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The Phenotypic Continuum of ATPLA3-Related Disorders

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ABSTRACT

BACKGROUND AND OBJECTIVES

ATP1A3 is associated with a broad spectrum of predominantly neurological disorders, that continues to expand beyond the initially defined phenotypes of Alternating Hemiplegia of Childhood (AHC), Rapid-onset Dystonia Parkinsonism (RDP) and Cerebellar ataxia, Areflexia, *Pes cavus*, Optic atrophy, Sensorineural hearing loss syndrome (CAPOS). This phenotypic variability makes it challenging to assess pathogenicity of an *ATP1A3* variant found in an undiagnosed patient. We describe the phenotypic features of individuals carrying a pathogenic/likely pathogenic *ATP1A3* variant and perform a literature review of all *ATP1A3* variants published thus far in association with human neurological disease. Our aim is to demonstrate the heterogeneous clinical spectrum of the gene and look for phenotypic overlap between patients that will streamline the diagnostic process.

METHODS

Undiagnosed individuals with *ATP1A3* variants were identified within the cohort of the Deciphering Developmental Disorders (DDD) study with additional cases contributed by collaborators internationally. Detailed clinical data was collected with consent through a questionnaire completed by the referring clinicians. PubMed was searched for publications containing the term “ATP1A3” from 2004 to 2021.

RESULTS

Twenty-four individuals with a previously undiagnosed neurological phenotype were found to carry 21 *ATP1A3* variants. Eight variants have been previously published. Patients experienced on average 2-3 different types of paroxysmal events. Permanent neurological features were common including microcephaly (7;29%), ataxia (13;54%), dystonia (10;42%) and hypotonia (7;29%). All patients had cognitive impairment. Neuropsychiatric diagnoses were reported in 16 (66.6%) individuals. Phenotypes were extremely varied and most individuals did not fit clinical criteria for previously published phenotypes.

On review of the literature, 1108 individuals have been reported carrying 168 different *ATP1A3* variants. The most common variants are associated with well-defined phenotypes, while more rare variants often result in very rare symptom correlations, such as are seen in our study.

CADD scores of pathogenic and likely pathogenic variants were significantly higher and variants clustered within six regions of constraint.

CONCLUSION

Our study shows that looking for a combination of paroxysmal events, hyperkinesia, neuropsychiatric symptoms, and cognitive impairment, as well as evaluating CADD score and variant location can help identify an *ATP1A3*-related condition, rather than applying diagnostic criteria alone.

INTRODUCTION

Throughout the last 20 years, pathogenic variants in *ATP1A3* have been discovered to cause an ever-expanding range of rare neurological phenotypes, affecting both children and adults. *ATP1A3* encodes the α3 subunit of a Sodium-Potassium-ATPase (NKA) present in excitable (neuronal and cardiac) cells. The α3-subunit has a relatively low Na+ affinity coupled with a high affinity to ATP[1,2]. Consequently, the NKA carrying the α3-subunit is ideally configured to clear high intraneuronal sodium concentrations occurring after intense neuronal firing, by being able to utilise the low concentration of ATP that will occur near the neuronal membrane shortly after an energy-demanding task.

In 2004, variants in *ATP1A3* were linked to Rapid-Onset Dystonia Parkinsonism (RDP)[3]. In 2012, Heinzen *et al*.[4] discovered such variants to also be a cause of Alternating Hemiplegia of Childhood (AHC). In 2014, the gene was linked to Cerebellar ataxia, Areflexia, *Pes cavus*, Optic atrophy, Sensorineural deafness (CAPOS) syndrome[5,6]. Since then many more phenotypes, including early infancy epileptic encephalopathy (EIEE)[7] sometimes accompanied by polymicrogyria (PMG)[8–10], relapsing encephalopathy with cerebellar ataxia (RECA)[11,12]/fever-induced paroxysmal weakness and encephalopathy (FIPWE)[13], childhood onset schizophrenia (COS)[14] and D-DEMØ, a phenotype characterized by dystonia, facial dysmorphism, encephalopathy, severe developmental delay, MRI abnormalities (including cerebellar hypoplasia) and lacking the AHC hallmark symptom of paroxysmal hemiplegia[15], were also attributed to *ATP1A3* variants. Table 1 summarizes phenotypes currently associated with *ATP1A3* variants. In a recent editorial, Salles *et al*[16] questioned the utility of describing evermore distinct phenotypes associated with *ATP1A3*, especially as some individuals do not fit any of the currently proposed phenotypes.

In this study, we aim to describe the phenotypic features of a cohort of previously undiagnosed individuals with developmental delay and a neurological presentation, carrying

a pathogenic/likely pathogenic *ATP1A3* variant and examine where they fit within the current spectrum of *ATP1A3*-related disorders. We also perform a literature review of all *ATP1A3* variants published thus far in association with human neurological disease. Our work clearly demonstrates the heterogeneous clinical spectrum associated with *ATP1A3* variants, as well as phenotypic overlap between patients, that will streamline the diagnostic process.

METHODS

SUBJECT COHORT

An application was made to the Deciphering Developmental Disorders (DDD) study[17] for a Complementary Analysis Project (CAP), allowing access to anonymised details of individuals with *ATP1A3* variants identified through this study (https://www.ddduk.org/). If variant analysis and phenotypic details made pathogenicity likely, responsible clinicians were contacted to invite patients and their families to study recruitment. Some of the contacted clinicians had further individuals with *ATP1A3* variants in their care, which they put forward as potential participants. Genomic diagnosis was reached through trio whole exome sequencing (WES) for the DDD participants and through either WES or diagnostic gene panels for the other study participants. Phenotypic details were collected using a standardized clinical proforma covering all symptoms previously reported in *ATP1A3*-related disorders, as well as MRI features.

We used the UpSetR package in R to visualize intersections of signs and symptoms, trying to identify common phenotypes amongst individuals. As the clinical proforma included a long list of symptoms and signs due to the phenotypic variability of *ATP1A3*-related conditions, we decided to also group symptoms into broader categories where possible. We formed 4 categories:

i. Paroxysmal events, including hemiplegic events, dystonic episodes, seizures, abnormal eye movements, apnoea and autonomic episodes

ii Persistent Movement disorders, including hyperkinetic phenotypes (dystonia and chorea) and ataxia

iii Cognitive impairment (mild, moderate and severe)

iv. Neuropsychiatric symptoms, including behavioural difficulties, ADHD, ASD and COS

In order to evaluate the pathogenicity of missense variants, *in silico* prediction tools were employed, including: SIFT (sorting tolerant from intolerant)[18], PolyPhen-2 (polymorphism phenotyping v2), PROVEAN[19], MutationTaster[20] and CADD scores[21]. The presence of

variants in the healthy population (141456 individual exomes/genomes in the Genome Aggregation Database v2.1.1 (gnomAD, gnomad.broadinstitute.org/) was determined and conservation across 14 species evaluated, as well as variant segregation within each family. A literature search was performed to determine if variants were recurrent or previously reported. American College of Medical Genetics and Genomics (ACMG) classification criteria[22] were used to assess pathogenicity. Individuals with class 4 or 5 variants (likely pathogenic and pathogenic variants based on ACMG variant classification, respectively) were included in this study. Informed consent was obtained from all patients and/or their guardians.

