RESEARCH ARTICLE

The Complex Genetic Landscape of Hereditary Ataxias in Turkey and Implications in Clinical Practice

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Relevant conflicts of interest/financial disclosures: Nothing to report.

Funding agencies: This work was supported by funds from Suna and İnan Kıraç Foundation, Koç University, Boğaziçi University.

Full financial disclosures and author roles may be found in the online version of this article.

Received: 13 October 2020; **Revised:** 6 January 2021; **Accepted:** 15 January 2021

Published online 24 February 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28518

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ABSTRACT: Background: The genetic and epidemiological features of hereditary ataxias have been reported in several populations; however, Turkey is still unexplored. Due to high consanguinity, recessive ataxias are more common in Turkey than in Western European populations.
Objective: To identify the prevalence and genetic structure of hereditary ataxias in the Turkish population.
Methods: Our cohort consisted of 1296 index cases and 324 affected family members. Polymerase chain reaction followed by Sanger sequencing or fragment analysis were performed to screen for the trinucleotide repeat expansions in families with a dominant inheritance pattern, as well as in sporadic cases. The expansion in the frataxin (*FXN*) gene was tested in all autosomal recessive cases and in sporadic cases with a compatible phenotype. Whole-exome sequencing was applied to 251 probands, selected based on the family history, age of onset, and phenotype.
Results: Mutations in known ataxia genes were identified in 30% of 1296 probands. Friedreich's ataxia was found to be the most common recessive ataxia in Turkey, followed by autosomal recessive spastic ataxia of Charlevoix–Saguenay. Spinocerebellar ataxia types 2 and 1 were the most common dominant ataxias. Whole-exome sequencing was performed in 251 probands with an approximate diagnostic yield of 50%. Forty-eight novel variants were found in a plethora of genes, suggesting a high heterogeneity. Variants of unknown significance were discussed in light of clinical data.
Conclusion: With the large sample size recruited across the country, we consider that our results provide an accurate picture of the frequency of hereditary ataxias in Turkey.

Key Words: ataxia; genetics; heterogeneity; whole-exome sequencing; Turkey

Hereditary cerebellar ataxias (HCAs) are a clinically and genetically heterogeneous group of disorders that are inherited in autosomal dominant (AD), autosomal recessive (AR), X-linked, or mitochondrial manners. The most common HCAs throughout the world are Friedreich's ataxia (FA), which is associated with GAA repeat expansions in the frataxin (*FXN*) gene, and spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, and 17 and dentatorubral-pallidoluysian atrophy (DRPLA), which are associated with CAG repeat expansions in the ataxin, calcium voltage-gated channel subunit alpha1 A (*CACNA1A*), TATA-box binding protein (*TBP*), and atrophin genes, respectively. Until recently, because of technical restrictions, a genetic diagnosis could be reached exclusively in patients carrying one of these mutations, leaving many familial and sporadic cases undiagnosed. However, as the next-generation sequencing (NGS)-based methods became more accessible and affordable, several other nonrepeat mutations in more than 140 genes associated with HCA were identified, and this number is increasing.[1] The yield of NGS technology improved our clinical insight and accuracy in HCAs in terms of understanding their underlying mechanisms and management.

Although individually rare, nonrepeat mutations are increasingly getting recognized as a common cause of both sporadic and familial ataxias. Initial studies in undiagnosed ataxia cohorts showed that NGS has a high diagnostic yield between 22% and 64%.[1-3] These studies also disclosed that mutations in several genes that were previously thought to be rare or restricted to specific geographical areas, including Sacsin molecular chaperone (*SACS*), Senataxin (*SETX*), Spectrin repeat-containing nuclear envelope protein 1 (*SYNE1*), and spastic paraplegia type 7 (*SPG7*), are, in fact, relatively frequent among European cases.[1,2] In addition, identification of pathogenic AARF domain-containing kinase 3 (*ADCK3*), Anoctamin 10 (*ANO10*), and Alpha-tocopherol transfer protein (*TTPA*) variants in patients has expanded the range of treatable HCAs potentially benefiting from replacement therapies.[4] A remarkable impact of NGS technologies on ataxia genetics is the recognition of the striking genetic overlap of HCAs with other neurodegenerative disorders, specifically with

hereditary spastic paraplegias. This overlap not only enabled an increase in the genetic diagnosis yield but also expanded the description of ataxia phenotypes.[5]

Here, we report the findings of a comprehensive study in a cohort of 1296 index cases and 324 affected family members (Fm) who were referred to our center for genetic analysis over a span of more than 20 years (1998–2020). The study provides the distribution of HCAs in Turkey as the first comprehensive report on the epidemiology of ataxias in Asia Minor, the Anatolian peninsula connecting Europe and the Middle East.

Subjects and Methods

Study Setting

The Neurodegeneration Research Laboratory (NDAL), a research center for the molecular analysis of neurodegenerative diseases, receives samples from specialist clinics and clinicians across the country. This study includes patients referred to us for the analysis of ataxias over a period of more than two decades. HCAs, which can be diagnosed by routine laboratory tests and radiological imaging, may not be exhaustively represented in this cohort because they were not consistently referred to molecular analysis (eg, Wilson's disease, vitamin E deficiency, fragile X tremor/ataxia syndrome). Demographic information, including patients' birth date, sex, age of onset (AO), parental consanguinity, pedigree, and detailed clinical description, were obtained from patients and clinicians. Informed consent was obtained from all individuals included in the study, which was approved by the Ethics Committee of Boğaziçi University, where the study was initiated.

