UNIVERSITA' DEGLI STUDI DI PAVIA

PhD IN BIOMEDICAL SCIENCES
DEPARTMENT OF BRAIN AND BEHAVIORAL SCIENCES
UNIT OF NEUROPHYSIOLOGY

Deep phenotyping in brain malformations: from disease trajectories to the development of new networks of rare neuropediatric diseases

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Declaration

I hereby declare that the present thesis is related to the scientific works authored by the PhD candidate. Most of the studies have led to scientific publications and will be presented along with the results of ongoing research.
All the projects presented in this thesis were carried out at IRCCS Mondino Foundation, in Pavia.
RENDER network project, as detailed along, was carried out in collaboration with other Italian researchers.

Ithaka gave you the marvelous journey
Without her you wouldn't have set out
But she has nothing left to give you

'Ithaka', Constantino Kavafis

Abstract

Deep phenotyping is pivotal in the study of rare neuropediatric diseases, as it enables a precise and comprehensive characterization of clinical features and improves disease understanding.

Subjects with rare neuropediatric diseases exhibit unique and highly heterogeneous functioning, and therefore require individualized interventions, updated outcome evaluation, and adapted tools. By leveraging advanced imaging, detailed neuropsychological assessments, and integrative bioinformatics, deep phenotyping may serve as a link to specific genotypes, enhancing diagnostic accuracy and promoting the discovery of disease mechanisms and therapeutic targets.

On the premise that current research in the field of rare neuropediatric diseases should not overlook deep phenotyping, my PhD program was structured around the development of an innovative tool for deep phenotyping and the characterization of neuroradiological and behavioral patterns associated with specific brain malformations.

Grounded in a constant interplay between clinical care and research, the development of an innovative registry for diagnosed and undiagnosed rare neuropediatric diseases was undertaken within the framework of a multicenter network project. Chapter II outlines the development process, the structure of the platform, and its underlying philosophy.

Chapter III presents all the studies conducted, focusing on the deep phenotyping of midline and posterior cranial fossa malformations. Regarding the former, biological pathways leading to septo-optic dysplasia were analyzed, and both subjective and objective assessments of sleep were performed in patients with septo-optic dysplasia and corpus callosum agenesis. Concerning the latter, abnormal fetal neuroradiological findings are correlated with long-term clinical outcomes, and the phenotypic patterns of posterior cranial fossa malformations are described and linked to both clinical prognosis and genotype.

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I. Introduction

1.1 Rare neuropediatric diseases: frontiers and the need of innovative tools

The study of rare neuropediatric diseases (RND) is an evolving field that faces numerous challenges, from diagnosis to identification of disease trajectories, from knowledge of disease mechanisms to availability of therapeutic strategies, from patients' quality of life improvement to accessibility to targeted and comprehensive healthcare services. Due to the rarity of each condition, people living with rare diseases face unique challenges, including lack of knowledge and understanding from health and social care professionals. As in a Rubik's cube, the many faces must be put together, because the complexity of rare diseases can only be addressed through multistakeholders collaboration and networking.

Rare neuropediatric diseases are a meaningful subgroup of rare diseases, considering that 50% of rare diseases have a neurological involvement and 70% have a pediatric onset. The definition of a condition as a rare disease varies between jurisdiction and organizations, leading to disparities in health policy, research focus and access to treatment. The World Health Organization (WHO) defines a rare disease as one affecting less than 1 in 2,000 people within a WHO region. The Orphan Drug Act, the law passed by the United States (US) in 1983 with the aim to promote and incentivize drug development for rare diseases, defines a rare disease as one affecting less than 200,000 people in the US. The European Union defines a rare disease as one with a prevalence of less than 5 per 10,000.

