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ORIGINAL COMMUNICATION

Validation of a clinical practice-based algorithm for the diagnosis of autosomal recessive cerebellar ataxias based on NGS identified cases

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Abstract Establishing a molecular diagnosis of autosomal recessive cerebellar ataxias (ARCA) is challenging due to phenotype and genotype heterogeneity. We report the validation of a previously published clinical practice-based algorithm to diagnose ARCA. Two assessors performed a blind analysis to determine the most probable mutated gene based on comprehensive clinical and paraclinical data, without knowing the molecular diagnosis of 23 patients diagnosed by targeted capture of 57 ataxia genes and high-throughput sequencing coming from a 145 patients series. The correct gene was predicted in 61 and 78 % of the cases by the two assessors, respectively. There was a high inter-rater agreement [$K = 0.85$ (0.55–0.98) $p < 0.001$] confirming the algorithm's reproducibility. Phenotyping patients with proper clinical examination, imaging, biochemical investigations and nerve conduction studies remain crucial for the guidance of molecular analysis and to interpret next generation sequencing results. The

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proposed algorithm should be helpful for diagnosing ARCA in clinical practice.

Keywords Recessive ataxia · Next generation sequencing · Neurogenetics · Electromyography

Introduction

Autosomal recessive cerebellar ataxias (ARCAs) are heterogeneous and complex inherited neurodegenerative disorders that affect the cerebellum, the spinal cord and the peripheral nerves. Molecular diagnosis of the ARCAs is challenging due to both phenotypic and genetic heterogeneity [1]. It was formerly a step by step approach with serial sequencing of several genes by the Sanger technique. The advent of affordable next generation sequencing (NGS) technologies allows now sequencing of exomes or large gene panels for diagnosis of rare neurological diseases such as ARCAs [2]. However, massive amount of data with multiple rare variants in several genes in a single patient increases the complexity of the analysis. A collaborative cross-talk between molecular geneticists and clinicians is even more necessary than before for NGS diagnosis validation in clinical practice. Age of onset, ataxia progression rate and careful clinical examination combined with laboratory, morphological and neurophysiological investigations [including vitamin E, alpha-fetoprotein (AFP), albumin, cholestanol, brain MRI, nerve conduction studies (NCS)] are useful for reaching a diagnostic conclusion. A new classification of ARCAs also has been established comprising 3 groups: ARCA with pure sensory neuropathy, ARCA with sensorimotor axonal neuropathy and ARCA without neuropathy. An algorithm for the diagnosis of ARCAs based on these items has been proposed by our group according to personal experience and literature data [3] (Fig. 1). We conducted a blind study to validate this clinical practice-based algorithm on a series of patients with molecular diagnosis of ARCA. These patients were part of a cohort of patients consecutively investigated with targeted capture sequencing of a panel of 57 ataxia genes.

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Patients and methods

Between 2010 and 2012, 145 unrelated index patients were recruited in 12 tertiary centers for movement disorders: 130 in France and 15 in Algeria. Inclusion criteria for the NGS analysis were the combination of: (1) progressive cerebellar ataxia; (2) age at onset before 60 years; (3) molecular analysis negative for Friedreich ataxia and other investigations depending on clinical assessment; (4) recessive inheritance or sporadic cases. Written informed consent was obtained from all participants and local ethics committee approved the study. Hundred and forty-five consecutive patients were analyzed by a targeted exon-capture strategy coupled with multiplexing and high-throughput sequencing of 57 genes causing ataxia when mutated (listed in supplementary file-A). Library preparation, targeted capture and sequencing were realized as previously reported [4]. NGS analysis is detailed in supplementary file-B [5].

Hundred and thirty-four patients were presenting ataxia starting before 40 years and 11 patients had late onset ataxia, starting after 40 years. Our cohort was mainly comprised of sporadic cases: 85 patients (59 %) had neither familial history of ataxia nor consanguinity. Fifty-four patients (37 %) had a recessive pedigree (2 or more affected in the kindred or isolated case with parental consanguinity). A molecular diagnosis was made in 27/145 patients (19 %) with mutations in ARCA genes. Among the 27 patients with ARCA molecular diagnosis, 4 were excluded due to the lack of available data. Molecular data of these 4 patients and clinical data of 118 patients without diagnosis are summarized in supplementary file C [6] and D, respectively.