Study Cohort

A total of 1296 index patients with ataxia and their affected Fm were referred to our laboratory for genetic analysis (Fig. 1). Patients were categorized according to their inheritance patterns. If one of the parents of the patients and/or a person in the upper generation was affected, an AD inheritance was considered (19%). In case of consanguinity between parents and/or if they were from the same/neighboring villages and/or if Fm only from the same generation were affected, an AR inheritance was assumed (46%). In case no consanguinity was described and no other affected Fm were known, patients were considered sporadic (20%). In some earlier patients, we did not have adequate information on parental consanguinity status; thus, they were classified as of unknown inheritance pattern (15%).

Demographic and clinical characteristics of the patients are compiled in Table 1. Only families with reliable information were included. Mean ± standard deviation (SD) for AO was 34.2 ± 16.5 years (range 0–75 years, n = 224) for AD patients and

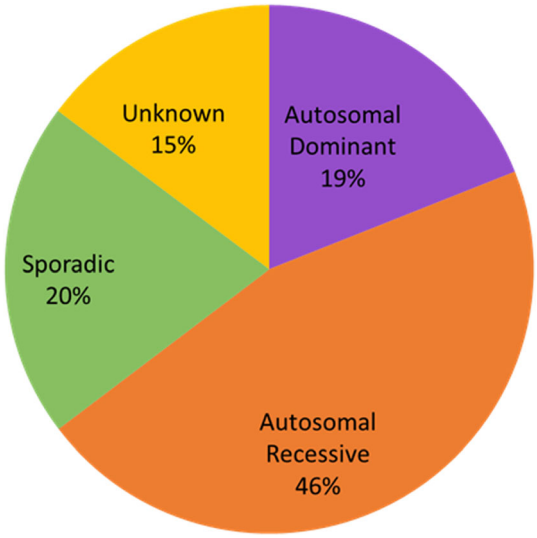

FIG. 1. Distribution of index patients (n = 1302) according to their inheritance patterns. [Color figure can be viewed at wileyonlinelibrary.com]

21.2 ± 15.2 years for AR patients (range 0–75 years, n = 529). AO was younger than 18 years in 40.3% of patients and ≥40 years in 24.9%. A definitive AO could not be obtained in 171 patients. There was at least one additional affected Fm in 194 cases. Parental consanguinity was present in 574 of 1116 (51.4%) cases, and there was no reliable information in 93. Family history suggested an AD inheritance in 19% and AR inheritance in 46%. The remaining 20% were classified as sporadic (Table 1).

Strategy of Genetic Analysis

A stepwise genetic analysis was performed according to the referring clinicians' initial diagnosis and the inheritance pattern obtained from the pedigree. All singlet cases with a negative history of parental consanguinity were considered as sporadic. In recessive and sporadic cases, analysis of GAA repeat expansion for FA was routinely carried out. In dominant families, CAG repeat expansions associated with the six most common SCAs (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17) were screened first. In cases with suggestive clinical findings, testing for DRPLA, SCA8, SCA12, and SCA36 followed. In addition, SCA6 was analyzed in all late-onset sporadic cases, whereas all other dominant SCAs were screened only when required by the clinician.

The recently discovered Replication factor C subunit 1 (*RFC1*) repeat expansion was evaluated by flanking PCR and repeat-primed polymerase chain reaction (PCR), primarily in patients with a late-onset ataxia and additional reports of sensory neuropathy and/or vestibular involvement.

When no mutations could be identified by conventional methods, whole-exome sequencing (WES) was

TABLE 1. Demographic features of the index patients under study

Inheritance pattern	AD (n = 246)	AR (n = 592)	Sporadic (n = 266)	Not known (n = 192)	Total (n = 1296)
Age (yr), mean ± SD (n)	51.7 ± 15.6 (238)	39.0 ± 14.9 (578)	45.9 ± 17.3 (263)	49.2 ± 16.7 (132)	43.6 ± 16.4 (1211)
AO (yr), mean ± SD (n)	34.2 ± 16.5 (224)	21.2 ± 15.2 (529)	30.9 ± 18.1 (249)	29.0 ± 18.7 (93)	26.7 ± 17.3 (1095)
Disease duration (until 2020), yr, mean ± SD (n)	17.4 ± 10.4 (221)	17.4 ± 9.3 (529)	14.7 ± 9.0 (249)	17.6 ± 8.0 (97)	16.8 ± 9.4 (1096)
Consanguinity, n (%)	71/231 (30.6)	487/582 (83.8)	0/266 (0)	15/37 (40.5)	578/1122 (51.5)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; SD, standard deviation; AO, age of onset.

performed in selected cases, in the presence of a high number of affected members, consanguinity in the upper generations, and the same parental origin indicating familial transmission; also, 30 sporadic cases were subjected to WES.

Conventional Analysis for Trinucleotide-Repeat Ataxias

Polyglutamine SCAs and DRPLA encompass the most common and best studied dominantly inherited ataxias. The number of CAG repeats was determined by PCR-based Sanger sequencing or fragment analysis using FAM-labeled primers. The results were evaluated with the Finch TV version 1.4.0 and Peak Scanner Software 1.0, respectively. The relationship between AO and CAG repeat length was assessed by a linear regression in R (version 3.5.1). Age of disease onset and natural log-transformed AO were modeled as a function of CAG repeat length of the expanded alleles in Ataxin 1 (*ATXN1*) and Ataxin 2 (*ATXN2*).

Flanking-PCR followed by repeat-primed PCR was performed to detect the hexanucleotide repeat associated with SCA36. Long-range PCR was applied to screen the patients for FA. Because most of the cases were tested by long-range PCR and very seldomly Southern blot analysis was applied, the long, medium, and small GAA repeat expansions were not distinguishable.