LITERATURE REVIEW

A PubMed search was performed for all papers that included the term *"ATP1A3"* from January 2004, the year of the first association of an *ATP1A3* variant with human disease (RDP), to August 2021. Included in this study are all publications in English and one in Spanish language (where phenotypic and genomic information were included in the English abstract) reporting individuals carrying a heterozygous *ATP1A3* variant considered to be pathogenic. One publication in Japanese, one in Russian and 3 in Chinese were not included. Only publications with sufficient details about the variant (nucleotide change and/or amino acid change and gene transcript) and patient phenotype were included. All individuals published were counted, unless clearly stated that they had already been published elsewhere, in which case they were only counted once. However, it is possible that cohorts overlap. Although *ATP1A3* variants have been reported in different gene transcripts, in this article, all variant nomenclature adhere to transcript NM_152296 (isoform 1). Variants that were inconsistent with all available transcripts were presumed to have been reported incorrectly and excluded.

We calculated Combined Annotation Dependent Depletion (CADD) scores[21] for all missense *ATP1A3* variants collected from the literature, as well as for all missense variants reported within ClinVar as likely benign and benign and compared them. Unlike other genomic annotations, that tend to exploit a single information type (i.e. conservation), CADD is a framework that objectively integrates many diverse annotations into a single, quantitative score. The integrated annotations include conservation metrics, functional genomic data, transcript information and protein-level scores (Grantham, SIFT, PolyPhen). CADD calculates a raw score and a 'PHRED-scaled' score. 'PHRED-scaled' scores are normalized to all potential ~9 billion SNVs, thus providing a comparable unit for analysis. So, a 'PHRED-scaled' score of >=10 indicates a raw score in the top 10% of all possible reference

genome SNVs, a score of >= 20 or greater indicates a raw score in the top 1%, etc[23]. The developers of CADD do not suggest a rigid cutoff to suggest pathogenicity; however, looking at various HGMD molecular categories of 174,183 disease-associated deleterious mutations, Itan et al.[24] found mean CADD scores for pathogenic missense variants to be above 20. The tool is freely available on cadd.gs.washington.edu.

Constraint Analysis

Constraint analysis[25] was performed on all missense pathogenic variants in the study cohort, as well as for published cases and compared to reported benign missense variants in the population database GnomAD. The number of benign missense variants present within every 10 amino acid residues was plotted across the length of the gene and from this, the missense constraint heat map was generated using the following parameters: Dark green = >20 variants; light green = 11-20 variants; yellow = 8-10 variants; light red 1-3 variants; dark red 0 variants. Locations of pathogenic missense variants identified in the study cohort were also plotted across the length of the gene, visually highlighting regions of the gene that are tolerant and intolerant to missense variation.

Data Availability

All data relevant to our patient cohort is published within the text and tables of this article. The detailed data extracted from the literature to conduct the literature review may be shared at the request of any qualified investigator for purposes of replicating procedures and results.

Standard Protocol Approvals, Registrations, and Patient Consents

The DDD study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). The "Natural history in ATP1A3-related disease: a deep phenotyping-genotyping project" has also been granted UK Research Ethics Committee approval (18/LO/1169), including for collecting anonymized genotypic and phenotypic data from international collaborators. Informed consent for publication of clinical and molecular data was obtained from all patients included in this manuscript and/or their guardians.

Results

Subject Cohort

27 individuals with *ATP1A3* variants in the DDD cohort (nearly 14000 children recruited with their parents) were identified. In seven individuals, the *ATP1A3* variant was classified as class 2 (likely benign) due to either an incongruent phenotype or high prevalence in healthy populations. For the remaining 20 individuals, two clinicians did not report back, two

clinicians declined participation in the study and three individuals did not consent to study participation. As a result, 13 individuals from the DDD cohort were included in this study. Another 11 individuals with class 4 and 5 (likely pathogenic/pathogenic) *ATP1A3* variants were volunteered by collaborating clinicians and included as they fulfilled the study criteria. Three were family members of two separate DDD patients. Three individuals have been previously published in the literature (Patients 3, 5 and 14). Table 2 summarises the *ATP1A3* variants present in our cohort, including 13 variants that have not been published previously.

Patient Age and Disease onset

Median age of our cohort was 32.8 years (IQR15.5 years, range 3 to 52 years). 16 individuals (66.6%) were female. Age of onset of symptoms ranged from birth to 21 years with a median age of onset at 6 months (IQR = 9.94 months). The initial concern in order of frequency was developmental delay (n=8, 33.3%), epileptic seizures (n=6, 25%), dystonic events (n=3, 12.5%), abnormal eye movements (n=3, 12.5%), in one case accompanied by laryngomalacia and distal arthrogryposis all occurring at birth, autism spectrum disorder (ASD) (n=2, 8.33%), hemiplegic events (n=1, 4.17%) and torticollis (n=1, 4.17%). In one patient the disease started after an encephalopathic episode, in two after febrile episodes.

Paroxysmal Events

All individuals except for two (siblings 19a and 19b) had at least one type of paroxysmal event in their clinical history. On average, individuals had 2-3 different types of paroxysmal events. The most common were dystonic events (n=14), followed by abnormal eye movements (n=12) and epileptic seizures (n=12). Hemiplegic episodes were less common in this cohort (n=9). Autonomic episodes, such as tachycardia, mydriasis, vomiting, were reported in nine and apnoeic events in four patients.

Epilepsy

Recurrent epileptic seizures were reported in 12 individuals. In six (50%) this was supported by epileptiform features on EEG. Seizure types were reported as focal seizures (n=6), generalized tonic-clonic seizures (GTCS) (n=5) and absence seizures (n=3). Four individuals had two seizure types and seven individuals had a history of status epilepticus (SE).

