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Research Paper

Clinical Characterization of a Multicenter International Cohort of Patients With Aicardi-Goutières Syndrome Homozygous for the *RNASEH2B*:p.Ala177Thr Variant: Early Clinical Markers of Disease Severity

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ABSTRACT

Background: Aicardi-Goutières syndrome (AGS) is a rare monogenic leukodystrophy belonging to type I interferonopathies caused by alterations in one of nine genes. Among them, homozygous *RNASEH2B*: c.529G>A(p.Ala177Thr) is the most common variant worldwide and associated to AGS2. This variant

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Keywords:
Aicardi-Goutières syndrome
RNASEH2B
Type I interferonopathy
IFN signature
Disease severity

typically leads to severe phenotypes, but individuals with later onset or milder clinical manifestations have been described, with recent finding of asymptomatic homozygous individuals. However, the cause for this intragenotypic clinical variability is unclear, as well as developmental trajectories and early prognostic factors. Our study objective is the description of phenotypic variability in patients with AGS2 and the identification of early clinical markers of prognosis.

Methods: A multicenter international retrospective natural history study was carried out by recruiting patients with AGS homozygous for p.Ala177Thr variant. Patients were categorized into three groups based on the clinical severity through the composite functional severity score, although comparison was made with the more recently introduced AGS severity score. Disease onset was divided into neonatal, infantile, and later onset. Demographic, clinical, and laboratory data were collected and compared between these groups.

Results: Irritability at onset correlates significantly to the three functional categories. Early age at onset and presence of extrapyramidal signs correlate to functional outcomes when comparing mild with severe patients. Furthermore, retrospective application of AGS severity score correlated well with the commonly used composite functional severity score.

Conclusion: The authors observed irritability, early onset, and extrapyramidal signs not to be exclusive to the severe group, hence the need for creation of a composite predictive biomarker for prognosis accuracy.

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Introduction

Aicardi-Goutières syndrome (AGS) is a rare monogenic leukodystrophy first described by Jean Aicardi and Françoise Goutières in 1984.[1] Owing to its underlying pathophysiology, AGS is listed among type I interferonopathies.[2] To date, pathogenic variants in nine genes—*TREX1, RNASEH2B, RNASEH2C, RNASEH2A, ADAR1, SAMHD1, IFIH1, LSM11,* and *RNU7-1*—have been identified.[3,4] Among them, the homozygous p.Ala177Thr in *RNASEH2B* is the most prevalent globally and particularly frequent in the Italian population[5,6] and associated to AGS2. It is estimated that the prevalence of AGS2 due to biallelic p.Ala177Thr mutations alone is one in 120,000 in the European population to one in 250,000 in the general population.[7]

AGS encompasses a wide range of neurological and extra-neurological manifestations, ranging from mild motor signs with preserved cognitive function to severe intellectual disability and spastic tetraparesis.[3,5] Although some genotype-phenotype correlation is recognized and described, only one study[8] identified possible prognostic factors within genotypes, highlighting the potential role of microcephaly, age at onset, and presenting symptoms. However, significant variability persists both between and within genotypes. Notably, p.Ala177Thr mutation in *RNASEH2B* is associated with lower mortality rates and, in some cases, negative interferon scores (which reflects the activation level of interferon-induced immune responses, calculated based on the expression of interferon-stimulated genes, and is often used as a biomarker in autoimmune or inflammatory diseases), suggesting a unique disease trajectory compared with other AGS genotypes.[5] This pattern raises the hypothesis that interferon score might be positive (with significant activation of interferon pathway) in the earlier phases and decline rapidly in these individuals. Indeed, along with typically severe phenotypes, this specific mutation appears to be among the most commonly associated with later onset[9] and milder manifestations, with some subjects having relatively preserved intellectual function, communication skills, and fine motor abilities.[8] However, reasons for this intragenotypic phenotypic variability have not been elucidated so far.

To address this gap of knowledge, the primary aim of this study was to characterize the phenotypic variability in an international cohort of patients with AGS homozygous for the *RNASEH2B* p.Ala177Thr mutation. Our secondary objective was to identify early clinical features that may predict long-term neurological outcomes within this genotype subgroup.

Materials and Methods

Study design

This is a retrospective natural history study, designed to describe a cohort of patients with homozygous p. p.Ala177Thr *RNASEH2B* with variable degrees of phenotypic expression. The study was approved by the Ethical Committee of Mondino Neurological Institute (approval no. 3549/2009 of September 30, 2009, and December 11, 2009; n. 20170035275 of October 23, 2017; and n. 25039/22 of May 6, 2022). Additional patients were obtained from the institutional review board–approved CHOP Myelin Disorders Bioregistry and Natural History Project (IRB: 14-011236). Informed consent was obtained for all participants, by participants themselves when able to provide, or parents or legal guardians.