Whole-Exome Sequencing and Bioinformatics Analysis

WES was performed by Macrogen Inc. (Seoul, Korea). Bioinformatics evaluation of the data was conducted using the SEQ Platform (https://seq.genomize.com/), developed by Genomize (Istanbul, Turkey). Only destructive (frameshift insertion/deletion, in-frame insertion/deletion, splicing variants, non-sense) and missense variants were included. Among these, retained variants were either rare (minor allele frequency, MAF < 1%) or absent in the major population-based databases [gnomAD (https://gnomad.broadinstitute.org) and 1000 Genomes Project (https://www.internationalgenome.org)] and in our NDAL database. Variants were then classified according to the recommendations of the American College of Medical Genetics and Genomics: (1) variants that showed evidence of pathogenicity and were described as disease causing in literature and in clinical databases; (2) variants classified as pathogenic or likely pathogenic by the American College of Medical Genetics and Genomics; and (3) variants that have strong deleterious effects according to functional and conservation prediction tools were categorized as pathogenic or likely pathogenic. Novel variants or variants classified as variants of unknown significance (VUSs) were further evaluated based on the clinical examination, core phenotype associated to the gene, family segregation, and functional and conservation predictions tools. Family segregation of the variants standing out as candidates was validated using Sanger sequencing.

Results

Frequency and Distribution of Ataxias in Turkey

An underlying genetic mutation could be identified in 393 of 1296 (30%) index cases using either conventional methods or NGS. The ratio was higher among cases with a positive family history (43.5%) compared with sporadic cases (23%). Overall, 43.7% of AD, 39.0% of AR, and 36.6% of apparently sporadic cases were solved.

AD Cerebellar Ataxias Caused by Repeat Expansions

Phenotypic and genetic features of patients with AD cerebellar ataxias (ADCAs) are shown in Table 2. Among the 93 genetically diagnosed ADCA probands, SCA2 was the most common (54.8%, n = 51), followed by SCA1 (25.8%, n = 24), DRPLA (6.5%, n = 6), SCA8 (5.4%, n = 5), SCA3 (2.2%, n = 2), SCA17 (2.2%, n = 2), SCA36 (2.2%, n = 2), and SCA6 (1.1%, n = 1). SCA7 and SCA12 were not present in our cohort. In seven families, AD inheritance was not apparent because of misleading high consanguinity and/or absence of disease in the upper generations due to shorter repeat numbers in the above generations.

TABLE 2. Phenotypic and genetic features of dominant ataxia patients with repeat expansions

	SCA1	SCA2	SCA3	SCA6	SCA8	SCA17	SCA36	DRPLA
No. of index cases (%)	24 (25.8)	51 (54.8)	2 (2.2)	1 (1.1)	5 (5.4)	2 (2.2)	2 (2.2)	6 (6.5)
No. of affected Fm	7	50	6	2	6	2	2	6
AO (yr), mean ± SD	34.6 ± 9.3 (n = 24)	29.8 ± 12.8 (n = 78)	33.0 ± 8.9 (n = 8)	34 (n = 1)	29.9 ± 20.4 (n = 7)	34.5 ± 11.7 (n = 4)	54.7 ± 8.8 (n = 3)	27.7 ± 13.6 (n = 10)
AO (yr), range	18–50	5–73	17–46	–	0–53	17–50	47–67	3–44
Disease duration (until 2020) (yr), mean ± SD	14.9 ± 8.6 (n = 24)	17.0 ± 9.2 (n = 77)	11.7 ± 7.0 (n = 8)	12 (n = 1)	25.6 ± 16.6 (n = 7)	19.3 ± 8.1 (n = 3)	11.0 ± 2.2 (n = 3)	15.4 ± 5.4 (n = 9)
Repeat length, range								
Expanded allele	43–63	32–84	63–73	23	65–250[a]	53–54		58–80
Short allele	28–33	17–24	15–27	13	13–19	37–38		16–23

Age of onset (AO) applies for both index patients and family members (Fm).
[a]One of the patients has 103 versus 250 CAG repeats.
Abbreviations: SCA, spinocerebellar ataxia; DRPLA, dentatorubral-pallidoluysian atrophy; SD, standard deviation.

Length of repeat expansions was inversely correlated with the AO in SCA1 and SCA2, explaining 67% and 57% of the variance of the log-transformed AO, respectively (Supporting Information Fig. S1). In at least five patients with SCA1, the CAG repeats were interrupted by one to two CAT triplets, shifting the AO toward older ages in these families.

Friedreich's Ataxia

FA is the most common hereditary ataxia worldwide with a high phenotypic variability that is partly based on the size of GAA triplet[6,7]; shorter GAA repeats explained 30%–50% of AOs in FA. In our cohort, FA comprises 41.9% of all genetically diagnosed index cases. The homozygous intronic GAA repeat expansion was detected in 97.6% of patients with FA; in the remaining four patients, three had a heterozygous GAA expansion with a heterozygous missense or splice junction mutation. In one patient, a 25-base pair insertion in the trans allele to the GAA triplet was present; these results were confirmed in the parents of the patients. Consanguinity was common among FA families (63.3%); 70 families (47.3%) harbored more than one patient. Approximately 19% of FA cases were apparently sporadic. Mean AO of patients with FA was 15.2 ± 7.0 years (range 3–44 years, n = 143). In our series, at symptom onset, 7% of patients were equal to or younger than 5 years, 26% were between 6 and 11 years, and 56% of the probands were in the typical puberty range. Only 11.2% of patients had a late-onset ataxia at older than 25 years, with only one patient having an onset in the fifth decade of life. Disease onset in compound heterozygous patients was not markedly earlier than GAA homozygotes and variable between 11 and 25 years.