Neurological Features

Most individuals had complex neurological phenotypes with 1-7 key neurological features (median: 2.5, min: 0, max 7, IQR:2). Ataxia was the most frequently reported feature (n=13), followed by dystonia (n=10), spasticity (n=8), microcephaly (n=7), decreased muscle tone/hypotonia (n=7), pyramidal signs (n=5), dysarthria (n=5), dysphagia (n=4), chorea (n=3), fluctuating muscle tone (n=3) and increased muscle tone (n=2). No individual had

areflexia . Eight individuals reported worsening of their neurological symptoms over time. Two individuals had no significant neurological co-morbidity.

NEUROIMAGING

MRI was available in 18 individuals and abnormal findings reported in 11. These were most commonly cerebellar atrophy (n=7, 38.9%), followed by hippocampal sclerosis (n=3, 16.7%), cerebral atrophy (n=2, 11.1%), thin corpus callosum (n=2, 11.1%) and delayed myelination (n=1, 5.6%). In one individual with MRI scans available at age four years and 11 years, the cerebellar atrophy progressed with age.

DEVELOPMENT

Cognitive impairment was reported in all individuals, but severity was extremely varied. Motor delay was reported in 20 (83.3%) individuals, with walking age ranging from normal at 13 months to some individuals not having learnt to walk by 18 years. Language delay was also reported in 20 (83.3%) individuals. Communication skills were very varied, ranging from starting to communicate at nine months to not having aquired language at age 20 years. Grade of cognitive impairment was reported in 17 individuals and classified as mild in five individuals (29.4%), moderate in five individuals (29.4%), and severe in seven individuals (41.2%). In five individuals (21%) regression of skills was reported, occurring either steadily over time (n=3) or in association with a clear trigger (fever, infection, status epilepticus) (n=2).

NEUROPSYCHIATRY

16 (66.7%) individuals had at least one neuropsychiatric diagnosis. 13 (54%) had behavioural difficulties, seven (29%) had a diagnosis of ASD, five (21%) a diagnosis of attention deficit and hyperactivity disorder (ADHD) and one individual a diagnosis of childhood onset schizophrenia (COS).

SYMPTOM COMBINATIONS

Looking at the combination of symptoms in our 24 patients, phenotypes were extremely varied. Across all 22 neurological signs and symptoms we collated, no two individuals shared the same combination (figure 1A). Looking only at the 11 more common signs and symptoms, again there was little overlap, with only two individuals sharing the same features (figure 1B). However, looking at the broader symptom categories paroxysmal events, movement disorders, cognitive impairment and neuropsychiatric symptoms, almost half of the cohort (45.8%, n=11) had at least one symptom from each of all four categories whilst most individuals (91.6%, n=22) had at least one symptom from three categories (figure 1C).

GENOTYPE-PHENOYPE CORRELATION IN INDIVIDUALS SHARING THE SAME *ATP1A3* VARIANT

13 of the 21 variants reported in our cohort have not previously been published in the literature. In three individuals, the variants previously published refer to the same patients as in our cohort [9,14,26]. The remaining previously published five variants (in different patients) are c.958G>A (p.Ala320Thr)[27], c.967C>T (p.Pro323Ser)[28], c.1073G>A (p.Gly358Asp)[29], c.2116G>A (p.Gly706Arg)[30–32] and c.2839G>A/C (p.Gly947Arg). Table 3 summarizes the individuals' phenotypes.

In our cohort, there are two families. They harbour two novel variants c.2393T>A (p.Leu798His) and c.2839G>T (p.Gly947Trp). The boy and girl (patients 19a and 19b), sharing the c.2393T>A (p.Leu798His) variant have both inherited the variant from their mosaic mother. They have similar phenotypes, presenting with severe cognitive impairment, ataxia, and spasticity. Patient 19b developed secondary microcephaly. In our second family, a pair of brothers (21a and 21c) have inherited variant c.2839G>T (p.Gly947Trp) from their symptomatic mother (patient 21b). Individuals 21a and 21b both had a late onset presentation with dystonic episodes presenting at 14 and 21 years respectively. Both are described as having mild cognitive impairment. The mother's neurological phenotype has further deteriorated, and she is now ataxic and has non-paroxysmal dystonia. The older brother (Patient 21c) has a much more severe phenotype, presenting much earlier at 6 months with torticollis and going on to develop hemiplegic episodes, dystonic episodes, abnormal eye movements and epilepsy. He has moderate cognitive impairment.

ATP1A3 VARIANT REVIEW

The literature search yielded 349 publications. Of these, 134 reported individuals with heterozygous *ATP1A3* variants and included genotypic and phenotypic details and were included in this review. After translating all variants into transcript NM_152296, 168 different *ATP1A3* variants in total were found in the literature to date, corresponding to 1108 reported patients. 144 variants were missense variants, 15 small intragenic deletions (one frameshift, 14 in frame), two small intragenic in frame deletion/insertions, three small intragenic in frame duplications and four splice site variants. Reviewing the number of individuals published with each variant demonstrated that almost half the patients (42.8%) carry one of the two most common variants c.2401G>A (p.Asp801Asn) and c.2443G>A (p.Glu815Lys). Two thirds (65.2%) of the cases (n=721) carry one of eight reported variants. These are c.2401G>A (p.Asp801Asn) (n=293), c.2443G>A (p.Glu815Lys) (n=180) and c.2839G>A/C (p.Gly947Arg) (n=77) the three most common variants associated with AHC,

followed by c.2452G>A (p.Glu818Lys) (n=53), the single variant associated with CAPOS. Next in frequency are variants c.1838C>T (p.Thr613Met) (n=49) and c.2273T>G (p.Ile758Ser) (n=27), the most common genotypes associated with RDP, and finally c.2267G>A (p.Arg756His) (n=26) and c.2267G>T (p.Arg756Cys) (n=16), the variants at the 756th amino acid (AA) residue causing RECA/FIPWE (Figure 2). The remaining 160 variants only account for 34.8% of the published individuals. For 84 variants, only one individual has been reported to date.

We attempted to assign phenotypes to all published cases. Mostly this was already provided by the authors but when not, we used the information available in the manuscript to determine phenotype. If we were unable to assign one phenotype (for example, if the patient had features of several *ATP1A3*-associated phenotypes) we used the term intermediate phenotype (IP). Although this approach is limited by the quality of published data, it nevertheless provided an overview of the most common *ATP1A3* phenotypes and associated genotypes. AHC was by far the most commonly reported phenotype, with 817 reported patients. A further 140 patients with RDP, 53 with CAPOS, 45 with RECA/FIPWE, 31 with EIEE, 13 with IP, four with D-DEMØ, three with isolated ataxia and two with COS have also been reported (Figure 2). Variants were generally phenotype-specific, except for six variants (c.2305A>C (Thr769Pro), c.2767G>A (Asp923Asn), c.1109C>A (Thr370Asn), c.1790G>C (Arg597Pro), c.829G>A (Glu277Lys), c.2401G>T (Asp801Tyr)), that were associated with either RDP or AHC. The phenotypes CAPOS and RECA/FIPWE are mutation-specific, with all described patients with CAPOS carrying variant Glu818Lys and all except one patient with RECA/FIPWE carrying a variant involving AA residue 756.