We selected the remaining 23 ARCA patients with an established molecular diagnosis to assess the validity of the clinical practice-based algorithm. Two movement disorders specialists (MA, CT) performed independently a blind analysis based on the clinical (age at onset, current age, current disability based on scale of assessment and rating of ataxia (SARA) [7] score and/or spinocerebellar degeneration functional score (SDFS) [1], exhaustive clinical examination abnormalities including ocular motor signs, movements disorders, pyramidal signs, mental retardation) and paraclinical (biomarkers—especially vitamin E, AFP, albumin, cholestanol-, brain MRI, NCS findings) but molecular data: each patient had to be categorized in one of the three ARCA groups as described above and ranking of

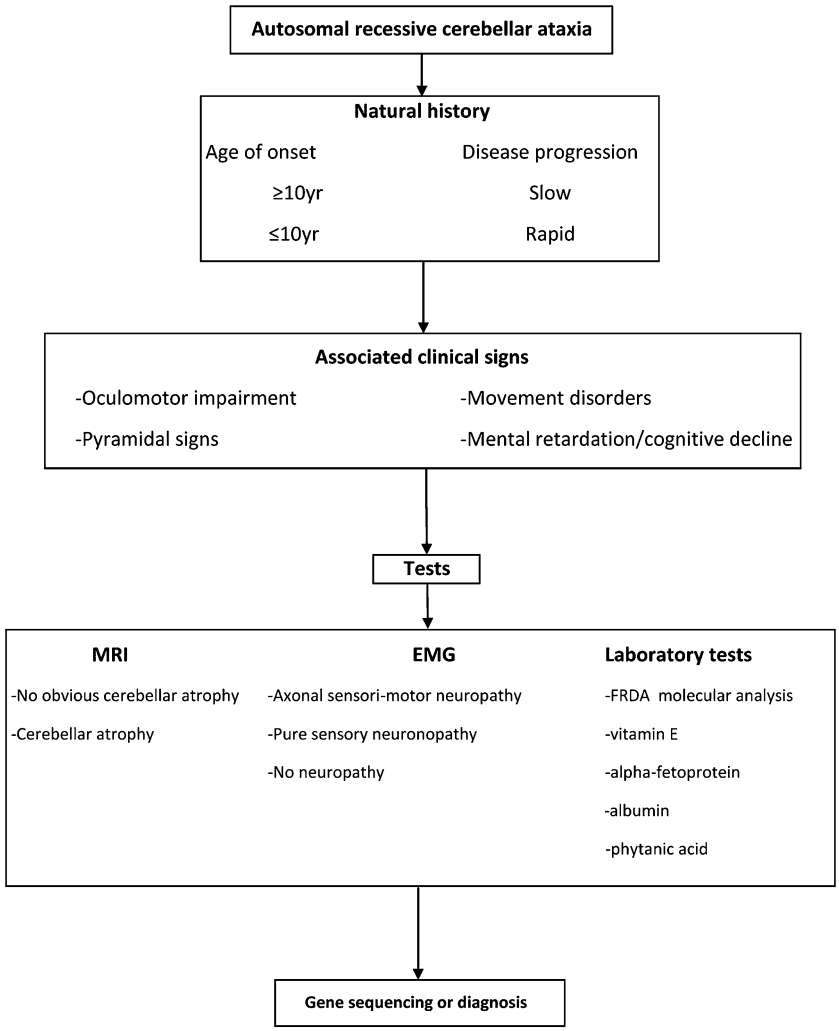

Fig. 1 Algorithm for the diagnosis of autosomal recessive cerebellar ataxias (simplified and adapted [3]). The combination of natural history, associated clinical signs and paraclinical data (such as brain MRI, nerve conduction studies and several biomarkers) leads to one or a few diagnosis. *yr* years, *EMG* electroneuromyography

