The Phenotypic Spectrum of Xia-Gibbs Syndrome

2/22/2108

Dr Nadja Ehmke, M.D.
Communicating Editor
American Journal of Medical Genetics

Re: 17-1067 (revised)

Dear Dr. Ehmke

Thank you for the reviews and consideration of our submission of the manuscript 'THE PHENOTYPIC SPECTRUM OF XIA-GIBBS SYNDROME' by Jiang et al., for publication in the American Journal of Medical Genetics. We have revised the manuscript following the guidance of the reviewers and hope that it is now view favorably by the Journal.

One suggestion by Reviewer two was to provide a single large table with all clinical data for all 20 patients and a summary column. In doing this, we have endeavored to minimize the size of the table, yet it remains quite large. If it is too large for the main text we will be pleased to further modify by returning some data to the supplement.

Please note we added one author since the initial submission – David R. Murdock.

Thanks again for all your efforts.

Sincerely

Richard Gibbs

Response to Reviewer's Comments:

THE PHENOTYPIC SPECTRUM OF XIA-GIBBS SYNDROME, Jiang et al.

Comments to the Author

This is a very interesting report on 20 patients with Xia-Gibbs syndrome, a condition originally identified by NGS analysis of a large heterogeneous cohort, in which subjects with truncating mutations in the same gene were identified and subsequently grouped together for analysis. With the advent of large scale NGS analysis of clinical populations this represents what is likely to become the norm in future. The original clinical descriptions were based on only 4 affecteds so clear information about the clinical nature of the disorder were lacking. The present publication provides clinical data on 20 patients and so makes a valuable contribution to the literature on this disorder. I think this data is a valuable addition to the sparse literature on this disorder, but I think some attention needs to be paid to the presentation.

Thank you for the positive view of the significance of the study.

There is very little novel molecular data in this manuscript, other than the reports of new mutations. There is no functional data to support any interpretation of the pathology or pathophysiology of the condition.

I feel the majority of the interest here lies in the clinical descriptions.

We agree that the clinical data provide the majority of the interest and recognize the reviewers point. We chose the title 'The Phenotypic Spectrum of Xia-Gibbs Syndrome' to reflect this and in revision, we have endeavored to more fully emphasize the clinical observations throughout.

We also respectfully point out that the data also show (i) the preponderance of de novo truncating mutations, (ii) additional sites of mutation and (iii) the additional 'length' of the region spanned by these mutations. While secondary, we believe the mutation data do add novelty. Hence, while we reduced the discussion of mutation data and removed the Supplementary Figure S1 (see below), we have still retained some of the data and discussion.

The original publications concerned 4 patients (Xia et al 2014) and 5 patients (Yang et al 2015). These publications highlighted different aspects of the phenotype, Xia stressing the presence of anatomic upper airways abnormalities, and subsequent sleep apnea. Yang et al (2015) stressed the presence of ataxia in their group of five patients.
The present manuscript reports on 20 patients., but does not directly address the issues of ataxia or anatomic airways abnormalities highlighted in the original reports. I feel that this should be addressed.

We have added more about ataxia and upper airway issues in the text. Ataxia is also added to the new Table One, describing all patient data.

Other comments:

(1) it is not completely clear exactly how the clinical data was obtained. It appears to me that no patients underwent clinical review in person, it seems the clinical data was obtained from case note review. This needs to be made explicitly clear.

We apologize for the confusion. The reported data here are composite clinical data from:
(i) Assessment in our clinic (2 individuals),
(ii) Observation by clinicians (MFW, JRL, JEP) at the face to face family meeting (11 individuals),
(iii) review of medical records, including genetic reports (18 individuals), and
(iv) the clinical survey with follow up (20 individuals)

To clarify we have modified the text and in addition we added Supplementary Table S2 (see below) indicating specifically how each patient was assessed.

Patient	Assessment in Clinic	Face to face meeting	Medical Record Review	Clinical Survey
1		X	X	X
2		X	X	X
3	X	X	X	X
4			X	X
5		X	X	X
6			X	X
7		X	X	X
8			X	X
9		X		X
10		X	X	X
11			X	X
12		X		X
13			X	X
14			X	X
15			X	X
16	X	X	X	X
17			X	X
18		X	X	X
19		X	X	X
20			X	X

(2) The description of the clinical phenotypes is spread across table 1, and supplementary tables 1 and 2. I feel that all of this data could be amalgamated into a single table of clinical features,

with summary numbers in a final column. This is a fairly standard way of presenting such data, and would complement fig 4. More attention needs to be given to clinical neurological findings, to extend the observations of Yang et al, and to confirm the presence/absence of spasticity (Yang et al) and the risk of seizures. These are important clinical features and will be of great value in the clinical setting. The MRI findings are based on review of 6 patients, but the increased volume of extra-axial space in the posterior fossa and hypoplasia of the corpus callosum are well demonstrated and should be included.

We have followed this reviewer's guidance and combined the previous Tables 1 (main text) and supplementary tables 1 and 2 into a single new supplementary Table. The new table is now quite large (the reason that we previously divided the data) and we hope that the journal will permit its inclusion in the main text as the new Table 1. We also modified the text to include statements regarding seizure and ataxia:

(3) There is detailed description of speech delay in terms of M-CHAT scores. The M-CHAT score is a tool in large measure used for case finding for children at high risk of ASD diagnoses. Despite the lengthy description of M-CHAT the authors then go to say that 5/20 children had diagnoses of autism, no mention is made of the methodology whereby this diagnosis was arrived at. I am not sure that the lengthy description of M-CHAT sits well with the paucity of the data on the ASD diagnosis.

The Autism diagnoses in those 5 individuals was derived from the primary care clinicians and were communicated to us in clinical survey data and notes. We considered the heterogeneity of methods used by different referring physicians for reporting an ASD diagnosis in the context of an international study and therefore used M-CHAT as an independent source of standardized criteria. In the text, we endeavor to more clearly point out the relationship between the prior clinical assessment and the M-CHAT data.

(4) Figure S2 illustrates relationships between age/sex and chat score and sex and speech. There is a clear difference in the acquisition of speech between males and females, but this could be easily included in the text, there is an obvious difference on X-squared testing. It is not clear what methodology is being used in the left-hand panel, I do not feel this contributes to understanding and should be left out, as well as comments accompanying it in the text.

We agree with the reviewer and have deleted Figure S2. Text mentioning Figure S2 was also deleted accordingly.

(5) Genotype-phenotype correlations are discussed on page 8 lines 48 et seq, it is not clear to me that any conclusions can be drawn with such small numbers. Supplementary Fig S3 seeks to illustrate this, but does not clarify mattes further. It is not clear what methodology is being used in this analysis, this figure could be omitted.

We agree that the overall study is not statistically significant, given the small number of patients. However, we respectfully disagree as to the value of the discussion – with appropriate qualifiers - and propose to continue to include these data to stimulate thoughts and hypotheses

on the possible genetic/phenotypic associations. We also note that patients with same genotype do not necessarily share the same phenotypes in our comparison.

We agree that Fig S3 can be removed and we changed the section in the text mentioning Fig S3 to 'data not shown'.

(6) Page 9 line 50 et seq speculates on the absence of missense mutations in this gene so far in the literature. This is supported by unpublished data from the authors lab. Given the difficulty of assigning pathogenicity to missense variation, the comments are reasonable, but hardly novel. This section could be omitted without detracting from the manuscript overall.

We note the reviewer's points and indeed similar debates of the value of this discussion have occurred here. In submission, we opted to maintain this part of the discussion as we are aware of other investigators rushing to assign pathogenicity to missense de novo variants in this gene. Of course, we will delete this entire paragraph if the reviewer insists – but meantime have abbreviated it.

(7) A single cartoon of mutations would be useful, I am not sure fig S4 is necessary.

Figure S4 (now Figure S1, after original S1, S2 and S3 were removed) illustrates the domain where the mutations are located at and the conservation of these mutation sites. Additional text edits were also added to illustrate this point. We feel this is an important new observation for readers to be aware of.

Reviewer: 2

Comments to the Author
In the article „The phenotypic spectrum of Xia-Gibbs syndrome "the authors describe the largest cohort of individuals with AHDC1 mutations identified by WES. This article is of interest to the readers of this journal, I have some major and minor comments, which are listed below.