The biallelic point mutation recently described in the *FXN* of a Turkish family with three affected offspring, presenting with a Charcot–Marie–Tooth disease-like phenotype rather than ataxia, gave rise to very early onset disease in all three siblings (AO: 4–6 years). This is, to the best of our knowledge, the first description of a biallelic point mutation in the *FXN* gene that is compatible with life.[8]

Cerebellar Ataxia, Neuropathy, Vestibular Areflexia Syndrome

The biallelic expansion in the Alu element of the *RFC1* gene has been recently described to give rise to a late-onset ataxia, cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS), which often presents with a triad of symptoms.[9] CANVAS is worldwide the second most prevalent recessive ataxia after FA.[10] In the Turkish cohort under study, 15 index patients and 3 affected Fm of 202 were found to carry the pathogenic intronic expansion, ranking CANVAS

in our cohort at the third position after FA and autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS). Turkish patients with CANVAS presented with a late-onset ataxia and an unexplained dry cough described to begin at young ages. The triad of symptoms, including cerebellar ataxia, neuropathy, and vestibulopathy, was present in eight patients; vestibulopathy was, in the majority of cases, detected after a detailed ear, nose, and throat examination, and thus easily can be missed in routine neurological examination.[10]

Nonrepeat Dominant and Recessive Ataxia Cases Identified by WES

WES was performed in 251 probands, 45 with AD and 176 with AR inheritance; 30 were sporadic, of whom 26 had an AO of ≤40 years. Disease-causing variants in genes associated with dominant ataxias were identified in nine families, corresponding to a diagnostic yield of 19.6%. This number was significantly higher in recessive ataxias (50.2%), as well as in apparently sporadic cases (36.6%). In WES analysis, the most common ataxias after FA were ARSACS (n = 23), SPG11 (n = 11), ataxia with oculomotor apraxia type 2 (AOA2) (n = 10), and SPG7 (n = 10) (Supporting Information Fig. S2). Forty-eight of these variants were novel. All other ataxia subtypes, including the dominant ones, are generally rare, very rare, or even family-private (Supporting Information Table S1).

Here, we briefly present a few families of interest (Supporting Information Table S1).

A novel pathogenic variant in *CACNA1A* classified initially as VUS was identified in a 65-year-old patient, who presented with episodic ataxia that lasts for 10 minutes. The patient had a slow progression between the relapses and responded well to diazomide. The phenotype of the patient is in accordance with his genotype.

All three index cases from independent families with Niemann–Pick disease type C (NPC) had juvenile-onset NPC presenting with cognitive decline and ataxia. Two had vertical supranuclear gaze palsy, one patient had spasticity, and one had hearing loss. Family segregation confirmed in two families the pathogenicity of the variants, and clinician review in all three favor a correct phenotype–genotype correlation.

An 18-year-old patient with the typical clinical picture of AOA2 presented with gait ataxia, dysarthria, and severe sensory axonal polyneuropathy (PNP); he did not have oculomotor apraxia at the age of 21 years but did have mildly elevated alpha-fetoprotein (AFP) (9 ng/mL). The variant identified in the aprataxin gene, giving rise to ataxia with oculomotor apraxia type 1 (AOA1), highlights the overlap between AOA1 and AOA2 and emphasizes the importance of genetic analysis.

Two unrelated SCA42 families were identified, both harboring several patients with the same *CACNA1G* variant. All patients had a slowly progressive cerebellar ataxia, AOs varied between 11 and 50 years, confirming the high intrafamilial AO variability seen in SCA42. Due to consanguinity, the index cases in both families have biallelic mutations and an earlier onset (11 and 14 years).

Apparently Sporadic Cases: Genetic Mutations Identified in Cases With No or Missing Family Information

All singlet cases without parental consanguinity were considered as sporadic (Table 1). Male-to-female ratio was 1.2. Eighty-eight of these patients had an AO ≥40 years (sporadic late-onset ataxia, 34.5%), 82 had an AO between 18 and 40 years (32.2%), and another 82 had an AO before 18 years (32.2%). All sporadic patients were tested for FA, and 31 had a positive result (11.7%). Three of these patients had an AO later than 24 years, classified as late-onset FA. SCA6 was negative in all sporadic cases. Two SCA2 and one SCA8 expansion were identified despite an undefined mode of inheritance. This was before the WES era, where we have screened for all SCAs upon the request of the clinician. Finally, of 30 apparently sporadic probands subjected to WES, 11 were solved (36.6%).

Discussion

In this study, the frequency and distribution of hereditary ataxias in a large Turkish cohort, a population underrepresented in current databases, were investigated; family history suggested AD inheritance in 19.0% and AR inheritance in 45.8% of families. The remaining 20.4% were sporadic and 14.8% were not of well-defined inheritance pattern. Consanguinity was present in more than half of our cohort (51.5%). Screening for CAG-repeat expansions revealed the underlying mutation in 36.8% of all AD families. Worldwide, the distribution of SCAs differs among various populations, and SCA3 is the most frequent type, followed by SCA2 and SCA6.[11-13] In our cohort, SCA2 is found to be the predominating ADCA (54.8%), followed by SCA1 (25.8%) and DRPLA (6.5%). DRPLA was first described in the Japanese population (9.7%),[14] although it is rare in Europe.[15] DRPLA should be considered in Turkey as a possible diagnosis among ADCAs, especially in combination with an early-onset progressive myoclonus epilepsy or later-onset chorea. In contrast, SCA6 seems to be rare in the Turkish population, including late-onset sporadic patients. Finally, SCA36, a rare hexanucleotide repeat