the two most probable disease causing genes was achieved. Inter-rater agreement was assessed by computing a weighted Kappa coefficient (K) with '"squared"' weights. All mutated genes affecting the 23 patients were ordered according to the data of the literature as followed on a ordinal scale to take account of the degree of disagreement [group without neuropathy: (1) *SYNE1* (2) *ANO10*, (3) *ADCK3*; group with pure sensory neuronopathy: (1) *POLG*, (2) *TTPA*; group with axonal sensorimotor neuropathy (1) *APTX*, (2) *SETX*, (3) *CYP27A1*, (4) *SACS*]. Genes that showed larger number of associated phenotypic differences were separated by greater distance on the ordinal scale. Confidence interval was calculated using the adjusted bootstrap percentile (BCa) method based on 10,000 replicates. A "z" test was performed to assess if the classification which produced the Kappa statistic is significantly better than a random result ($K = 0$). A p value <0.05 was considered statistically significant. Analyses were performed using R software version 3.1.0 (R Project for Statistical Computing) with the "irr" package.

Results

Twenty-three patients were investigated with the clinical practice-based algorithm. The age of onset ranged from 1 to 47 years (median 16). Genetic analysis identified two pathogenic mutations in *ANO10* (6 patients), in *SETX* (4), in *SYNE1* and *ADCK3* (3 each), in *SACS* and *APTX* (2 each) and in *TTPA*, *CYP27A1*, *POLG* (1 each) (Table 1). The correct ARCA group was found in all patients by the two assessors. The gene ranked first by the first assessor (MA) was correct in 14/23 cases (61 %) and 18/23 cases (78 %) for the second assessor (CT). The most frequent error (11 errors/14) was misdiagnosis within the ARCA group without neuropathy probably due to the closely overlapping phenotypes of pure cerebellar ataxias (especially ARCA1, ARCA3 and to some extent ARCA2), with a wide range of age at onset. Five errors were shared by both experts. Considering the two most probable genes according to the assessors, the correct diagnosis was identified in 18/23 (78 %) and 21/23 (91 %), respectively.

Table 1 Gene ranking based on the clinical practice-based algorithm

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Geographical origin	French	French	Algerian	French	French	Portuguese	French	French
Gender	F	M	F	M	M	M	M	F
Age of onset (years)	27	27	7	10	5	15	33	37
Age (years)	62	43	32	41	18	46	44	44
SDFS (/7)	3	3	3	2	2	3	4	3
SARA (/40)	18	13	21	12	ND	10	14	6,5
Upper motor neuron dysfunction	−	−	−	−	−	−	+	−
Others symptoms	Scoliosis	Square wave jerks, horizontal ophtalmoparesis, unilateral ptosis	Slow evolution, pes cavus	Mild developmental delay	Dysarthria, seizures, delayed growth	Mild developmental delay	Left hypoacousia	Tongue fasciculations, gaze evoked nystagmus
Others individuals affected in the family	−	+	−	−	−	−	−	−
Reference			[6]			[17]	[8]	[8]
Nerve conduction studies	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Cerebellar atrophy	+	NA	+	+	+	+	+	+
Other MRI findings	–	NA	–	Global brain atrophy	–	–	–	–
Biomarkers	–	–	–	–	Lactate, pyruvate, CK, AFP normal	–	–	–
Mutated gene	*SYNE1*	*SYNE1*	*SYNE1*	*ADCK3*	*ADCK3*	*ADCK3*	*ANO10*	*ANO10*
Zigosity	homo	homo	homo	homo	het	het	het	het
Variant n°1/ cDNA variant/protein change	Nonsense c.6978G>A p.Trp2326* [exon 47]	Donor splice site c.12315+1G>A p.Ile4047_Lys 4105del59 (predicted) [intron 76]	Nonsense c.3736G>T p.Glul246* [exon 30]	Missense c.911C>T p.Ala304Val [exon 7]	Missense c.8950T p.Arg299Trp [exon 7]	Missense c.895C>T p.Arg299Trp [exon 7]	Missense c.685G>T p.Gly229Trp [exon 6]	Frameshift c.l214delT p.Leu405* [exon 7]
Variant n°2/ cDNA variant/protein change					Missense C.1651G>A p.Glu551Lys [exon 14]	Frameshift c.l358delT p.Leu453Arg fs*24[exon 11]	Nonsense c.1291C>T p.Gln431* [exon 8]	Donor splice site c.1476+1 G>T; p.Ser432_Leu49 2del61 [exon 9]
Results first assessor/gene n°1	***SYNE1***	***SYNE1***	*ANO10*	*ANO10*	***ADCK3***	*ANO10*	*SYNE1*	*SYNE1*
Results first assessor/gene n°2	*ANO10*	*ANO10*	*ADCK3*	*SYNE1*	*ANO10*	*SYNE1*	*ANO10*	*ANO10*
Results second assessor/gene n°1	***SYNE1***	***SYNE1***	*ADCK3*	*ANO10*	***ADCK3***	*SYNE1*	***ANO10***	***ANO10***
Results second assessor/gene n°2	*ANO10*	*ANO10*	*ANO10*	*ADCK3*	*ANO10*	*ANO10*	*SYNE1*	*SYNE1*