Major comments:

Abstract:
The abstract does not reflect the article very well and is imprecise concerning some points. For example, it suggests that all individuals show thinning of the corpus callosum. Self-injury is mentioned as a feature, but not discussed properly in the manuscript. I suggest rewriting/rewording of the abstract.
0

Thank you for pointing this out – we have modified the abstract to indicate that only six of the individuals had MRIs that were available for re-review, to be shown to have thinning of the corpus callosum. We deleted the reference to self-injury in the abstract.

All individuals were diagnosed by WES. I strongly suggest including a supplemental table listing all other possibly pathogenic variants identified in all individuals.

We have primary whole exome data from 5 of the 20 patients. Data and genetic information for the rest of the patients were from genetic reports or physician letters from outside institutions (all were de novo except for one who was assumed de novo, wildtype only confirmed in mother). Supplementary table S1 was added listing other possible gene candidates for some of the patients.

Minor comments:

Some sentences are difficult to understand. For example in the Introduction: The long sentence starting on page 3, line 21: … the term "the suggestion of aggressive behavior" does not make sense.

(line 25 instead) changed to " indication of aggressive behavior"
I changed to "presence"

Results: First sentence of this section (it contains "molecular" twice). Please read the manuscript carefully to correct mistakes.

We have removed the second 'molecular'

Results:
Page 6, line 3: What does M-CHAT stand for? Please include this information.

M-CHAT has been spelled out as 'Modified checklist for Autism in Toddlers'

Page 6, line 24: Six out of 20 patients are 30%, not 31.6%. Please change.

Corrected

Discussion:
Page 10, second and third paragraph: Please reword the last sentences of these paragraphs: .. suggest … significantly… Data on significance for male patients and the likelihood of being non-verbal is included, but there is not data on the significance of the risk of developing scoliosis.

Data indicating the significance of the risk of developing scoliosis is in page 7, line 26, p=0.018, compared to up to 5.2% prevalence in general population.

Page 10, line 50: please reword the last sentence of this paragraph: … "for symptoms that may be greater in individual cases" … what does that mean?

'greater' has been changed to 'more severe'

Figure legends:
Figure 1: The figure legend includes the description of an asterix, but I cannot find it in the figure (?). Please change the figure accordingly.

These have been been added to Figure 1

Figure 2: Please include that M-CHAT scores indicate risk for autism. It is not mentioned in the figure legend.

M-CHAT scores with high risks are being indicated in the legend.

Figure 4: Please include a short description of some overlapping facial features.

A short description of some overlapping facial features are included: 'overlapping facial features include broad forehead, hypertelorism, flat nasal bridge and thin upper lip'.

Supplementary table 1: Which height measurement is used? Please include explanation. No height available for patient 12 and 20?

Height was shown in percentage (WHO child growth standards 2-5yo http://www.who.int/childgrowth/standards/height_for_age/en/ and WHO growth references for 5-19yo http://www.who.int/growthref/en/, percentage for two adult patients are assessed as 19 year olds). The above information has been added to the table legend. Heights for patients 12 and 20 were not available at the time of manuscript submission. Height information for 20 was currently available and has been added (0.01).

THE PHENOTYPIC SPECTRUM OF XIA-GIBBS SYNDROME

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ABSTRACT:

Xia-Gibbs syndrome (XGS: OMIM # 615829) results from *de novo* truncating mutations within the AT-Hook DNA Binding Motif Containing 1 gene (*AHDC1*). To further define the phenotypic and molecular spectrum of this disorder, we established a Xia-Gibbs Syndrome Registry and recruited patients from the worldwide pool of approximately 60 probands. Additional *de novo* truncating mutations were observed among 25 individuals, extending both the known number of mutation sites and the range of positions within the coding region that were sensitive to alteration. Detailed phenotypic examination of 20 of these patients via clinical records review and data collection from additional surveys showed a wider age range than previously described. Data from developmental milestones showed evidence for delayed speech and that males were more severely affected. Neuroimaging from six available patients showed an associated thinning of the corpus callosum and posterior fossa cysts. An increased risk of both scoliosis and seizures relative to the population burden was also observed. Data from a modified autism screening tool revealed that XGS shares significant overlap with autism spectrum disorders. These details of the phenotypic heterogeneity of XGS implicate specific genotype/phenotype correlations and suggest potential clinical management guidelines.

INTRODUCTION:

Xia-Gibbs syndrome (XGS: OMIM #615829) is a newly described disorder characterized by developmental delay, hypotonia, speech delay, sleep apnea and seizures(Xia et al., 2014). Patients with XGS have *de novo* truncating mutations within a critical region of the *AT-Hook DNA Binding Motif Containing 1* gene (*AHDC1*). Probands present with features that are associated with several different syndromes and are observed in many undiagnosed patients who likely harbor other genetic disorders, making DNA testing and the establishment of molecular diagnosis the primary diagnostic tool for XGS. Indeed, the original identification of the disorder depended on the identification of molecular variation in *AHDC1,* rather than recognition of a distinctive set of shared clinical features. This initial report of XGS focused on four probands and their relatives who were clinically assessed after identification of *AHDC1* truncating alleles, revealing a shared set of phenotypic features(Xia et al., 2014). Subsequently, an additional seven patients were described with features consistent with the original four that were reported(Yang et al., 2015). These published cases each have truncating *AHDC1* mutations, but display clinical heterogeneity, including the variable occurrence of seizures, the extent of anomalies detectable by brain MRI, differences in the overall levels of cognitive function, the age of language development and the possible presence of aggressive or unruly behavior in some cases(Xia et al., 2014; Yang et al., 2015). Whether this reflects allelic heterogeneity and if specific genotype/phenotype correlations can be discerned, remains unknown.

Approximately 60 probands worldwide have now been identified through additional publication(Bosch et al., 2016; Garcia-Acero & Acosta, 2017; Miller et al., 2017; Park, Kim, Jang, & Jang, 2017; Quintero-Rivera et al., 2015), self-referral, physician contact, and social media. Xia-Gibbs syndrome is, therefore, an example of a disorder where the diagnostic impact of whole-exome sequencing, when combined with social media, has allowed families to rapidly build networks, establish support groups and participate as stakeholders in research(Enns et al., 2014).

To more systematically evaluate the consequences of *de novo* truncating mutations in this gene, and to provide a resource for the future study of XGS and *AHDC1*-related disorders,

we established a Xia-Gibbs Syndrome Registry to gather genotype and phenotype data from multiple families. The detailed evaluation of the molecular and clinical spectrum of 20 cases provides a basis for both improved forecast of prognoses and support for research studies.

METHODS:

Identification of AHDC1 Families: Sixty families were identified worldwide via self-reporting, physician referral, and social media.

Registry: A Registry was established utilizing RedCap (Research Electronic Data Capture), which is a secure, web-based application designed to support data capture for research studies(Harris et al., 2009). This application was initially housed in a HIPAA compliant environment hosted by Amazon Web Services (AWS) and later migrated to a local, compliant host environment to allow all the records in the registry to be maintained in a confidential environment.

Ethics, Consent, and Permissions: The infrastructure for the Registry and outreach to identified probands and families was approved by the Baylor College of Medicine Institutional Review Board. Invitation emails were sent to parents and caretakers, and positive responders were provided informed consent for Registry participation. The Registry includes full contact information, detailed patient records from each participant's clinical consultations and parent responses to a questionnaire designed to capture clinical and developmental features of the affected child. A second IRB approved Protocol was used to further consent individuals who were in the Registry, for whom there were additional requests for research participation.

Clinical Ascertainment: Parents of probands consented to their participation in the registry, together with their affected children. Participants join the registry by completing an initial survey and by providing their contact information and a genetic testing report documenting a pathogenic variant in the *AHDC1* gene. A further clinical survey was then administered (complete survey details are in the supplementary materials). In addition to the clinical survey, 14 probands and their families attended a clinical and family conference held in April of 2017, in Houston, Texas. At that conference, clinical observations from the survey data were clarified by the family and physicians. Additional medical records and MRI images were also obtained for some of the subjects. Available MRI images were independently reviewed by a pediatric neuroradiologist (JVH).

Facial Feature Analysis: Facial feature analyses were performed using the Facial Dysmorphology Novel Analysis software (FDNA, Inc. Boston, MA) and facial images provided by families. A mask depicting the composite, characteristic appearance of XGS was created.