CADD SCORES

The mean CADD score for missense *ATP1A3* variants published in the literature as likely pathogenic/pathogenic was 26.5 (SD: 2.04). The mean CADD score for variants reported in ClinVar as likely benign/benign was 7.729 (SD: 5.27). This difference was statistically significant ($p < 3.49e\text{-}85$). The CADD scores of the novel variants identified in our patient cohort were similar to the variants published (mean: 25.98, SD: 1.65) (Figure 3).

CONSTRAINT ANALYSIS AND MUTATION CLUSTERS

The majority of pathogenic and likely pathogenic variants identified both in the literature and this study lie within six clusters, that correspond to benign missense variant deserts from the gnomAD database and vice versa (figure 4). Benign missense constraint analysis identified gene deserts displayed as a heatmap. The six regions of constraint in which missense variation leads to pathogenicity are p.123-154, p.264-382, p.578-613, p.706-818, p.854-867

and p.887-955. These regions include key protein domains such as the transmembrane helices and the cytoplasmic P and N domains.

DISCUSSION

In this study we describe the genetic and clinical features of a heterogeneous cohort of individuals with pathogenic or likely pathogenic variants in the *ATP1A3* gene. Previous studies have mostly recruited patients with a specific *ATP1A3*-related phenotype such as AHC, RDP, CAPOS or RECA, resulting in the description of clinically homogeneous cohorts. The starting point of this study differed in that patients were recruited based on the detection of *ATP1A3* variants within a whole exome sequencing study aimed at using advanced genomics to diagnose individuals with previously undiagnosed developmental delay. The resulting cohort is clinically very heterogeneous, apart from a degree of cognitive impairment present in all patients. Only two individuals in our cohort, that were recruited through a collaborator rather than the DDD study, met Aicardi's diagnostic criteria for AHC, and no individual met diagnostic criteria for either RDP or CAPOS. We believe that our dataset represents a growing reality commonly faced by clinicians: the increasing phenotypic pleiotropy associated with *ATP1A3* variants and identification of variants of uncertain significance (VUS) poses a number of diagnostic challenges.

In addition to the broad phenotypic spectrum, our cohort was also genetically heterogeneous, with shared variants only seen amongst the members of the two participating families (individuals 19a and 19b and individuals 21a, 21b, and 21c). The genetic heterogeneity of the cohort might be reflective of its phenotypic heterogeneity, as some phenotype-genotype association is evident for *ATP1A3*-related phenotypes. As discussed, all 53 CAPOS individuals published so far are linked to the variant Glu818Lys and 45 published RECA/FIPWE cases are linked to variants involving substitution of arginine at residue 756. Within the AHC phenotype, it is also well documented that individuals with variant c.2443G>A (p.Glu815Lys) are more severely affected than individuals with variant c.2401G>A (p.Asp801Asn), whilst the third most common variant c.2839G>A/C (p.Gly947Arg) results in a milder phenotype[33,34]. The eight pathogenic variants most reported in the literature are associated with one specific phenotype (figure 1).

Looking at the phenotypes of individuals sharing the same *ATP1A3* variant, it seems that, whilst some genotypes are strongly associated with specific phenotypes, there are others that result in more phenotypic variability, such as those associated with c.2116G>A

(p.Gly706Arg) and c.2839G>T (p.Gly947Trp). However, this is an observation based on a very small number of individuals and it might be that given the opportunity to look at larger cohorts of the rarer *ATP1A3* variants, mutation-specific phenotypes will arise, as they have for c.2452G>A (p.Glu818Lys) and variants at amino acid residue 756. This is important information to gather as it may help clinicians provide families with more accurate prognosis after diagnosis.

Fifty percent of our patients were reported to have a history of epileptic seizures. In half of these, epileptiform features were seen on EEG. The epilepsy phenotype varied amongst patients with some having focal seizures, whilst others had generalised epilepsy. This variability in epilepsy phenotype has been described previously in a study of 51 patients with an AHC phenotype[35] where 32 individuals had an epilepsy diagnosis, 18 focal and 11 generalized. Twenty-nine percent of our patients had a history of status epilepticus. Epilepsy treatment in this patient group is challenging. Many paroxysmal events are misdiagnosed as epilepsy and treated with antiepileptic medications with no benefit, whereas missing the epilepsy diagnosis in these patients might put them at risk of status epilepticus which has been associated with severe aggravation of symptoms[36]. It is important to correctly classify the events patients are experiencing and have a low threshold to conduct Video-EEG investigations if uncertain of the nature of occurring events. This is especially important in individuals with a higher risk of a more complex epilepsy phenotype, such as those carrying variant c.2443G>A (p.Glu815Lys).

Earlier studies of patient cohorts with a clinical phenotype of AHC mostly reported normal MRIs[37]. More recently, however, as the diverse phenotypes associated with *ATP1A3* are evolving, several reports of abnormal neuroimaging have also been published including cerebellar atrophy[38] and polymicrogyria[8–10]. Eleven of our patients also had abnormal MRI, most commonly with cerebellar atrophy, in one case proven to be progressive.

A large European cohort study of 155 individuals with a clinical diagnosis of AHC conducted in 2010 reached the conclusion that AHC is a non-progressive disease[39]. However recent publications dispute this at least for some patients with an AHC phenotype[36,40] Little long-term data has been published for patients with other *ATP1A3*-related phenotypes. In our cohort 21% (n=5) of individuals had regression of skills over time. Although further longitudinal data are required, when counselling families, it may be important to discuss the risk of neurological regression over time in *ATP1A3*-related disorders.