Recruitment and inclusion criteria

This is a part of a multicenter international study involving homozygous *RNASEH2B* p.Ala177Thr patients longitudinally followed at different institutions (IRCCS Mondino Foundation, Spedali Civili di Brescia, V. Buzzi Children's Hospital, IRCCS Stella Maris Foundation, IRCCS Bambino Gesù Children's Hospital of Rome, University Hospital Città della Salute e della Scienza, and Children's Hospital of Philadelphia).

Inclusion criteria were as follows:

- Homozygous p.Ala177Thr mutation in *RNASEH2B* gene.
- Proved parental heterozygosity for the mutation.
- Presence of neurological and neuroradiological signs and symptoms classifiable as AGS according to Orcesi et al.[10] and Tonduti et al.[11] AGS is defined by the presence of
  1. Early-onset encephalopathy with psychomotor delay, spasticity, extrapyramidal signs, and microcephaly, the last appearing in the course of the first year of life.
  2. Calcifications particularly visible at the basal ganglia level (putamen, pallidus and thalamus) but also extending to the periventricular white matter.

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3. Cerebral white matter abnormalities.
4. Cerebral atrophy.
5. Exclusion of pre-/perinatal infections, in particular the TORCH complex (toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus).

These criteria are considered necessary to establish the clinical diagnosis of AGS, and they previously needed to be associated with cerebrospinal fluid inflammatory alterations, nowadays replaced by the less-invasive interferon signature and its relative score, which, however, may be negative in some individuals. Extra-neurological symptoms and genetic confirmation of disease are considered supportive but not necessary for a clinical diagnosis of AGS.

Neurological and extraneurological phenotype

We retrospectively collected demographic, clinical neurological and extraneurological, and laboratory data from patients' medical records and through direct contact, as detailed in Table 1/ Supplementary Table 1.

Symptom onset in relation to age was defined based on Livingston et al.[3] In particular, neonatal onset was intended within the first four weeks of life, infantile onset was defined between four weeks of life and age 12 months, and later onset was defined as an onset before the first year of life.

The presence of irritability was considered when we found reference in medical records to inconsolable crying or fussiness without apparent cause, difficulty in being calmed and consoled, and signs of distress without an obvious cause. Assessment was based on clinical observation and caregiver reports.

Global functional impairment was assessed using a composite severity score, used as an indicator of global clinical severity,[8] derived from

- the Gross Motor Function Classification System,[12]
- the Manual Ability Classification System,[13]
- the Communication Function Classification System.[14]

The resulting composite functional severity score ranged from 3 (meaning fully preserved motor and communicative function) to 15 (extremely severe impairment of motor and communicative function) and defined phenotypic severity as

- mild: <6
- moderate: 6-12
- severe: >12.

In parallel, the novel "neurologic severity score for AGS" developed by Adang and colleagues,[15] (here referred as the novel AGS score for simplicity), based on 11 key items reflecting the severity of AGS across gross motor, fine motor, and cognitive skills, was applied retrospectively for each patient. This final AGS scale score ranges from 0 (no neurodevelopmental acquisition) to 11 (preserved neurological function).

Neuroradiological images were retrospectively reviewed according to the approach described by La Piana and colleagues[16] and detailed in Supplementary Table 2.

Interferon-stimulated gene score

Interferon-stimulated gene (ISG) scores were registered when available as the first value and a value at follow-up of at least six months. The scores were derived from the mRNA measurement (extracted from the peripheral blood sample) of six interferon-inducible genes (*IFI2J, IFI44L, IFIT1, ISG15, RSAD2,* and *SIGLEC1*) and four housekeeping genes (*ALAS1, HPRT1, TBP,* and *TUBB*) as previously described by Armangue et al.[17] Interferon score was considered "positive" when above the score in healthy control subjects.

TABLE 1.
Demographics and Clinical Data of the Cohort (n = 42)

Gender	
Male	55% (23)
Female	45% (19)
Age (years)	
>18	19% (8)
14-17	17% (7)
6-13	38% (16)
<5	26% (11)
Disease severity	
Mild	19% (8)
Moderate	29% (12)
Severe	52% (22)
Age at onset (years)	
Neonatal	17% (7)
Infantile	62% (26)
Late onset	21% (9)
Age at diagnosis (years)	
<1	22% (9)
1-5	61% (25)
6-13	5% (2)
14-17	10% (4)
>18	2% (1)

Statistical analysis

Descriptive statistics was used to summarize demographic, clinical, and laboratory features.

Associations between clinical/laboratory features and neurological impairment (categorized by composite severity score) were analyzed using chi-square and Kruskal-Wallis tests. Where significant, posthoc analyses were performed using chi-square and Dunnett tests, with Bonferroni correction applied. P value ≤ 0.05 was considered statistically significant. We also reported results with P value ≤ 0.1, since we could not exclude that the P values obtained were dependent on the small sample size.

To assess the relationship between the composite severity score and the novel AGS score:

- AGS scores were compared across severity categories using Kruskal-Wallis tests.
- A nonparametric correlation analysis (Spearman correlation) was also conducted.