Data Analysis: Statistical analyses were performed using R. The Fisher exact test was used to compare the language ability between male and female patients. A binomial test was used to compare the prevalence of scoliosis in the patients with the frequency in the general population.

RESULTS:

Overview of the patients, gene, and *AHDC1* mutations:

Twenty individuals with *de novo* truncating *AHDC1* mutations had detailed clinical assessments and were included for the phenotypic comparison described in this study. These individuals represent a subset of the 60 families described worldwide as of November 2017, for whom our review of the available records of mutation data was consistent with a molecular XGS diagnosis in 34 families. Of these 34 families, 25 joined the registry and all but five families provided sufficient clincial data for inclusion.

The 20 described individuals include six previously published and 14 novel cases. Medical records were sought and successfully obtained for 18 of the probands, while two others were determined to have already provided sufficient clinical information. MRI images and reports were collected and independently reviewed by a pediatric neuroradiologist, for six cases.

We directly confirmed the mutational data by DNA sequencing in 13 individuals, where samples were available to be tested. The confidence in the accuracy of the mutation data from the remaining seven cases was based upon the availability of clinical diagnostic reports from major providers. Figure one shows the position of all 25 mutations from XGS patients in the Registry. In five of the cases described here, we also had data from whole exome sequencing and we considered other gene candidates as contributing to disease, apart from *AHDC1*. We were able to exclude all such candidates based on phenotype mismatch, frequency, or lack of a second variant contributing to an established recessive mode of inheritance (Table S1). A *de*

novo NF1 variant reported in one individual was excluded as contributing to the clinical profile, due to the phenotype mismatch.

In addition to the more severely affected individuals with truncating mutations who are included in the Registry, one less severe case has been putatively associated with a *de novo* heterozygous missense mutation in *AHDC1*. This patient is diagnosed with autism spectrum disorder, without other reported features of XGS. Confirmation of missense mutations leading to mild phenotypes would be of considerable interest for delineating gene function and disease pathology. At this time, however, it is impossible to eliminate mutations in other loci as the cause of this patient's disorder. A search for mutations contributing to this individual's phenotype is underway, and so these data are not included in the present report.

Mutation position:

The previously reported cases represented nine distinct mutations that spanned the amino acid positions in the AHDC1 gene product from codon 375 to 1270 (Figure 1). In the present report, we now describe an additional 14 cases, including 11 novel *AHDC1* truncating mutations, extending this range from codon 262 to 1330 and doubling the number of known pathogenic mutation sites of *AHDC1*. There are three recurring mutations in our cohort, including the previously reported p.Cys791Trpfs*57 (c. 2373_2374delTG), which is now known to be present in 4/20 (20%) of these unrelated patients. Two additional newly observed mutations, p.Arg925* (c.2773C>T) and p.Gln970* (c.2908C>T), each occurred in two patients.

The mutations in the 20 probands all are predicted to lead to premature chain termination (PCT) during translation and include stop-gain changes (9 sites) or frameshifts leading to downstream truncation events (11 Sites). The truncation peptide sequences predicted by conceptual translation to arise as a result of the frameshift mutations did not show high homology to other proteins. Two different mutations gave rise to similar truncation peptides and, with one exception, all truncation products terminate within the boundaries of other non-sense mutation sites.

Patient phenotype:

The current ascertainment of the clinical spectrum associated with XGS is described in Table 1. Ascertainment for the reported cases includes data from initial clinical visits, family

survey, and subsequent consultancy (see supplementary Table S2 for details). The 20 cases include 11 males and nine females, with a median age of 8 years old. Two of the participants were more than 18 years old.

Language and Cognitive ability:

Overall Cognitive Ability: A diagnosis of autism, or autism spectrum disorder, had previously been reported in 5 of the 20 patients described here (25%). Methods for assessing autism spectrum disorder can be heterogeneous, hence we used the Modified Checklist for Autism in Toddlers (M-CHAT) as a standardized method to assess cognitive ability in the individuals in the cohort (Robins et al., 2014). This tool is widely used as a preliminary screen for children to identify increased autism risk, for which a formal, full neuropsychological evaluation would be recommended. In general practice, those who have a score indicating medium or high risk of autism will receive further testing. The 20 patients had a median M-CHAT score of 8 (0 ~ 14). Of these, 5 (25%) had an M-CHAT score less than 2, which is considered low risk for autism, 4 (20%) had an M-CHAT score between 3~7, at medium risk for autism, and 11 (55%) had an M-CHAT score between 8~20 and were at high risk for autism. All five individuals who had been diagnosed with autism, or autism spectrum disorder prior to this study, had an M-CHAT score higher than 8 (Figure 2A).

In addition to M-CHAT, the patients were assessed on their language skills and on the capability to follow simple commands (Figures 2B, 2C). A total of 18 (90%) of 20 patients were reported as able to follow simple commands, with a median age of 3.9 (1.5~8) years (Figure 2C).

Timing of Speech: Eight out of 20 patients (40%) were reported to have no speech, four (20%) were reported to use less than 50 words, two (10%) were reported to have a vocabulary of more than 50 words but do not speak in full sentences, and six (30%) patients were reported to use complete sentences with a vocabulary of more than 200 words (Figure 2B and Supplementary Figure S2B). Among the 12 patients who use at least one word other than ‘mama’ or ‘dada’, the median age for using one word is 2.75 (1~5) years, while the median age for using two words together is 3.5 (2~6) years (Figure 2C). These data are consistent with a relationship between gender and speech as male patients are significantly more likely to be non-verbal than female patients ($p < 0.01$). One of the male patients is two years old, which is

younger than the median age at which first words were spoken, however, the p-value is still <0.01 when he is removed from this analysis. It is noteworthy that all the patients reported with no speech are male (Figure 2B).

Correlation of M-CHAT and Speech: Multilinear linear regression showed that a linear relationship exists between the M-CHAT score, the square root of age and language capability ratings (0=nonverbal; 1=using less than 50 words; 2=not using sentence but using more than 50 words; 3=using sentences with a vocabulary of more than 200 words). The equation is: M-CHAT score = 1.99* sqrt(age) – 2.27 * language capability rating (adjusted R^2 = 0.42). This indicates that the M-CHAT score increases with age, but the rate of increase slows down with the gain of age. It also suggested for patients of the same age, for every point increase in language capability rating, the M-CHAT score decreases by approximately 2 points.

Neurology:

Abnormal MRI: An abnormal MRI was reported in 12 out of 20 (60%) patients who have had MRI performed. MRI images for re-review were available for six patients. We found that 6/6 had thinning of the corpus callosum (Figure 3, compare B-F to "control" individuals A and D). In addition, several subjects were found to have a cyst in the posterior fossa (Subject 4, Figure 3B, Subject 3 Figure 3E, Subject 7, Figure 3H).

Seizures: Seizures were reported in six out of 20 (30%) patients. The median onset age of seizure is four years old (9 months ~ 12 years old). An abnormal EEG was reported in three out of these six (50%) patients.

Timing of Walking: Independent walking is reported in 16 out of 20 patients (80%), with median beginning walking age of 2.5 (1.5~6) years. All the patients who are not able to walk independently are male. However, there is no significant difference between the ability to walk independently between male and female patients (p = 0.09). Hypotonia is reported in 19 out of 20 patients (95%).

Other significant features:

Scoliosis: Scoliosis was reported in four of 20 (20%) patients (ages 10~21 years), and three had received surgery for the condition. Compared to a general population prevalence of idiopathic scoliosis of 0.47 to 5.2% with an age of onset of 10 to 15 years, the risk of scoliosis in XGS

individuals is significantly increased (p = 0.018, compared to 5.2% prevalence)(Konieczny, Senyurt, & Krauspe, 2013; Mirtz, Thompson, Greene, Wyatt, & Akagi, 2005). No sex bias was apparent.

Airway/Sleep: Sleep apnea was reported in eight out of 20 patients (40%), three of these eight patients (37.5%) use respiratory support during sleep.

Vision: Strabismus was reported in eight out of 20 patients (40%).

Dysmorphic features: Facial features (Figure 4A) consistently revealed dysmorphic features such as broad forehead, hypertelorism, flat nasal bridge, and thin upper lip (Table 1). These features are also shown in the composite mask depicting the characteristic appearance of XGS patients (Figure 4B). To date, no patient has been clinically diagnosed based on a recognizable pattern of facial dysmorphisms, or a facial gestalt.