The epidemiology of rare neuropediatric diseases is a challenging subject due to the low prevalence but also underdiagnosis of these conditions. Due to the rarity of diseases, and the inconstant disposal of comprehensive tools for epidemiological data track and disease knowledge advancement, obtaining accurate population-based data is not obvious. And the lack of comprehensive natural history data delays and complicates crucial aspects of trial design, such as selecting appropriate endpoints. Natural history studies (NHS) play a vital role in the research setting for a rare disease. Based on the relevant US Food and Drug Administration (FDA) guidance: "Rare Diseases: Natural History Studies for Drug Development", NHS can accelerate drug development by four main contributions: identification of patient population; identification or development of clinical outcome assessments; recognition of biomarkers, and provision of datasets for externally controlled trials.

While rare diseases are by definition infrequent, put together, their impact on affected families and healthcare systems is substantial, and thus, innovative and translational research and dedicated clinical programs are essential to improve early detection, accurate diagnosis, targeted therapeutic intervention, and outcome measurement. A multistakeholder approach that actively involves patients or patients' representatives in research is essential for producing high quality, relevant, and ethically sound research. Patients and caregivers indeed bring unique perspectives that help shaping research questions, improving study designs, and enhancing the implementation of research findings.

Below are resumed some of the key obstacles faced in the field of research in rare neuropediatric diseases:

1. **Heterogeneity of Disease Presentation**
   RND are characterized by a high degree of phenotype heterogeneity, with presentation that can vary significantly between patients, within the same genetic condition. This variability complicates early diagnosis and may delay the initiation of appropriate interventions. Furthermore, the progression of many of these conditions is often unpredictable, making it difficult for researchers to establish patterns of disease evolution. Collecting broad data and achieving a deep phenotyping are key points to improve disease knowledge and establish the underlying pathogenetic mechanisms.
2. **Small Patient Populations**
   The rarity of these conditions means that clinical trials and research studies often have small sample sizes, reducing statistical power. Additionally, scarcity of eligible subjects means that it can be difficult to launch enough a clinical trial. That's the reason why, for instance, N-of-1 trials have been implemented in RND: it's about personalized, single-patient studies designed to assess the effectiveness of a specific treatment or intervention even on a single patient, considering all possible bias and influences of outcome. By focusing on individual tailored therapeutic option, N-of-1 trials represent important opportunities to achieve valuable insights into treatment responses and understanding.
3. **Difficulty in Diagnosing and Characterizing Disease Mechanisms**
   Diagnosing RND can be a lengthy and complex process, often requiring concomitant advanced genetic testing, neuroimaging, and specific testing. Moreover, in the majority of cases the molecular and genetic mechanisms underlying many of these diseases are not fully understood.

4. **Limited Access to Therapeutic Options**
   The great majority of RND currently have no specific treatment target, and treatment options are often limited to symptom management. This is partly due to the lack of comprehensive clinical trials and research focused on developing disease-specific therapies. Nevertheless, known and already available potential treatment in use in other diseases has been adopted for 'new' targeted population.
5. **Challenges in Collaboration and Data Sharing**
   Due to the fragmented nature of research on rare diseases, there is often a lack of collaboration between researchers and institutions. In this framework, specific networks such as ERN (European reference networks) represent extremely virtuous example. Still, the absence of centralized data can hinder progress in understanding disease natural history and in identifying trial ready populations. Efforts to promote data sharing and collaboration are underway, but challenges related to data privacy, standardization, and accessibility are still an issue.
6. **Funding Constraints**
   Research into rare diseases often receives limited funding. In this direction the active role of patients' association and the favorable meeting of multiple stakeholders are considered positive boost leading to financial support.

1.2 Disease trajectories and registries

One critical tool in advancing research and improving patient outcomes is the creation of disease registries. By definition, a patient registry is "an organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition, or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes" (Gliklich R, 2014). The development of dedicated disease registries and related biosamples' collection represent a common priority of the majority of RND. The gathering of patients sharing the same rare genetic diagnosis and a standardized longitudinal collection of their clinical and instrumental data are essential steps towards understanding the disease history, mechanisms and genotype-phenotype correlates, which are prerequisites towards the development of therapies. This is even more important for rare and ultra-rare neuropediatric diseases, where the number of affected individuals is extremely small. Therefore, only collaborative efforts and sharing makes it possible to achieve sufficient caseloads for the advancement of scientific knowledge, yet the development of individual registries for each rare disease is clearly an impracticable task.