Table 1 continued

	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
Geographical origin	French	French	French	French	Algerian	French	Lebanese	Algerian
Gender	F	F	F	M	M	M	M	M
Age of onset (years)	30	32	32	30	17	47	16	7
Age (years)	33	61	37	34	23	60	51	15
SDFS (/7)	3	4	3	4	3	3	5	4
SARA (/40)	10	ND	10.5	16	ND	11.5	ND	ND
Upper motor neuron dysfunction	−	−	+	+	−	+	+	−
Others symptoms	Hypermetric saccades, ankle clonus	Diplopia, dysphagia, gaze evoked nystagmus, increased reflexes	–	Focal epilepsy since 18 with secondary generalization, mild dysexecutive syndrome	Head tremor, cerebellar and proprioceptive ataxia, abolished reflexes	Cerebellar and proprioceptive ataxia, external ophtalmoplegia, abolished reflexes, absence of vibration sense	Decreased vibration sense, abolished reflexes, intellectual deterioration, abnormal ocular movements	Oculomotor apraxia
Others individuals affected in the family	−	+	−	+	−	+	+	−
Reference	[8]	[8]	[8]		[6]		[20]	[6]
Nerve conduction studies	ND, no clinical signs of neuropathy	Normal	Normal	Normal	Normal	Axonal sensory neuropathy	Mild axonal sensorimotor neuropathy with sensory predominance	Axonal sensory neuropathy
Cerebellar atrophy	+	+	+	+	−	+	+	NA
Other MRI findings	–	–	–	Left parietooccipital porencephalic cyst	−	−	Vermis atrophy	−
Biomarkers	–	AFP : 12ng/ml	–	–	NA	–	Hypercholesterolemia	–
Mutated gene	*ANO10*	*ANO10*	*ANO10*	*ANO10*	*TTPA*	*POLG*	*APTX*	*APTX*
Zigosity	homo	het	het	het	homo	het	homo	homo
Variant n°1/ cDNA variant/protein change	Deletion of exon 12	Missense c.1009T>G p.Phe337Val [exon 6]	Missense c.512T>C p.Phel71Ser [exon 5]	Missense c.1009T>G p.Phe337Val [exon 6]	Frame shift c.744delA p.Glu249Asnfs* 15 [exon 5]	Missense c.l880G>A pArg627Gln [exon 10]	Missense c.781C>T p.Leu261Phe [exon 6]	Nonsense c.837G>A p.Trp279* [exon 6]
Variant n°2/ cDNA variant/protein change		Frameshift c.l32dupA, p.Asp45Argfs*9 [exon 2]	Frameshift c.l32dupA, pAsp45Argfs*9 [exon 2]	Frameshift c.l32dupA, p.Asp45Argfs*9 [exon 2]		Missense c.2243G>C p.Trp748Ser [exon 13]		
Results first assessor/gene n°1	*SYNE1*	***ANO10***	***ANO10***	*ADCK3*	***TTPA***	***POLG***	*POLG*	*ATM*
Results first assessor/gene n°2	*ANO10*	*ATM*	*SYNE1*	*POLG*	*POLG*	*TTPA*	*ANO10*	*APTX*
Results second assessor/gene n°1	***ANO10***	***ANO10***	***ANO10***	*ADCK3*	***TTPA***	***POLG***	*POLG*	***APTX***
Results second assessor/gene n°2	*SYNE1*	*SYNE1*	*SYNE1*	*ANO10*	*POLG*	*SETX*	*APTX*	*ATM*