Aggressive Behaviors: Aggressive behavior in at least one patient was suggested by Yang et al.(Yang et al., 2015). We were unable to ascertain any history of overtly aggressive behavior in the reported cases, although several parents described a history of self-injury.

Genotype and phenotype association:

To explore the correlation between the specific site of mutation and phenotype, we compared three sets of patients who share the same mutations. Patients 1, 10, 18 and 19 share mutation p.Cys791Trpfs*57, patients 6 and 17 share mutation p.Arg925* and patients 9 and 12 share mutation p.Gln970*. For the mutation p.Cys791Trpfs*57, all four patients are verbal and reported to have sleep apnea and abnormal brain MRI. Seizures were reported in three of the four patients. Strabismus and a high-risk MCHAT score were reported in two of the four patients. It is challenging to compare the two patients with mutation p.Arg925* as one of them (patient #6) is only three years old, and is yet to reach some developmental milestones. Patients 9 and 12 were each reported to be verbal, using sentences with a vocabulary of more than 200 words. They were both reported to have normal MRI and EEG. They achieved developmental milestones, such as using words and walking, at similar ages. Strabismus was reported in patient 9 but not in patient 12.

To further explore correlations between the mutations and phenotypes, multiple phenotypes were plotted against the mutation sites (data not shown). No significant association was observed.

DISCUSSION:

The ability to aggregate data from the observation and care of individuals with XGS is a key step for clinical management, family counseling and molecular research into the condition. A concerted effort to identify patients worldwide has identified approximately sixty families, of whom 20 are described in detail here, including 14 not previously reported. The ascertainment combined records from initial clinical visits and subsequent clinical and family-reported findings.

Previously known truncating mutations in AHDC1 leading to XGS were observed to cluster around the middle of the coding region (from p.Gly375Argfs∗3 to p.Gln1270Argfs∗75)2, and this report expands these positional boundaries (from p.Gln262* to p.Ser1330*, Figure 1). Importantly, the number of different known mutations increased, revealing the sensitivity of the protein to truncating events throughout much of its length. All mutations discussed here are *de novo* in the proband. The mutations each are truncating and lead to a shorter predicted protein.

The *AHDC1* gene contains a single coding exon. Although the newly reported mutations do not shed further light on the putative functional regions of the AHDC1 protein, our database searches reveal, for the first time, domains shared with REV3L (DNA polymerase zeta catalytic subunit) and/or KIAA2022, a target for truncating mutations that cause intellectual disability(Gan, Wittschieben, Wittschieben, & Wood, 2008; Van Maldergem et al., 2013) (Figure 1; Supplementary Figure S2). The cross-species alignments reveal striking conservation across multiple domains with both REV3L and KIAA2022. REV3L is the catalytic subunit of polymerase zeta and is implicated in genome stability and DNA translesion repair[13]. It is therefore conceivable that AHDC1 may play a role in complexes that involve DNA repair through its ability to interact with the DNA via the AT-hook domains. Previous work has shown the interaction of AHDC1 with Tax Interaction Protein 1 which was proposed to occur *in vivo* under DNA damaging conditions(Shalaby, Hampson, Oliver, & Hampson, 2012).

The preponderance of *de novo* truncating pathogenic *AHDC1* alleles could indicate that missense mutations do not cause disease. Alternatively, the absence of pathogenic missense variants may simply reflect the early period of discovery and reduced ascertainment due to a potentially milder phenotype. A similar, illustrative example is Bainbridge-Ropers Syndrome (BRS: OMIM #615485), where severe phenotypes resulting from *de novo* truncating mutations in four cases led to the initial disease gene identification of *ASXL3*(Bainbridge et al., 2013) and follow up discovery of additional truncating mutations(Balasubramanian et al., 2017). Recently, however, compound missense heterozygous alleles in *ASXL3* have been associated with mild forms of BRS(Giri et al., 2017) and by analogy, future observation of missense mutations in *AHDC1* leading to pathogenicity cannot be discounted.

The absence of well-characterized cases in this cohort with large deletions that either interrupt or overlap the gene may reflect a dominant negative mechanism of pathogenesis or the difficulties of precise ascertainment of the disorder. We note twelve patients with copy number loss or gain covering regions of the genome ranging from 39kb to 19Mb at or near *AHDC1*, recorded in the DECIPHER database(Firth et al., 2009; https://decipher.sanger.ac.uk/search?q=AHDC1#consented-patients/results). Seven of these share similar phenotypes with XGS patients such as developmental delay, hypotonia, and dysmorphic features, one has 'ankyloglossia' and four have no phenotype information available. The interpretation of the phenotypes of these patients is confounded by the presence of genes near *AHDC1* for which mutation can independently result in developmental disorders(Rocha, Vasques, Santos, & Paiva, 2016). These cases, therefore, warrant further investigation, including the precise mapping of the boundaries of the lesions.

Some *AHDC1* mutation positions correlate with clinical observations and warrant follow up studies with much larger sample sizes to establish robust genotype-phenotype correlations. Patients with mutations occurring near the C-terminus of the protein (p.Ser1258*, p.Gln1270Argfs*75 and p.Ser1330*) are non-verbal with high M-CHAT scores, while the patient with the early stop-gain mutation p.Gln262* uses sentences and has an M-CHAT score of 1, which may suggest milder features. This may indicate that nonsense and frameshift variants closer to the C-terminus have a more severe clinical impact. Comparison of individuals with

identical mutations suggests that there is heterogeneity among patients, even with the same alteration in *AHDC1* gene. To further establish the correlation between phenotype and specific mutation site, larger numbers of patients will be needed to be analyzed.

The participating families indicated a wider range of patients' age than previously recognized. The median age was eight years – but two of the patients were adults (nineteen or older). As this is a newly described syndrome and younger children are more likely to seek genetic diagnosis, the true age distribution in the population is not known. This is a particularly important question for families as they plan ongoing care. The parents of older children reported challenging behavioral issues during puberty but declined to describe the patients as aggressive.

Approximately one-half of the patients either had a diagnosis of autism, autism spectrum disorder or were considered at high risk due to their M-CHAT assessment. The precise relationship between ASD and ID is controversial and complex(Matson & Shoemaker, 2009). In general, the younger children in this group exhibit hallmarks of ID, however, the relative cognitive abilities of older children reveal higher function and therefore are more likely to suggest ASD. Relatedly, ASD has an elevated frequency in males(Matson & Shoemaker, 2009), and the data here from the assessment of language capabilities suggest that male XGS patients are significantly more likely to be non-verbal than female patients. This feature warrants further investigation.

Previous reports on XGS have focused on somewhat different clinical features. In particular, clarification of the neurological features was more in-depth from this larger group of subjects. We observed that six patients (30%) developed seizure s, while 13 (65%) had ataxia, in line with previous reports, further reinforcing the CNS involvement in XGS.

Upper airway abnormalities also appear to be a common feature in XGS. In the original report, 3 of 4 probands had obstructive sleep apnea, and the 4th had suspected tracheomalacia in infancy (Xia et al., 2014). Sleep apnea was present in 40% of the XGS cases described here, with many requiring continuous positive airway pressure (CPAP) at night. This suggests careful monitoring of airway function is essential and there should be a low threshold for referral to pulmonology.

A surprising finding from this detailed assessment was the frequency of scoliosis among XGS individuals. Scoliosis was not included as a characteristic phenotype in previous publications (only reported in patient#2 in Yang et al.)(Yang et al., 2015). However, the usual age-at-onset of the feature is 10~15 years old(Konieczny et al., 2013) while the median age for the 11 patients reported here was five years (1.5 ~ 16) at the time of publication. Our study, therefore, suggests that patients are at significantly increased risk for developing scoliosis and would benefit from an orthopedic referral when appropriate.

The evaluation here suggests several features of XGS for which early diagnosis and regular surveillance can be of clinical benefit. All individuals should undergo detailed assessment by a physician who is familiar with the condition, with annual (more frequent during the first years of life) assessments of growth including height, weight, and head circumference to identify those at risk of failure to thrive or short stature necessitating intervention, and clinical evaluation for scoliosis. Neurodevelopmental delay is a ubiquitous feature of this condition, and regular childhood developmental assessments with early initiation of therapies will maximize developmental potential. Given the high frequency of structural brain anomalies, a baseline brain MRI should be considered, particularly if neurologic abnormalities such as hypotonia are present on exam. Neurologic and ophthalmologic involvement in some individuals may warrant consultation by the appropriate specialists. An evaluation for obstructive sleep apnea as well as an upper airway assessment should also be considered. Together, these practices can both provide better clinical diagnosis and suggest management strategies for symptoms that may be more severe in individual cases.

