n.a.	(5,1,2,1)
$MC^{SARA<3}$	14	42±13.3 [24-65]	6/8	1.21±0.9 [0-2.5]	0.21±0.4 [0-1]	0.7±0.7 [0-2]	9.5 [0.4-20.3]	6.07 [0.9-15]	(5,2,3,4)
$MC^{SARAp\&g=0}$	8	44.9±15.3 [24-65]	5/3	0.5±0.46 [0-1]	0 [0-0]	0.8±0.8 [0-2]	8.25 [0.4-12]	7.6 [1.6-15]	(3,0,1,4)
nMC	8	43.8±16.3 [20-60]	4/4	0.56±0.7 [0-2]	0 [0-0]	0.5±0.5 [0-1]	n.a.	n.a.	n.a.
HC	23	40.5±13 [20-66]	11/12	0.33±0.5 [0-2]	0 [0-0]	n.a.	n.a.	n.a.	n.a.

#: Number of subjects in each group. $SCA^{SARA3-8}$: patients with SCA 1,2,3 or 6 in the early clinical stage of the disease with a SARA score of 3-8; $MC^{SARA<3}$: *pre-clinical* mutation carriers of SCA 1,2,3 and 6 with a SARA score of <3; $MC^{SARAp\&g=0}$: subgroup of the *pre-clinical* mutation carrier group, all with a $SARA_{posture\&gait}$ subscore=0 and SARA total score ≤1, HC: healthy controls, nMC: subgroup of healthy, age-matched controls consisting of first degree relatives of SCA patients tested negative for the mutation. n.a,, not applicable. Ataxia symptoms were clinically assessed using the scale for the assessment and rating of ataxia (SARA). SARA covers a range from 0 (no ataxia) to 40 (most severe ataxia). The SARA score includes eight items: three items rating gait and posture, one item for speech disturbances and four items for limb-kinetic functions. The three items rating gait and posture are summarized in the gait&posture subscore ($SARA_{p\&g}$). Given are mean values and standard deviation. INAS: Inventory of Non-Ataxia Symptoms[27] (see supplement for details of scores). $eT2DO^{CAG}$: genetically estimated timespan (years) to clinical disease onset, according to [28]; $\mathbf{eT2DO^{CAG}_{par}}$: genetically estimated time to clinical disease onset, adjusted by parental disease of onset (see equation 1). SCA(1,2,3,6): Number of subjects with spinocerebellar ataxia type 1,2,3,6 Given are averages, standard deviations (±) and ranges []. The groups $MC^{SARA<3}$ and HC differed significantly in SARA scores (p=0.002). In contrast, the $MC^{SARAp\&g=0}$ and nMC groups did not differ in SARA score (p=0.97), INAS score (p=0.5) or age (p=0.98), and in both groups none of the subjects showed clinical gait and posture abnormalities. For individual INAS scores, see Supplement 3.

Figure Legends

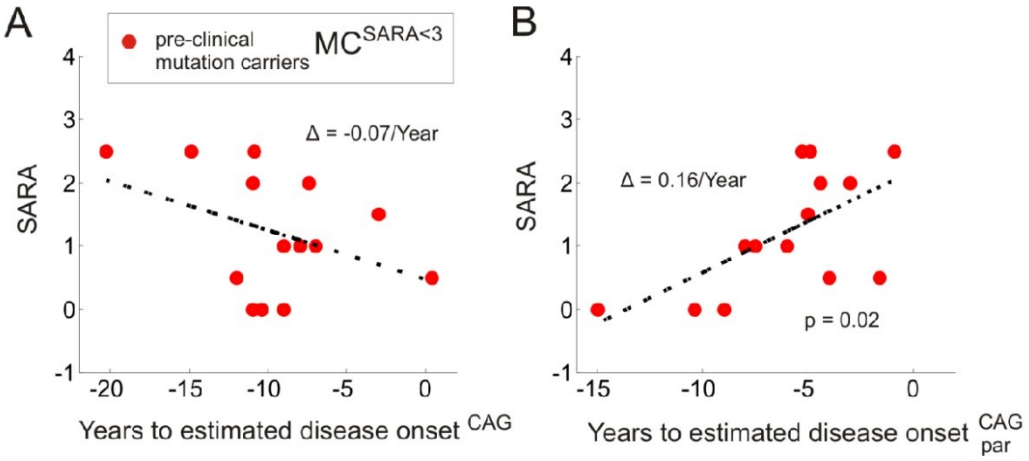


Figure 1. Relationships between estimated time to disease onset and the SARA scores in the $MC^{SARA<3}$ cohort, with time to disease onset calculated by the unadjusted model [28] (A) and the parentally adjusted model (B)(see equation 1). The adjusted model modifies the estimated onset in particular for those subjects with >10 years to disease onset and incipient clinical signs (SARA>1). The black lines denote linear fits of the data; the averaged changes per year are indicated by the symbol (Δ). The p-value indicates a significant correlation between durations to estimated disease onset and SARA score.

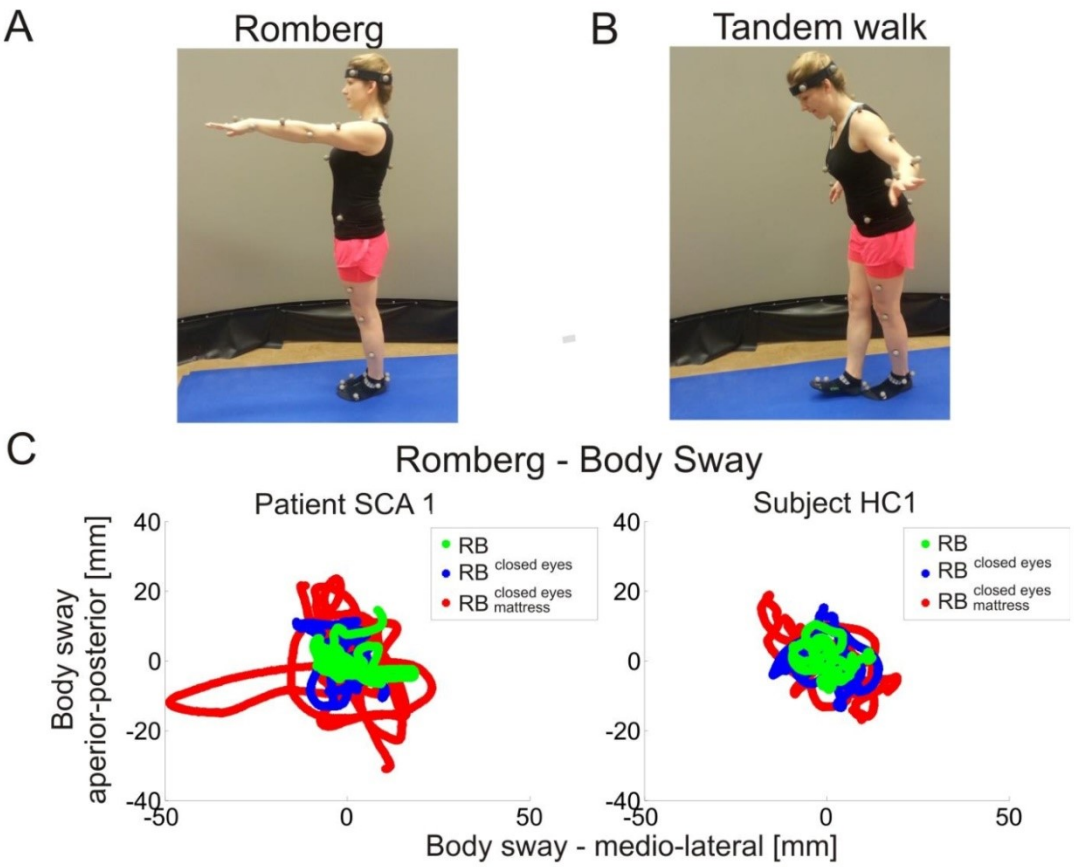


Figure 2. Snapshots of a healthy subject performing Romberg test (A) and tandem walk (B) on a 3cm thick mattress. (C) Illustrating of the body sway determined by the path of the cog (centre of gravity) for the different Romberg conditions. Both an exemplary SCA patient from the $SCA^{SARA3-8}$ group and a healthy control subject show an increased body sway in the conditions with closed eyes and in particular with closed eyes on a mattress, yet increases are larger in the patient.

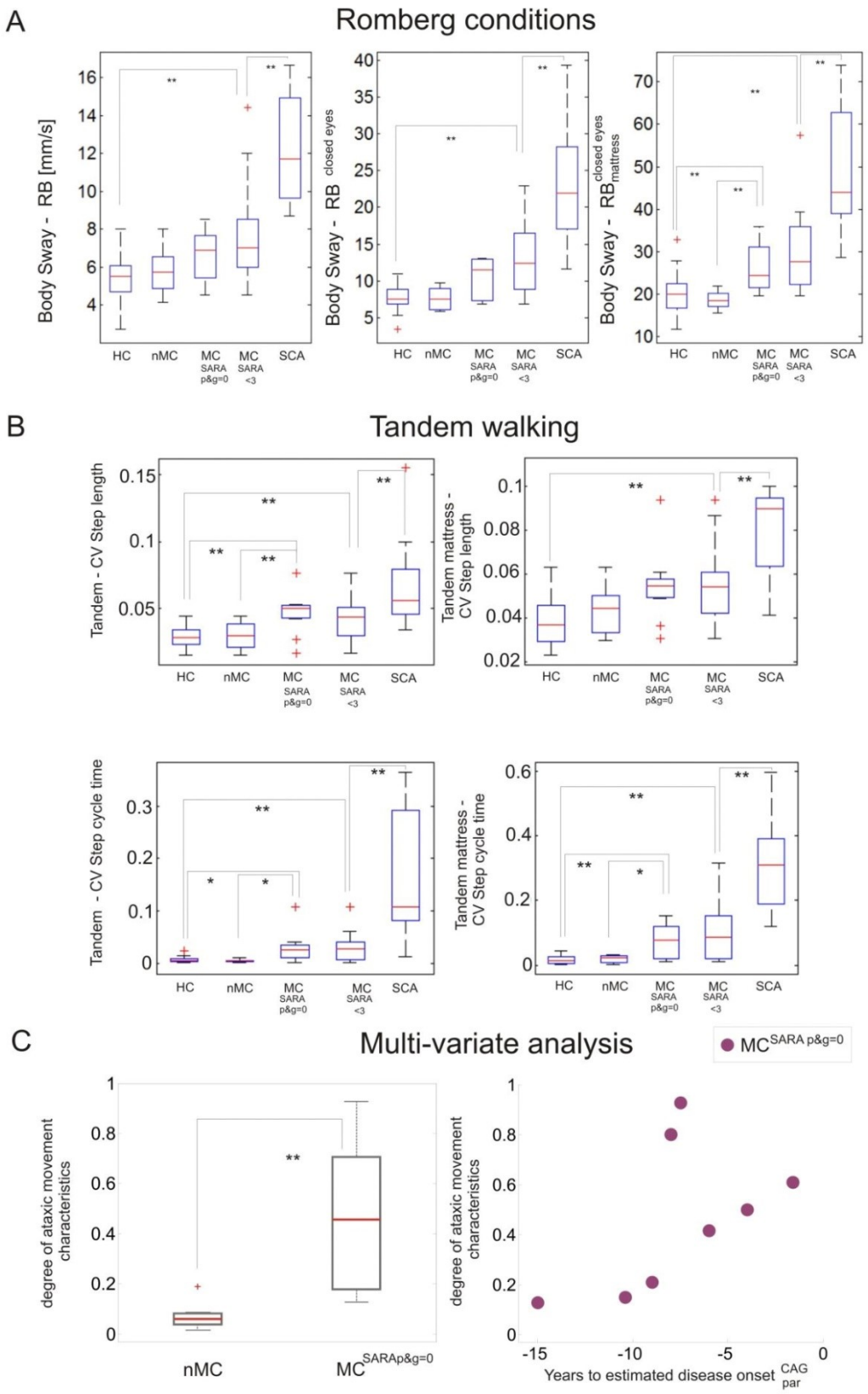

A
Romberg conditions
Body Sway - RB [mm/s]
Body Sway - RB closed eyes
Body Sway - RB closed eyes mattress
B
Tandem walking
Tandem - CV Step length
Tandem mattress - CV Step length
Tandem - CV Step cycle time
Tandem mattress - CV Step cycle time
C
Multi-variate analysis
MC SARA p&g=0
degree of ataxic movement characteristics
nMC
MC SARA p&g=0
Years to estimated disease onset CAG par
HC
nMC
MC SARA p&g=0
MC SARA <3
SCA

Figure 3. Group results from quantitative movement analysis for the different Romberg stance conditions (A) and tandem walking conditions (B). Stars indicate significant differences between groups (*≡ $p<0.05$, **≡ Bonferroni-corrected significance levels, see methods). Group descriptions: HC, healthy controls ; nMC, healthy subgroup of blood-related non-mutation carriers; $MC^{SARAp\&g=0}$, pre-clinical mutation carriers without clinical signs in posture and gait ; $MC^{SARA<3}$, pre-clinical mutation carriers with SARA score <3; SCA, SCA patients with SARA 3-8 ; CV: coefficient of variation (see methods). (C) Left panel: results from the output of the logistic regression model for the feature set [age, body sway in $RB_{mattress}^{closed\ eyes}$, variability in step cycle time for tandem walk]. The model output interpreted as degree of ataxic movement characteristics differed significantly between groups nMC and $MC^{SARAp\&g=0}$ ($p=0.006$). Right panel: Analysing model outputs in relation to the estimated duration to disease onset revealed that all mutation carriers with an estimated duration to disease onset < 10 years show a model output greater than all nMC subjects. Only the two mutation carriers with duration to disease onset > 10 years reduce the specificity of the categorization.