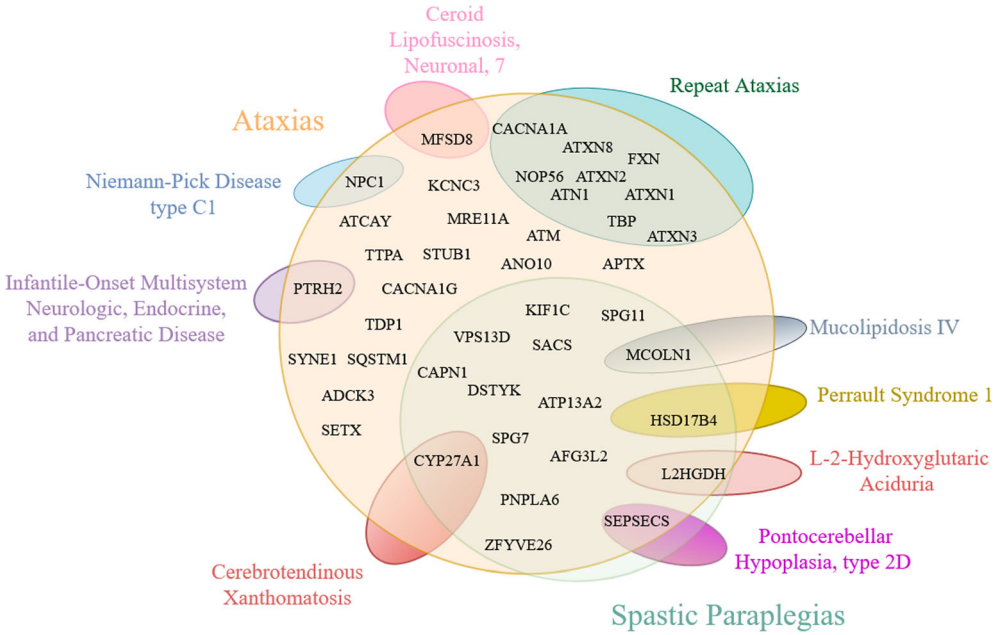

FIG. 2. Genes giving rise to dominant and recessive ataxias in our cohort. Venn diagram points to genetic heterogeneity and pleiotropy and to overlapping genes in phenotypes classified as different diseases. Larger font sizes emphasize the higher frequencies of ataxias and hereditary spastic paraplegias. [Color figure can be viewed at wileyonlinelibrary.com]

expansion ataxia, was identified in two unrelated Turkish families with four affected members. The mean AO for SCA1 and SCA2 is in accordance with the world average of around 30–35 years,[16-18] with the repeat sizes accounting for 67% and 57% of the AO variability of SCA1 and SCA2, respectively.

The abundance of recessive families in our cohort is the result of first-cousin marriages (>60%) as traditionally common in the Turkish culture.[19] The most common genetic ataxia in our population, comprising 41.9% of all genetically confirmed ataxias, is FA; this is in line with Europe and the Middle East. FA was diagnosed in 19.9% of all recessive and in 11.7% of sporadic cases. The mean AO of around 15 years is conforming to the literature,[20,21] whereas early-onset (≤11 years) patients comprising almost 30% of all FA cases are higher than reported.[6]

We have extensively used WES for understanding the genetic causes of a significant number of non-FA recessive pedigrees, with the diagnostic yield reaching 50.2%. The frequency and distribution of the more common non-FA autosomal recessive cerebellar ataxia (ARCA) in Turkey are similar to the recently published data from European centers.[1-3,22] ARSACS is the most common non-FA ARCA in our cohort, followed by CANVAS, SPG11, AOA2, SPG7, and AOA1. In addition, several rare ataxia-causing genes were also identified (Supporting Information Table S1), some of which were already described in the context of other neurological disorders with genetic basis, pointing to overlapping pathological mechanisms. Importantly, some of these rare ataxias, for example, vitamin E deficiency and COQ8A, are amenable to treatment by supplementing the relevant drugs; this points to the necessity of a firm genetic diagnosis, which also shortens the long diagnostic journey.

The phenotypic overlap among HCA is amazing, when the large differences in the relevant disease proteins are considered and thus justify the search for shared molecular mechanisms. Interestingly, but not unexpectedly, the multitude of genes and variants identified through WES in ARCA cases converge on common denominators, clustering in certain molecular pathways. The most common pathways involved in ataxias are: (1) mitochondrial metabolism [*SACS*, *SPG7*, *ADCK3*, L-2-hydroxyglutarate dehydrogenase (*L2HGDH*)]; (2) DNA repair (*SETX*), Aprataxin (*APTX*), MRE11 homolog A, double-strand break repair nuclease (*MRE11A*), *ATM*, Tyrosyl-DNA phosphodiesterase 1 [*TDP1*]); (3) lipid metabolism [NPC intracellular cholesterol transporter 1 (*NPC1*), cytochrome P450 family 27 subfamily A member 1 (*CYP27A1*), hydroxysteroid 17-beta dehydrogenase 4 (*HSD17B4*), Patatin-like phospholipase domain-containing 6 [*PNPLA6*]); and (4) autophagy/lysosomal activity [ATPase 13A2 (*ATP13A2*), *SPG11*, Zinc finger FYVE-type-containing 26 (*ZFYVE26*), Vacuolar protein sorting 13 homolog D [*VPS13D*]) (Supporting Information Table S1).[22-24]

Mechanisms of genetic pleiotropy include different downstream effects of mutations within the same gene,