XGS has multiple features overlapping with autism, suggesting that XGS should be on the differential for unexplained intellectual disability and developmental delays. There are likely older children, adolescents, and adults previously diagnosed with an autism spectrum disorder or intellectual disability who have XGS and would benefit from a clinical genetics referral.

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Figure legends:

Figure 1: Schematic representation of mutations identified in 25 XGS patients that have participated in the XGS Registry. Mutations in five individuals who are not clinically assessed in detail, in this study, are indicated by an asterisk. Previously reported variants are indicated below the line, newly reported mutations above. The x-axis shows amino acid positions in the encoded protein, pink rectangles indicate AT-Hook domains, green rectangles indicate REV3L homology domains, purple rectangle indicates KIAA2022 homology domains and grey rectangle indicates REV3L and KIAA2022 homology domains. Green circles indicate frameshift variants, yellow indicates nonsense variants (see Supplementary Material for further description of homologies and conservation).

Figure 2: A) M-CHAT score of the 20 patients. Patients with M-CHAT scores 8~20 are considered high risk and those who scored 0~7 are considered to have medium-low risk. B) Language capability distributed by age and sex. C) Age at onset distribution of patients who achieve different milestones (from up to bottom): independent walking, using first word, using two words together and following simple commands. For patients who have not reached any of the milestones, their current ages are shown in red in each panel.

Figure 3: Brain MRI features of the subjects, unless otherwise indicated a Sagittal T1 image is shown

A. A control subject (a clinical MRI for history of minor head trauma) in a subject at age 13 months showing a typical corpus callosum (red arrow)
B. Subject 4 at age 6 months, shows thinning of the corpus callosum posteriorly (red arrow) in addition there is a large extra-axial fluid space posterior to the cerebellum
C. Subject 2 at age 6 months, although the young age limits the assessment, the corpus callosum appears thin (red arrow)
D. A control subject (a clinical MRI for history of seizure) in a subject at age 3 years showing a typical corpus callosum (red arrow)
E. Subject 3 at age 10 years, shows thinning of the corpus callosum (red arrow), particularly in the posterior portion, there is also a large extra-axial fluid space posterior to the cerebellum

F. Subject 2 (also shown in C) now at age 1 year, a later study confirms the suspicion of the 6-month study suggesting thinning of the corpus callosum
G. Subject 7 at age 5 years, a T2 axial image shows the extra-axial fluid space around the cerebellum is larger than normal and dysmorphic in appearance
H. Subject 7 at age 5 years, a T1 sagittal image shows thinning of the corpus callosum and a large extra-axial fluid space posterior to the cerebellum.

Figure 4: (A) Frontal face images of some of the patients (accession number in this study are shown at top left corner) (B) A computer generated masking depicting the characteristic features of Xia-Gibbs syndrome. This image was generated using the FDNA software. Overlapping features include a broad forehead, hypertelorism, flat nasal bridge and thin upper lip.

List of Supplementary Materials:

Supplementary Figures:

Figure S1: Protein alignments illustrating conservation domains between human AHDC1 and its orthologs and homologs.

Supplementary Tables:

Table S1: Other possible gene candidates for some of the patients.

Table S2: Clincial/phenotype assessment method for each patient.

Other Supplementary Files:

Clinical survey 1of2: general clinical history and developmental questions

Clinical survey 2of2: M-CHAT R/F (Robins et al., 2014)

TABLES

Table One: Detailed patient phenotypes. Height was shown in percentage (WHO child growth standards 2-5yo http://www.who.int/childgrowth/standards/height_for_age/en/ and WHO growth references for 5-19yo http://www.who.int/growthref/en/, percentage for two adult patients are assessed as 19-year-olds).

Footnotes:

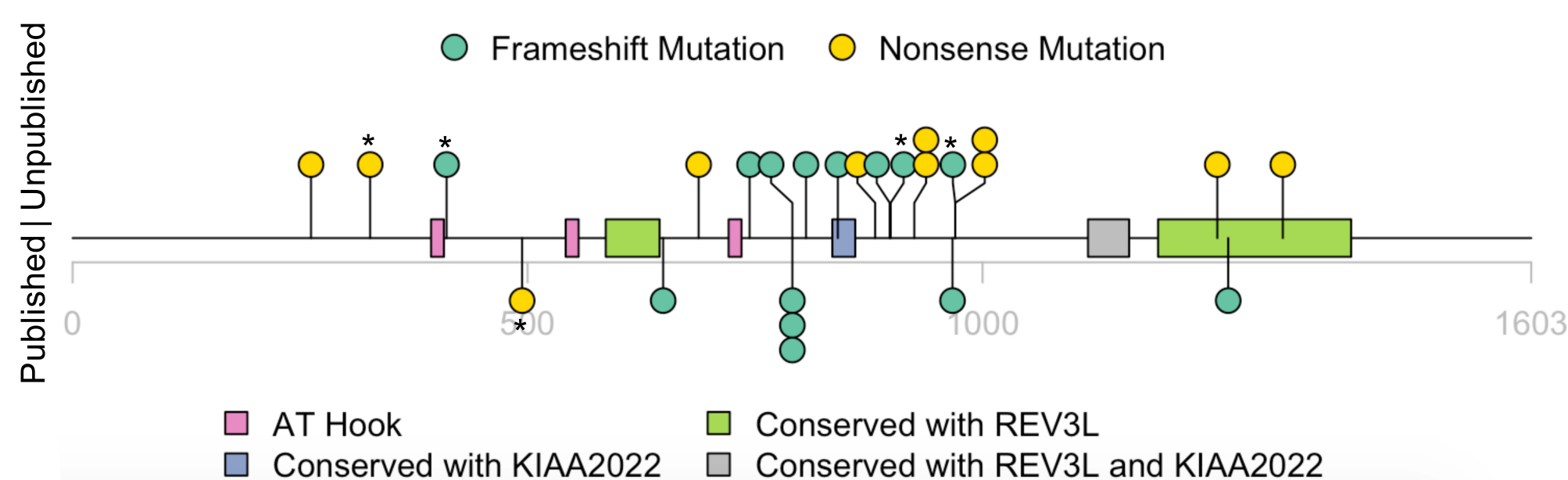
*References: 1, Xia et al., 2014; 2, Yang et al. 2015.