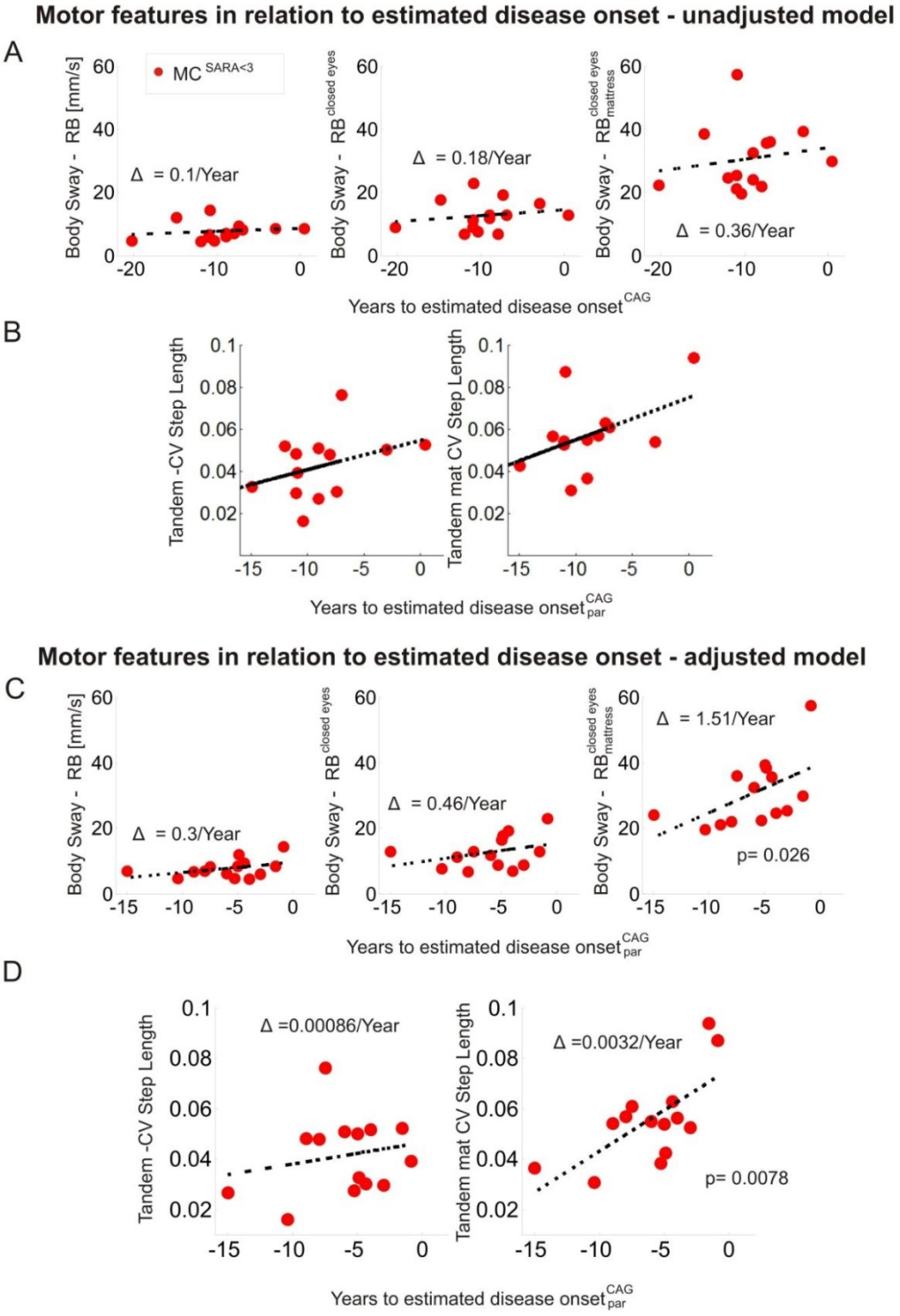


Figure 4 Relationship between estimated time to disease onset and motor features for the pre-clinical mutation carriers $MC^{SARA<3}$. Shown are relationships for estimates of onset according

to the unadjusted model [28] (A,B) and according to the adjusted model (C, D). Each circle represents one subject. (A+C) Relationships between estimated time to onset and body sway in the different Romberg (RB) conditions. (B+D) Relationship between estimated time to onset and step length variability in tandem gait conditions with and without mattress. The black lines represent a linear fit of the data; the average changes per year are indicated by the symbol (Δ). A steeper increase of change can be seen in the most complex stance and gait tasks, respectively, namely $RB^{\text{closed eyes}}_{\text{mattress}}$ and tandem gait on a mattress. P-values indicate significant correlations between durations to estimated disease onset and movement parameters.

Supplemental Material

Supplement 1: Technical details and additional analyses of the quantitative movement assessment

a) Technical details

Motor performance was determined by quantitative movement analysis using a VICON MX motion capture system with 10 cameras. The three-dimensional movement trajectories of the subjects were recorded at a sampling rate of 120 HZ, using 41 reflecting markers. The marker and angle trajectories were smoothed with a Savitzky-Golay polynomial filter (order 4 and with a window size of 41 sampling points). Gait cycles were automatically determined from the trajectories by detection of heel-strike events, based on the vertical components of the heel marker positions. Results of the automatic detection were verified manually using a stick figure animation in order to correct for different types of foot placement. Subjects were performing all stance and gait tasks barefoot.

b) General relation between clinical phenotype and movement parameters

Cerebellar ataxia presents with a lack of control in coordinatively demanding movements, in particular of stance and gait, which is specifically characterized on a movement analysis level by an increased variability in spatial and temporal movement parameters [1]. To unravel first pre-clinical changes in SCAs, we thus employed a battery of coordinatively demanding stance and gait tasks, predicting that motor abnormalities might unravel in particular with increasing motor demands, and in particular in measures of motor variability.

c) Further details on the condition "tandem walk on the mattress" and exclusion of subjects unable to perform this task

In the mattress conditions, the underground consisted of a 3cm thick soft plastic mattress (AIREX™). Three subjects from the $SCA^{SARA3-8}$ group were unable to perform the tandem walk on the mattress, i.e. they showed a very high number of mis-steps as well as several interruptions of the gait trial. These patients were excluded from the analysis of this specific task, including the analysis of this task within the multi-variate analysis.

d) Analyses of the influence of gait asymmetry, speed differences, and gender

Step variability measures were calculated based on all left and right steps jointly, i.e. we did not calculate the variance of each the left and the right steps first and then combine the results. To ensure that variability measures are not dominated from asymmetry, we tested for group differences (HC, nMC, $MC^{SARAp\&g=0}$, $MC^{SARA<3}$ and $SCA^{SARA3-8}$) on spatial and temporal step asymmetry in gait and tandem gait by the non-parametric Kruskal-Wallis–test (using the definition of gait asymmetry from [2]). We found no group differences in asymmetry in either condition ($p>0.18$). Thus, putative differences in asymmetry cannot explain our group findings.

Furthermore, to control for the possibility that gait variability measures might be influenced by gait speed[3], we tested for speed differences. We tested for group differences (groups HC, nMC, $MC^{SARAp\&g=0}$, $MC^{SARA<3}$ and $SCA^{SARA3-8}$) on speed in gait and tandem gait by the non-parametric Kruskal-Wallis test. In normal gait, we found no group difference ($p>0.48$). In contrast, Kruskal-Wallis test yielded a significant effect in speed for tandem and tandem on a

mattress ($p<0.003$). Post-hoc analysis revealed a decreased speed of the SCA patient group ($SCA^{SARA3-8}$) for both tasks ($p<0.01$), but, importantly, no differences in speed between all pre-clinical mutation carrier groups (nMC, $MC^{SARAp\&g=0}$, and $MC^{SARA<3}$, respectively) versus healthy controls ($p>0.21$). Thus, putative differences in gait speed cannot explain our group findings.

Also gender effects cannot explain our group findings. We did not find any gender effects in groups SCA, $MC^{SARA<3}$ and HC for all Tandem and Romberg conditions.

e) Analysis of the influence of sensory abnormalities

The INAS scores (see supplement 3) revealed for 5 out of 14 preclinical mutation carriers ($MC^{SARA<3}$) abnormalities in the sensory item, i.e. the item on reductions in vibration sense. To control for the possibility that our gait and stance results might be due to an sensory impairment in the preclinical mutation carriers, namely a deficit in afferent peripheral or spinal tracts, rather than a primary cerebellar deficit, we tested for differences in measures for stance and tandem gait between the subgroup with vibration deficits vs. the subgroup without vibration deficits (i.e. group with INAS sensory item =1 versus group with INAS sensory item=0). We found no group differences for body sway (Romberg conditions) or spatio-temporal gait variability (tandem gait) ($p>0.28$). This makes it less likely that our results in the preclinical mutation carrier group are mainly due to afferent deficits.

Supplement 2: Step cycle timing variability in relation to estimated disease onset

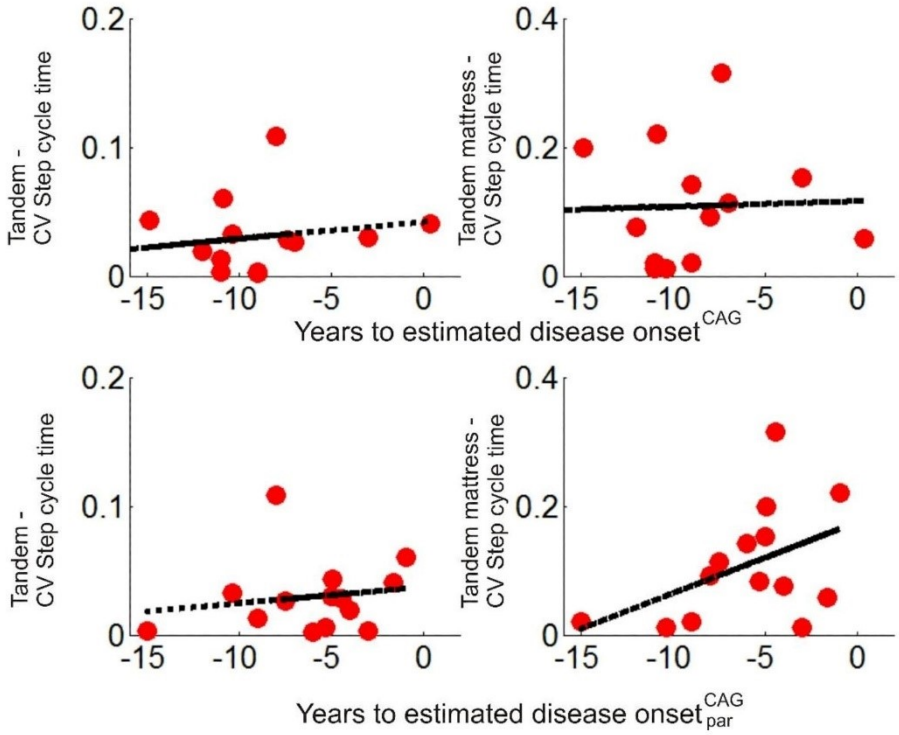


Figure Supplement 2 Relationship between estimated time to onset and step cyle timing variability in tandem gait conditions with and without mattress for the pre-clinical mutation carriers MC$^{SARA<3}$. Shown are the relationships of step cyle timing variability with both types of disease onset estimates: the disease onset estimate according to the unadjusted model (upper row), and the disease onset estimate according to the adjusted model (lower row). Each circle represents one subject. The black lines represent a linear fit of the data. A steeper increase of change can be seen in the most complex stance gait task, namely tandem gait on a mattress, in particular for the adjusted model of estimated disease onset (p=0.1 for the adjusted model).

Supplement 3: Inventory of Non-Ataxia Symptoms (INAS)

A systematic clinical characterization of non-ataxia signs was performed by means of the Inventory of Non-Ataxia Signs (INAS) [4]. The INAS has 30 items comprising of 29 items related to 16 symptoms and one open question (item # 30 "other abnormal clinical findings or reported abnormalities"). In line with previous studies applying the INAS[4, 5], only the presence or absence (but not the degree) of the respective INAS sign was noted in this study. The number of INAS signs was counted in each patient, yielding the semi-quantitative INAS count, a dimensionless value with a range from 0 (absence of non-ataxia symptoms) to 16 (most severe extra-cerebellar involvement).

Supplement 3, Table 1: Grouping of the 16 variables from the INAS form

variable # INAS count	symptom	item of the INAS inventory
1	Areflexia	1,2,3
2	Hyperreflexia	1,2,3
3	Extensor plantar	4
4	Spasticity	5
5	Paresis	6
6	Muscle atrophy	7
7	Fasciculations	8
8	Myoclonus	9
9	Rigidity	10
10	Chorea/dyskinesia	11
11	Dystonia	12
12	Resting tremor	13
13	Sensory symptoms	14
14	Urinary dysfunction	28
15	Cognitive dysfunction	29
16	Brainstem oculomotor signs	20, 21, 22

Supplement 3, Table 2: Detailed listing of non-ataxia symptoms for the individual subjects of the group $MC^{SARA<3}$ and its subgroup $MC^{SARAp\&g=0}$.

This table shows that mainly hyperreflexia and sensory system abnormalities are found in the pre-clinical SCA mutation carriers. However, as shown by Supplement 3, Table 3, these two non-ataxia features are also frequently altered in non-mutation carriers, indicating that they are unspecific findings that can be found in both preclinical SCA mutation carriers as well as healthy controls. mc, preclinical mutation carrier (individual subject), $MC^{SARAp\&g=0}$, group of pre-clinical mutation carriers without clinical signs in posture and gait ; $MC^{SARA<3}$, group of pre-clinical mutation carriers with a total SARA score of <3.

Mutation carriers														
$MC^{SARAp\&g=0}$	**mc1**	**mc2**	**mc3**	**mc4**	**mc5**	**mc6**	**mc7**	**mc8**						
$MC^{SARA<3}$									**mc9**	**mc10**	**mc11**	**mc12**	**mc13**	**mc14**
SCA type	**6**	**6**	**6**	**1**	**1**	**6**	**3**	**1**	**3**	**1**	**3**	**2**	**2**	**1**
INAS Symptoms														
Areflexia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyperreflexia	0	0	1	1	0	0	0	0	0	1	0	0	0	0
Extensor plantar	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spasticity	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paresis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle atrophy	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fasciculations	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myoclonus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rigidity	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chorea/dyskinesia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dystonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Resting tremor	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sensory Systems	1	0	0	1	1	0	1	0	0	0	0	0	0	1
Urinary dysfunction	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cognitive dysfunction	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brainstem oculomotor signs	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Total INAS score	2	0	1	2	1	0	1	0	0	1	0	1	0	1

Supplement 3, Table 2: Detailed listing of non-ataxia symptoms for the individual subjects of the group NMC. This table shows that hyperreflexia and sensory system abnormalities are also a recurrent finding in healthy subjects without SCA mutations (=non-mutation carriers, NMC).

Non-Mutation carriers	**nmc1**	**nmc2**	**nmc3**	**nmc4**	**nmc5**	**nmc6**	**nmc7**	**nmc8**
INAS Symptoms								
Areflexia	0	0	0	0	0	0	0	0
Hyperreflexia	0	0	1	0	0	0	1	0
Extensor plantar	0	0	0	0	0	0	0	0
Spasticity	0	0	0	0	0	0	0	0
Paresis	0	0	0	0	0	0	0	0
Muscle atrophy	0	0	0	0	0	0	0	0
Fasciculations	0	0	0	0	0	0	0	0
Myoclonus	0	0	0	0	0	0	0	0
Rigidity	0	0	0	0	0	0	0	0
Chorea/dyskinesia	0	0	0	0	0	0	0	0
Dystonia	0	0	0	0	0	0	0	0
Resting tremor	0	0	0	0	0	0	0	0
Sensory Systems	1	1	0	0	0	0	0	0
Urinary dysfunction	0	0	0	0	0	0	0	0
Cognitive	0	0	0	0	0	0	0	0

dysfunction								
Brainstem oculomotor signs	0	0	0	0	0	0	0	0
Total INAS score	1	1	1	0	0	0	1	0

Supplement 3, Table 4: Detailed listing of non-ataxia symptoms for individual subjects of the group SCA$^{SARA3-8}$. In the group with SCA mutations at the early stage of the disease (SARA score between 3 to 8 points) non-ataxia features increase. They here include not only sensory abnormalities and hyperreflexia, but also wide range of variable other non-ataxia symptoms.