Traditionally, patients with an *ATP1A3*-related phenotype, such as AHC or RDP, have been diagnosed by utilising clinical diagnostic criteria[41,42]. In recent years with the association of

ATP1A3 variants with a broadening clinical spectrum, this approach is not feasible for all patients, as many do not fulfil classic phenotypic criteria. Also, with broad genetic testing (gene panels, WES, whole genome sequencing (WGS)) being brought into the diagnostic process at a much earlier stage, clinicians are often faced with an *ATP1A3* variant in an undiagnosed patient, trying to decide whether it is responsible for the phenotype, rather than having already reached a clinical diagnosis and using genetic investigations to confirm or inform it. We compared the phenotypic characteristics of our cohort to the diagnostic criteria published for AHC, RDP, CAPOS, D-DEMØ (Table 1). Using the classic diagnostic criteria for AHC first introduced by Aicardi[43] and subsequently used as inclusion criteria in clinical studies of patients with AHC[39], only two patients in our cohort and none of our DDD cohort achieve a clinical AHC diagnosis. Very recently these criteria have been revised to include presence of an *ATP1A3* variant, and relaxed to describe a wider clinical spectrum of patients[41]. Applying the new criteria, one patient recruited through the DDD study and another seven patients of our extended cohort are clinically diagnosable with AHC. None of our patients met criteria for either RDP or CAPOS, and one patient met criteria for D-DEMØ. Overall, we found that most of our patients cannot be grouped into any of the existing described phenotypes. Rosewich et al.[44] published major and minor criteria to support a diagnosis of an *ATP1A3*-related condition. The authors identified five major and five minor criteria for patients with infantile and early childhood onset, six major and six minor criteria for patients with childhood and adult onset, and seven major and seven minor criteria applicable for patients presenting at any age. So, for early onset there are 12 major and 12 minor criteria overall, whilst for late onset there are 13 major and 13 minor criteria. No cut-off is given as to how many criteria should be fulfilled to establish an *ATP1A3*-related condition diagnosis. In our cohort, 22 individuals had an onset in infancy/ early childhood, and two had an onset in later childhood or adulthood. All patients met at least three minor criteria. The only criteria met by all individuals in this cohort were cognitive impairment and negative family history or history suggesting autosomal dominant inheritance (both minor criteria). On average, patients met 3.4 major and 5 minor criteria. This approach of defining a spectrum of associated symptoms seems to be preferable for patients with *ATP1A3*-related disorders, if the threshold for number of criteria needing to be fulfilled to prompt testing is kept low.

In addition to this, we found that in our cohort looking for a combination of broad symptom categories, namely paroxysmal symptoms, hyperkinetic symptoms, neuropsychiatric symptoms, and cognitive impairment, rather than specific symptom combinations was more

likely to identify patients with *ATP1A3*-related disorder. A CADD-score above 20 and a variant located within the mutation clusters in regions of constraint further support diagnosis of an *ATP1A3*-related disorder.

There are limitations to our study. The phenotypic information was collected in retrospect through patient interview or case note review, rather than prospective evaluation. The phenotypic information available for published cases is variable and sometimes limited; patients are reported at different ages, clinical information is collected retrospectively, and different authors focus on different symptoms.

CONCLUSION

Two thirds of all published individuals with *ATP1A3*-related disorders carry one of the eight most common variants and display one of the four most common phenotypes: AHC, RDP, CAPOS or RECA/FIPWE. However, the remaining third of individuals carry another 160 *ATP1A3* variants and their phenotypes are very variable forming a phenotypic continuum of paroxysmal, neurological, developmental, and neuropsychiatric features. Nowadays clinicians are often faced with novel variants of uncertain significance in genes associated with rare diseases. Looking for a combination of paroxysmal events, hyperkinesia, neuropsychiatric symptoms, and cognitive impairment, as well as evaluating CADD score and variant location, can aid diagnosis of an *ATP1A3*-related disorder. The ongoing collection of phenotypic information of individuals carrying rarer variants will help us discover further mutation-specific phenotypes and aid disease prognosis and management.

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Table 1: *ATP1A3* associated phenotypes.

Phenotype	Rapid-Onset Dystonia Parkinsonism (**RDP**)	Alternating Hemiplegia of Childhood (**AHC**)	Cerebellar Ataxia, Areflexia, Pes Cavus, Optic Atrophy, Sensorineural Hearing Loss Syndrome (**CAPOS**)	Relapsing Encephalopathy with Cerebellar Ataxia (**RECA**) / Fever-induced Paroxysmal Weakness and Encephalopathy (**FIPWE**)	Early Infantile Epileptic Encephalopathy (**EIEE**) +/- Polymicrogyria (**PMG**)	Symptom onset on day 1 of life, Dystonia, Dysmorphism of the face, Encephalopathy (Global Delay), MRI abnormalities (Cerebellar Hypoplasia), Absence of Hemiplegia (**D-DEMØ**)	Childhood Onset Schizophrenia (**COS**)
Number of patients published 2004-2021	140	817	53	45	31	4	2
Common associated *ATP1A3* variants (> 15 cases published)	p.Thr613Met p.Ile758Ser	p.Asp801Asn p.Glu815Lys p.Gly947Arg	p.Glu818Lys	p.Arg756His p.Arg756Cys			
Diagnostic criteria/ supportive clinical features	Diagnostic criteria[37]: - Dystonia - Absence of motor symptoms < age 18 months - *ATP1A3* mutation Supportive features: - Rapid onset of dystonia with features of parkinsonism over a few minutes to 30 days - Bulbar findings on examination - Executive	Diagnostic criteria[36]: Essential criteria: - Paroxysmal episodes of Hemiplegia that alternate between sides and/or quadriplegia - Abnormal neurological development Major criteria: - Onset < age 18 months - Dystonic episodes - Different types of episodes occurring independently or together at the same time with evolution from one or more symptoms to	Clinical Features[6]: - Onset 6 months – 4 years - Episodes of ataxic encephalopathy and/or weakness during or after a febrile illness - Usually 1-3 separate episodes with residual symptoms that can slowly progress over time Residual symptoms: - Cerebellar Ataxia	Clinical Features[12]: - Onset 8 months – 10 years - Episodes of ataxic encephalopathy and/or weakness during or after a febrile illness - Patients experience 2 episodes on average - Slow recovery and residual symptoms - No progression in Adulthood Residual symptoms: - Cerebellar Ataxia - Dysarthria - Hypotonia	Clinical Features: - Severe develop mental delay - Epilepsy Onset in Infancy - Polymicr ogyria present in some patients.	Clinical Features[15]: - Onset day 1 of life - Dystonia - Dysmorp hism of the Face - Global Developm ental Delay - Cerebellar Hypoplasi a - No Hemipleg ic episodes	Diagnostic Criteria (DSM IV-TR): - 2 or more of the following: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour, negative symptoms (flattening, alogia, avolition) - Social Dysfunction - Persistence of symptoms for at least 6 months - Exclusion of

	dysfunction (Haq et al. 2019)	others during that one episode - Paroxysmal abnormal eye movements, such as nystagmus and especially monocular nystagmus - *ATP1A3* mutation - Plegia spells improve with sleep Minor criteria: - Epileptic seizures - Episodes of altered consciousness - Abnormal motor function (hypotonia or dystonia that can coexist, ataxia, choreoathetosis, oral motor control - Episodic autonomic dysfunction 2 essential criteria + 3 major criteria OR 2 essential criteria + 2 major and 3 minor criteria needed for diagnosis	- Areflexia - Pes Cavus (few patients described) - Optic Atrophy - Sensorineural Hearing Loss	- Dystonia - Choreiform movements - Cognitive Delay - Oculomotor symptoms			Schizoaffective and Mood Disorder - Exclusion of Substance Abuse or General Medical Condition - If an Autistic Disorder or other Pervasive Development al Disorder is present the additional diagnosis of COS can only be made if prominent delusions or hallucinations are also present for at least 1 month. - Age of onset < 13 years