** Language: 1. No words or few words; 2. no sentence but >50 words; 3. fun sentence > 200 words

PATIENT	1	2	3	4	5	6	7	8	9	10
Reference*	#1 (patient 1)	#2 (patient 5)					#2 (patient 1)			#1 (patient 3)
Mutation										
Nucleotide change	c.2373_2374delTG	c.3809delA	c.2644C>T	c.2520del	c.2229delG	c.2773C>T	c.1945delG	c.2062C>T	c.2908C>T	c.2373_2374delTG
Protein change	p.Cys791Trpfs*57	p.Gln1270Argfs*75	Gln882*	Arg841Alafs*91	Ser744Profs*188	Arg925*	Ala649Profs*83	Arg688*	Gln970*	Cys791Trpfs*57
Age	5 yo	12 yo	10 yo	9 yo	13 yo	3 yo	4	20	6	12 yo
Gender	F	M	M	M	M	M	M	F	F	M
Ethnicity	White	white/asian	white	white	white	white	white	white	white	white
Growth										
Height	0.05	0.01	0.05	0.15	0.03	<0.01	<0.01	>0.99	(0.05,0.15)	0.05
Scoliosis	N	N	Y	N	Y	N	N	Y		N
Language										
M-CHAT Score	12	6	9	13	10	5	9	10	1	2
Prior Autism diagnosis	Y	N	Y	N	N	N	Y	Y	N	N
Current language**	1	0	0	0	0	0	0	2	3	3
Age at first word	4 yo	NA	NA	NA	NA	NA	NA	5 yo	2.5 yo	12 mo
Age, two words together	NA	NA	NA	NA	NA	NA	NA	6 yo	3.5 yo	24 mo
Age following command	2 yo	8 yo	8 yo	NA	4 yo	23 mo	3 year 9 mo	4 yo	3-4 yo	18 mo
Mobility										
Hypotonia diagnosis	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
Independent walking	Y	Y	Y	N	Y	N	Y	Y	Y	Y
Age independent walking	3 yo	6 yo	3 yo	NA	3 yo	NA	3 year 5 mo	22 mo	2.5 yo	18 mo
Sleep/airway										
Sleep apnea	Y	N	N	N	Y	Y	Y	N	N	Y
Breathing support	Y	N	N	N	N	N	N	N	N	Y
Neuro										
MRI	abnormal	abnormal	abnormal	abnormal	normal	normal	abnormal	abnormal	normal	abnormal
EEG	normal	NA	normal	normal	NA	normal	NA	normal	normal	abnormal
Seizure	N	N	Y	N	N	N	N	Y	N	Y
Age at first seizure			9 yo					9 mo		3 yo
Ataxia	Y	Y	N	Y	N	N	Y	Y	Y	Y
Vision										
Glasses/contacts	Y	No	N	Y	N	N	Y	Y	N	N
Vision acuity	n.asc.	n.asc.	n.asc.	mild myopia	n.asc.	n.asc.	slight astig.	n.asc.	n.asc.	prismatic lenses
Strabismus	Y	N	Y	N	N	Y	N	N	Y	N
Dysmorphic features										
Small ear lobes	N	NA	Y	N	N	N	N	N	N	Y
Upturned earlobes	N	NA	N	Y	N	N	Y	N	N	N
Low-set ears	N	NA	N	N	N	N	N	Y	N	N
Protuberant ears	N	NA	Y	N	N	N	N	N	Y	Y
Deep-set eyes	N	NA	N	N	Y	Y	N	Y	N	N
Upslanting palpebral fissures	N	NA	N	Y	N	N	N	N	Y	N
Downslanting palpebral fissure	N	NA	N	Y	Y	N	Y	N	N	N
Mild ptosis	N	NA	N	Y	N	Y	N	N	N	N
Esotropia	N	NA	N	Y	N	Y	N	Y	Y	N
Hypertelorism	N	NA	Y	Y	N	N	Y	Y	Y	N
Flat nasal bridge	N	NA	N	N	N	Y	N	N	N	N
Micrognathia	Y	NA	Y	Y	Y	Y	Y	Y	Y	Y
Thin upper lip	Y	NA	Y	Y	N	Y	Y	Y	Y	N
Broad forehead	Y	NA	N	Y	N	Y	Y	Y	Y	N

1

Patient	11	12	13	14	15	16	17	18	19	20	SUMMARY
Reference*						#1 (patient 2)		#2 (patient 6)			
Mutation											
Nucleotide change	c.2691delA	c.2908C>T	c.784C>T	c.3989C>A	c.2415delG	c.2898delC	c.2773C>T	c.2373_2374delTG	c.2373_2374delTG	c.3773C>G	
Protein change	V898Wfs*34	Gln970*	Gln262*	Ser1330*	Leu806Trpfs*126	Tyr967Thrfs*175	Arg925*	Cys791Trpfs*57	Cys791Trpfs*57	Ser1258*	
Age	4 yo	11 yo	6 yo	17 yo	6 yo	8 yo	6 yo	8 yo	21yo	2yo	8yo (2~21yo)
Gender	F	F	F	M	F	F	F	M	M	M	Female 9 (45%)
Ethnicity	white	white	white	white	white	Asian	white	white	white	white	White 18 (90%)
Growth											
Height	<0.01	NA	0.05	<0.01	(0.25,0.5)	<0.01	(0.15,0.25)	0.15	<0.01	0.01	
Scoliosis	N	N	N	N	N	N	N	N	Y	N	4 (20%)
Language											
M-CHAT Score	0	9	1	14	0	4	8	4	10	13	8.5 (0~14)
Prior Autism diagnosis	N	N	N	Y	N	N	N	N	N	N	5 (25%)
Current language**	1	3	3	0	3	1	1	3	2	0	1 (0~3)
Age at first word	2.5 yo	2 yo	4 yo	NA	around 3 yo	UKN	around 2.5-3 yo	2 yo	5 yo	NA	2.75yo (1~5yo)
Age, two words together	3.5 yo	3 yo	5 yo	NA	3 yo	4 yo	NA	2 yo	7 yo	NA	3.5yo(2~7yo)
Age following command	2 yo	6 yo	5 yo	5 yo	2 yo	6 yo	3 yo	4 yo	3 yo	NA	3.875(1.5~8yo)
Mobility											
Hypotonia diagnosis	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	19 (95%)
Independent walking	Y	Y	Y	Y	Y	Y	Y	Y	N	N	16 (80%)
Age independent walking	2.5 yo	2 yo	3 yo	19 mo	2 yo	2yo	2 year 4 mo	4 year	NA	NA	2.5yo (1.5~6yo)
Sleep/airway											
Sleep apnea	N	N	N	N	N	Y	N	Y	Y	Y	8 (40%)
Breathing support	N	N	N	N	N	N	N	N	Y	N	3 (15%)
Neuro											
MRI	abnormal	normal	normal	normal	normal	normal	abnormal	abnormal	abnormal	abnormal	12 (60%)
EEG	normal	normal	normal	NA	normal	abnormal	NA	abnormal	abnormal	normal	4 (20%)
Seizure	N	N	N	N	Y	N	N	Y	Y	N	6 (30%)
Age at first seizure					2 yo			5 yo	12yo		4 yo (9mo ~ 12yo)
Ataxia	Y	Y	N	N	Y	N	Y	Y	Y	N	13(65%)
Vision											
Glasses/contacts	N	N	Y	N	N	N	Y	N	N	N	6 (30%)
Vision acuity	NA	sunglasses	40/70	20/20	100%	NA	6/9.5	NA	NA		
Strabismus	Y	N	Y	N	N	N	Y	N	Y	N	8 (40%)
Dysmorphic features											
Small ear lobes	NA	NA	N	NA	Y	N	N	N	N	NA	
Upturned earlobes	NA	NA	Y	NA	N	Y	N	N	N	NA	
Low-set ears	NA	NA	Y	NA	N	N	N	N	N	NA	
Protuberant ears	NA	NA	N	NA	N	Y	N	N	N	NA	
Deep-set eyes	NA	NA	N	NA	N	N	N	N	N	NA	
Upslanting palpebral fissures	NA	NA	Y	NA	N	N	N	N	N	NA	
Downslanting palpebral fissure	NA	NA	N	NA	N	N	N	N	N	NA	
Mild ptosis	NA	NA	Y	NA	N	Y	N	N	Y	NA	
Esotropia	NA	NA	Y	NA	N	N	N	N	N	NA	
Hypertelorism	NA	NA	Y	NA	N	N	Y	N	Y	NA	
Flat nasal bridge	NA	NA	N	NA	N	N	N	N	Y	NA	
Micrognathia	NA	NA	N	NA	N	N	N	N	Y	NA	
Thin upper lip	NA	NA	Y	NA	Y	Y	Y	N	Y	NA	
Broad forehead	NA	NA	Y	NA	Y	N	Y	N	Y	NA	


Published | Unpublished
Frameshift Mutation
Nonsense Mutation
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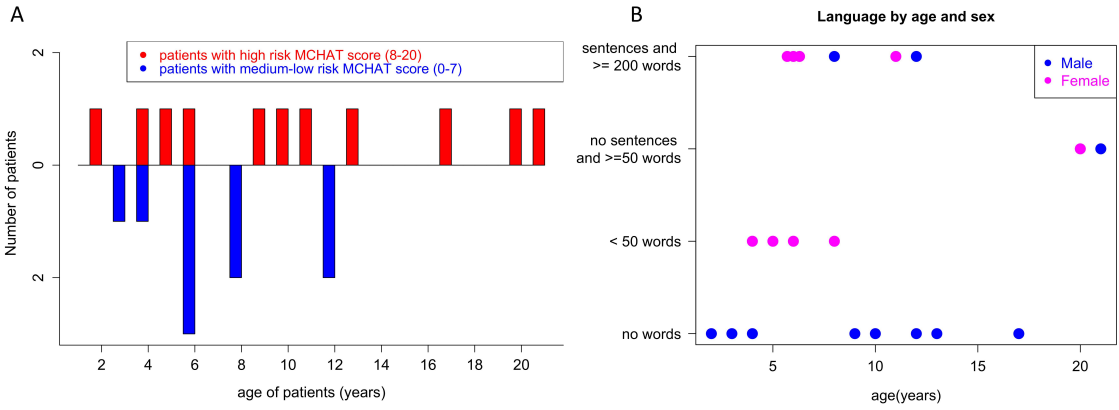
Conserved with KIAA2022
Conserved with REV3L
Conserved with REV3L and KIAA2022