Results

Population

Our cohort included 42 individuals: 19 (45%) females and 23 (55%) males (Table 1, Supplementary Tables 1 and 2). An overview of clinical and demographic data of our international cohort is summarized in Table 1 and Supplementary Table 1. Neuroradiological data are collected in Supplementary Table 2.

Mean age at enrollment was 7.5 years (S.D., 6 years; range, 19 months-27.5 years). Regarding age at enrollment, 19% (8 of 42) were older than 18 years, 17% (7 of 42) were aged between 14 and 17 years, 38% (16 of 42) were aged 6-13 years, and 26% (11 of 42) were younger than five years.

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Mean age at diagnosis was 3.8 years (S.D., 5 years; range, 4 months-19 years). Parental consanguinity was reported in 9.5% (4 of 42) of patients.

Concerning ethnicity, 81% (34 of 42) of patients in our cohort were of Caucasian origin, 5% (2 of 42) were of African origin, 5% (2 of 42) were of Asian origin, and 9% (4 of 42) were bi- or multiracial.

Age at disease onset and severity

Age at disease onset was within the first year of life in 79% (33 of 42) of patients, with 17% (7 of 42) presenting neonatally and 62% (26 of 42) during infancy. Late-onset disease was observed in 21% (9 of 42) of the cohort. When using the composite functional severity score, 19% (8 of 42) of patients in our cohort fell within the mild group and 29% (12 of 42) in the moderate group, whereas 52% (22 of 42) presented with a severe phenotype. Age at presentation related to severity was heterogeneous: 25% (two of eight) of patients in the mild group had neonatal onset, 25% (two of eight) had infantile onset, and 50% (four of eight) had late onset. In the moderate group, 67% (8 of 12) had infantile onset and 33% (4 of 12) had late onset. The severe group predominantly exhibited infantile onset (73%, 16 of 22), with 23% (5 of 22) presenting neonatally and only 4% (1 of 22) later in life (Fig 1).

Clinical symptoms at onset

As far as symptoms at disease onset are concerned (Fig 2), recurrent fever was the most common symptom at presentation, reported in 57% (24 of 42) of cases. Feeding difficulties were noted in 29% (12 of 42), sleep disorders in 40% (17 of 42), and irritability in 45% (19 of 42). Notably, irritability was absent in all individuals within the mild group but was present in 58% (7 of 12) of the moderate and 55% (12 of 22) of the severe group (Fig 2).

Neurological features

Neurological function, including postural, motor, and communication milestones, varied significantly with disease severity (Table 2). Head control was achieved by 100% (eight of eight) of patients in the mild group and 92% (11 of 12) in the moderate group but only 18% (four of 22) in the severe group. Independent sitting was achieved by all individuals in the mild and in 92% (11 of 12) of the moderate group but was absent in the severe group. Independent walking occurred only in 75% (six of eight) of patients in the mild group.

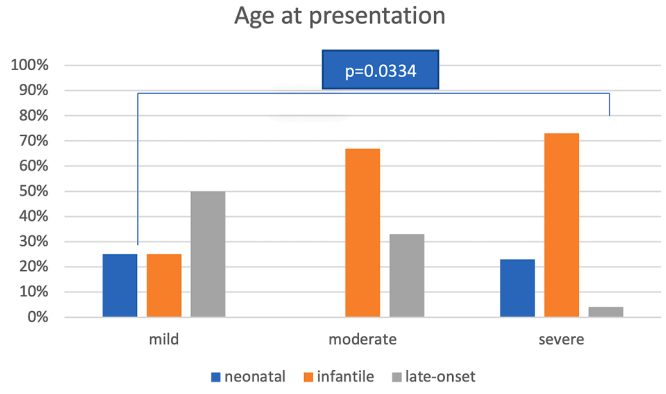

FIGURE 1. Age at disease onset by severity, Symptoms of onset, calculated by severity are depicted in Fig 2 . The color version of this figure is available in the online edition.

Language development also varied: whereas 88% (seven of eight) of patients with mild disease formed phrases, this was reduced to 33% (four of 12) in the moderate group and was absent in the severe group. Babbling and use of single words were more commonly observed in moderate and severe groups, indicating partial communication development.

Startle responses were present in 54% (22 of 41, one missing datum) of patients in our sample. Their distribution into groups of severity varied: none of the patients in the mild group, 50% (six of 12) in the moderate group, and 76% (16 of 21, one missing) in the severe one experienced startles (Fig 3).

Pyramidal signs were nearly universal (98%, 41 of 42), whereas extrapyramidal signs were present in 60% of patients (25 of 42). None of the subjects in the mild group presented with extrapyramidal signs, whereas 67% (eight of 12) and 77% (17 of 22) showed extrapyramidal signs in the moderate and severe groups, respectively (Fig 4).

Microcephaly was observed in 40% (17 of 42) of subjects. Stratifying into group of severity, we observed that 12.5% (one of eight) patients in the mild group, 25% (three of 12) in the moderate group, and 59% (13 of 22) in the severe one showed microcephaly. Epilepsy was present in 21% (nine of 42) of patients in our cohort, equally distributed in the moderate and severe groups, where it appeared in 25% (three of 12) and 27% (six of 22) of patients, respectively.