Disease registries should help in data collection and centralization, providing a valuable resource for researchers, clinicians, and policymakers. By aggregating deep phenotyping clinical, genetic , and outcome data, registries help identifying trends, facilitating better diagnostic protocols, and promoting the development of targeted treatments. In Italy there are at about 80 national registries for rare diseases, of which 20 for neurological diseases. The European Community is strongly promoting the creation of networks, and with this in mind it has set up the European Research Networks (ERNs) to connect the various centres that, in European countries, deal with specific rare pathologies (e.g. EPICARE for epilepsy, or ITHACA for congenital malformations and rare intellectual disabilities). However, these huge networks suffer from considerable organizational complexities, and currently do not offer effective sharing tools, especially for national realities. In Italy, there are some registries dedicated to specific neuropediatric diseases, e.g. the Italian neuromuscular registry supported by Telethon and UILDM, or registries for Dravet syndrome or GLUT1 syndrome, just to mention a few. However, most rare and ultra-rare neuropediatric diseases are not included in specific registries, and the collection of clinical and instrumental data remains limited to the Centre to which the patient belongs or to a restricted network of collaborators. Finally, biorepositories of samples from patients with such ultra-rare neuropediatric diseases have not been established so far.

In the realm of rare pediatric neurological diseases, disease-specific registries are particularly vital. Registries foster collaboration across institutions, allowing for more comprehensive datasets that can drive larger-scale studies and clinical trials. In addition, they play a crucial role in raising awareness about many emerging conditions, rendering them trial ready once eligible.

The exact roles of disease registries and their regimentation however are not yet fully defined, and their implementation often fails to keep pace with technology and, today, with the prospects continually offered by the opening up of artificial intelligence. The four principles of findability, accessibility, interoperability and reusability (FAIR) aimed to make order in the expanding big data and electronic data capturing in research have also been adopted in rare disease registries. Although several guidelines for rare disease registries have been attempted, quality remains a challenge.
The study of Schoenmakers et al (Schoenmakers 2024), provides a practical framework to support the creation and implementation of patient registries, focusing on RND, to guide registry holders. The framework addresses common challenges and elaborates on possible approaches for building a data infrastructure with maximum impact on patient care by serving research, drug development, and regulatory and reimbursement decision making.

II RENDER project

2.1 History and participants

RENDER (RaRE Neuropediatric DiseasE Registry) is the result of a pilot network project funded by the Mariani Foundation, that involved a broad team of child neuropsychiatrists and clinical geneticists from different Institutions (Fondazione IRCCS Istituto Mondino , Università degli studi di Pavia, IRCCS Istituto Gaslini di Genova; Università Sapienza di Roma; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano; Università di Verona).

The mentioned Institutions participated to the Mariani scientific ban of 2021 with diverse individual project design in the single centers, namely based respectively on intellectual disability, Sotos syndrome and generalized overgrowth, childhood neurodegenerative disorders, movement disorders, ataxia. The different proposals had a common denominator which consisted of the identification of new harmonized tools to collect broad and high-quality data on potentially diverse rare neuropediatric conditions. Mariani Foundation decided to promote the network project with the ultimate objective of developing a unique platform able to centralize and harmonize the collection of clinical, instrumental and genetic data of patients with rare neuropediatric diseases. Such an ambitious goal was conducted searching to keep in mind a very wide range of disease presentation and etiologies in the neuropediatric area. RENDER model is unprecedented, but still, it can be in part viewed and explained within a process of innovative and broad spectrum disease registry development. Since the recent paper of Schoenmakers et al (Schoenmakers, 2024), provides an updated practical framework to support the creation and implementation of patient registries, focusing on RND, the presentation of RENDER registry will follow this theoretical referral to tackle the potential and readiness of the tool.