Figure 2


A
patients with high risk MCHAT score (8-20)
patients with medium-low risk MCHAT score (0-7)
Number of patients
age of patients (years)
B
Language by age and sex
sentences and >= 200 words
no sentences and >=50 words
< 50 words
no words
Male
Female
age(years)

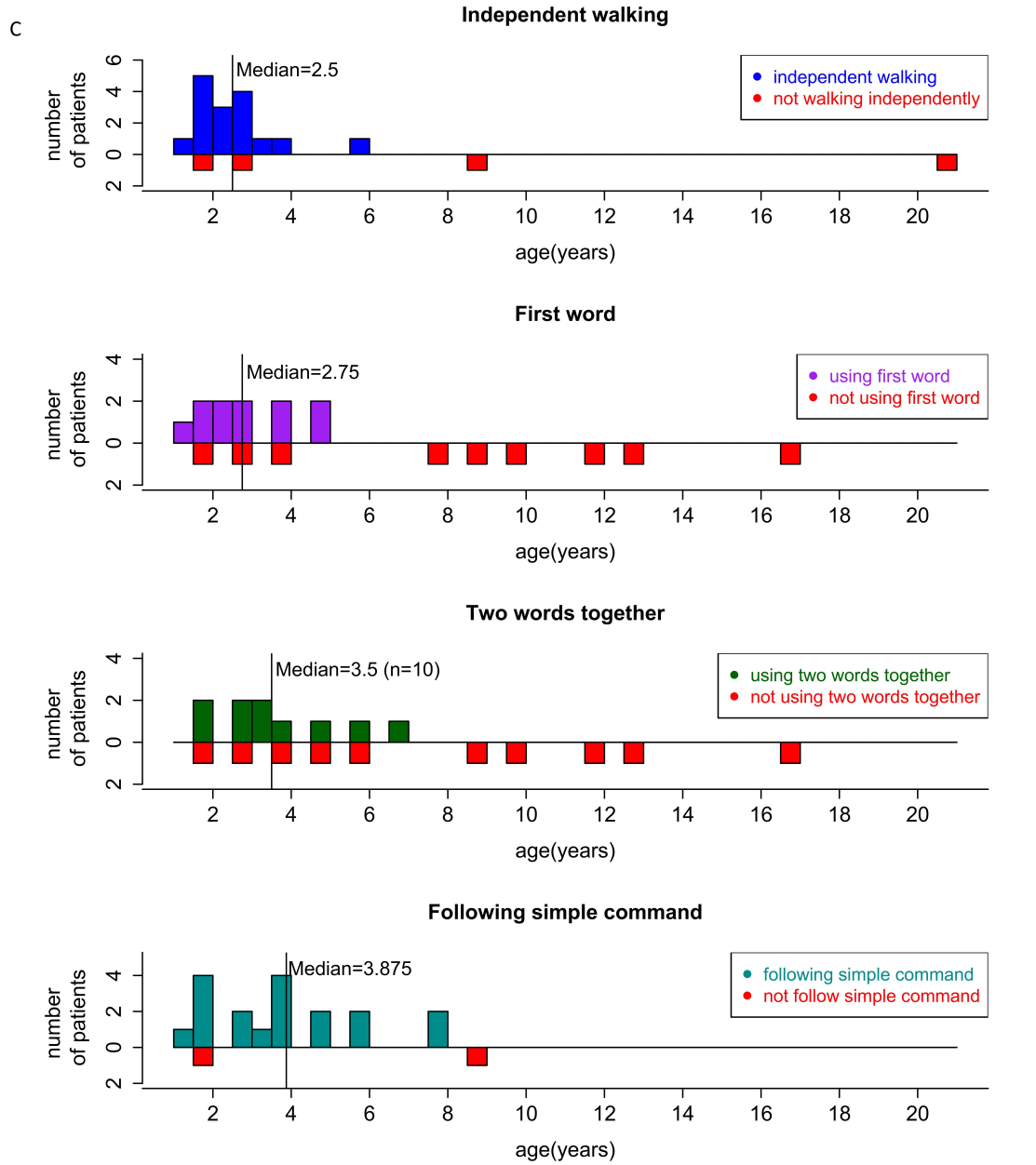
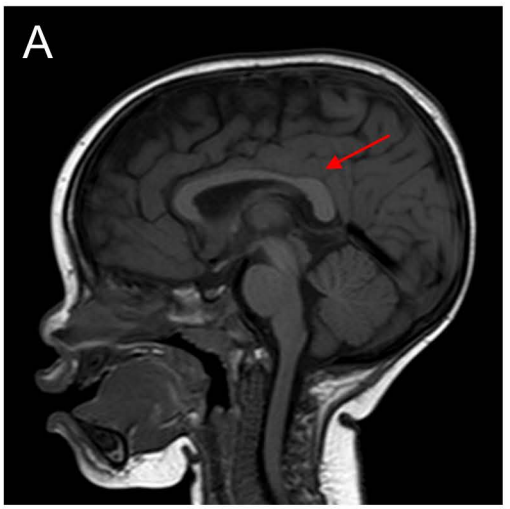
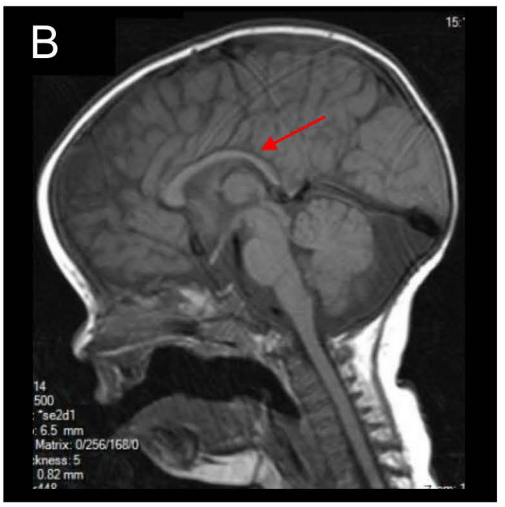

C
Independent walking
Median=2.5
independent walking
not walking independently
First word
Median=2.75
using first word
not using first word
Two words together
Median=3.5 (n=10)
using two words together
not using two words together
Following simple command
Median=3.875
following simple command
not follow simple command
number of patients
age(years)