TABLE 3. "Presumed disease-causing" VUSs

HGVSc (Varsome) HGVSp	Gene	Mutation Type	Phenotype (ataxia +)	Family Segregation	Clinician Review	ACMG Varsome	SIFT	PolyPhen	DANN	CADD (phred)	GERP++_RS	gnomAD_AF (exomes)
ENST00000382298.3: c.11766A>T ENSP00000371735.3:p. Leu3922Phe[a]	*SACS*	MS		+	+	VUSs: PM2, BP1	de (0.01)	prob_dam (0.997)	0.985	15.9	−8.06	–
ENST00000382298.3:c.7940T>A ENSP00000371735.3:p. Ile2647Asn[a]		MS		+	+	VUSs: PM2, PP3, BP1	de (0)	prob_dam (0.931)	0.992	27.9	5.35	–
ENST00000382292.3:c.8315G>C ENSP00000371729.3:p. Gly2772Ala		MS	PNP	+	+	VUSs: PM2, PP3, BP1	tol (0.06)	pos_dam (0.755)	0.996	23.8	5.2	5.181e−5
ENST00000382292.3: c.7732_7734delGAT ENSP00000371729.3:p. Asp2578del[a]		Del	PNP	+	+	VUSs: PM2, PM4, PP3	–	–	–	–	–	–
ENST00000382292.3:c.3767A>G ENSP00000371729.3:p. Tyr1256Cys[a]		MS	PNP	+	+	VUSs: PM2, PP3, BP1	de (0)	prob_dam (0.947)	0.997	27.4	6.06	–
ENST00000382292.3:c.4192T>C ENSP00000371729.3:p. Cys1398Arg		MS	PNP	+	+	VUSs: PM2, PP3, BP1	de (0)	be (0.418)	0.996	24.4	6.06	–
ENST00000382292.3: c.11542_11544delATT ENSP00000371729.3:p. Ile3848del		Del	PNP	+	+	VUSs: PM2, PM4, PP3	–	–	–	22.0	–	–
ENST00000382292.3: c.11274_11276delAAC ENSP00000371729.3:p. Ile3758_Thr3759delinsMet		nFS INDEL	PNP, VI, SNHL	+	+	VUSs: PM2, PM4, PP3	–	–	–	–	–	–
ENST00000268704.2:c.1972G>A ENSP00000268704.2:p.Ala658Thr	*SPG7*	MS	UMN, CI, DCI	+	+	VUSs: PM2, PP2, PP3	Tol (0.18)	prob_dam[1]	0.999	25.0	5.06	–
ENST00000268704.2:c.2096T>C ENSP00000268704.2:p. Met699Thr[a]		MS	UMN	+	+	VUSs: PM2, PP2, PP3	de (0)	pos_dam (0.579)	0.993	25.9	5.21	–
ENST00000372169.2:c.6686T>C ENSP00000361242.2:p. Met2229Thr	*SETX*	MS	Tr	+	+	VUSs: PM2, PP3, PP5, BP1	de (0)	pos_dam (0.86)	0.995	27.1	5.69	3.977e−6
ENST00000379819.1:c.731T>G ENSP00000369147.1:p. Val244Gly[a]	*APTX*	MS		+	+	VUSs: PM2, PP2, PP3	de (0)	pos_dam (0.906)	0.997	26.0	5.59	1.591e−5
ENST00000379819.1:c.731T>G ENSP00000369147.1:p. Val244Gly[a]		MS	PNP	+	+	VUSs: PM2, PP2, PP3	de (0)	pos_dam (0.906)	0.997	26.0	5.59	1.591e−5

(Continues)

TABLE 3. Continued

HGVSc (Varsome) HGVSp	Gene	Mutation Type	Phenotype (ataxia +)	Family Segregation	Clinician Review	ACMG Varsome	SIFT	PolyPhen	DANN	CADD (phred)	GERP++_RS	gnomAD_AF (exomes)
ENST00000379819.1:c.635C>T ENSP00000369147.1:p. Ala212Val[a]		MS	CI, PNP, EPS	+	+	VUSs: PM2, PP2, PP3	de (0)	prob_dam (0.999)	0.998	23.6	4.9	1.193e−5
ENST00000379819.1:c.635C>T ENSP00000369147.1:p. Ala212Val[a]		MS	PNP, UMN, OA	+	+	VUSs: PM2, PP2, BP4	de (0)	prob_dam (0.999)	0.998	23.6	4.9	1.193e−5
ENST00000379819.1:c.902T>G ENSP00000369147.1:p. Phe301Cys[a]		MS	PNP	+	+	VUSs: PM2, PP2, PP3	de (0)	prob_dam (0.999)	0.992	33	6.01	–
ENST00000323929.3:c.464G>C ENSP00000325863.3:p. Arg155Pro[a]	*MRE11A*	MS	PNP, OA	+	+	VUSs: PM2, PP3, BP1	de (0.01)	prob_dam (0.979)	0.997	28.8	5.54	–
ENST00000269228.5:c.2257G>A ENSP00000269228.4:p. Val753Met	*NPC1*	MS	UMN	+	+	VUSs: PM1, PM2, PP2, BP4	tol (0.1)	be (0.063)	0.894	62	−11.8	0.0001909
ENST00000504811.1:c.425A>G ENSP00000420914.1:p. Asp142Gly[a]	*HSD17B4*	MS	HL	+	+	VUSs: PM1, PM2, PP3, BP1	de (0.01)	prob_dam (0.98)	0.998	34	6.02	–
ENST00000504811.1:c.425>G ENSP00000420914.1:p. Asp142Gly[a]		MS	PNP, UMN	+	+	VUSs: PM1, PM2, PP3, BP1	de (0.01)	prob_dam (0.98)	0.998	34	6.02	–
ENST00000261866.7:c.3809T>A ENSP00000261866.7:p. Val1270Asp	*SPG11*	MS	PNP	+	+	VUSs: PM2, PP3	de (0)	prob_dam (0.996)	0.988	28.3	6.03	–
ENST00000261866.7:c. 6886_6900dupA AGTTGATAACTCTG ENSP00000261866.7:p. Lys2296_Leu2297insLysLeulle ThrLeu[a]		Ins	UMN	+	+	VUSs: PM2, PM4, PP3	–	–	–	–	–	–
ENST00000261866.7:c.6886_ 6900dupAAGTTGATAACTCTG ENSP00000261866. 7:p.Lys2296_Leu2297 insLysLeulleThrLeu[a]		Ins	UMN	+	+	VUSs: PM2, PM4, PP3	–	–	–	–	–	–
ENST00000326735.8:c.1970C>T ENSP00000327214.8:p. Pro657Leu[a]	*ATP13A2*	MS	UMN, EPS	+	+	VUSs: PM2, PP3, BP1	de (0.01)	prob_dam (1)	0.998	24.9	5.57	8.167e−6