Table 2: ATP1A3 variants present in our cohort. All variants are missense and absent from the population database gnomAD version 2.1.1

Case nr	1	2	3	4	5	6	7	8	9	10	11	12
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	unknown	de novo
Chr19(GRCh37)	g.42492179C>G	g.42492179C>A	g.42490354C>T	g.42489219T>A	g.42489116C>A	g.42489105C>T	g.42489099C>T	g.42489096G>A	g.42489071G>A	g.42486215C>A	g.42486179C>T	g.42486176G>T
cDNA change	c.266G>C	c.266G>T	c.385G>A	c.844A>T	c.947G>T	c.958G>A	c.964G>A	c.967C>T	c.992C>T	c.1037G>T	c.1073G>A	c.1076C>A
Protein change	p.(Gly89Ala)	p.(Gly89Val)	p.(Val129Met)	p.(lle282Phe)	p.(Gly316Val)	p.(Ala320Thr)	p.(Val322Ile)	p.(Pro323Ser)	p.(Thr331Ile)	p.(Cys346Phe)	p.(Gly358Asp)	p.(Ser359Tyr)
SIFT	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)
MutationTaster	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)
Polyphen 2 HumDiv	Probably damaging (score 0.999)	Probably damaging (score 1)	Probably damaging (score 0.999)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 0.999)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 0.998)
PROVEAN	-4.489 (deleterious)	-6.732 (deleterious)	-2.374 (neutral)	-3.392 (deleterious)	-6.873 (deleterious)	-3.427 (deleterious)	-0.848 (neutral)	-6.947 (deleterious)	-5.042 (deleterious)	-9.292 (deleterious)	-5.913 (deleterious)	-5.069 (deleterious)
CADD	23.2	23.7	25.3	26.5	26.2	26.6	25.3	25.4	27.2	27.5	25.7	25.7
Conservation (14 species)	complete	complete	complete	complete	complete	complete	complete	complete	complete	complete	complete	complete
Novel variant	yes	yes	no[12]	yes	no[8]	no[26]	yes	no[24]	yes	yes	no[27]	yes
Case nr	**13**	**14**	**15**	**16**	**17**	**18**	**19a**	**19b**	**20**	**21a**	**21b**	**21c**
Inheritance	de novo	de novo	de novo	de novo	de novo	paternal (mosaic father)	maternal (mosaic mother)	maternal (mosaic mother)	unknown	maternally inherited	unknown	maternally inherited
Chr19(GRCh37)	g.42482377G>A	g.42480621C>T	g.42479928C>T	g.42479882C>T	g.42479831A>C	g.42479793C>T	g.42474565A>T	g.42474565A>T	g.42471896C>T	g.42471896C>A	g.42471896C>A	g.42471896C>A
cDNA change	c.1732C>T	c.2041G>A	c.2116G>A	c.2162G>A	c.2213T>G	c.2251G>A	c.2393T>A	c.2393T>A	c.2839G>A	c.2839G>T	c.2839G>T	c.2839G>T
Protein change	p.(Leu578Phe)	p.(Ala681Thr)	p.(Gly706Arg)	p.(Gly721Glu)	p.(Met738Arg)	p.(Gly751Arg)	p.(Leu798His)	p.(Leu798His)	p.(Gly947Arg)	p.(Gly947Trp)	p.(Gly947Trp)	p.(Gly947Trp)
SIFT	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)	Deleterious (score 0)
MutationTaster	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)
Polyphen 2 HumDiv	Benign (score 0.386)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 0.976)	Benign (score 0.203)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)	Probably damaging (score 1)
PROVEAN	-3.267 (deleterious)	-3.035 (deleterious)	-6.208 (deleterious)	-5.999 (deleterious)	-4.490 (deleterious)	-5.827 (deleterious)	-5.180 (deleterious)	-5.180 (deleterious)	-4.310 (deleterious)	-4.673 (deleterious)	-4.673 (deleterious)	-4.673 (deleterious)
CADD	26.7	25.9	25.1	27.3	24.2	25	26.6	26.6	27.7	28.9	28.9	28.9
Conservation (14 species)	complete	complete	complete	complete	complete	complete	moderate	moderate	moderate	moderate	moderate	moderate
Novel variant	yes	no[23]	no[28,29,30]	yes	yes	yes	yes	yes	no[4]	yes	yes	Yes

Table 3: Phenotype of patients with variants previously published in the literature compared to phenotypes of patients with the same variants in our cohort. Published phenotypic descriptions were limited for variants p.Ala320Thr and p.Pro323Ser. Our patient with variant p.Gly947Arg has an AHC phenotype, as expected. For variants p.Gly358Asp and p.Gly706Arg we highlight symptoms previously described in red.

***ATP1A3* variant**	**Phenotype of patient in our cohort**	**Phenotype of patients previously published**		
p.Gly358Asp	Patient 11 • Age: 3 years • Onset age 2 years • Initial concern: Hemiplegic Episodes • Dystonia • Dysarthria • Dysphagia • Mild Developmental Delay: walks independently and talks in 2-word sentences.	Pereira et al.[30] • Age: 5 years • Onset age 3 years • Initial concern: Rapid-onset left brachial-predominant dystonia • Dysarthria • Dysphagia • Action tremor • Hemiplegic episodes • Normal Development		
p.Gly706Arg	Patient 15 • Age: 27 years • Onset in infancy • Initial concern: Developmental Delay • Moderate cognitive impairment • Epilepsy • Ataxia • Chorea • Autistic features • MRI: bilateral temporal sclerosis	Yang et al.[31] • Onset age: 2 months • Initial concern: Abnormal Eye Movements • Hemiplegic Episodes • Quadriplegic Episodes • Dystonia • Developmental Delay	Holze et al.[33] • Onset age: 9 months • Initial concern: Apnoeic events (? Epileptic) • Mild Developmental Delay • Lost to follow up age 2 years	Hully et al.[32] (siblings) • Onset age: 2 months and 4.5 months • Initial concern: Epileptic seizures • Severe developmental and epileptic encephalopathy • Autistic features • Abnormal eye movements • MRI: bilateral temporal sclerosis
p.Ala320Thr	Patient 6 • Age: 5 years • Onset age day 1 of life • Initial concern: distal arthrogryposis, laryngomalacia, abnormal eye movements. • Severe developmental	Trump et al.[29] • Seizures • Developmental Delay • Alternating Hemiplegia		