Interferon score

ISG expression data were available for 83% (35 of 42) of patients. Of these, 80% (28 of 35) had a positive interferon signature. A second ISG was available for 52% (22 of 42), of which 64% (14 of 22) remained positive.

Statistical correlations

- Neonatal onset was significantly more frequent in the severe group compared with the mild group ($P = 0.0334$). A trend toward significance was also observed between the moderate and severe groups ($P = 0.0968$) (Fig 1).
- A statistically significant difference in terms of irritability was observed between the mild and the severe groups ($P = 0.03$) and between the mild and moderate groups ($P = 0.044$) (Fig 2), being less probably present at onset in the mild group.
- The presence of startle significantly differed between the mild and severe groups ($P < 0.001$) (Fig 3), being more probably present in the severe group.
- As expected, a statistically significant difference ($P < 0.01$) was observed between different severity groups in head control, ability to maintain sitting position, ability to walk, and language abilities.
- Differences in extrapyramidal involvement were statistically significant between mild and moderate groups ($P = 0.0141$), and mild and severe groups ($P < 0.001$) (Fig 4), being less probably present in the mild group.
- Although in the pairs comparison no statistically significant difference was observed, with the exception of a trend toward significance in the mild versus moderate groups (P value $= 0.1$), at Pearson chi-square test a statistical significance appeared (P value $= 0.03$), thus being less probably present in the mild group.
- A statistically significant association was found between disease severity and the number of positive ISG tests ($P = 0.02$), with a higher likelihood of persistent positivity in the severe group.

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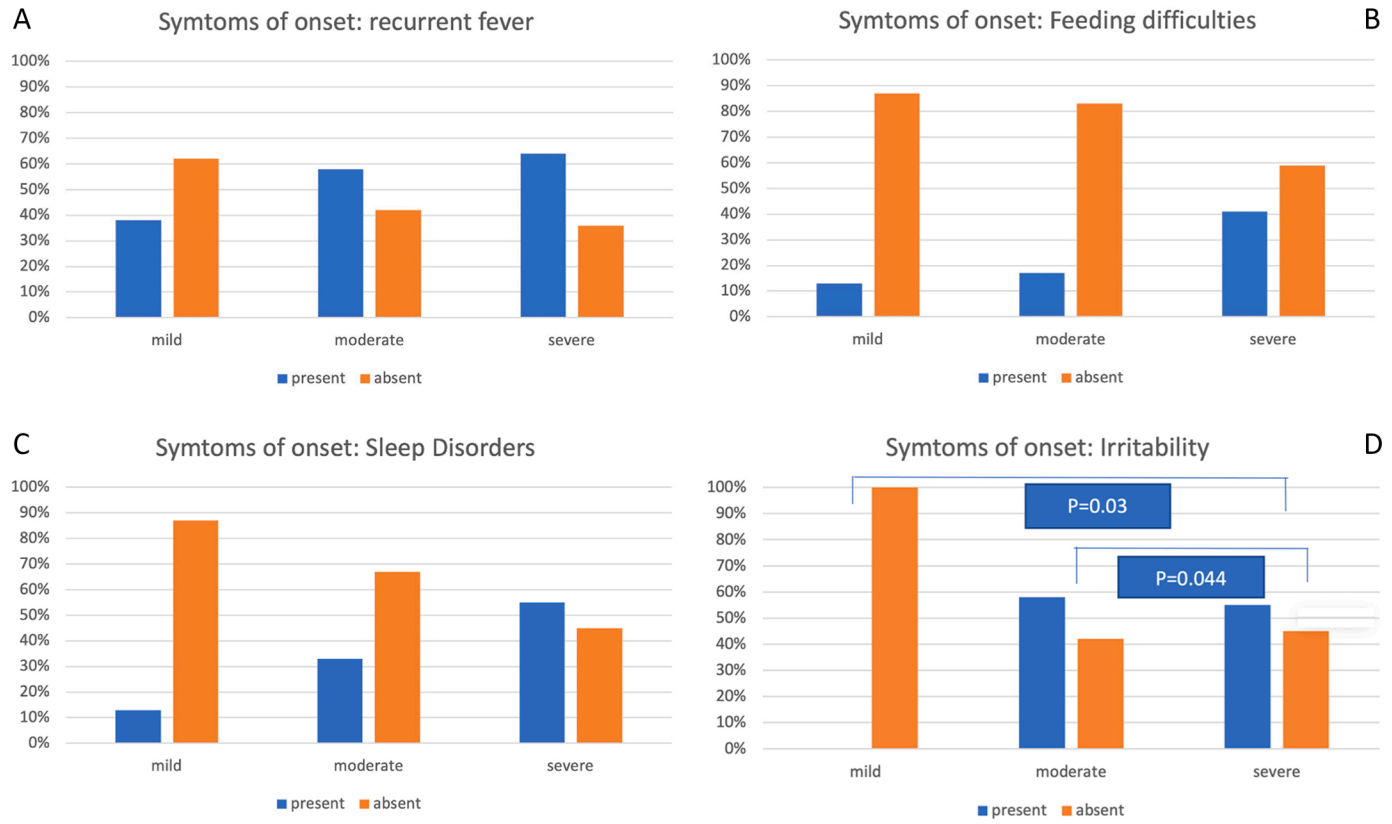