Table 1 continued

	Patient 17	Patient 18	Patient 19	Patient 20	Patient 21	Patient 22	Patient 23
Geographical origin	French	French	French	French	French	French	Algerian
Gender	M	M	M	M	M	F	F
Age of onset (years)	15	13	Adolescence	12	29	1	3
Age (years)	25	26	67	33	33	42	39
SDFS (/7)	4	4	6	3	5	5	4
SARA (/40)	ND	21,5	ND	ND	7, 5	ND	12
Upper motor neuron dysfunction	−	−	−	−	+	+	−
Others symptoms	Oculomotor apraxia	Oculomotor apraxia	–	Oculomotor apraxia	Developmental delay, epilepsy since early childhood	–	Cataract at 37, spasticity in lower limbs
Others individuals affected in the family	+	–	+	−	−	−	−
Reference					[21]		
Nerve conduction studies	Axonal sensorimotor neuropathy with sensory predominance	Axonal sensorimotor neuropathy	Axonal sensorimotor neuropathy	Axono–myelinic sensorimotor neuropathy	Axonal sensorimotor neuropathy	Axonal sensorimotor neuropathy	Demyelinating sensorimotor neuropathy
Cerebellar atrophy	+	+	NA	+	−	+	NA
Other MRI findings	−	−	NA	−	Cerebellar white matter abnormalities	−	NA
Biomarkers	AFP: 50ng/ml	AFP: 13ng/ml	AFP: 32ng/ml	AFP : 90ng/ml	Increased cholestanol	–	–
Mutated gene	*SETX*	*SETX*	*SETX*	*SETX*	*CYP27A1*	*SACS*	*SACS*
Zigosity	het	het	homo	het	homo	het	homo
Variant n°1/ cDNA variant/protein change	Nonsense C.4075C>T p.Glnl359* [exon 10]	Missense c.986G>C p.Arg329Pro [exon 6]	Frameshift c.5075delT p.Leul 692Cysfs* 15 [exon 8]	Nonsense C.4087C>T p.Argl363* [exon 8]	Missense C.1016C>T p.Thr339Met [exon 5]	Nonsense C.12973C>T p.Arg4325* [exon 10]	Missense C.12220G>C p.Ala4074Pro [exon 10]
Variant n°2/ cDNA variant/protein change	Missense C.6694C>T pArg2232Cys [exon 21]	Missense c7331G>A p.Arg2444His [exon 24]		Nonsense c.5617G>T p.Glu1873* [exon 11]		Frameshift c.l358delG p.Gly453Valfs* 25 [exon 8]	
Results first assessor/gene n°1	***SETX***	***SETX***	***SETX***	***SETX***	***CYP27A1***	***SACS***	***SACS***
Results first assessor/gene n°2	*ATM*	*ATM*	*ATM*	*ATM*	*POLG*	*ATM*	*CYP27A1*
Results second assessor/gene n°1	***SETX***	***SETX***	***SETX***	***SETX***	***CYP27A1***	***SACS***	***SACS***
Results second assessor/gene n°2	*ATM*	*ATM*	*ATM*	*ATM*	*POLG*	*APTX*	*CYP27A1*

The clinical features of the 23 ataxic patients presented in Table 1 were available for the two assessors for a blinded study, in a random way. The first selected gene is *bolded* when correctly ranked by the assessor. Some patients were already published: patients 3, 13 and 16 [6], patient 6 (patient 4 [17]), patients 7-8-9-10-11 (case 2, 4, 5, 6, 9, respectively [8]), patient 15 (case 28 [20]), patient 21 (case 13 [21]) and patient 1, 2, 3 and 25 (case 16-1, 15-1, 17-1 and 20-1 [22]). In biomarkers section, "–" means no abnormalities in biomarkers tested. Biochemical tests were performed prior to NGS analysis

het compound heterozygous, *homo* homozygous, *M* male, *F* female, *SARA* scale for the assessment and rating of ataxia, *SDFS* spinocerebellar degeneration functional score, *AFP* alpha-fetoprotein (normal range is less than 7 ng/ml), *NA* not available

There was a high inter-rater agreement [$K = 0.85$ (0.55–0.98) $p < 0.001$] on the first gene ranked by the assessors confirming the algorithm's reproducibility.