(Continues)

TABLE 3. Continued

HGVSc (Varsome) HGVSp	Gene	Mutation Type	Phenotype (ataxia +)	Family Segregation	Clinician Review	ACMG Varsome	SIFT	PolyPhen	DANN	CADD (phred)	GERP++_RS	gnomAD_AF (exomes)
ENST00000533820.1:c.994G>A ENSP00000435272.1:p. Gly332Arg	*CAPN1*	MS	Keratoconus, UMN	+	+	VUSs: PM1, PM2, PP3, BP1	de (0)	prob_dam (1)	0.999	31	4.63	8.112e−6
ENST00000367255.5:c.3371A>G ENSP00000356224.5:p. Asp1124Gly[a]	*SYNE1*	MS	UMN	+	+	VUSs: PM2, PP3	de (0)	prob_dam (0.999)	0.998	25.4	5.78	–
ENST00000320785.5:c.38G>A ENSP00000320821.5:p.Arg13Gln	*KIF1C*	MS	UMN, DCI, CI	+	+	VUSs: PM1, PM2, PP3, BP1	de (0)	be (0.013)	0.999	23.4	2.02	0
ENST00000320785.5:c.38G>A ENSP00000320821.5:p.Arg13Gln		MS		+	+	VUSs: PM1, PM2, PP3, BP1	de (0)	be (0.013)	0.999	23.4	2.02	0
ENST00000320785.5:c.866A>C ENSP00000320821.5:p. Gln289Pro[a]		MS	UMN	+	+	VUSs: PM1, PM2, PP3, BP1	de (0.03)	prob_dam (0.954)	0.992	33	5.66	0.001109
ENST00000296468.3:c.479C>A ENSP00000296468.3:p. Thr160Asn	*MFSD8*	MS	Epilepsy, EPS, VI	+	+	VUSs: PM2, PP3, PP5, BP1	de (0)	prob_dam (0.983)	0.990	23.6	4.86	–
ENST00000358136.3: c.12629C>T ENSP00000350854.3:p. Ala4210Val	*VPS13D*	MS	HL, UMN	+	+	VUSs: PM2, PP3, PP5, BP1	de (0)	prob_dam (0.986)	0.999	33	6.16	4.057e−5
ENST00000382103.2:c.1321G>A ENSP00000371535.2:p. Gly441Arg	*SEPSECS*	MS	UMN, PR	+	+	VUSs: PM2, PP3, PP5, BP1	de (0)	prob_dam (0.996)	0.999	28.8	5.32	7.97e−6
ENST00000367162.3:c.1169G>A ENSP00000356130.3:p. Arg390His[a]	*DSTYK*	MS	UMN, PNP	+	+	VUSs: PM2, PP3, BP1	de (0)	prob_dam (0.999)	0.999	32	5.68	3.978e−6
ENST00000219548.4:c.146A>G ENSP00000219548.4:p.Tyr49Cys[a]	*STUB1*	MS	CI, UMN, PNP	+	+	VUSs: PM2, PP2, PP3	de (0)	prob_dam (1)	0.993	24.6	2.57	–

[a]Novel variants.

Abbreviations: HGVS, Human Genome Variation Society (c for cDNA and p for protein); DANN, Deleterious Annotation of genetic variants using Neural Networks; CADD, Combined Annotation-Dependent Depletion; SIFT, Scale-invariant feature transform; GERP, Genomic Evolutionary Rate Profiling; VUS, variant of unknown significance; ACMG, American College of Medical Genetics and Genomics; MS, missense; de, deleterious; prob_dam, probably damaging; PNP, polyneuropathy; tol, tolerated; pos_dam, possibly damaging; be, benign; Del, deletion; nFS INDEL, nonframeshifting insertion deletion; VI, visual impairment; SNHL, sensory neural hearing loss; *SPG7*, spastic paraplegia type 7; UMN, upper motor neuron involvement; CI, cognitive impairment; DCI, dorsal cord involvement; Tr, tremor; *SETX*, Senataxin; *APTX*, Aprataxin; EPS, extrapyramidal system involvement; *MRE11A*, MRE11 homolog A, double-strand break repair nuclease; OA, oculomotor apraxia; *NPC1*, NPC intracellular cholesterol transporter 1; HL, hearing loss; *SPG11*, spastic paraplegia type 11; Ins, insertion; *SYNE1*, Spectrin repeat-containing nuclear envelope protein 1; *KIF1C*, kinesin family member 1C; *MFSD8*, major facilitator superfamily domain-containing 8; *SEPSECS*, Sep (O-phosphoserine)-tRNA:Sec (selenocysteine)-tRNA synthase; PR, psychomotor retardation; *DSTYK*, dual serine/threonine and tyrosine protein kinase; *STUB1*, STIP1 homology and U-box-containing protein 1.

modifier genes, and oligogenic inheritance. The best-known example of pleiotropy in ataxias is *CACNA1A* mutations that can present as SCA6 in combination with a trinucleotide repeat expansion, episodic ataxia type 2 with a point mutation in the calcium channel gene, and familial hemiplegic migraine, due to different functional downstream mechanisms[25] (Fig. 2).

A WES-based molecular diagnostic approach allows the classification of genes under shared mechanisms guiding the clinician to targeted strategies for therapies. A major question arising, however, is the increasing number of patients with VUSs, because correct interpretation of a previously undescribed variant is of prime importance for the clinical management of patients. Intrafamilial allele segregation and genotype–phenotype correlations are major elements in defining the causality of VUSs; thus, precise clinical information through a tight collaboration with expert clinicians and reverse phenotyping are helpful to evaluate novel variants.