FIGURE 2. (A) Recurrent fever calculated by severity, (B) feeding difficulties calculated by severity, (C) sleep disorders of onset calculated by severity, and (D) irritability of onset calculated by severity. Vertical axis represents the percentage of patients in each category (mild, moderate, or severe group, displayed on longitudinal axes) either showing (present, in blue) or not showing (absent, in orange) that sign/symptom. The color version of this figure is available in the online edition.

Comparison between the composite functional severity score and the novel AGS score

The novel AGS severity score was compared between the composite score categories; it showed clear stratification across severity groups.

Mild: Mean 10.87 (range 10-11)

Moderate: Mean 8 (range 4-10)

Severe: Mean 1 (range 1-4)

The Kruskal-Wallis test confirmed significant differences between all groups ($P < 0.001$), supporting the concordance between the novel AGS score and the composite functional severity classification (Figs 5 and 6).

Discussion

Our cohort represents the biggest cohort of patients with AGS with homozygous p.Ala177Thr variants in *RNASEH2B* gene. This specific variant represents the most common one found in national and international cohorts. p.Ala177Thr has a broad ethnic and geographic distribution. As a confirmation of its widespread distribution, it has been identified across ethnic and geographic groups (Algerian, Moroccan, Irish, Italian, French Canadian, German, Egyptian, North African, and Indian backgrounds). In a previous study form our group from an Italian cohort of patients with AGS, a slightly higher prevalence of *RNASEH2B* homozygous variants was found in the Italian cohort when compared with international cohorts.[5,6] Our findings also confirm the significant intragenetic phenotypic variability among individuals with the p.Ala177Thr variant. Clinical variability in our cohort ranges from severe neonatal forms to milder atypical cases with preserved motor function.[7,8,15,18,19] This variability highlights the complexity of genotype-phenotype correlations in AGS and the need for tailored clinical evaluation and counseling.

Moreover, based on the broadening phenotype and the predicted prevalence of the variant in the general population, our data support prior suggestions that individuals with p.Ala177Thr may have been historically misdiagnosed with conditions such as

TABLE 2.
Summarized Neurological (Postural, Motor, and Communication) Acquisition of Our Cohort

Developmental Milestones	Mild (n = 8)	Moderate (n = 12)	Severe (n = 22)	Total (n = 42)
Head control	100% (8)	92% (11)	18% (4)	55% (23)
Independent sitting	100% (8)	92% (11)	0% (0)	45% (19)
Walk with aid	25% (2)	42% (5)	0% (0)	16% (7)
Independent walking	75% (6)	0% (0)	0% (0)	14% (6)
Babbling	0% (0)	17% (2)	27% (6)	19% (8)
Single words	22% (1)	50% (6)	5% (1)	19% (8)
Phrases	88% (7)	33% (4)	0% (0)	26% (11)

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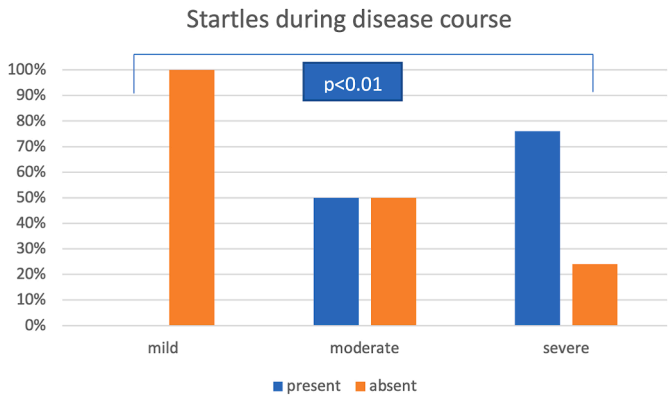

FIGURE 3. Presence of startles calculated by severity. Vertical axis represents the percentage of patients in each category (mild, moderate, or severe group, displayed on longitudinal axes) either showing (present, in blue) or not showing (absent, in orange) startles. The color version of this figure is available in the online edition.