Patients	**sca1**	**sca2**	**sca3**	**sca4**	**sca5**	**sca6**	**sca7**	**sca8**	**sca9**
SCA type	**1**	**1**	**3**	**1**	**6**	**1**	**1**	**3**	**2**
INAS Symptoms									
Areflexia	0	0	0	0	0	0	0	0	1
Hyperreflexia	0	0	0	0	0	1	0	0	0
Extensor plantar	0	0	0	0	0	0	0	0	1
Spasticity	1	0	1	0	0	1	0	0	0
Paresis	0	0	0	0	0	1	0	0	0
Muscle atrophy	0	1	0	0	0	1	0	1	0
Fasciculations	0	0	0	0	0	1	0	0	0
Myoclonus	0	0	0	0	0	0	1	1	0
Rigidity	0	0	0	0	0	0	0	0	0
Chorea/dyskinesia	0	0	0	0	0	0	0	0	0
Dystonia	0	0	0	0	0	0	0	0	0
Resting tremor	0	0	0	0	0	0	0	0	0
Sensory Systems	1	0	1	1	1	1	1	1	0
Urinary	0	0	0	0	0	1	1	0	1

dysfunction									
Cognitive dysfunction	0	0	0	0	0	0	0	0	0
Brainstem oculomotor signs	1	0	1	0	0	1	0	1	0
Total INAS score	3	1	3	1	1	8	3	4	3

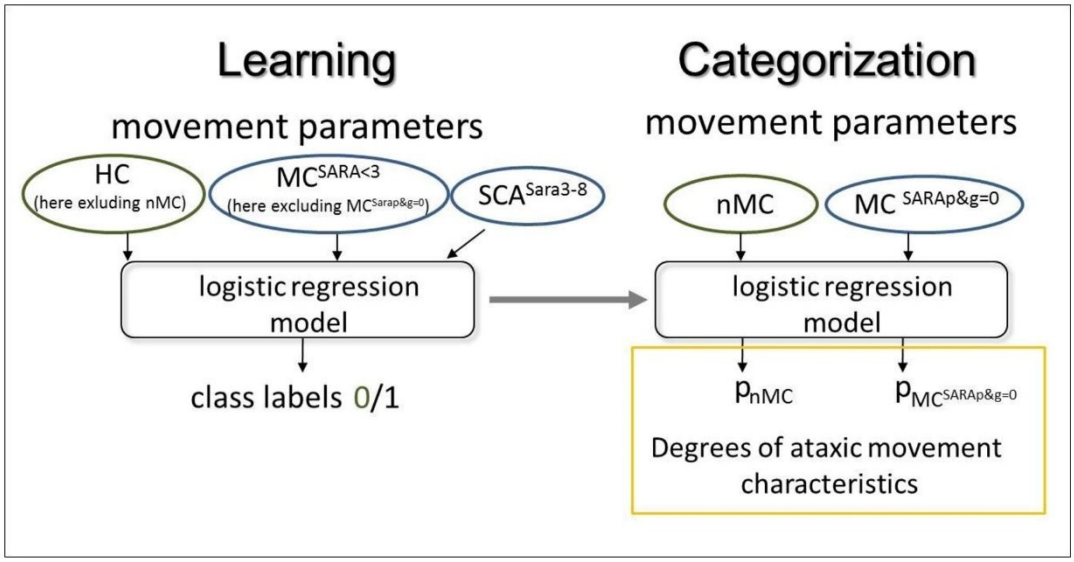
Supplement 4: mean, standard deviation and range of CAG repeats

	number of subjects	Mean		Standard deviation		Range	
		Expanded allele	Shorter allele	Expanded allele	Shorter allele	Expanded allele	Shorter allele
SCA 1	5	43	29.8	3.6	0.8	[40-47]	[29-31]
SCA 2	2	38	23	0	0	[38-38]	[23-23]
SCA 3	3	66	21.3	3.4	7.0	[64-70]	[14-28]
SCA 6	4	21.25	10.7	0.5	0.95	[21-22]	[10-12]

Supplement 4, Table 1. Mean, standard deviation and range of CAG repeats for each preclinical SCA group (SCA 1, SCA2, SCA3 and SCA6) investigated in this study. The size of the expansion is known to be negatively correlated with age at onset in SCAs[6] . No descriptive single subject data about individuals' allele sizes and their genotypes are shown, given that this would allow individual study participants to re-identify themselves and to recognize her/his own genotype status.

Only the CAG repeats of the respective SCA gene known to be mutated in the family were investigated, not the CAG repeats in other SCA and non-SCA repeat expansion genes, which might also influence disease penetrance and disease manifestation[6].

Supplement 5: Multi-variate Analysis



Supplement 5, Figure 1: Differences in multi-variate analysis combining different movement features and the factor "age" were determined using multi-variate logistic regression models[7] for nMC and $MC^{SARAp\&g=0}$. To generate and validate the logistic regression models, we used a three-step procedure. 1.) Models were established to discriminate between non-mutation carriers and mutation carriers using feature sets from groups $MC^{SARA<3}$ (here excluding subgroup $MC^{SARAp\&g=0}$) and $SCA^{SARA3-8}$ as prototypes for mutation carriers (class label 1) as well as from healthy controls (HC) (here excluding subgroup nMC) as prototypes for non-mutation carriers (class label 0) . 2.) In the test step we determined model outputs as the degree of ataxic movement characteristics for the groups $MC^{SARAp\&g=0}$ and nMC. 3.) We analysed the generated outputs of groups nMC and $MC^{SARAp\&g=0}$ in respect to (a) group differences and (b) categorization capabilities by using them as input into a ROC (receiver operating characteristic) analysis[8] in order to determine the accuracy of the identification of pre-clinical mutation carriers $MC^{SARAp\&g=0}$ on a single subject level. HC, healthy controls (here excluding the nMC group) ; nMC, subgroup of

healthy controls, comprising of blood-related non-mutation carriers from SCA families; $MC^{SARAp\&g=0}$, pre-clinical SCA mutation carriers without clinical signs in posture and gait ; $MC^{SARA<3}$, pre-clinical SCA mutation carriers with a total SARA score of <3; $SCA^{SARA3-8}$ (here excluding the $MC^{SARAp\&g=0}$ group), clinically symptomatic SCA mutation carriers with a SARA score between 3 to 8 ; p, degree of ataxic movement characteristics.

Supplement 6 – Details of genetic analysis

All SCA subtypes studied here share the same genetic mechanism, namely CAG trinucleotide repeat expansions which are fully penetrant if exceeding a certain CAG repeat threshold of the respective SCA gene. All SCA mutation carriers studied here carried repeat expansions in the clearly pathological, fully penetrant range.

CAG repeat length was analysed in DNA extracted from EDTA blood samples. All genetic analyses were performed at the same lab, namely the Institute of Medical Genetics and Applied Genomics, University of Tübingen with established and standardised methods[9, 10]. In the context of the study, genetic tests in all pre-clinical subjects were done anonymously, and results were not disclosed to the participants or to the investigators that were performing the assessments (i.e. the SARA and movement recordings). However, independent from this study, all patients with manifest SCA and also some individuals at risk received genetic diagnostics for SCA repeats. 100% of the individuals in the the $SCA^{SARA3-8}$ group, 14% of the $MC^{SARA<3}$ group, and 0% of the nMC group knew their genotype status (carrier vs. non-carrier).

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2.2 Real-time use of audio-biofeedback can improve postural sway in patients with degenerative ataxia

This chapter refers to the following study, including the supplementary information that has been published in the online version:

Fleszar, Z., Mellone, S., Giese, M., Tacconi, C., Becker, C., Schöls, L., Synofzik, M. and Ilg, W. (2019), Real-time use of audio-biofeedback can improve postural sway in patients with degenerative ataxia. Ann Clin Transl Neurol, 6:285-294. doi:10.1002/acn3.699

2.2.1 Introduction

The quantitative analysis of movements under different conditions has provided fundamental insights into the role of the cerebellum in motor control. Such findings have had implications on rehabilitation strategies for patients who suffer from cerebellar damage. Individualized physiotherapy using whole-body controlled videogames for cerebellar movement deficits is an example for such a translational application.[96, 125] Quantitative movement analysis has also been shown to be a sensitive method to objectively assess and quantify fine-grained effects of such treatment strategies in intervention trials.[66-69] Analysing movement patterns has helped to form the scientific consensus that the underlying contributions of the cerebellum to motor control are based on computations of internal feed-forward models that predict the sensory consequences of a motor command and allow for rapid adjustments of ongoing movements (chapter 1.5). Some studies that have investigated the consequences of cerebellar damage on motor control observed an increased reliance of patients on sensory feedback information, possibly as a compensation strategy for deficient prediction output. However, the processing of some sensory feedback channels, e.g. proprioception or vestibular information, can be itself impaired in cerebellar patients, leading to a predominant dependence on visual cues. These findings suggest that the augmentation of an external feedback source might help patients to improve cerebellar movement deficits, such as poor postural control. If proven so, this

could present a potential assistive strategy for cerebellar patients. To provide first proof-of-concept evidence for the effectiveness of such an approach, we used quantitative movement analysis to assess the effects of an augmented acoustic feedback signal of trunk accelerations that might function as a real-time corrective tool for cerebellar patients to improve balance.

The study procedures were approved by the ethics committee of the University of Tübingen (602/2012BO1).

2.2.2 Original Publication

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RESEARCH ARTICLE

Real-time use of audio-biofeedback can improve postural sway in patients with degenerative ataxia

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[a]Joint last authors.

Abstract

Objective: Cerebellar ataxia essentially includes deficient postural control. It remains unclear whether augmented sensory information might help cerebellar patients, as the cerebellum underlies processing of various sensory modalities for postural control. Here, we hypothesized that patients with cerebellar degeneration can still exploit audio-biofeedback (ABF) of trunk acceleration as a real-time assistive signal to compensate for deficient postural control. **Methods**: Effects on postural sway during stance were assessed in an ABF intervention group versus a no-ABF disease control group (23 vs. 17 cerebellar patients) in a clinico-experimental study. A single-session ABF paradigm of standing plus short exergaming under ABF was applied. Postural sway with eyes open and eyes closed was quantified prior to ABF, under ABF, and post ABF. **Results**: Postural sway in the eyes closed condition was significantly reduced under ABF. Both benefit of ABF and benefit of vision correlated with the extent of postural sway at baseline, and both types of sensory benefits correlated with each other. Patients with strongest postural sway exhibited reduced postural sway also with eyes open, thus benefitting from both vision and ABF. No changes were observed in the no-ABF control group. **Interpretation**: Our findings provide proof-of-principle evidence that subjects with cerebellar degeneration are still able to integrate additional sensory modalities to compensate for deficient postural control: They can use auditory cues *functionally similar* to vision in the absence of vision, and *additive* to vision in the presence of vision (in case of pronounced postural sway). These findings might inform future assistive strategies for cerebellar ataxia.

Introduction

The use of augmented sensory modalities (e.g., auditory, vibro-tactile, or electro-tactile/lingual) has been shown to reduce postural sway in stance and gait in subjects with balance deficits due to aging, vestibular loss, Parkinson's disease, or Progressive Supranuclear Palsy[1–9] (for reviews, see 10,11). However, such approaches have not yet been systematically tested in patients with cerebellar dysfunction, e.g. degenerative cerebellar ataxia. The effects of augmented sensory information on improving postural control here seem questionable, as the cerebellum underlies processing of various sensory modalities for postural control, including vestibular,[12] proprioceptive,[13] and visual sources[14] (for review, see 15). Moreover, the cerebellum is involved in multimodal sensory integration, for example, to provide estimates of body movement based on proprioceptive or vestibular information.[16,17] Such multisensory representations together with motor efferences are suggested to form internal forward models within the cerebellum, predicting the outcome of motor actions and subserving the calibration of motor actions including postural responses[18] and the adaptation to changing environments.[19,20]

In accordance with these hypotheses on the functional role of the cerebellum in postural control, patients with cerebellar dysfunctions show substantially increased postural sway in different posture conditions like normal stance, and stance with narrow feet position or on soft ground,[21–25] which becomes particularly pronounced with closed eyes.[21,26] At the same time, these observations also already indicate that cerebellar patients might still be able use information from one sensory modality – here: vision – to partly compensate for deficits in other sensory modalities.[15,26] Further hints for the hypothesis that reweighting of different sensory modalities might still be partly preserved in cerebellar patients comes from a psychophysics study on the estimation of hand positions which indicates that these patients might still be able to perform sensory realignment and short-term reweighting.[27]

Based on these first hints, we here hypothesized that cerebellar patients can still exploit auditory biofeedback (ABF) signals of trunk acceleration as an assistive signal to compensate for their deficient processing of proprioceptive and vestibular signals in postural control. This finding would provide proof-of-principle evidence for the notion that - despite progressive cerebellar damage - the brain is still able to act according to the principles of cue integration and sensory reweighting, namely to improve postural control by adding/increasing the weight of one additional sensory cue (here: auditory signals) and change the relative weight of the remaining sensory modalities.[28,29,30,31]

Methods

Patients

40 consecutive patients with degenerative cerebellar ataxia were recruited from the Ataxia Clinic of the Center for Neurology, Tübingen, Germany, from February 2014 until May 2016. Patients were included based on following g inclusion criteria: (1) progressive degenerative cerebellar ataxia in the absence of any signs of secondary CNS disease; (2) age between 18 and 75 years; (3) SARA (Scale for the Assessment and Rating of Ataxia) total score >3, but SARA gait and stance subscores each <4 (i.e., walking and standing possible without support),[32] thus ensuring sufficient capacity to benefit, but also to complete the tasks. The exclusion criteria were: (1) clinical signs or mutations known to cause afferent ataxia (e.g., Friedreich's ataxia) (2) severe visual or hearing disturbances, cognitive impairment, predominant nonataxia movement disorders, or orthopedic constraints. The experimental procedure was approved by the local ethics committee. All subjects gave their informed consent prior to participation.

The intervention group receiving ABF comprised of a consecutive series of $n = 23$ subjects with cerebellar ataxia (=ABF group). To control for the effects seen in the ABF group, we subsequently recruited a consecutive series of $n = 17$ subjects with cerebellar ataxia (same inclusion criteria as for the intervention group) who performed the same tasks as the ABF group, but without auditory feedback in any of the conditions (= CON group) (for group characteristics, see Table 1; for detailed patient descriptions, see Data S1 Patients Description). This block assignment of two strictly consecutive series of cerebellar ataxia patients into the ABF and then the CON group was geared to reduce selection bias. Subjects who received ABF were also assessed by quantitative vibration testing by a Rydel-Seiffer tuning fork to determine the degree of possible vibration sense impairments and their relation to ABF effects.