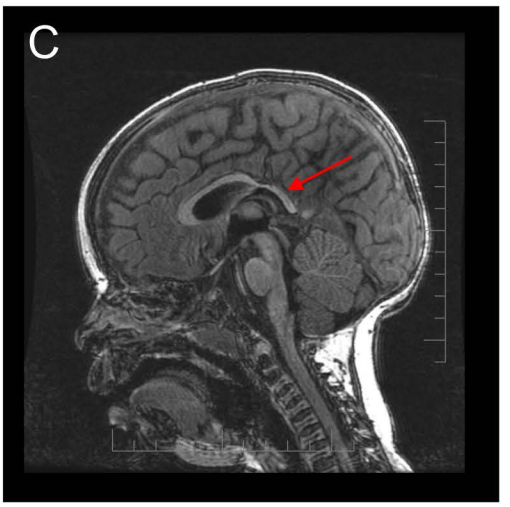
Figure 3



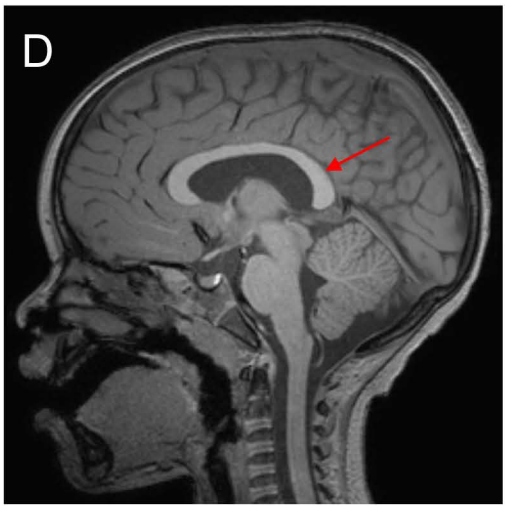
Sagittal T1 Control at 13 months



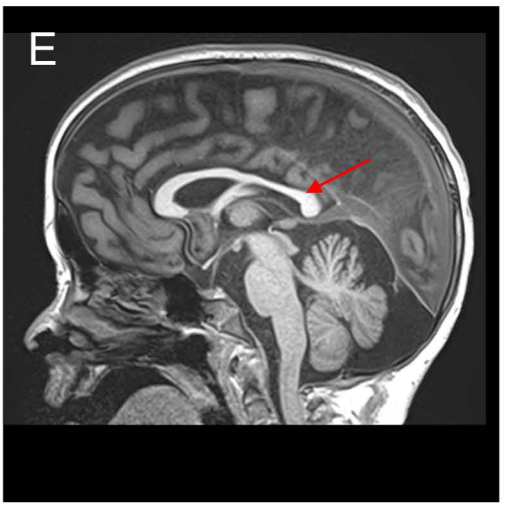
Sagittal T1 Subject 4 at 6 months



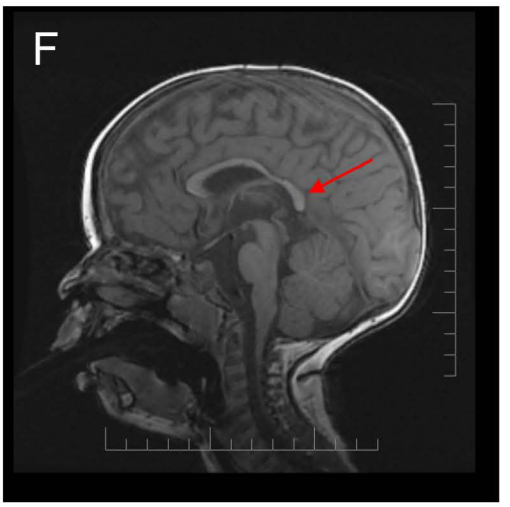
Sagittal T1 Subject 2 at 6 months



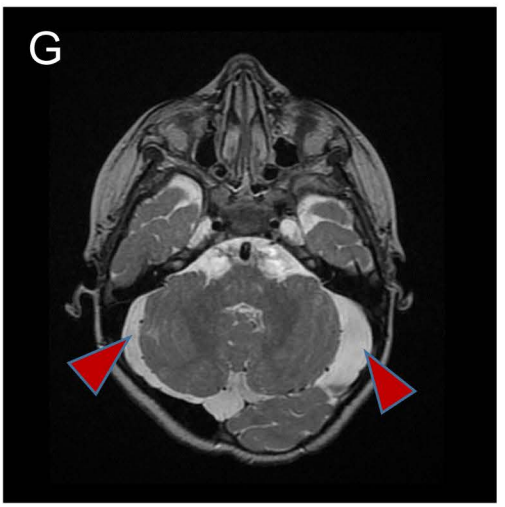
Sagittal T1 Control at 3 years



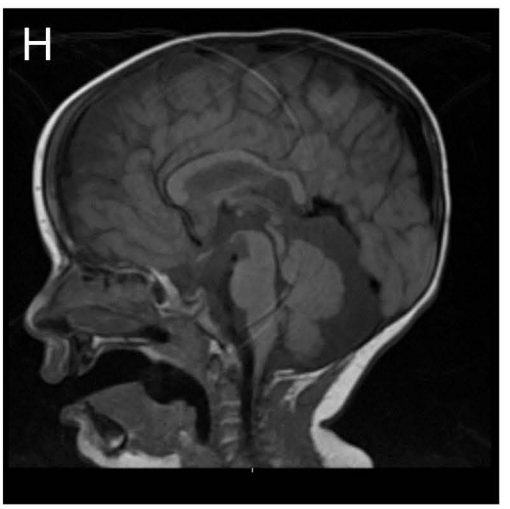
Sagittal T1 Subject 3 at 10 years



Sagittal T1 Subject 2 at 1 year



Axial T2 Subject 7 at 5 years



Sagittal T1 Subject 7 at 5 years

Figure 4

A

B

SUPPLEMENTARY MATERIALS:

THE PHENOTYPIC SPECTRUM OF XIA-GIBBS SYNDROME. by Yunyun Jiang[1,2], Michael F. Wangler[2,3], Amy L. McGuire[4], James R. Lupski [1,2,5,6], Jennifer E. Posey[2], David R. Murdock[1,2], Michael M. Khayat[1, 2], Luis Sanchez-Pulido[7], Chris P. Ponting[7], Fan Xia [2], Jill V. Hunter[3], Qingchang Meng[1,2], Mullai Murugan[1,2], *Richard A. Gibbs[1,2]

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CONTENTS:

PATIENT	GENE	MUTATION	cDNA	PROTEIN	gnomAD	LOCAL	PHENOTYPE	INHERITANCE	FAMILY	COMMENTS
Patient 3	KAT6B	10:76602922C>T	c.307C>T	p.Arg103Cys	45/277194	5/29642	Genitopatellar syndrome	AD	NA - no trio	
Patient 3	SERPING1	11:57379324G>A	c.1164G>A	p.Met388Ile	31/277218	7/29642	Hereditary angioedema	AD, AR	NA - no trio	
Patient 3	PKP2	12:32949166A>G	c.2366T>C	p.Ile789Thr	10/277068	0/29642	Arrhythmogenic right ventricular dysplasia	AD	NA - no trio	
patient 6	NF1		c.3457_3460delCTCA	p.Leu1153Metfs*4					de Novo	From supplied genetic report
Patient 10	GGN	19:38876427T>G	c.1475A>C	p.Gln492Pro	213/134120	278/29642	None known	NA	de Novo	
Patient 10	LAMB3	1:209823322G>A	c.170C>T	p.Thr57Ile	30/277180	9/29642	Epidermolysis bullosa	AR	Father	
Patient 10	LAMB3	1:209803163C>T	c.1051G>A	p.Glu351Lys	262/277236	33/29642	Epidermolysis bullosa	AR	Mother	
Patient 14	ARID1A	1:27106208C>T	c.5168C>T	p.Pro1723Leu	2/246262	1/29642	Coffin-Siris syndrome	AD	NA - no trio	
Patient 14	OPHN1	X:67283825G>T	c.2029C>A	p.Leu677Met	248/198097	24/29642	Mental retardation, X-linked, with cerebellar hypoplasia and distinctive facial appearance	XLR	NA - no trio	
Patient 14	AIPL1	17:6329101C>T	c.834G>A	p.Trp278Ter	91/269044	9/29642	Cone-rod dystrophy	AR	NA - no trio	
Patient 14	ELN	7:73482987G>A	c.2075G>A	p.Gly692Asp	818/276770	134/29642	Cutis laxa	AD	NA - no trio	
Patient 16	CD96	3:111319710C>T	c.1036C>T	p.Pro346Ser	55/276876	9/29642	Opitz trigonocephaly	AD	NA - no trio	
Patient 16	FKRP	19:47259227A>T	c.520A>T	p.Ser174Cys	503/161236	42/29642	Congenital Muscular Dystrophy	AR	NA - no trio	

TABLE S1: Additional candidate variants in considered in 5 patients. 'GNOMAD' indicates number of alleles reported in the gnomAD (Genome Aggregation Database: http://gnomad.broadinstitute.org); LOCAL indicates allele counts within internal Human Genome Sequencing Center databases.

TABLE S2: Sources of data for clinical ascertainment

Patient	Assessment in Clinic	Face to face meeting	Medical Record Review	Clinical Survey
1		X	X	X
2		X	X	X
3	X	X	X	X
4			X	X
5		X	X	X
6			X	X
7		X	X	X
8			X	X
9		X		X
10		X	X	X
11			X	X
12		X		X
13			X	X
14			X	X
15			X	X
16	X	X	X	X
17			X	X
18		X	X	X
19		X	X	X
20			X	X

FIGURE S1: Conserved regions of *AHDC1*: Protein alignments illustrating conservation domains between human AHDC1 and its orthologs and homologs