2.2 Methods

Team organization

The included researchers (clinicians and geneticists), PIs and collaborators, have been guided by a coordination team that included the main Principal investigator, a data manager and a clinician. The project was finalized in collaboration with a Bioengineering and Medical Informatics Team with expertise in the development of platforms for clinical studies and pathology registries.
The data manager got involved in almost all the meetings with the clinicians and geneticists and with the informatics, coordinating the long-term work.

Timeline

- First Two-year period: the clinic and genetic working groups designed the electronic Case Report Form (eCRF) through serial online and in person meetings.

- Second Two-year period: Optimization and harmonization of the tool; Beta testing and RENDER promotion

Procedures

By first using excel files to present structure and content proposal, once an agreement was found on a specific single form, a meeting to discuss contents and finalize the most suitable implementation on RedCap was finalized.
The individual forms were developed for maximum flexibility in compilation to allow data entry of patients with different neuropediatric conditions, of varying degrees of depth, and their periodic updating. Closed answers instead of open fields were favored in the great majority of cases, with each field being coded using a universally recognized language based on HPO (human phenotype ontology) terms. Once an open field was required, the entry data was anyhow bound to a linked database (e.g. OMIM). With the exception of the Patient reported outcome measures (PROMs) section, all the forms are in English language.

Specifically, the General and Neurological section were developed by Child Neuropsychiatrists with experience in rare diseases, while the Organ-specific and Genetic-instrumental areas were developed by clinical geneticists and reviewed by specialists in the field. The neuroimaging form was developed with the special collaboration of a neuroradiologist expert in neuropediatric diseases and brain malformations. Once the vast majority of forms was implemented, the eCRF were reviewed by the coordination team to test for homogeneity and duplicates and congruence.
Subsequently, RENDER has been tested locally at each institution, though insertion of heterogeneous and complex clinical cases. Young residents in child neuropsychiatry and genetics were involved to assess the accessibility, comprehensiveness and reliability of the tool. In a second phase of beta testing, experts in the field outside RENDER network were involved.

Information technology infrastructure

RENDER was developed on a modular RedCap-based electronic Case Report Form (eCRF), one of the most internationally used web-based platforms in the medical field. RENDER fully complies with the FAIR (Findability, Accessibility, Interoperability Reusability) principles.
The choice of REDCap is strategic due to the presence of built-in functions able to simplify phases of data quality control and definition of statistical analysis models. These features include: i) low-level quality checks; ii) logging and tracing of changes made to the eCRF fields; iii) custom reporting capabilities; iv) data export functionality; v) implementation of the most informative statistical indices for the description of collected data.
REDCap also keeps track of all input activity and of any changes made to the data, allowing targeted checks on the data source to quickly trace possible sources of error.
A REDCap module (Data Exports, Reports and Stats) allows the creation of advanced reports whose content can be analysed through a simple interface. It is possible to generate reports in different formats and to choose the variables to export. When exporting or sharing data, de-identification options allow removing identifier fields during data export (e.g. note or date fields) or shifting such fields in time. Data are usually exported in CSV format (comma separated), but can be also exported in PDF format if needed. Other export format options will be available, which will generate data already compatible with popular statistical analysis software (SPSS, R, Stata, SAS).

Currently and for the next 5 years, RENDER will be hosted on a reliable external server , Consorzio di Bioingegneria Informatica Medica (CBIM), which will provide all the technical documentation related to maintaining the platform, ensuring data security (backup systems, procedures in place to prevent data loss, disaster recovery plan, etc.). CBIM focuses on the integration of bioengineering and medical informatics to advance healthcare technologies. As CBIM develops and deploys innovative solutions in areas such as medical data analysis and digital health, it faces critical challenges related to data protection. The sensitive nature of medical data, including patient information and clinical research results, necessitates the implementation of rigorous data protection measures. RENDER adheres to strict privacy regulations, such as the General Data Protection Regulation (GDPR). GDPR in the European Union, is crucial for ensuring that personal health information remains secure. To protect against unauthorized access, breaches, or misuse, CBIM must adopt advanced encryption techniques, secure data storage solutions, and access control mechanisms, all while maintaining compliance with both national and international data protection standards.