Table 1. Characteristics of subject groups.

Group	Number of subjects	Gender F/M	Age, y	Disease Duration, y	SARA
ABF	23	8/15	51.2 (14.5)	13 (9.2)	11 (3.1)
CON	17	7/10	54.5 (11.5)	9.4 (6.3)	9.9 (3.3)

Given are mean values and standard deviations. ABF and CON did not differ in age ($P = 0.58$), disease duration ($P = 0.33$), or SARA score value ($P = 0.25$). ABF, feedback intervention group; CON, cerebellar ataxia control group, controlling for the ABF group. SARA, Scale for the Assessment and Rating of Ataxia.

Study design overview

The study was designed as a clinico-experimental study aiming to deliver proof-of-principle evidence that cerebellar patients are able to perform short-term multisensory integration and profit from ABF as a real-time assistive signal. We designed a single-session ABF paradigm, which provided the subjects of the ABF group with acoustic feedback of trunk acceleration during consecutive stance and exergaming conditions, allowing to test for improvements in postural control assessed at stance conditions. Effects were tested both *within* the intervention group (pre-post within-group control design) as well as *between* the intervention group and the control group (between-group control design).

Audio biofeedback device

We used a wearable ABF system as established previously.[33] It consists of two main components: (1) an inertial sensor node capturing trunk accelerations based on a 3D-accelerometer, -gyroscope, and -magnetometer, and (2) a smartphone-based application receiving trunk acceleration information via Bluetooth™ 2.1 connection. Audio signals are delivered via headphones (see Fig. 1 and Data S2 for technical details). Before the intervention, subjects familiarized with the ABF signal for 2 min.

Experimental Procedures

Subjects completed a sequence of quiet stance conditions, each of them lasting 30 seconds. During stance conditions subjects stood on a firm surface (=the floor) without footwear, arms loosely hanging down on the lateral sides of their body (Fig. 1). Feet were placed closely together. Two types of stance conditions were exploited: (1) standing with eyes open (EO) and (2) standing with eyes closed (EC).

The feedback intervention paradigm was structured into five consecutive phases: (1) *PreABF*, (2) *Training I*, (3) *Training II*, (4) *TestABF*, and (5) *PostABF* (for an overview of the experimental trial design, see Fig. 2). The stance conditions EO and EC were provided at the phases *PreABF, Training I, TestABF, and PostABF*. *PreABF* comprised of both stance conditions, each condition performed once, without ABF. These trials served to assess each subject's extent of trunk sway at baseline prior to training. In *Training I*, subjects completed each stance condition four times under the presence of ABF. The order of the two conditions was balanced between subjects, thus reducing possible order effects. *Training II* consisted of an exergaming period, exploring actively the ABF-sensorimotor mapping. Here, subjects played a Microsoft Xbox Kinect® balance game ("Slip Slide", Ice Age: Continental Drift, by Activision (R), Santa Monica, CA) for 10 min, controlling an avatar by quick eccentric trunk movements. This period of ABF served to provide subjects with the opportunity to exploit the acoustic signal during a full range of active trunk movements, facilitating the mapping of ABF signals to trunk movements. Such a period of active movements has been proposed to facilitate an auditory-sensorimotor mapping processes compared to standing alone.[34] *TestABF* was almost identical to *Training I*; that is, subjects performed both stance conditions with ABF, and in the same order as in *Training I*, but only two stance tasks per condition. *TestABF* served as the critical phase to test whether ABF has led to an effect on postural sway. *PostABF* comprised of both stance conditions, each performed once without ABF. This phase served as a *within* group control to rule out that possible effects seen in *TestABF* might just be due to unspecific effects, for example, due to prolonged standing, task repetition, or exergaming during the experimental phases.

Quantitative movement analysis

Balance performance was evaluated by quantitative movement analysis using a VICON motion capture system (Oxford Metrics, UK). For a detailed description of the system, recording procedure, and analysis of stance, see 35–37. The extent of trunk sway was determined by the path length of the center of gravity (COG) during each stance trial in [mm/sec]. For exemplary subject results illustrating this measure, see Fig. 1C and D). For a comparison of this sway measure with the method of elliptical area fits[38] see Data S4.

Statistical analysis

For comparison of both within-group and between-group differences in trunk sway, we pooled each (1) the trials 1–4 of *Training I* and (2) the two stance trials of *TestABF* to an average value. Averaging was performed for the EO and the EC condition separately. Before pooling, we controlled for significant differences within these trials using the nonparametric Friedman test (χ^2, $P > 0.2$) (for details of statistical methods and analyses without pooling see Data S3, confirming the results of the pooled data).

In order to examine whether in particular subjects with large body sway profit from ABF, we subclassified subjects according to their individual extent of postural sway at baseline. Subjects with an individual postural sway in the top tertile of the whole group in the EO condition at baseline, that is, with the highest postural sway ($ABF_{>66\%}$ subgroup; postural sway >13.5 mm/sec, $n = 8$; $CON_{>66\%}$ subgroup; postural sway >11.3 mm/sec, $n = 6$), were separated from subjects in the lower two tertiles, i.e. with less individual postural sway ($ABF_{<66\%}$ subgroup $n = 15$, $CON_{<66\%}$ subgroup $n = 11$, see Fig. 3H). Statistical analysis was performed using the software package MATLAB.

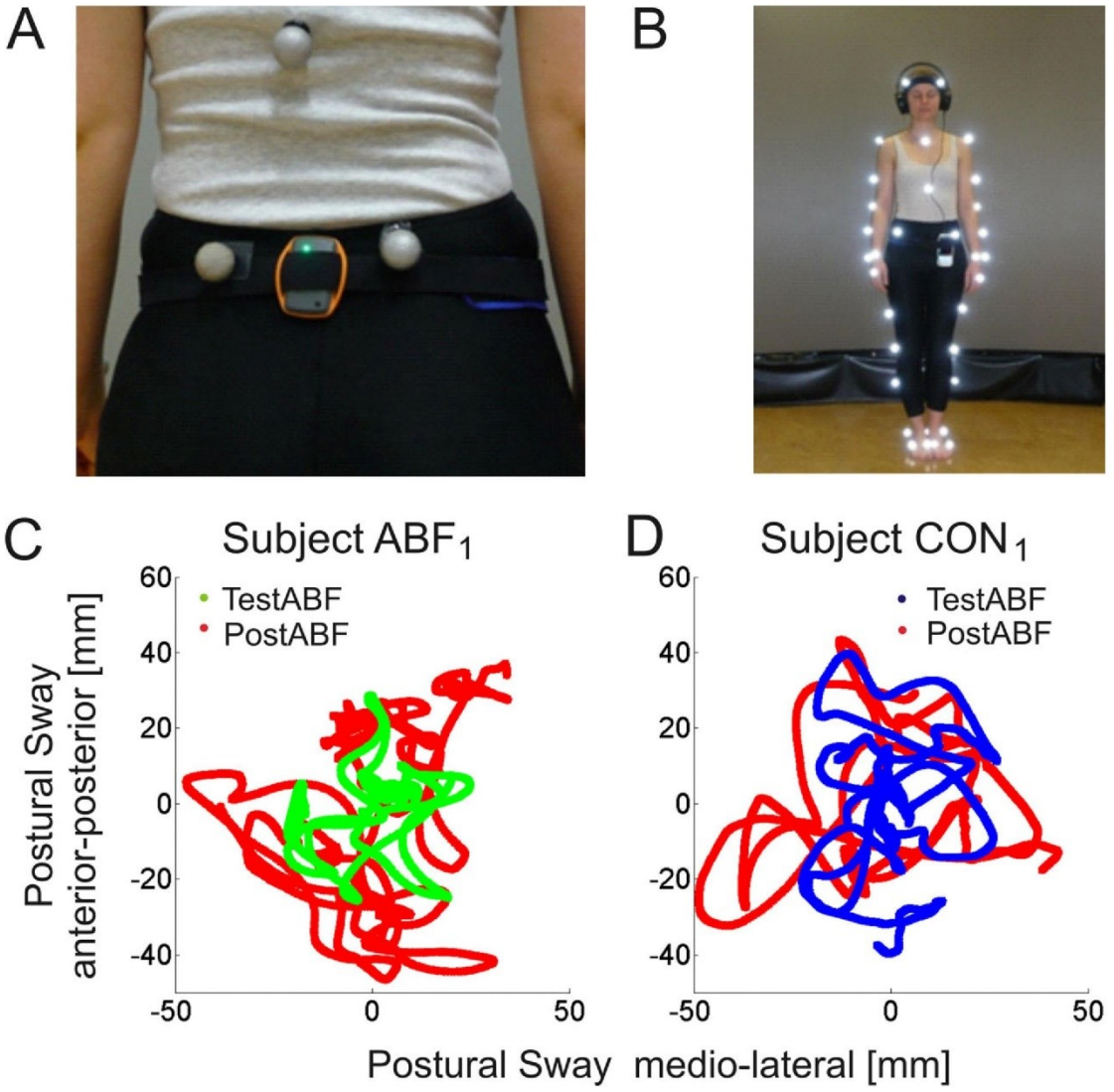

Figure 1. Experimental equipment for ABF (A + B). Subjects wore the sensor node (black sensor) mounted with a Velcro belt at L4/L5 (A). The sensor is linked to a smartphone tightly attached with the Velcro belt, which generated the ABF of sensor-recorded trunk acceleration. The ABF is transmitted to the subject via headphones (B). In parallel, a VICON Motion Capture System was used to quantitatively assess trunk sway across the experimental trials, with reflective markers being attached to predefined body positions. Shown is an exemplary subject in stance position in the eyes closed condition. Postural sway in stance tasks (C+D). Shown are the paths of the centre of gravity (COG, projection of the center of mass on the floor) during stance tasks in anterior-posterior and medio-lateral direction from an exemplary subject of the ABF group (subject ABF_1, left) and of the CON group (subject CON_1, right). The ABF subject showed an improvement in postural sway with ABF in the *TestABF* phase (green) compared to the trial with no ABF in the *PostABF* phase (red), while the CON subject without ABF showed no difference in the corresponding trials.

Results

The disease control group CON did not differ from the ABF group in: (1) ataxia severity as determined by the SARA (ABF: 11 ± 3.13; CON: 9.85 ± 3.33; $r = 0.22$, $P = 0.25$), (2) age (ABF: 51.2 ± 14.5 years; CON: 54.5 ± 11.5 years; $r = 0.11$,$P = 0.584$), (3) disease duration (ABF: 12.7 ± 9.42 years; CON: 9.06 ± 6.33 years; $r = 0.13$, $P = 0.328$), or (4.) extent of postural sway at baseline in either of the two conditions (EC: ABF:

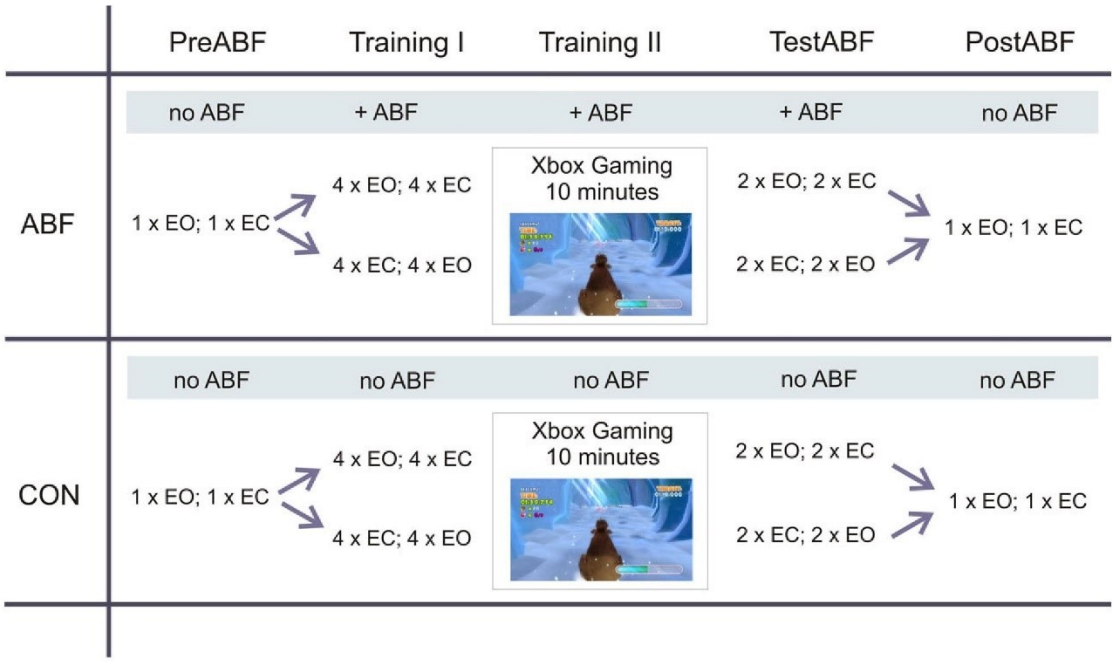

Figure 2. Experimental design: Combined between- and within-group control design with five experimental phases. ABF: feedback intervention group; CON: control group. *Between-group control:* Both groups executed the same protocol including stance trials as well as a 10 min exergame exploration period playing a postural controlled exergame. Only the ABF group received ABF (+ABF). The CON groups performed all the trials without ABF. Within-group control: Effects of the ABF phases were also tested within the intervention group by comparing the *TestABF* phase with the *PreABF* as well as the *PostABF* phase. EO: stance task with eyes open; EC: stance task with eyes closed.

25.8 ± 20.8 mm/sec; CON: 22.2 ± 15.9 mm/sec; $r = 0.24$, $P = 0.25$; EO: ABF: 12.7 ± 5.5 mm/sec; CON: 11.1 ± 5.1 mm/sec; $r = 0.22$,$P = 0.27$). This demonstrates that the serial block assignment led to a good matching between the two groups.

Benefits of vision

Both groups ABF and CON revealed a significantly increased postural sway in the EC compared to the EO condition at baseline (*PreABF*; $r > 0.87$, $P < 0.0001$), at *TestABF* ($r > 0.76$, $P < 0.0003$) and at *PostABF* ($r > 0.76$, $P < 0.0002$), indicating a benefit of vision on postural control (Fig. 3A + F). In both groups, the amount of postural sway in the EC condition correlated with reduction of sway by vision in the EO condition: the larger the sway in EC, the larger the reduction of postural sway by visual information in EO ($r > 0.59$, $P < 0.008$, Fig. 3 D, red dots).