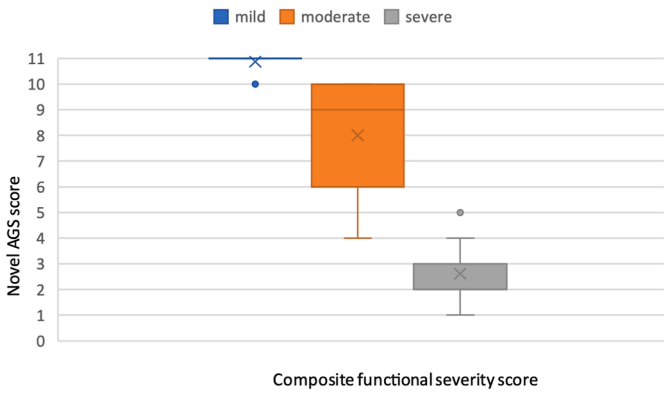

FIGURE 5. Correlation analysis between novel AGS score and composite functional severity score among groups of severity. The color version of this figure is available in the online edition.

cerebral palsy.[7,20] Advance of genetic testing, the increasing use of next-generation sequencing, and particularly exome sequencing, have led to the identification of more patients with milder or atypical presentations, thereby broadening the recognized phenotypic spectrum associated with this pathogenic variant.[7,18,19] Although the causes of such phenotypic variability are still unknown, we can argue the fundamental interplay of so far unidentified or not fully elucidated factors, including environmental and other complementary genetic and epigenetic factors that might be involved and influence the age of presentation, symptom manifestation, and overall clinical outcome.

Compared with pathogenic variants in other AGS-related genes,[8,9] the proportion of atypical or mild presentations appears to be higher. This finding raises important implications for clinical prognosis and family counseling, especially when diagnoses are reached early through genomic screening. Although earlier age at onset appeared to be more commonly associated with worse outcome, we suggest being extremely cautious in communicating the prognosis to families, since the developmental trajectory of patients affected by the specific p.Ala177Thr variant in *RNASEH2B* can be extremely heterogeneous and multifaceted and is difficult to predict based on knowledge available at the present time.

In our case series, in which patients with AGS2 were included only if they presented a homogeneous clinical picture classified as AGS according to Orcesi et al.[10] and Tonduti et al.,[11] only few and

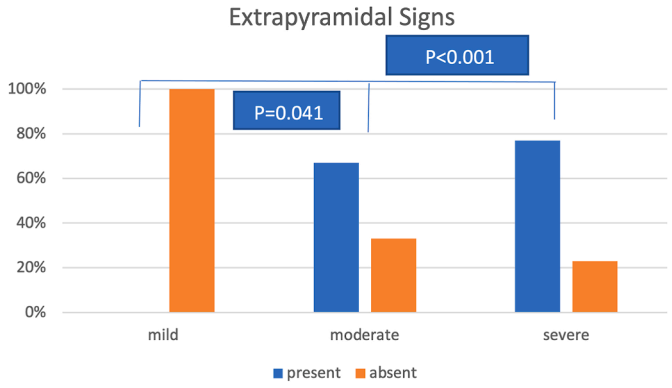

FIGURE 4. Extrapyramidal signs calculated by severity. Vertical axis represents the percentage of patients in each category (mild, moderate, or severe group, displayed on longitudinal axes) either showing (present, in blue) or not showing (absent, in orange) extrapyramidal signs. The color version of this figure is available in the online edition.

nonspecific characteristics have been found to be related to prognosis. Irritability at onset emerged as the only characteristic significantly associated with different outcome categories. However, irritability is a common and highly nonspecific symptom in patients with acute and subacute neurological disorders, whose etiology is complex and multifaced.

Other features—such as the presence of startle reactions and extrapyramidal signs—were more frequently observed in the severe group and may reflect more widespread cortical and subcortical dysfunction.[21] Microcephaly, although only trending toward significance in our cohort, has been previously identified as a negative prognostic factor in AGS[8] and may further contribute to clinical stratification.

The role of interferon signature in AGS2 remains uncertain. Literature reports that interferon signature is not always positive in patients with AGS2,[5] and our results confirm this observation. In the specific subset of our patients carrying the homozygous *RNASEH2B* p.Ala177Thr variant, interferon signature is positive in 80% of patients. However, in our cohort it appeared to be not significantly related to severity outcome. Although limited by the relatively small number of repeated measurements, our data seemed to show that interferon signature tends to be positive more often in severe patients, thus suggesting a pathogenetic process that lasts longer and therefore causes a more serious clinical picture. More natural history longitudinal data and other biomarkers accurately reflecting brain disease[22] would be needed to eventually confirm this hypothesis.

As a side comment, we believe noteworthy the observation that in our sample the retrospective application of the AGS severity score proposed by Adang and colleagues[15] correlated well with the commonly used composite functional severity score. Our observation seems to confirm the possibility of applying, even retrospectively, the novel AGS severity score to classify and monitor the clinical severity of patients with AGS, with the possibility of providing a more accurate grading of disease severity, even among similar clinical pictures.

Taken together, our data underline the urgent need for the identification of composite early biomarkers that could better predict disease severity. Until such tools are available, clinicians should exercise caution when providing prognostic information to families, especially in cases diagnosed early through genetic screening. The wide range of possible outcomes—from minimal disability to severe impairment—underscores the unpredictable nature of the condition.