2.3 RENDER utilization

Appropriate comments, reminders, and fill-in examples are included in each form to assist the compiler in data entry and minimize error. Please find below general information to be confident with RENDER utilization:

Date of compilation: the date to be inserted is the one corresponding to evaluation date; an automatic generation of age at examination will follow

Status of compilation: At the end of each form, the compiler can specify whether the form is complete, incomplete, unverified *(to be checked/ revised).* The legend is available in the 'Record home page'

Order of compilation: Personal data form has to be compiled first, then the order suggested should follow the form list but it is not compulsory. The clinician can choose to open only the forms that fit with the clinical picture of the patient.

To facilitate the compilation, sometimes **red flags** will appear to address the clinician in the wright direction.

For example in the 'behavioral form' whether a 'low adaptive functioning' is selected, the flag '***REMINDER: compile the "Scale" form'*** will appear to orient the detailed insertion of the referral scale.

Form typology: A single form can be REPEATABLE (e.g. the 'general and neurological examination' form will be performed different times; for each single neurological examination a different form can be compiled. After the compilation of the first form, the subsequent form will automatically contain the content of the previous, to be eventually modified) and UPDATABLE (e.g. the 'psychomotor development' form once compiled for the first time, can be modified later.

Language: The language utilized utilizes HPO terms to be consistent. Only some scales are set in Italian in order to allow self-compiling.

Open fields: Almost always the open fields are linked to HPO database through search option, when not the compiler can add a free text. If the information to be inserted here are already comprised in previous closed questions, it is suggested to leave the open field empty since the information put here won't be searchable by the system later.

Searching tool: It is possible to search for a variable that the compiler can't find. Press the button "Coodbook" and start searching!

Data retrieval

Having completed data entry in the platform, it is possible to proceed with data visualization and export for eventual data analysis. This is feasible through the "Data Export, Reports, and Stats" function, which allows intuitive visualization of data within reports, containing graphs and descriptive statistics. It also gives the option of exporting data to Excel, Stata, SAS, SPSS, or R for analysis.
To create a report, it is necessary to proceed with data filtering, selecting the patients, observations or laboratory data to extract, with the possibility of also adding a brief description of the report itself. The results of the report containing the information of interest are downloadable in pdf format with graphs and descriptive statistics of the selected observations, or can be exported for later analysis.

2.4 RENDER platform structure

At the end of the pilot project, RENDER is composed of 32 modules with a structure that is both cross-sectional (to ensure flexibility in the inclusion of patients with different pathologies so that, depending on the disease and clinical picture, it may be required to fill some eCRFs but not others) and longitudinal (to allow recording of follow-up data over time). Some modules record instrumental data (e.g. electrophysiology; neuropathology; biochemical testing) and treatments; others are devoted to record quantitative scales and functional measures, which are pivotal to collect quantitative outcome measures across many distinct areas.

Here is the complete form list: 1) personal data; 2) family history; 3) family member; 4) pregnancy and delivery; 5) psychomotor development; 6) general and neurological examination; 7) behavioral assessment; 8) movement disorders; 9) epilepsy ; 10) auxological parameters and growth; 11) head and neck; 12) limbs; 13) axial; 14) skin; 15) auditory system; 16) ocular system; 17) endocrine system; 18) cardiovascular and respiratory system; 19) gastrointestinal system; 20) genitourinary system; 21) imaging; 22) histopathology; 23) genetics; 24) electrophysiology; 25) samples and biomarkers; 26) scales; 27) testing; 28) diagnosis; 29) medications and therapies ; 30) biochemistry; 31) metabolism.