Benefits of ABF in the eyes closed condition

All subjects were able to complete the tasks and all subjects from the ABF group reported that interacting with the ABF system was well feasible. In the EC condition differences in postural sway for the ABF group were found across phases (Friedman-test, $X^2 = 79.6$, $P = 0.047$, Fig. 3A). Post-hoc analysis showed a significant reduction of postural sway in *TestABF* phase compared with *PreABF* phase (*TestABF* vs. *PreABF*: $r = 041$, $P = 0.045$). Comparison of *Training I* with *PreABF*, did not reveal any significant reduction in postural sway (*Training I* vs. *PreABF*: $r = 0.12$,$P = 0.563$), indicating that the *Training I* phase alone was not sufficient to yield a training effect.

After the exergaming period in Training phase II, comparison of phases with ABF (*TestABF*) versus without ABF (*PostABF*) revealed a significantly smaller postural sway in the ABF condition compared to the subsequent condition without ABF (*TestABF* vs. *PostABF*: $r = 0.53$, $P = 0.011$, Fig. 3A).

In contrast, the CON group did not show any differences in postural sway across stance phases for any of the two conditions (EO: X^2=17.3, $P = 0.63$; EC: X^2=21.5, $P = 0.541$, see Fig. 3B).

In the ABF group, the difference of postural sway between *TestABF* versus *PostABF* was highly correlated with the extent of postural sway at *PreABF* ($r = 0.65$,

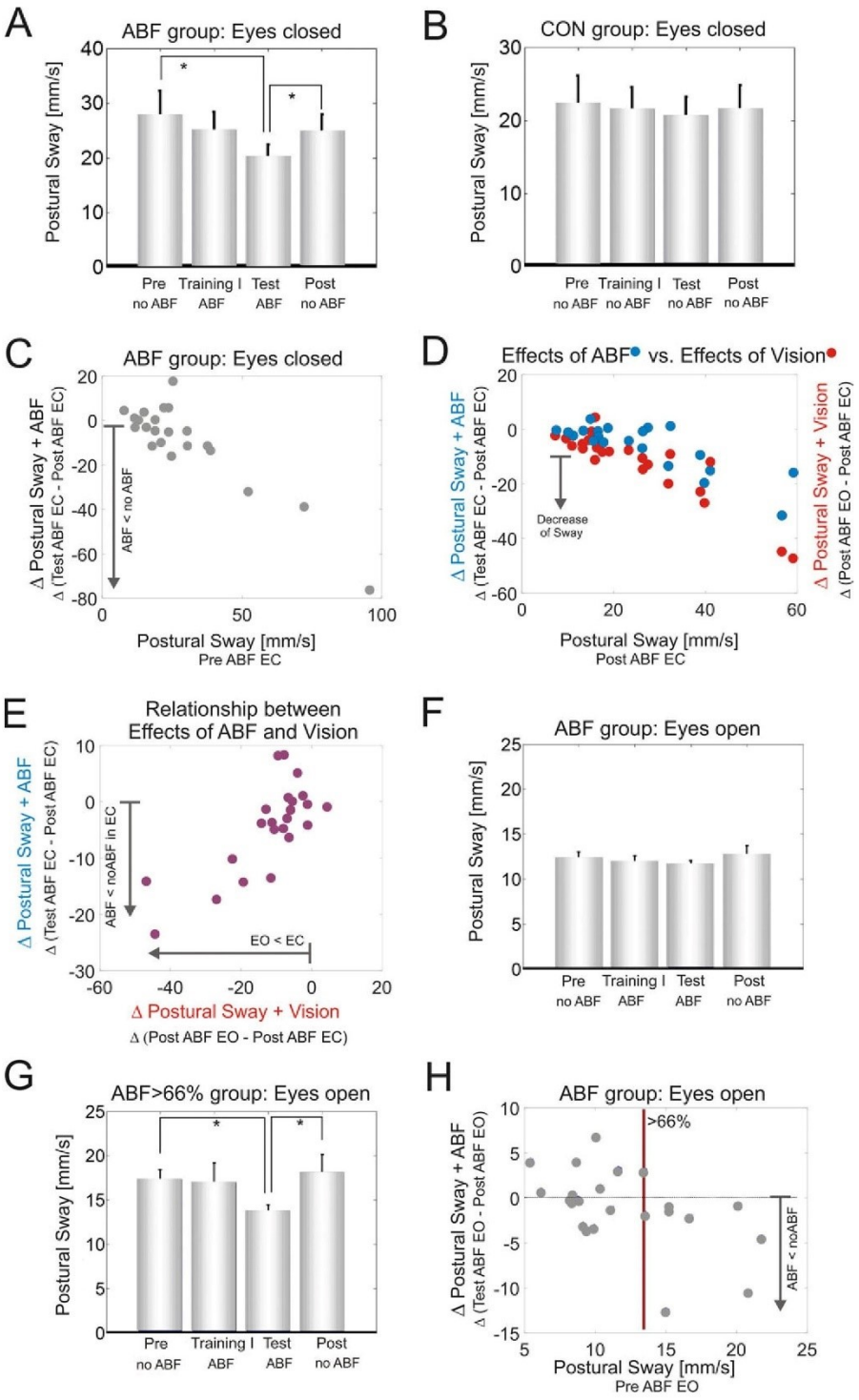

Figure 3. (A) Postural sway during Romberg stance in the ABF group in the eyes closed condition during the different experimental phases. The four bars indicate the consecutive experimental phases: *PreABF*, *Training I*, *TestABF,* and *PostABF* comparing trials with ABF (ABF) and without ABF (no ABF). (B) Postural sway during Romberg stance in the CON group in the eyes closed condition during the different experimental phases. (C) Relationship between baseline performance (*x*-axis) and difference (Δ) of postural sway between the *TestABF* and the *PostABF* phase (*y*-axis) for the ABF group in the eyes closed condition; (D) The effects of ABF in the closed eyes condition (in blue) compared to the effects of vision comparing the differences between conditions EO and EC (in red). (E) Difference in postural sway between eyes closed and eyes open without ABF (*x*-axis) in relation to the improvement in postural sway under ABF (*TestABF- PostABF*) in the closed eye condition (*y*-axis). Stars indicate significant differences (*$P < 0.05$) between different phases. (F) Postural sway during Romberg stance in the ABF group in the eyes open condition (EO); (G) Postural sway in the $ABF_{>66\%}$ subgroup in the eyes open condition during the different experimental phases. (H) Relationship between the *PreABF* baseline performance (*x*-axis) and difference (Δ) of postural sway between the *TestABF* and the *PostABF* phase (*y*-axis) for the ABF group in the eyes open condition. The red vertical line demarcates the top tertile of postural sway at baseline (>13.5 mm/sec), categorizing a subgroup $ABF_{>66\%}$ ($n = 8$) with increased postural sway.

$P = 0.001$ see Fig. 3C). No such a correlation was observed in the CON group ($r = 0.14$, $P = 0.65$).

Comparing the effects of vision and acoustic feedback

We next analyzed the relationship between the effects of vision and of acoustic feedback. The effect of vision on postural control was determined by comparing *PostABF* EO versus *PostABF* EC; the effect of ABF by comparing *TestABF* versus *PostABF* in the EC condition. Both sensory modalities yielded a similar, functionally almost equivalent benefit on postural control, as shown by the large overlap in Figure 3D. This relationship was analyzed in more detail by a correlation analysis, confirming a positive correlation between adding vision and adding auditory feedback (Fig. 3E). That is, those subjects benefiting most from vision (i.e., with the most pronounced difference between eyes open vs. eyes closed) benefited to a similar extent from the ABF in the EC conditions ($r = 0.53$, $P = 0.03$). Neither baseline performance nor ABF or vision effects were related to tuning fork measures of vibration sense (see Data S1 and S5 for details).

Benefits of ABF in the eyes open condition

In the EO condition, subjects of the ABF group did not show a significant group difference in postural sway between trials with and without ABF (Friedman-test, $X^2=12.8$, $P = 0.734$, see Fig. 3F). However, again a significant correlation was observed between the extent of postural sway at baseline (*PreABF*) and the difference of postural sway between *TestABF* versus *PostABF* in the ABF group ($r = 0.55$, $P = 0.007$). This indicates that, also in the EO condition, subjects with more pronounced postural sway benefit from the augmented sensory signal. No such correlation was observed in the CON group ($r = -0.17$, $P = 0.52$).

To further explore this correlation we performed a subgroup analysis of the tertile of subjects with the most pronounced postural sway at baseline ($ABF_{>66\%}$ subgroup, see Methods). This tertile showed a significant reduction in postural sway in the *TestABF* phase compared to both *PreABF* and *PostABF* (Friedman-test, $X^2=6.75$, $P = 0.08$, *PreABF* vs. *TestABF*: $P = 0.023$, *TestABF* vs. *PostABF*: $P = 0.023$, see Fig. 3G + H), indicating that these subjects profit from ABF also in the EO condition. No such change was seen in the CON group (neither overall CON group nor $CON_{>66\%}$ subgroup, Friedman-test, $X^2=0.2$, $P = 0.97$).

Discussion

Here, we provide proof-of-principle evidence that cerebellar patients can still exploit augmented sensory information to partly compensate for their impairment in processing proprioceptive and vestibular signals in postural control. The reductions in postural sway were observed only in the ABF intervention group after ABF training, as shown by our combined between-group and within-group control design. This demonstrates that the improvements were induced by exploitation of the ABF and were not merely due to unspecific non-ABF related factors, for example, exercise effects. Such a disease control group was missing in most other neurological conditions where bio-feedback has been explored.[1,3,6,39,40]

ABF-induced benefits are particularly pronounced in cerebellar patients with large postural sway

If it was indeed the deficient postural control which drives the integration of ABF, then in particular those patients with larger postural sway should show larger benefit by ABF. In line with this prediction, we observed that the larger the extent of body sway prior to ABF, the larger the ABF benefit (Fig. 3C). Such a correlation was seen not only in the EC condition ($P = 0.001$), but also in the EO condition ($P = 0.007$). Correspondingly, the subgroup of patients with the most pronounced sway showed a

benefit of ABF on postural control also in the EO condition ($P = 0.02$, Fig. 3G).

In contrast, ABF might be of limited benefit for subjects with less postural sway with eyes open. If vision is available, these only mildly affected subjects do not need to rely on acoustic signals, but the use of visual signals suffices to maintain a sufficient level of postural stability.

Preserved sensory integration to compensate for deficient postural control: the use of vision and auditory feedback

The process of sensory reweighting in posture control has been characterized by changing the relative contribution of the sensory systems depending on their availability and reliability,[28,29,41] thus allowing to constantly adjust sensory integration and subsequent postural control during the changing conditions of everyday living. According to this notion, those subjects who benefit most from vision for stabilizing postural control should rely most on the augmented sensory input (like auditory cues) - when visual cues are less reliable or even absent.

Correspondingly, our results show a correlation between the benefit by ABF in EC and the benefit by vision in EO, and both types of benefits correlate with the amount of postural sway at baseline. This observation supports the hypothesis that, in the absence of vision, cerebellar patients can use auditory cues functionally similar to vision to compensate for deficient postural control. That is, the more severe the damage to processing of proprioceptive and vestibular signals, as indicated by an increased degree of postural sway, the more the patients integrate and reweight one of these two additional sensory modalities.

Thus, the correlation between the benefit of vision and auditory cues also indicates that a similar mechanism might underlie the integration of vision and auditory cues. This supports the hypothesis that indeed sensory reweighting might be the mechanism underlying the effects observed here (although other functional mechanisms might also add to the improvements observed here, e.g. cognitive alert mechanisms based on the auditory signal; for a discussion of sensor augmentation mechanisms see 10). Our results moreover show that cerebellar patients can use auditory cues and vision not only *in substitution*, but also *in combination* to yield a more stable postural control. In the EO condition, the $ABF_{>66\%}$ subgroup showed a benefit from both types of sensory information, namely visual information (*TestABF* EO vs. *TestABF* EC:$P = 0.03$) and ABF (TestABF vs. PostABF in EO:$P = 0.02$, Fig. 3G). These results suggest that even these patients with pronounced impairments in postural control are capable to exploit the integration of both sensory signals.

These findings substantially extend the existing classical clinical observation that patients with sensory ataxia (e.g. Friedreich's Ataxia) - and partly also with cerebellar ataxia - profit from *visual* information in the Romberg test.[21,26,42] Moreover, on the level of functional mechanisms, our results deliver additional pieces of evidence for the hypothesis that the process of sensory integration and reweighting is not necessarily dependent on the integrity of the cerebellum, thus corroborating findings from an earlier psychophysics study on the estimation of hand positions.[27]

Preserved sensory reweighting on a short time-scale

Our protocol used a short-term familiarization program of less than one hour, demonstrating that cerebellar patients are able to exploit sensory information and to perform sensor reweighting even on a rapid time scale. Such rapid reweighting might enable cerebellar patients to profit from ABF as a real-time assistive signal in everyday life, for example, when walking in rooms with mixed light zones and poor visibility which is known to facilitate falls.[43]

Limitations of the Study

Our short-term protocol does not allow to test for retention and carry-over of effects after removing ABF as a potential rehabilitation device, which would require longer multisession protocols (e.g., see 8). In addition, although we used a short exergaming period for familiarization, the focus of this study was not to examine the facilitation of training effects by sensor augmentation (for review, see 44). These limitations point to interesting directions for further research.

Conclusion and outlook

Our findings provide proof-of-principle evidence that – despite intricate cerebellar damage – patients with degenerative cerebellar ataxia still have a preserved capacity to exploit ABF as a real-time assistive signal to compensate for deficient postural control. In fact, they seem to be able to use auditory cues *functionally similar* to vision in the absence of vision, and *additive* to vision in case of pronounced postural sway. Future studies are warranted to transfer these proof-of-principle results to balance control also during walking and possibly also to other bio-feedback signals being more suitable for daily application (e.g. vibro-tactile feedback[7] or bone conduction).

Finally, follow-up studies testing the feasibility and effectiveness of sensory augmentation on walking and in longer

clinical trials are required to confirm the clinical effectiveness of this translational work, ideally performed in a multicenter health-care setting and utilizing additional patient reported and functional outcomes. These examinations might inform future assistive strategies for balance control in cerebellar patients.

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Author Contributions

Mrs Fleszar: design and conceptualization of the study, acquisition of data, analysis of the data, drafting the manuscript. Dr. Mellone and Dr. Tacconi: design, development, and implementation of ABF application used in the study, interpretation of the data, revising the manuscript. Dr. Giese, Dr. Schöls and Dr. Becker: interpretation of data, revising the manuscript. Dr. Synofzik and Dr. Ilg: design, conceptualization and supervision of the study, acquisition of data, analysis of the data, drafting the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Detailed patient characteristics.
Data S2. Details of audio biofeedback device.
Data S3 Statistics.
Data S4 Details of movement analysis.
Data S5 Relationship of Vibration Sensing on posture control capabilities.