	delay • Epilepsy • Apnoeic Episodes • Hemiplegic Episode (only occurred once at age 10 years) • Dysarthria • Chorea	
p.Pro323Ser	Patient 8 • Age: 18 years • Onset age day 1 of life • Initial concern: Epileptic seizures (resolved at 18 months) • Severe developmental delay • Dystonia • Abnormal eye movements • Microcephaly • Dyskinesia • No Hemiplegic Episodes	Moya-Mendez et al.[27] • 2 patients with a clinical diagnosis of AHC, no further phenotypic details given
p.Gly947Arg	Patient 20 • Age: 43 years • Onset age: 6-12 months • Initial concern: Moderate Developmental Delay • Hemiplegic episodes • Quadriplegic Episodes • Abnormal Eye Movements • Autonomic Episodes • Dystonia • Dysarthria • Spasticity with Pyramidal Signs	One of the 3 most common variants associated with a relatively mild phenotype of AHC (n=77 patients published)

FIGURE LEGENDS

Figure 1: Symptom combinations in our subject cohort

A: Phenotypes in our cohort are extremely variable, with none of the patients sharing a combination of neurological signs and symptoms.

B: Looking only at the most common 11 signs and symptoms, only 2/24 have overlapping features.

C: If we group symptoms into 4 categories: 1. neuropsychiatric symptoms, 2. hyperkinetic movement disorders, 3. paroxysmal episodes and 4. cognitive impairment almost half our cohort (n=11) has a phenotype combining all four categories and almost all individuals (22/24 individuals) have a phenotype combining three out of the four categories.

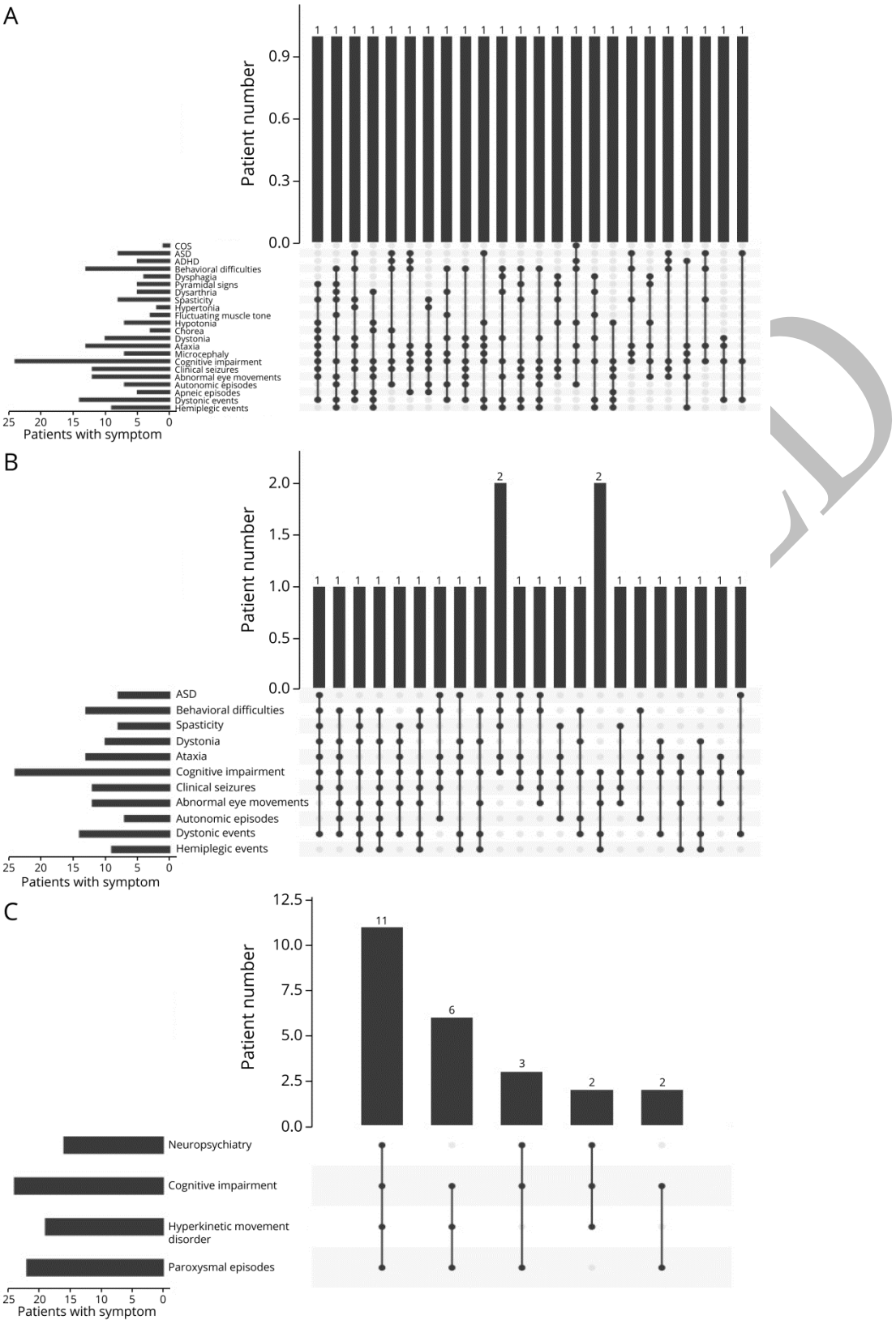

Figure 2: *ATP1A3* variants

A: Distribution of variants across the α3 subunit of the sodium/potassium transporting ATPase. Location of protein domains is shown across the protein: turquoise 1-10 transmembrane domains, red Actuator domain, orange Phosphorylation site, green Nucleotide binding site. Variants are color-coded per phenotype. AHC: Alternating Hemiplegia of Childhood, RDP: Rapid-Onset Dystonia Parkinsonism, CAPOS: Cerebellar Ataxia, Areflexia, Pes Cavus, Optic Atrophy, Sensorineural Hearing Loss Syndrome, RECA: Relapsing Encephalopathy with Cerebellar Ataxia, D-DEMØ: dystonia, dysmorphism of the face, encephalopathy with developmental delay, brain MRI abnormalities always including cerebellar hypoplasia, no hemiplegia (Ø) and neonatal onset of symptoms, EIEE: Early Infant Epileptic Encephalopathy, COS: Childhood Onset Schizophrenia, IP: Intermediate Phenotype.