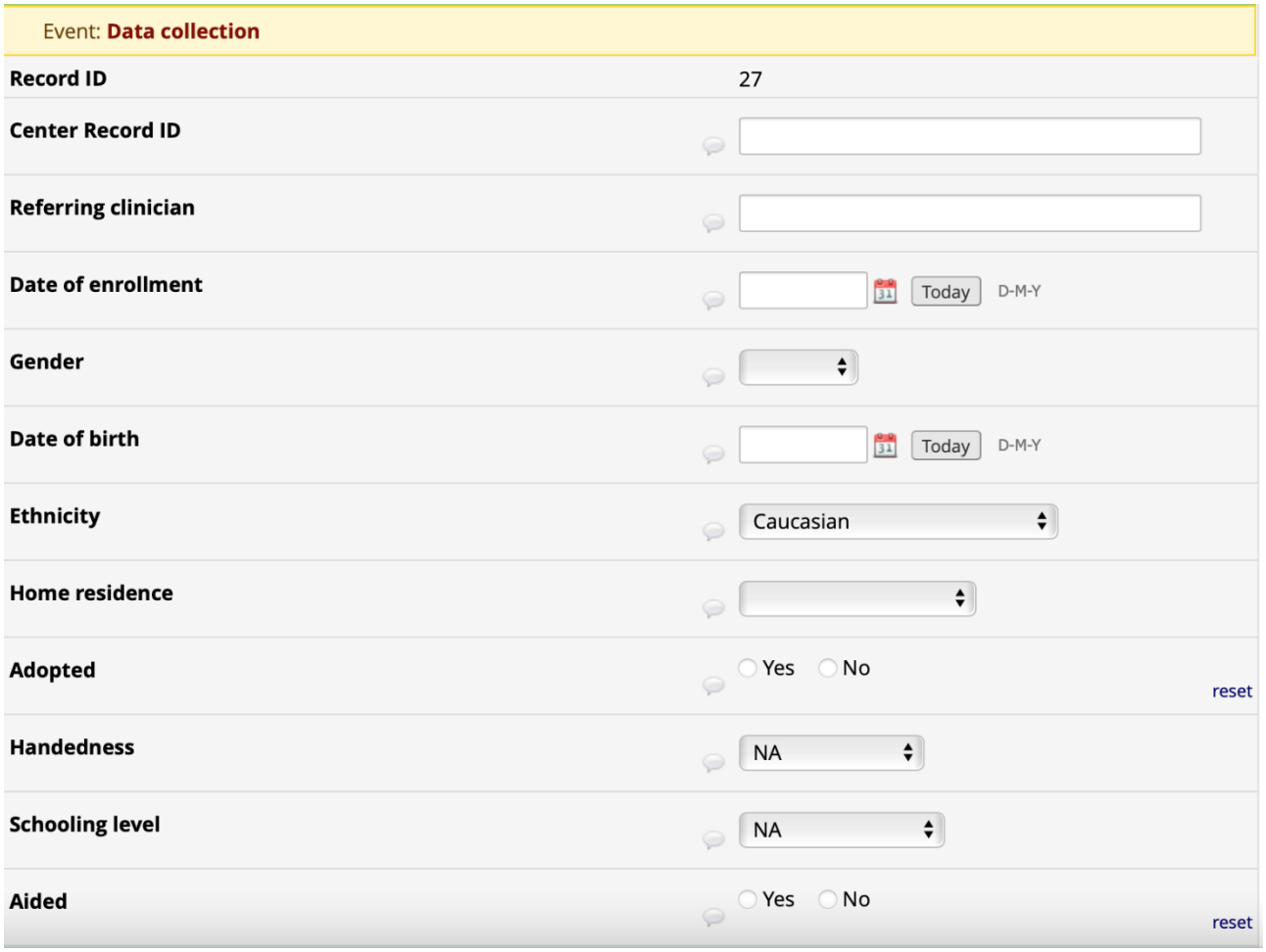
To further implement this eCRF, additional modules can be built, which, as the others, will be optional (to be filled or not according to the phenotype) and repeatable (for follow-up data collection).