Supplementary Information

S1 - Detailed patient characteristics

Table 1 **Detailed patient characteristics of the acoustic biofeedback group (ABF group).** Given are averages or mean values and standard deviations. Ataxia symptoms were clinically assessed using SARA [1]; ISCA: idiopathic sporadic cerebellar ataxia; ADCA: autosomal dominant ataxia; SCA 1-14: spinocerebellar ataxia type 1,2,3,6, 14; SYNE1: Autosomal recessive cerebellar ataxia type I, AOA2: Ataxia with oculomotor apraxia type 2. VIB: Vibration sense as determined by clinical assessment with a Rydel-Seiffer tuning fork (RSTF) [2]

Pat ID	Gender F/M	Diagnosis	Age, y	Disease Duration, y	SARA	VIB
ABF1	M	SYNE1	31	10	13	7/8
ABF2	M	ISCA	69	10	6	4/8
ABF3	F	SCA3	55	14	8.5	6/8
ABF4	M	ISCA	59	3	13.5	7/8
ABF5	M	ISCA	54	4	14	7/8
ABF6	M	ISCA	52	11	15.5	7/8
ABF7	M	ISCA	38	6	6.5	7/8
ABF8	M	ISCA	30	9	13	6/8
ABF9	M	AOA2	27	1	4.5	5/8
ABF10	F	SYNE1	22	4	9.5	5/8
ABF11	F	ISCA	74	10	12	3/8
ABF12	M	ISCA	48	6	11	5/8
ABF13	M	ADCA	47	34	10.5	5/8
ABF14	M	ADCA	45	25	14.5	5/8
ABF15	F	ISCA	55	14	8	6/8
ABF16	F	SCA6	56	20	15.5	7/8
ABF17	F	ISCA	66	6	8.5	5/8
ABF18	F	SCA2	57	19	15	8/8
ABF19	M	ADCA	53	13	12	5/8
ABF20	M	ADCA	54	24	12.5	4/8
ABF21	M	ISCA	73	5	9	3/8
ABF22	M	SCA1	69	9	9.5	4/8
ABF23	F	SCA28	43	33	10	7/8
	8/15		**51.2 (14.5)**	**13 (9.2)**	**11 (3.1)**	**5.6(1.4)/8**

Table 2 **Detailed patient characteristics of control group (CON group).** Ataxia symptoms were clinically assessed using SARA [1]; ISCA: idiopathic sporadic cerebellar ataxia; ADCA: autosomal dominant ataxia. SCA 1-14: spinocerebellar ataxia type 1,2,3,6, 14.SYNE1: Autosomal recessive cerebellar ataxia type I, AOA2: Ataxia with oculomotor apraxia type 2.

Pat ID	Gender F/M	Diagnosis	Age, y	Disease Duration, y	SARA
CON1	F	ISCA	50	15	11
CON2	F	SCA6	63	15	7
CON3	M	ISCA	61	10	9.5
CON4	M	ISCA	50	11	9
CON5	F	ISCA	63	1	7.5
CON6	F	ISCA	68	2	5.5
CON7	M	ISCA	70	2	7.5
CON8	M	SCA1	41	10	4
CON9	M	ADCA	46	11	8
CON10	M	ISCA	48	12	14
CON11	M	SYNE1	33	12	14.5
CON12	F	SCA3	42	7	16.5
CON13	M	ISCA	47	3	8.5
CON14	M	ADCA	73	18	10.5
CON15	M	SCA14	58	23	12
CON16	F	SCA6	53	2	9.5
CON17	F	ADCA	57	6	13
	7/10		**54.5 (11.5)**	**9.4 (6.3)**	**9.9 (3.3)**

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Supplementary Information

S2-Audio biofeedback device

The wearable ABF system[1] consists of two main components: (i) an inertial sensor node (EXL-s1 by EXEL, Bologna, Italy) capturing trunk accelerations based on a 3D-accelerometer, -gyroscope, and -magnetometer, and (ii) a smartphone (Galaxy SIII by Samsung, Seoul)-based application receiving this trunk acceleration information via Bluetooth™ 2.1 connection.The sensor unit was attached on subjects' lower back at L5 near the center of body mass.

Signals are sampled at 100Hz. The smartphone application processes the information sent by the sensor unit and modulates a continuous stereo sound in pitch, volume and balance, mapping the acceleration and bi-dimensional position (which is estimated based on the acceleration) of the user. The sound (sampling frequency 22050 Hz) was provided to the subjects via headphones (511 by AKG Acoustics, Vienna). Subjects were instructed to maintain a stereo, low-volume (9% of the volume range) and pure tone (f=400 Hz) with perfect balance (50% right and left earphone, respectively) indicating that their postural sway was within a stable reference region. The reference region is considered to represent an area in which subtle sway and small accelerations permanently appear, even in healthy individuals [1]. These reference regions were determined on an individual basis before the experiment with subjects standing 20 seconds with feet in a comfortable distance without relevant sway.

When subjects exceeded the reference region during the experimental trials, the volume was modulated by means of a sigmoid function, while frequency modulation following a linear law. Accelerations in medio-lateral direction lead to an increased volume in the corresponding headphone side (up to 100% of the volume range) while decreasing in the opposite side (down to 0%). Excessive sway in anterior-posterior

direction increased volume equally for both headphones sides (up to 100% of the volume range). Outside the reference region frequency decreased down to f=150 Hz when subjects leaned backwards and increased up to f=1000 Hz when subjects leaned forwards.

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Supplementary Information

S3-Statistics

Statistical Methods

Repeated measurement analyses were performed using the non-parametric Friedman test (χ^2,p-values) to determine *within-group differences* in sway between stance trials at *PreABF* , *Training I*, *TestABF* and *PostABF*. When the Friedman test yielded a significant effect ($p<0.1$), post hoc analysis was p erformed using a Wilcoxon signed-rank test (p-values) for pair-wise comparisons between stance trials. Effect sizes of the Wilcoxon signed-rank test are given by r-values, which can be calculated for subject groups $n>15$. *Between-group* differences (ABF group vs. CON group) in postural sway were determined by the non-parametric Kruskal-Wallis-test. When the Kruskal-Wallis-test yielded a significant effect ($p<0.1$), post-hoc analysis was performed using a Mann-Whitney U-test for comparison between groups. Spearman rho was used to examine the correlation between the benefit of ABF (i.e. the difference in postural sway *TestABF - PostABF*) with the amount of postural sway prior to training (*PreABF*).

Statistical analysis without pooling

In order to confirm the results of analysis of the pooled data, we also performed analyses without pooling stance trails over phases, Instead, the last trials of the *Training I* phase (i.e. the fourth trial of *Training I*) and of the *TestABF* phase (i.e. the second trial of *TestABF*) were taken for statistical analysis.

<u>Eyes closed condition (EC)</u>

- Differences in body sway for the ABF group were found across phases (Friedman-test, $X^2=9.3$, $p=0.025$).

- Post-hoc analysis showed a significant reduction of postural sway in ***TestABF* phase** compared with ***PreABF*** phase (***TestABF*** versus ***PreABF***: p=0.03).
- In the *PostABF* phase without ABF, trunk sway in the EC condition significantly increased again compared to *TestABF* (*TestABF* vs. *PostABF*: p = 0.005),

Eyes open condition (EO)

In consistency with the analysis of the pooled data, single trial analysis revealed no with-in group difference in postural comparing trials with and without ABF for the whole group (Friedmann Test p=0.43).

Subgroup analysis of the upper tertile of patients with the largest postural sway at baseline ($ABF_{>66\%}$ subgroup, see Methods) shows a reduced postural sway in *TestABF* compared to *PreABF* and *PostABF*, respectively (Figure 4B) (Friedman-test, X^2=8.85, p=0.03, *PreABF* vs. *TestABF*: p = 0.023, *TestABF* vs. *PostABF*: p = 0.007), indicating that these patients profit significantly from ABF even in the EO condition.

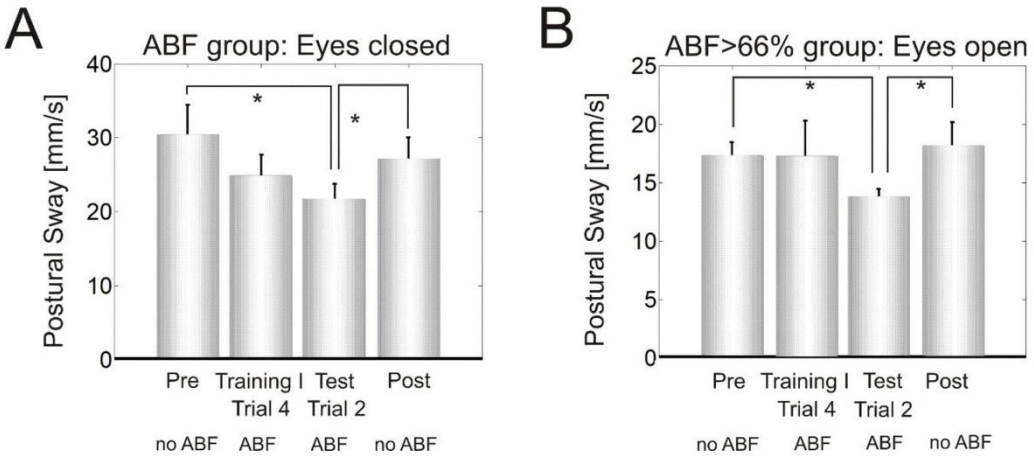

Figure 1 : Group results of the ABF group from movement analysis quantifying body sway during Romberg stance (RB) presenting an analysis of single trials from the respective condition without pooling. The four bars indicate the consecutive experimental phases: *PreABF*, *Training I*, *TestABF* and *PostABF* comparing trials with ABF (ABF) and without ABF (no ABF). In the Training I and the *TestABF* phase, only the last trials were analyzed, i.e. only

the 4th trial in Training I and only the 2nd trial in *TestABF*. A: Group results of AFB group in the eyes closed condition. B: Group results of the $ABF_{>66\%}$ subgroup in the eyes open condition. Stars indicate significant differences (*:$p<0.05$) between different phases.

Supplementary Information

S4 Movement Analysis

Motor performance was determined by quantitative movement analysis using a VICON MX motion capture system. The three-dimensional movement trajectories of the subjects were recorded at a sampling rate of 120Hz. The marker trajectories were pre-processed using the commercial software provided by VICON. This software fits a clinically evaluated kinematic model to the marker trajectories and extracts velocities, joint angles, and the course of the centre of mass (CoM).

Stance tasks: In stance all conditions, subjects had to stand for 30 seconds in Romberg position with their feet tightly together and arms outstretched 90° in front of their body. We determined body sway by measuring the path length of the centre of gravity.

In order analyze this measure also in comparison with other common sway measures, we compared it with the commonly used measure elliptical area fits[1]. Figure S4 shows the comparison of both sway measures. The analysis demonstrates a very strong correlation between our path-length based measure with this other standard measure (r =0.8779, p = 2.9028e-38), thus demonstrating that our measure is well compatible with other standard measures in the field. We now added a diagram to the Supplement S4 showing both measures for 230 stance trials with open and closes eyes, as well as with and without ABF.

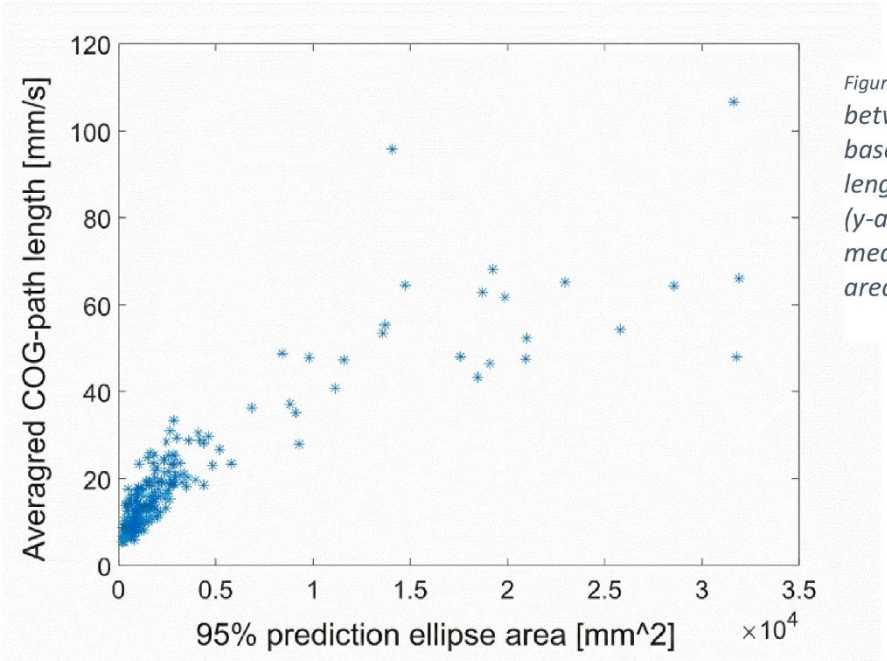

Figure S4 Comparison between sway measures based on the path length of the COG-path (y-axes) and the measure of elliptical area fits (x-axes).

References

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Supplementary Information

S5 Relationship of Vibration Sensing on posture control capabilities

We examined the influence of impairments in proprioception on (i) our stance tasks and (ii) the benefits of ABF. Subjects who received ABF were assessed by quantitative vibration testing by a Rydel-Seiffer tuning fork (scale 0-8)[2] to determine the degree of possible vibration sense impairments and their relation to ABF effects.

No correlations were observed between the results of the vibration test and the postural sway in either of the two conditions (eyes open and closed). Neither did we find a significant group difference in vibration test results between the two subgroups with $ABF_{>66\%}$ (large body sway) and $ABF_{<66\%}$ in the EO condition.

Baseline performance

No correlation between vibration sense and body sway in EO ($r= -0.1$, $p= 0.64$)

No correlation between vibration sense and body sway in EC ($r= -0.16$, $p=0.46$)

Benefits from vision

No correlation between vibration sense and benefits from vision ($r=-0.12$, $p=0.56$)

Benefits from ABF in EC and EO

No correlation between vibration sense and benefits from ABF in EC ($r=-0.09$, $p=0.66$)

No correlation between vibration sense and benefits from ABF in EO ($r=-0.13$, $p=0.52$)

Examining of vibration sense in subgroups $ABF_{<66\%}$ (small body sway) vs. $ABF_{>66\%}$ (large body sway)

No group difference ($p=0.57$) between $ABF_{<66\%}$ (vibration sense: 5.8±1.7) and $ABF_{>66\%}$ (vibration sense: 5.5±1.3)

At least from these results, no obvious influence of the vibration sense on the benefit from ABF can be concluded. However, one has to keep in mind that this single clinical standard test of vibration is still very crude in itself. More dedicated studies specifically designed to test this question are needed to fully address this question, using a more fine-grained and comprehensive test battery to fully assess functioning of the peripheral (and central dorsal column) sensory system, and relate it to benefits from augmented sensory feedback.