B: Frequency of phenotypes among the 1000+ reviewed patients reported in the literature from 2004-2021.

C: Eight most frequent variants responsible for ATP1A3-related disorders in the 1000+ reviewed patients reported in the literature from 2004-2021. Each variant leads to a specific phenotype.

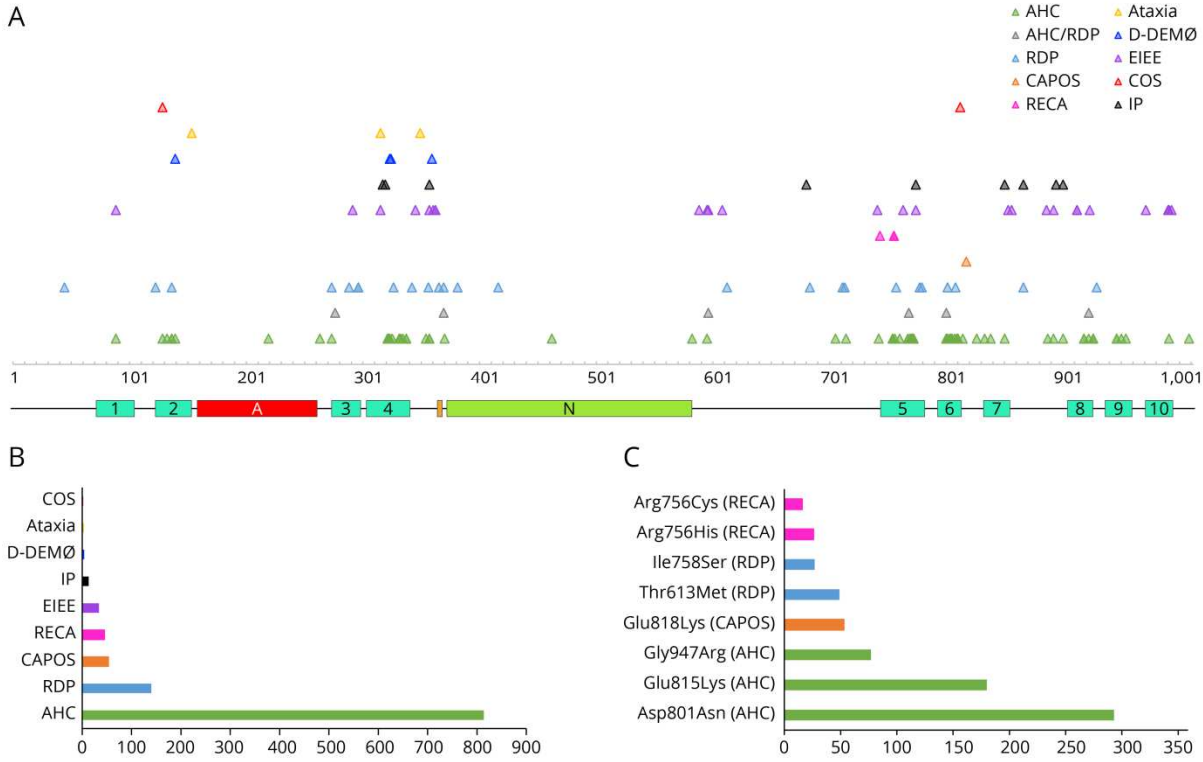

Figure 3: CADD scores

CADD scores associated with benign/ likely benign ATP1A3 variants published in ClinVar (green) are significantly lower than the novel ATP1A3 variants in our patient cohort (orange) (p= 2.94e-39), and ATP1A3 variants published as pathogenic in the literature (purple) (p=1.05e-84). There is no significant difference between the novel and published pathogenic variants (p=0.167).

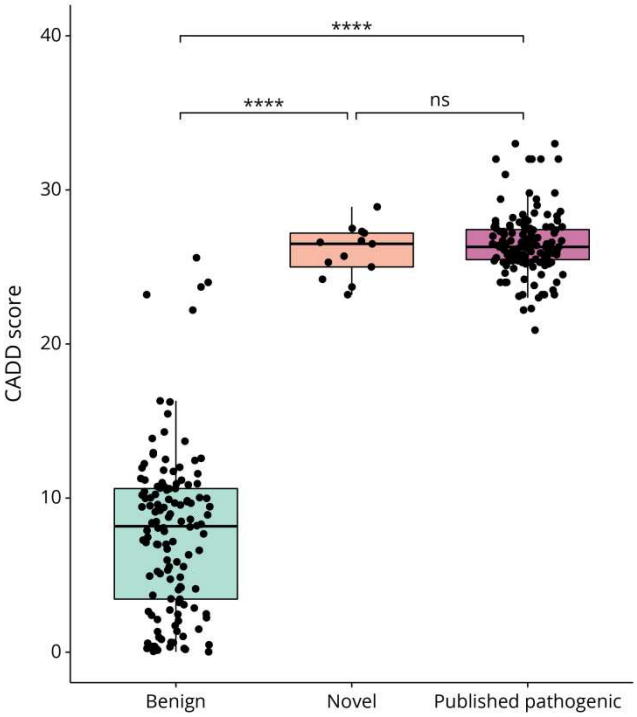

Figure 4: Constraint Analysis

Missense allele counts for all ATP1A3 missense variants were obtained from gnomAD v2.1.1. Missense amino acid substitutions are represented in grey (top section). We constructed a heatmap representing intolerance to missense changes, ranging from dark green through yellow, orange and red with increasing intolerance (dark green = >20 variants; light green = 11-20 variants; yellow = 8-10 variants; light red 1-3 variants; dark red 0 variants). All pathogenic mutations from the literature (missense in green, small deletions/duplications in pink and a sole frameshift mutation at residue 89 in red) and our cohort (all missense in yellow) and their distribution across the protein are shown (bottom section). The grey and white vertical shading represent mutation clusters and deserts respectively. Highly constrained regions encompass transmembrane domains 2-9 (turquois), the phosphorylation site (orange) and the end of the nucleotide binding site (green), whilst the actuator domain (red) is situated in a mutation desert.