Discussion

We report on the validation of a clinical practice-based algorithm in a series of patients, based on a blind analysis of clinical and paraclinical data. Given the high percent of correct diagnosis in our study (the assessors were able to find the good molecular diagnosis in 2/3 and 3/4 of cases, respectively), it is expected that this algorithm will be useful in clinical practice for neurologists and geneticists. Moreover, the algorithm allowed us to identify nine distinct entities, including entities that belong to the three different groups of the new classification of ARCAs [3]. One hundred forty-five patients suspected with ARCA were included in the study which is a high number since Friedreich ataxia was previously excluded. Therefore, the evaluation of the 23 patients with a molecularly confirmed diagnosis is relevant.

Confirmation of diagnosis by NGS can be straightforward in presence of clear-cut mutations, even with few clinical data. However, NGS data analysis frequently reveals several variants of unknown significance especially missense mutations in one or more ARCA-causing genes. In these difficult cases, proper phenotyping of ARCA patients, including precise clinical examination, biochemical investigations, brain imaging and NCS, is still necessary for guidance of genetic analysis and interpretation of the NGS data: these data have to be in agreement with an already described phenotype in order to confirm the variant's pathogenicity. Similarly, appropriate knowledge of the several entities and of their description in the literature is recommended for the best management of such scarce diseases.

Despite the relevance of the algorithm, it is not infallible since few errors were made. *ANO10* and *SYNE1* mutations are responsible for close phenotypes [8, 9] with pure cerebellar ataxia and slow progression and may be difficult to distinguish without genetic analysis especially because there is no biomarker. Some errors were therefore done during the blinded assessment regarding these entities. Such algorithm is limited by the presence of atypical phenotypes associated with mutations in known genes. For instance, most patients with autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS) experience their first symptoms before 5 years of age, but in very few patients the first signs may occur after 20 or 30 years. Atypical phenotypes have also been reported regarding peripheral neuropathy which was lacking in 1/11 patient in a recent series of ARSACS [10]. Lack of neuropathy is also a rare feature in ataxia with oculomotor apraxia type 2 (AOA2), representing only 2.5 % of cases in a series of 90 patients [11]. Only one patient with *ADCK3* confirmed mutations presented a mild axonal neuropathy (out of 34 patients described in the litterature [12–18]). In the same way, few patients with AOA2 or ataxia telangiectasia presented with the very unusual lack of elevated AFP serum level [11, 19]. However, the overall clinical and paraclinical assessment mostly lead to only a few possible diagnoses, confirming that the phenotype in one subtype of ARCA is relatively homogeneous. Few clinical findings (such as oculomotor apraxia, vertical supranuclear gaze palsy, spastic paraplegia, telangiectasia) as well as reliable biomarkers (such as vitamin E, AFP and albumin serum level) may also be suggestive of one or few diseases. The good results of our blinded assessment support this statement.

Herein, the percentage of positive diagnosis (19 %) is similar to previous studies on NGS in cerebellar ataxias [2] but remains low for several reasons including selection of patients with onset before 60 years of age (whereas ARCAs mostly occur before 30), exclusion of patients with Friedreich ataxia, absence of important ataxia genes in the gene panel, such as *WFS1* and *SPG7*, and the fact that many genes have not been identified yet. It is also possible that a few sporadic cases have in fact polyglutamine SCA due to marked anticipation, particularly for SCA2 and SCA7. Polyglutamine expansions should therefore also be tested, along with Friedreich ataxia expansions.

Web resources

UCSC Genome Browser: http://genome.ucsc.edu/index.html

Ensembl Genome Browser: http://www.ensembl.org/index.html

Exome Variant Server (EVS), NHLBI GO Exome Sequencing Project (ESP), Seattle, WA: http://evs.gs.washington.edu/EVS (June, 2013)

http://www.lbgi.fr/VaRank/.

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Compliance with ethical standards

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