Although outside the primary aim of this article, we wondered whether an expert consensus might discuss a revision of

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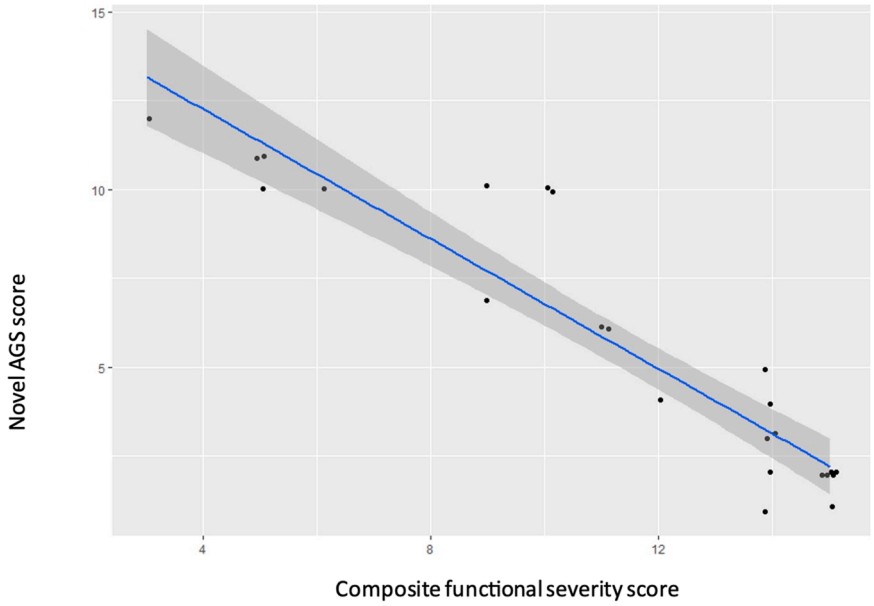

FIGURE 6. Correlation analysis between novel AGS score and composite functional severity score patient by patient. The color version of this figure is available in the online edition.

nomenclature when highly atypical features, symptoms overlapping with other forms of interferonopathies, or other phenotypic pictures (such as spastic paraparesis with normal neuroradiological findings or acute bilateral striatal necrosis without other symptoms/brain abnormalities) occur.

Our results should be read in the light of some limitations. Despite the effort to collect a homogeneous group of patients according to the diagnostic criteria for AGS, our study is retrospective in its nature. A certain degree of parental recall bias should be taken into consideration. This might have, at least in part, limited the collection of data regarding the earlier phases of the disease. Further prospective multicenter studies will be essential in the future to unravel in depth the role of early signs of the disease as prognostic factors.

Conclusions

In summary, we describe the largest reported cohort of patients with AGS homozygous for *RNASEH2B*:p.Ala177Thr, emphasizing the substantial clinical heterogeneity observed. The integration of multicenter data proved essential in characterizing this variability and advancing the understanding of AGS2. The biological underpinnings of this phenotypic diversity remain elusive and likely involve a combination of genetic, epigenetic, and environmental factors. Notably, recent work by Garau et al.[23] highlights the potential influence of differential DNA methylation in patients with this variant. Furthermore, the discovery of individuals carrying the homozygous p.Ala177Thr variant in *RNASEH2B* expands the genotype-phenotype correlation to include nonpenetrant individuals.[24]

Moving forward, large-scale genomic, transcriptomic, and proteomic studies will be critical to uncovering molecular modifiers of disease expression. Such research could pave the way for precision medicine strategies tailored to individual disease trajectories in AGS.

CRediT authorship contribution statement

Costanza Varesio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Davide Politano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Adang:** Writing – review & editing, Supervision. **Elena Ballante:** Writing – review & editing, Supervision. **Roberta Battini:** Writing – review & editing, Supervision. **Enrico Bertini:** Writing – review & editing, Supervision. **Renato Borgatti:** Writing – review & editing, Supervision, Funding acquisition. **Valentina De Giorgis:** Writing – review & editing, Supervision, Investigation. **Annamaria Del Boca:** Writing – review & editing, Supervision, Investigation. **Francesca Dragoni:** Writing – review & editing, Supervision. **Elisa Fazzi:** Writing – review & editing, Supervision, Investigation. **Jessica Galli:** Writing – review & editing, Supervision, Investigation. **Jessica Garau:** Investigation, Data curation. **Francesco Gavazzi:** Writing – review & editing, Supervision. **Alice Gardani:** Writing – review & editing, Supervision, Investigation. **Roberta La Piana:** Writing – review & editing, Supervision. **Isabella Moroni:** Writing – review & editing, Supervision, Investigation. **Francesco Nicita:** Writing – review & editing, Supervision, Investigation. **Anna Pichiecchio:** Writing – review & editing, Supervision, Investigation. **Antonella Pini:** Writing – review & editing, Supervision, Investigation. **Federica Ricci:** Writing – review & editing, Supervision, Investigation. **Stefano Sartori:** Writing – review & editing, Supervision, Investigation. **Davide Tonduti:** Writing – review & editing, Supervision, Investigation. **Adeline Vanderver:** Writing – review & editing, Supervision. **Simona Orcesi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Vanderver, Gavazzi, and Adang have licensed the AGS scale and received funding for AGS research from Biogen, Eli Lilly, and Boehringer Ingelheim without any personal compensation. Del Boca, Politano, Galli, Fazzi, Tonduti, and Orcesi participate in a clinical trial by Transposon Therapeutics, Inc for patients with AGS

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2025.07.011.