3 Discussion

3.1 Movement changes in preclinical spinocerebellar ataxia

3.1.1 Detection of subclinical motor changes in spinocerebellar ataxia before clinical disease onset

The success of future treatment trials in spinocerebellar ataxias will largely depend on their conduct in well-defined preataxic stages and on the availability of valid, reliable biomarkers that are sensitive enough to capture the presence, progression and potential reversibility of earliest disease-specific dysfunctions. In this study we focused on the detection of motor symptoms, since these are the unifying features across all SCAs and underly the severity grading of manifest disease. In accordance with previously reported onset symptoms, we systematically investigated posture and gait in preataxic mutation carriers of the most common SCA types, namely SCA1, SCA2, SCA3 and SCA6, as well as affected subjects with manifest SCA at early stages and age- and gender-matched healthy controls. Spatiotemporal motor features were used to analyse movement patterns during walking and Romberg stance tasks of increasing balance demand. Our findings demonstrate increased body sway in preataxic mutation carriers under different Romberg conditions compared to healthy controls. This is in line with the results of a study investigating postural stability in preclinical SCA2 mutation carriers using stabilometry.[94] The authors reported higher oscillation frequencies in the mutation carriers for the standing conditions ‘feet together’ and ‘tandem’ position compared to healthy controls. Abnormalities were detected in a range of up to 15 years to estimated disease onset, indicating postural instability to be one of the earliest detectable motor dysfunctions in SCA2.

In our work, we also detected differences in spatiotemporal variability measures between healthy controls and preataxic mutation carriers during tandem walking (both on firm surface and on a mattress), but not during straight walking at preferred pace. A study by Rochester and colleagues, on the contrary, reported abnormalities during straight walking in preclinical SCA6 mutation carriers.[70] However, an overall higher prevalence of clinically detectable gait ataxia

symptoms in their enrolled mutation carriers might explain these contrasting results.

Studies on movement analysis in preataxic SCA mutation carriers face inherent challenges in defining the threshold between preataxic and manifest ataxia stages.[70, 94, 95, 126] More recent studies have agreed on the validated cut-off value of 3 on the SARA scale to determine clinically present ataxia. Consequently, mutation carriers are often classified as 'preclinical' or 'presymptomatic', although showing mild abnormalities on clinical examination that result in SARA scores below 3. In fact, some studies reported significant differences in SARA scores between healthy controls and preataxic mutation carriers, such as our own study.[9, 94] However, not all studies disclosed this information and some did not clinically examine healthy controls, leaving it unanswered whether controls and 'preclinical' mutation carriers were distinguishable by clinical assessments.[70, 95] A promising aspect of quantitative movement analysis, nevertheless, lies in its application during disease stages when clinical scales fail to detect any abnormalities. To test for this notion, we performed a sub-group analysis for mutation carriers who were completely normal on the posture and gait subitems of the SARA score ($MC^{SARAp\&g=0}$). Compared to an adjusted healthy control group which consisted of mutation-negative family members of SCA patients (nMC), the $MC^{SARAp\&g=0}$ group differed in body sway and spatiotemporal features for the most challenging motor tasks (Romberg stance with eyes closed on a mattress and tandem walking on firm surface and on a mattress). Furthermore, our multivariate analysis allowed to increase the discriminatory power between $MC^{SARAp\&g=0}$ and nMC for selected motor features whilst controlling for age. Using logistic regression, we computed a classifier fed by this feature set that discriminated healthy controls and mutation carriers up to 10 years away from estimated disease onset with a 100% sensitivity. This is of specific interest, since outcome measures serving in treatment trials potentially applied at preataxic disease stages need to be sensitive enough to detect changes not only on a group level, but also on a single-subject level. Additionally, such a classifier might also be of particular use

for the stratification of patient eligibility, as trials will face challenges in defining the optimal point of treatment initiation.

Future work will be required to reproduce our findings and possibly detect motor changes at even earlier stages. In accordance with our results and studies investigating movement changes in preclinical subjects of other neurodegenerative conditions, e.g. Parkinson's disease or Fragile X-associated tremor-ataxia syndrome, complex motor tasks will be most promising in unravelling earliest movement changes.[127, 128, 129] Such tasks may include more dynamic assessments, for example turning or gait termination which have been found to be deficient in cerebellar patients.[130] Walking may be analysed under different speed conditions, e.g. on a treadmill. In fact, there is a strong body of evidence suggesting that cerebellar patients critically depend on self-selected gait velocity in order to minimize variability features and consequently increase stability.[62, 131] Analyses of upper body metrics during gait might reveal additional abnormalities in trunk variables. Manifest cerebellar patients have been shown to exhibit abnormally extensive trunk and head oscillations during gait that correlated with disease severity and variability measures.[75] Furthermore, higher spatiotemporal variabilities in trunk-thigh coordination were reported and possibly present a contributing factor to early gait abnormalities in preataxic individuals.[79]

3.1.2 Quantification of the preclinical course of spinocerebellar ataxia

To determine the sensitivity of our motor features to change across the preataxic course, we performed correlation analyses with the estimated time to onset. These showed significant results for body sway during Romberg stance on a mattress with closed eyes and step length variability in tandem walking on a mattress, indicating a potential suitability of these motor features as progression markers during the preataxic stage. The latter showed a particularly steep progression rate, confirming the notion that complex tasks are more sensitive to change in preataxic mutation carriers. Although mathematical estimations of the time to onset provide a useful tool to interpret detected dysfunctions in preataxic stages, conclusions need to be drawn with caution. It

has been demonstrated that the number of CAG repeats of the pathogenic allele which underlies the most commonly used prediction model for the age of onset, only accounts for about 60% of its variance.[25, 132] It is believed that a wide range of familial, population-specific and environmental factors act as modifiers of the age of onset, such as other (CAG)n-containing genes.[133-135] To take family-specific factors into consideration, we calculated an adjustment to the CAG-based estimated time to onset. This calculation was based on a corrective difference between the predicted and actual age of onset of the affected parent. Our adjusted prediction model yielded more plausible time to onset values for several of our mutation carriers, especially those that already showed detectable abnormalities on the SARA score. Nevertheless, our estimation model warrants further prospective longitudinal studies in larger SCA populations in order to be validated.

Longitudinal studies will also be needed to determine the actual sensitivity to change over time of our motor features. The only quantitative study to include longitudinal data during the preataxic course has been performed by Nanetti and colleagues.[95] Stance stability was measured in 'preclinical' SCA1 mutation carriers during a longitudinal 4 year follow up design with study visits at baseline, after 2 and 4 years. For SCA1 carriers with a range of up to 7 years to onset, postural instability differed significantly from controls for the most challenging stance conditions. It gradually increased over the study course, yielding statistical significance at the 4-year follow up visit compared to baseline performance. Although these results appear promising, it needs to be taken into account that several of the included subjects scored ≥3 on the SARA over the course of the follow up period and therefore, strictly speaking, did not qualify as 'preclinical'. Considering the small sample size, these subjects certainly drove the increased degree of postural instability on a group level and it remains unclear whether the 'actual' preataxic participants experienced progressive changes in the reported stability index. This addresses a fundamental limitation of our own study: while some of our movement variables correlated with estimated time to disease onset, their true responsiveness to change over time still needs to be established using prospective longitudinal study designs. Apart

from sufficient sample sizes necessary for such studies, challenges will lie in the translation of progression markers based on natural history studies to outcome measures capturing effects of treatment trials. In fact, there may be divergence in the sensitivities of different movement variables to natural decline and treatment. This may particularly hold true, if interventions aim to boost compensatory mechanisms, such as rehabilitation trials. Another limitation may lie in a non-linear progression of movement changes during the preataxic phase, as indicated in manifest SCA2 by non-constant clinical progression.[136] Further psychometric properties of our motor features would need to be investigated, such as validity and reliability. Inter-session reliability, for example, is crucial to ensure that observed changes are indeed due to natural decline or treatment and not caused by day-to-day variability. Facing the scarcity of spinocerebellar ataxias, and particularly, individuals at risk for SCAs, it is desirable for effect sizes of future outcome measures to be as large as possible to minimize necessary sample sizes in placebo-controlled treatment trials. Movement variables of gait and stance assessments alone may not provide sufficient statistical power in preataxic stages. Therefore, composite measures incorporating additional tasks of different functional domains need to be explored to reach satisfying effect size calculations. Such tests could include quantitative measures of ocular movements or motor adaptation tasks, as these have been repeatedly shown to be abnormal in preataxic mutation carriers of different SCA types.[23, 38, 126, 137, 138] It also remains to be elucidated whether assessments of other modalities, such as MRI, electrophysiological studies or fluid biomarkers, will provide potential candidates for such composite measures.

A further limitation of our study includes the analysis of preataxic mutation carriers across different SCA types, namely SCA1, SCA2, SCA3, and SCA6. Although cerebellar degeneration and, consequently, ataxia represent a unifying feature, these conditions are distinct in their molecular and pathophysiological signatures that affect different collateral brain regions. The involvement of other parts of the central nervous system ultimately affects movement patterns, e.g. an impairment of the pyramidal tract results in increased muscle tone and spastic movement features. Due to small sampling, an isolated analysis of each

preataxic SCA type was not possible, but this warrants further investigations in the future.

3.1.3 Conclusion

In our exploratory, cross-sectional study, we provide evidence for the notion that motor features of complex stance and gait tasks are able to discriminate between healthy controls and preataxic SCA mutation carriers, even in absence of clinically detectable abnormalities and on a single-subject level. Furthermore, our correlation analyses demonstrate that detected movement changes increase with proximity to estimated disease onset, thus, enabling a quantification of disease progression in the preataxic stage of the most common SCA types. Further studies, including longitudinal data, are necessary to establish the psychometric properties of these motor features prior to their application as outcome measures in intervention trials.

3.2 The benefit of audio-biofeedback in cerebellar ataxia

3.2.1 Audio-biofeedback-induced effects on postural sway in cerebellar ataxia

In our controlled intervention study, we show that patients with cerebellar degeneration can benefit from augmented audio-biofeedback (ABF) of trunk accelerations in postural control. Using quantitative movement analysis, we observed that the ABF group exhibited significantly reduced postural sway when receiving ABF in the eyes closed (EC) condition after the short ABF training phase. By contrast, our disease-control group did not show any differences between stance trials for both conditions. Based on the discrepancy between our ABF group and control group, we can verify that the observed reduction in postural sway was not merely due to ABF-unrelated factors, such as task repetition resulting in motor learning. This contrasts the design of most studies investigating the impact of augmented bio-feedback in neurological patients, as they did not include disease-control groups.[139-143] Our analysis revealed that the extent of postural sway at baseline (*PreABF*) was significantly

associated with the reduction of sway under ABF after the training phase (*TestABF)*. This correlation proved to be statistically significant for both eyes open (EO) and eyes closed (EC) conditions, indicating that deficient balance control is the driving factor behind the exploitation of the ABF signal. Indeed, our subgroup analysis demonstrated that the patients with the greatest sway at *PreABF* improved even under the EO condition in the *TestABF* trial. Contrary, milder affected subjects did not noticeably benefit from ABF during the EO condition at any point, suggesting that for these patients, vision presents a sufficiently strong cue to maintain balance.

Both ABF and control groups benefitted from vision during standing, as indicated by reduced body sway in the EO conditions compared to the EC conditions. Similar to the ABF conditions, the benefit of visual information on postural sway correlated with the extent of sway in the EC condition at baseline. Furthermore, the benefits of both sensory modalities correlated with each other, i.e. the more subjects improved their body sway by vision, the more they benefitted from ABF. Interestingly, the degrees of sway reduction induced by ABF and vision in the same individual were very similar. An explanation for this observation points to a similar mechanism by which visual and auditory information are integrated for postural control (see 3.2.2).

3.2.2 Preserved integration of augmented sensory information in cerebellar ataxia

Our results demonstrate that those patients who depended most on visual information to maintain balance, were more likely to exploit the augmented sensory channel (ABF) when vision became unavailable. In addition to this association between the induced benefits of vision and ABF, both sensory gains correlated with the extent of postural sway at baseline. This led to our assumption that those patients who are more severely affected by stance imbalance i) depend more on vision to maintain balance, ii) are more willing to rely on ABF *in the absence* of vision, and iii) are able to exploit ABF *in addition* to vision to optimize postural control. Thus, depending on the degree of postural instability (i.e. cerebellar dysfunction), ABF not only functions as a sensory

substitution when vision becomes unavailable, but also as a *supplementation* to vision. Models of postural control indicate that the integrity of sensory information (particularly proprioceptive, vestibular and visual) and its processing in the CNS play a crucial role for balance maintenance.[120, 144] The role of the cerebellum in such perceptual processes has only recently gained scientific interest with subsequent conceptualization attempts. Nonetheless, a growing body of evidence suggests that the cerebellum is involved in processing and integrating multimodal sensory information, possibly to generate body estimations for predictive motor control (on the role of the cerebellum in predictive motor control, see chapter 1.5). Supportive of this notion is, inter alia, a study by Brooks et Cullen which showed that proprioceptive and vestibular information is relayed to single cerebellar neurons.[104] It has been suggested that cerebellar feedforward control not only relates to movements, but also to sensory consequences of a motor command. Psychophysical studies demonstrated that perception, e.g. proprioception or force perception, depends on the integrity of the cerebellum during active but not passive limb movements.[109, 145] An explanation for this observation is that the acuity of perception during self-induced movements is enhanced by internal predictions. Simultaneously, these experiments suggest that the cerebellum might be less important for feedback-guided motor control. In fact, reactive movements based on feedback control appear to be more normal in cerebellar patients than movements that require predictions, such as adaptations to novel conditions. This theory has been experimentally supported in various studies involving different motor effector levels, mainly during arm reaching and standing tasks, but also in speech.[146-148]

The hypothesis that an increased reliance on sensory feedback might present a compensation strategy for cerebellar patients, is also supported by our data. The fact that our patients in both ABF and control group showed significantly increased body sway in eyes closed compared to eyes open conditions, points to an enhanced reliance on vision for postural control. But why do cerebellar patients rely more on vision? A reason might be that the processing of proprioceptive and vestibular signals for balance control relies more on an intact

cerebellum than in the case of visual or auditory cues. Our correlation analysis supports this notion, as it revealed that the greater patients swayed at baseline, i.e. the more deficient their processing of vestibular and proprioceptive cues was, the more they relied on vision and ABF during standing. This is in line with a previous study by Bunn and colleagues who demonstrated that patients with SCA6 seem to be particularly sensitive to visual distortions during balance control compared to proprioceptive or vestibular stimulation.[149] The visually induced balance perturbations correlated with disease severity, indicating an increasing gain of the visual channel during disease progression. The notion that visual motor control is less reliant on cerebellar activity is further supported by the results of an fMRI study investigating brain activity during a tool-use motor task.[150] Participants were assessed during two different conditions in which the reliability of either visual or somatosensory feedback was selectively reduced. Functional brain imaging data showed that reduced visual reliability resulted in expanded cerebellar activity, whereas decreased somatosensory reliability was characterised by absent activity of the cerebellum, but increased frontal, parietal and temporo-occipital brain regions. The use of the ABF signal during motor control possibly activates similar brain regions, which would support the thesis that i) auditory and visual feedback are integrated similarly for postural control and that ii) this process may be cerebellum-independent. In fact, findings from an electroencephalogram (EEG) study indicate that ABF use during postural tasks increases cortical activation, especially in the temporo-parietal area during eyes closed conditions and in the temporo-occipital area when eyes are open.[151]

According to the principle of cue optimization, our cerebellar patients were able to upregulate the contribution of one sensory modality (here: vision and ABF) for motor control to compensate for the variance of another channel (here: proprioceptive and vestibular cues). Furthermore, our patients were able to reweight the contributions of vision and ABF to a surprisingly similar extent, as indicated by comparable effects of vision and ABF on body sway. This indicates that the process of sensory reweighting may occur independently of cerebellar integrity. In fact, it has been previously proposed that it rather relies on other

brain regions, particularly the posterior parietal cortex that is known for multimodal integration.[152, 153] Although this notion has been mainly studied for arm reaching tasks, there is evidence that cerebellar patients can shift their relative sensory reliance during balance control.[123]

While sensory reweighting represents a plausible explanation for our results, it needs to be pointed out that the fundamental functionality of biofeedback systems is controversially discussed in the literature.[154] One theory, for example, states that increased cognitive attention may underly improved motor performance during feedback conditions. Sham trials, in which erroneous or meaningless 'feedback' information is augmented, might be useful to further explore this hypothesis.