Table 3 compiles variants that are highly likely to be converted from a VUS to a "presumed disease-associated variant" after thorough reevaluation of genotypic, *in silico*, and clinical data of the patients. Of 41 variants initially classified as VUSs (Supporting Information Table S1), 34 were found to be "likely disease associated" rather than VUSs. This result is based on more detailed re-review of the respective patients with their specialist clinicians and interpreting the family segregation analysis of the variants.

As opposed to European countries in which family sizes have been steadily decreasing in the last 60 years, Turkey is still very dynamic with a high crude birth rate (15/1000).[26] Half of Turkey's population is younger than 35 years. Large families consisting of several generations and with an impressive number of offspring are rather the rule than the exception, especially in the eastern Turkish provinces. With its unique geographical location as a crossroads between three continents, the Anatolian peninsula has served as the cradle of several civilizations since ancient times; thus, Turkey has an extremely wealthy historical background. Although the practice of consanguineous marriage is still a part of the Turkish culture (25%–36%), counteracting this high consanguinity is the ethnic admixture of the population, which is reflected in an extreme heterogeneity at the molecular level. This combination of ethnic heterogeneity on one hand and inbreeding on the other hand renders the Turkish peninsula very admixed on a macroscale and inbred on a microscale.

Population data for ataxias are primarily limited to the populations of Europe and North America. However, there is great variation in predominance of distinctive ataxic disorders in different ethnicities and geographical regions because founder effects and consanguinity can greatly influence the population prevalence.[13,27-30] This study aims to unravel the genetic epidemiology of HCA in a representative Turkish cohort. It further aims to raise awareness among young Turkish clinicians to facilitate the choice of genetic tests in clinical practice and to shorten the long diagnostic odyssey of patients with ataxia. The article also implies that in an era of emerging genetic therapies, the routine testing of patients with ataxia for their genetic background is not curiosity-driven research anymore but a prerequisite.

The implementation of countrywide screening programs to prevent inherited neurological diseases as successfully practiced in blood disorders in the Mediterranean basin should be among the main concerns of Turkish authorities. Indeed, educating families affected by inherited neurological diseases about their reproductive options, their risks, and their limitations should be offered by local health care providers. This study, compiling the results of more than 1600 patients with ataxia from different centers across Turkey over a long time span of 20 years, offers the data to pave the way for a comprehensive prevention program in common neurological disorders. A few limitations regarding the precise family history and full coverage of accurate clinical data have to be taken into account, especially in earlier cases, because these may not have been exhaustive; also, FMRP translational regulator 1 (*FMR1*) was not systematically tested. However, with the number of ataxia specialist clinicians across the country who have participated in this study and the wealth of samples tested, with conventional and NGS-based methods, we consider that our results give a highly accurate picture of the distribution of genetic ataxias in Turkey.

Acknowledgments: We would like to express our heartfelt thanks to Suna, İnan, and İpek Kıraç; none of our efforts would be possible without their vision, devotion, dedicated mentorship, and sustained support. We extend our deepest gratitude to our academic advisors Prof. Robert H. Brown (UMass Medical School, Worcester, MA, USA) and Prof. Jeffrey D. Macklis (Harvard Medical School, Boston, MA, USA) for supporting NDAL's growth and development over the years. We thank Prof. Murat Emre for encouraging and supporting us throughout the study, and Dr. Erşen Kavak and the Genomize team for their bioinformatics assistance and sincere friendship. Dr. Murat Çetinkaya is acknowledged for critical reading of the manuscript. The authors gratefully acknowledge use of the services and facilities of the Koç University Research Center for Translational Medicine (KUTTAM), funded by the Presidency of Turkey, Presidency of Strategy and Budget. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Presidency of Strategy and Budget.

Appendix

Definitions of American College of Medical Genetics and Genomics (ACMG) Classifications from ACMG Guideline: PVS1: pathogenic very strong; null variant [non-sense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion] in a gene where LOF is a known mechanism of disease. PS1: pathogenic/strong; same amino acid change as a

previously established pathogenic variant regardless of nucleotide change. PS2: pathogenic/strong; de novo (both maternity and paternity confirmed) in a patient with the disease and no family history. PS3: pathogenic/strong; well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product. PS4: pathogenic/strong; the prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. PM1: pathogenic/moderate; located in a mutational hot spot and/or critical and well-established functional domain (eg, active site of an enzyme) without benign variation. PM2: pathogenic/moderate; absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. PM3: pathogenic/moderate; for recessive disorders, detected in *trans* with a pathogenic variant. PM4: pathogenic/moderate; protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants. PM5, pathogenic/moderate; novel MS change at an amino acid residue where a different MS change determined to be pathogenic has been seen before. PM6: pathogenic/moderate; assumed de novo, but without confirmation of paternity and maternity. PP1: pathogenic/supporting; cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease. PP2: pathogenic/supporting; MS variant in a gene that has a low rate of benign MS variation and in which MS variants are a common mechanism of disease. PP3: pathogenic/supporting; multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.). PP4: pathogenic/supporting; patient's phenotype or family history is highly specific for a disease with a single genetic etiology. PP5: pathogenic/supporting; reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation. BA1: benign/standalone; allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. BS1: benign/strong; allele frequency is greater than expected for disorder. BS2: benign/strong; observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age. BS3: benign/strong; well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing. BS4: benign/strong; lack of segregation in affected members of a family. BP1: benign/supporting; MS variant in a gene for which primarily truncating variants are known to cause disease. BP2: benign/supporting; observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in *cis* with a pathogenic variant in any inheritance pattern. BP3: benign/supporting; in-frame deletions/insertions in a repetitive region without a known function. BP4: benign/supporting; multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.). BP5: benign/supporting; variant found in a case with an alternate molecular basis for disease. BP6: benign/supporting; reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation. BP7: benign/supporting; a synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site *and* the nucleotide is not highly conserved. ■

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Supporting Data

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