References

1. Aicardi J, Goutières F. A progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis. Ann Neurol. 1984;15:49–54.
2. Crow YJ, Manel N. Aicardi-Goutières syndrome and the type I interferonopathies. Nat Rev Immunol. 2015;15:429–440.
3. Livingston J, Crow Y. Neurologic phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1, and IFIH1: aicardi–goutières syndrome and beyond. Neuropediatrics. 2016;47:355–360.
4. Uggenti C, Lepelley A, Depp M, et al. cGAS-mediated induction of type I interferon due to inborn errors of histone pre-mRNA processing. Nat Genet. 2020;52:1364–1372.
5. Crow YJ, Chase DS, Lowenstein Schmidt J, et al. Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. Am J Med Genet A. 2015;167A:296–312.
6. Garau J, Cavallera V, Valente M, et al. Molecular genetics and interferon signature in the Italian Aicardi Goutières syndrome cohort: report of 12 new cases and literature review. J Clin Med. 2019;8:E750.
7. Svingen L, Goheen M, Godfrey R, et al. Late diagnosis and atypical brain imaging of Aicardi-Goutières syndrome: are we failing to diagnose Aicardi-Goutières syndrome-2? Dev Med Child Neurol. 2017;59:1307–1311.
8. Adang L, Gavazzi F, De Simone M, et al. Developmental outcomes of Aicardi Goutières syndrome. J Child Neurol. 2020;35:7–16.
9. Piccoli C, Bronner N, Gavazzi F, et al. Late-onset aicardi-goutières syndrome: a characterization of presenting clinical features. Pediatr Neurol. 2021;115:1–6.
10. Orcesi S, La Piana R, Fazzi E. Aicardi-Goutieres syndrome. Br Med Bull. 2009;89:183–201.
11. Tonduti D, Panteghini C, Pichiecchio A, et al. Encephalopathies with intracranial calcification in children: clinical and genetic characterization. Orphanet J Rare Dis. 2018;13:135.
12. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol. 1997;39:214–223.
13. Eliasson AC, Krumlinde-Sundholm L, Rösblad B, et al. The Manual Ability Classification System (MACS) for children with cerebral palsy: scale development and evidence of validity and reliability. Dev Med Child Neurol. 2006;48:549–554.
14. Hidecker MJC, Paneth N, Rosenbaum PL, et al. Developing and validating the communication function classification system for individuals with cerebral palsy. Dev Med Child Neurol. 2011;53:704–710.
15. Adang LA, Gavazzi F, Jawad AF, et al. Development of a neurologic severity scale for Aicardi Goutières syndrome. Mol Genet Metab. 2020;130:153–160.
16. La Piana R, Uggetti C, Roncarolo F, et al. Neuroradiologic patterns and novel imaging findings in Aicardi-Goutières syndrome. Neurology. 2016;86:28–35.
17. Armangue T, Orsini JJ, Takanohashi A, et al. Neonatal detection of Aicardi Goutières Syndrome by increased C26:0 lysophosphatidylcholine and interferon signature on newborn screening blood spots. Mol Genet Metab. 2017;122:134–139.
18. Videira G, Malaquias MJ, Laranjinha I, Martins R, Taipa R, Magalhães M. Diagnosis of aicardi-goutières syndrome in adults: a case series. Mov Disord Clin Pract. 2020;7:303–307.
19. Lambe J, Murphy OC, Mu W, Sondergaard Schatz K, Barañano KW, Venkatesan A. Relapsing–remitting clinical course expands the phenotype of Aicardi–Goutières syndrome. Ann Clin Transl Neurol. 2020;7:254–258.
20. Galli J, Gavazzi F, De Simone M, et al. Sine causa tetraparesis: a pilot study on its possible relationship with interferon signature analysis and Aicardi Goutières syndrome related genes analysis. Medicine (Baltimore). 2018;97:e13893.
21. Bakker MJ, van Dijk JG, van den Maagdenberg AMJM, Tijssen MAJ. Startle syndromes. Lancet Neurol. 2006;5:513–524.
22. Rodero MP, Decalf J, Bondet V, et al. Detection of interferon alpha protein reveals differential levels and cellular sources in disease. J Exp Med. 2017;214:1547–1555.
23. Garau J, Charras A, Varesio C, et al. Altered DNA methylation and gene expression predict disease severity in patients with Aicardi-Goutières syndrome. Clin Immunol Orlando Fla. 2023;249:109299.
24. Crow YJ, AGS group. Clinical non-penetrance associated with biallelic mutations in the RNase H2 complex. J Clin Immunol. 2023;43:706–708.

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