3.2.3 Outlook on future assistive strategies

To our knowledge, this is the first study providing proof-of-principle evidence for the notion that patients with degenerative cerebellar ataxia are able to exploit real-time augmented auditory bio-feedback to improve postural stability. Investigations on effects induced by augmented sensory input or bio-feedback are scarce in cerebellar patients and lack the necessary study designs to allow for meaningful conclusions.[143, 155, 156] One study investigated the effects of tongue electrotactile biofeedback of head motion on postural control in patients with degenerative cerebellar ataxia during a two-week rehabilitation program.[143] Although the feedback device seemed promising due to its practicality in daily life and reductions in postural sway were observed after the rehabilitation program, it is not possible to attribute the benefits to the biofeedback, given the absence of a control group. Instead, the observed effects may be merely a result of the high-intensity training. Interestingly, sway reduction was seen only in the eyes closed condition, but not during the eyes open task. However, pure sensory reweighting cannot explain these results, as benefits were still present after a 4-week retention phase. This points to a more likely mechanism by which the application of the electrotactile feedback boosted the exploitation of compensatory strategies when vision was absent. Similarly, two other studies tested the effects of a rehabilitation program combined with non-invasive focal

mechanical vibrations (NIFMV) in an adult and infant patient group with degenerative ataxia.[155, 156] NIFMV has been vaguely hypothesized to facilitate neuroplasticity and ultimately improve movement patterns by increasing proprioceptive input to the CNS. However, the induced muscle vibrations are not linked to a meaningful biological process (such as body movement) and instead, result in a constant stimulation of a sensory channel (here: proprioception). Effects were analysed on a variety of functional scores, such as the SARA, and spatiotemporal gait parameters. Although some benefits were reported, conclusions about the efficacy of the NIFMV device cannot be drawn due to the lack of control groups. Despite their methodological flaws, these studies point to the potential application of feedback devices in ameliorating rehabilitation benefits. As the short-term design of our study did expectedly not result in any retention effects, the notion that ABF potentially facilitates postural training warrants placebo-controlled multi-session trials in the future. Our ABF device was designed and tested only for static standing. More dynamic assessments that include walking and reflect more realistic daily life scenarios, require feedback devices that are able to capture and translate far more complex movement patterns into meaningful signals. Such feedback systems would need to minimize their interference with inherent senses, especially vision and hearing, when applied in real-life. Vibrotactile feedback, for example, represents a promising candidate for walking conditions and should be further explored in cerebellar patients for its benefits.[157]

3.2.4 Conclusion

In this controlled study we demonstrated that the exploitation of augmented auditory bio-feedback of trunk accelerations can improve postural control in patients with degenerative cerebellar ataxia. ABF-induced reduction of body sway i) correlated with the severity of postural instability at baseline, ii) occurred *in the absence* of vision, and iii) in most affected participants also *in addition* to vision. Patients relied on ABF to a functionally similar extent as on vision, indicating a cerebellar-independent underlying sensory integration process for visual and auditory cues in postural control. Further research is necessary to

test the benefit of feedback devices in more complex conditions, such as walking and in rehabilitation.

4 Summary

Degenerative ataxias are a heterogenous group of movement disorders defined by progressive ataxia due to a degeneration of the cerebellum and its associated tracts, often of genetic origin. Disease-modifying drugs are still lacking for degenerative ataxias, thus highlighting the need for paving the way for both interventional drug trials and for innovative neurorehabilitation approaches. Detailed quantitative movement analysis might hereby help to detect and grade cerebellar dysfunction, possibly even at the preataxic stages of the disease, thus helping to chart a promising window for early treatment interventions before clinical disease onset. Moreover, it might help to gain new insights into the functional role of the cerebellum in motor control and related sensory integration mechanisms, which might be used to inform future neurorehabilitation strategies. Correspondingly, we here hypothesized (1) that quantitative movement analysis allows to reveal early movement changes when clinical signs are still absent and to capture motor progression in subjects at the preataxic stage of spinocerebellar ataxia (SCA) (study #1). Moreover, we hypothesized (2) that quantitative movement analysis allows to identify the effects of a biofeedback intervention in patients with degenerative ataxia, where they might be able to exploit real-time acoustic bio-feedback signals (ABF) of trunk acceleration to compensate for impaired postural control (study #2).

Study 1: 46 participants (14 preataxic SCA mutation carriers [SCAs 1,2,3,6], 9 SCA patients at an early symptomatic stage; and 23 healthy controls) were analysed by quantitative movement analysis during stance and walking tasks of increasing complexity. We identified motor features that (i) differentiated between preataxic mutation carriers and healthy controls, even in absence of clinical signs and (ii) correlated with repeat expansion-based estimated time to disease onset. These results demonstrate that quantitative movement analysis in combination with tasks of rising difficulty levels allows to detect subclinical motor changes in spinocerebellar ataxia before clinical manifestation, which may enable the quantification of disease progression in the preclinical phase.

Study 2: Quantitative movement analysis was used to investigate the effects on postural sway during stance in a short-term ABF intervention group versus a no-ABF disease control group (23 and 17 cerebellar patients, respectively). Postural sway under the conditions ‘eyes open’ and ‘eyes closed’ was measured prior to ABF, under ABF, and post ABF. Our analysis revealed a significant reduction of body sway under ABF in the ‘eyes closed’ condition. Patients who had the largest extent of postural sway at baseline even improved their stability in the ‘eyes open’ condition under ABF. Correlations were found between the degree of postural sway at baseline and the benefits of both ABF and vision, and moreover, between the benefits of both sensory modalities (i.e. ABF and vision). The no-ABF control group did not exhibit any changes in sway across stance trials. These results provide proof-of-principle evidence that despite cerebellar degeneration, patients are still able to improve dysfunctional postural control by integrating augmented sensory cues: In absence of vision, the reliance on added auditory cues can be exploited to a similar extent as vision. In case of strong postural sway, augmented auditory information can be exploited also in combination with vision. These findings indicate promising compensatory strategies of cerebellar patients to maintain balance and might inform future assistive approaches.

5 German Summary

Bei den degenerativen Ataxien handelt es sich um eine heterogene Gruppe von Bewegungsstörungen, die durch eine Degeneration des Kleinhirns und assoziierten Nervenbahnen hervorgerufen werden. Die Ursache ist oftmals genetischen Ursprungs. Da bislang keine effektiven Therapien verfügbar sind, besteht die dringende Notwendigkeit die erforderlichen Voraussetzungen für Medikamentenstudien und neuartige Rehabilitationsansätze zu schaffen. Quantitative Bewegungsanalysen können dabei helfen, zerebelläre Funktionsstörungen zu erkennen und nach ihrem Schweregrad einzustufen, möglicherweise sogar in prä-ataktischen Krankheitsstadien. Damit könnte der Behandlungszeitpunkt in Zukunft vor Manifestation der Erkrankung beginnen. Darüber hinaus ermöglichen quantitative Bewegungsanalysen neue Einblicke in

die funktionelle Rolle des Kleinhirns in motorischer Kontrolle, sowie in ihre zugrundliegenden sensorischen Integrationsmechanismen. Diese könnten für künftige innovative Rehabilitationsstrategien von Nutzen sein. Basierend auf diesen Annahmen haben wir die Hypothese aufgestellt, dass (1) quantitative Bewegungsanalyse in der Lage ist, frühe Bewegungsveränderungen vor klinischer Krankheitsmanifestation aufzudecken und das Fortschreiten der motorischen Veränderungen in Personen zu erfassen, die sich im präataktischen Stadium der spinozerebellären Ataxien (SCA) befinden (Studie #1). Des Weiteren haben wir vermutet, dass (2) quantitative Bewegungsanalyse ermöglicht die Effekte einer Audio-Bio-Feedback (ABF) Intervention zu erfassen, bei der Patienten mit degenerativen Ataxien möglicherweise ein akustisches Signal nutzen können, das in Echtzeit Beschleunigungen des Körperstamms rückmeldet, um ihre fehlerhafte posturale Kontrolle zu kompensieren (Studie #2).

Studie 1: 46 Teilnehmer (14 präataktische SCA Mutationsträger [SCA1, 2, 3, 6], 9 SCA Patienten, die sich in einem frühen Krankheitsstadium befanden, und 23 gesunde Kontrollen) wurden mittels quantitativer Bewegungsanalyse während Stand- und Ganguntersuchungen von zunehmendem Schwierigkeitsgrad analysiert. Wir konnten Bewegungsmaße identifizieren, die (i) sogar in Abwesenheit von klinischen Zeichen einer Ataxie zwischen präataktischen Mutationsträgern und gesunden Kontrollen unterschieden und (ii) mit der Anzahl der Jahre bis zum geschätzten Krankheitsbeginn korrelierten. Diese Ergebnisse zeigen, dass die quantitative Analyse von Bewegungen während motorischer Aufgaben mit steigender Komplexität, subklinische Bewegungsauffälligkeiten vor Krankheitseintritt in spinozerebellären Ataxien erfassen kann. Künftig wird dies möglicherweise zu einer Quantifizierung der präataktischen Krankheitsprozesse bei SCAs beitragen.

Studie 2: Mittels quantitativer Bewegungsanalyse wurde der Einfluss von kurzzeitig eingesetztem ABF auf posturales Schwanken in einer Interventionsgruppe bestehend aus zerebellären Patienten untersucht und mit einer ‚Nicht-ABF', Gruppe verglichen (jeweils 23 und 17 zerebelläre Patienten).

Körperschwankungen wurden unter den Bedingungen ‚Augen offen' und ‚Augen geschlossen' vor, während und nach dem Einsatz von ABF gemessen. In unserer Auswertung konnten wir eine Reduktion der Körperschwankungen für die Bedingung ‚Augen geschlossen' während der ABF Applizierung entdecken. Diejenigen Patienten, die das größte Schwanken in der Ausgangsuntersuchung aufwiesen, profitierten von ABF sogar in der ‚Augen offen' Bedingung. Der Nutzen von ABF korrelierte mit dem Nutzen von Sicht, und die Schwankungsverringerungen, die durch beide sensorische Modalitäten induziert wurden, korrelierten mit dem Ausmaß von posturaler Instabilität in der Ausganguntersuchung. Die ‚Nicht-ABF' Kontrollgruppe zeigte keine Veränderungen im Schwankungsausmaß über alle Stehuntersuchungen hinweg. Diese Studie liefert einen Grundsatzbeweis dafür, dass Patienten trotz zerebellärer Degeneration in der Lage sind, ein künstlich hinzugefügtes sensorisches Signal zu integrieren, um ihre mangelhafte Standkontrolle zu verbessern. In der Abwesenheit von visuellem Input kann auf das neue auditive Signal zu einem funktionell ähnlichen Ausmaß wie bei Sicht zurückgegriffen werden. Bei ausgeprägterer posturaler Instabilität kann das auditive Signal sogar in Kombination mit visueller Information ausgenutzt werden. Diese Ergebnisse weisen auf vielversprechende Kompensationsstrategien von zerebellären Patienten bei der Erhaltung von posturaler Kontrolle hin und könnten zur Entwicklung von künftigen Hilfsmaßnahmen genutzt werden.

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7 Statement on contributions

7.1 Publication 2.1.2

Zofia Fleszar contributed to the conceptualization, design and organization of the study and to the recruitment of the study participants. She was solely responsible for patient assessment, the acquisition and first processing of the clinical and movement analysis raw data, and contributed to the statistical analysis of the data. Furthermore, she critically revised the manuscript. Winfried Ilg and Matthis Synofzik supervised the design and execution of the study and provided the theoretical study concepts. Winfried Ilg performed the primary statistical analysis of the data and wrote the first draft of the manuscript. Matthis Synofzik led the recruitment of the subjects for study inclusion, contributed to the acquisition and interpretation of the data, and wrote the first draft of the manuscript together with Winfried Ilg. Cornelia Schatton helped with the acquisition of the movement analysis data, whereas Holger Hengel contributed to the acquisition of the clinical data. Florian Harmuth and Peter Bauer were responsible for the genetic analysis of study participants at risk for SCA mutations and reviewed the manuscript. Dagmar Timmann contributed patients and reviewed both the data and the manuscript. Martin Giese contributed to the organization of the study, reviewed the data and the manuscript. Ludger Schöls provided critical thoughts on the conceptualization of the study, interpretation of the data and the manuscript.

7.2 Publication 2.2.2

Zofia Fleszar planned the design and organization of the study and contributed to its underlying conceptual framework. Furthermore, she was solely responsible for the assessment of the patients, and the acquisition and first processing of the raw data. She contributed to patient recruitment, the statistical analysis and interpretation of the data and wrote the first draft of the manuscript. Both Matthis Synofzik and Winfried Ilg supervised the conceptualization and organization of the study. Winfried Ilg performed advanced statistical analysis of the data, while Matthis Synofzik provided critical thoughts on interpretation of the data. Both Winfried Ilg and Matthis Synofzik drafted the final version of the

manuscript. Sabato Mellone and Carlo Tacconi designed, developed and implemented the ABF application that was used in the study and contributed the manuscript. Martin Giese, Ludger Schöls and Clemens Becker provided input to the interpretation of the data and revised the manuscript.

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Trying to make sense of the data during long hours in the motion lab and behind the desk not only required scientific advice, but also emotional support. I thank all my friends, flatmates and family members who patiently supported me in various ways and deserve a happy end to this journey.