2.4.1 General data

Personal Data (*updatable)*

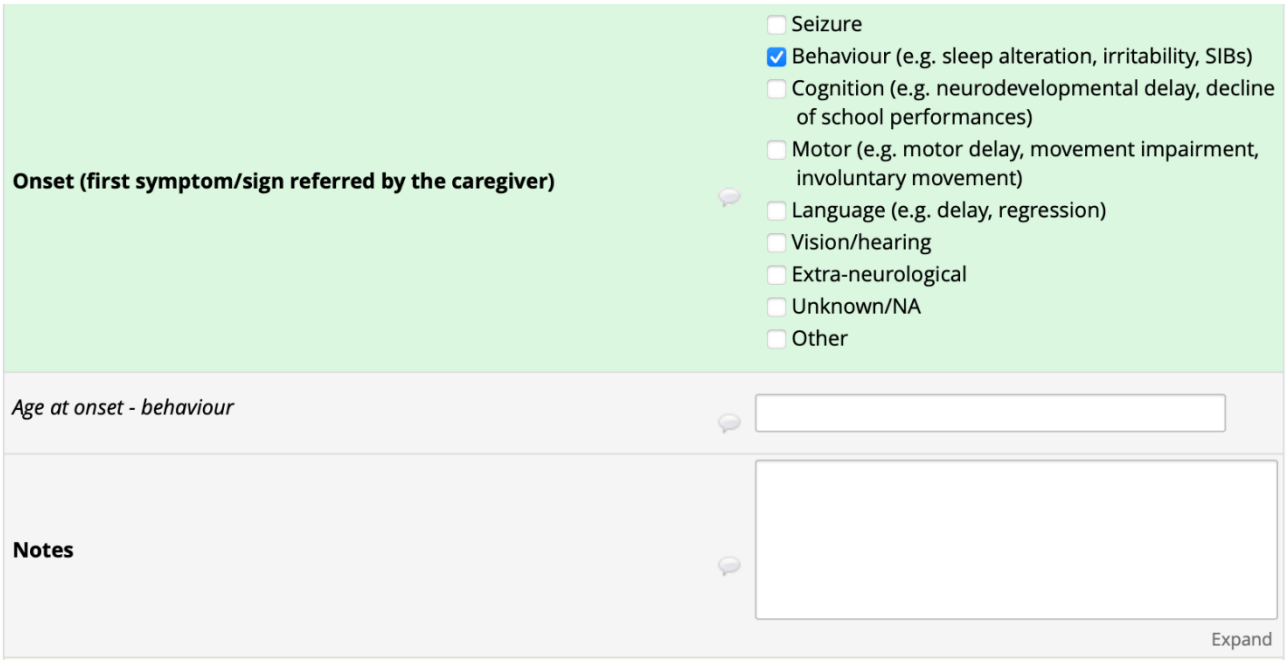
This form opens automatically on the first entry of each new subject. Here is where both patient ID record and referring clinician are recorded, alongside all demographic information: **date of birth, gender, ethnicity, home residence, handedness, schooling level**. Note, the **date of enrollment** must be the same of those of study enrollment, thus of those of consent form signature.

Fig. 1 Personal data



Moreover, it is also possible to register the first onset reported by the caregivers, in terms of typology (for example: behaviour) and the age at first symptom presentation.

Fig. 2 Personal data: onset symptom



Family history (*updatable)*

This form allows the family tree to be traced, especially in respect to the presence of a neurological disorder. This form can be compiled in respect to proband family history (Fig 3) or to the relative of enrolled proband family history (Fig 4). Composition and history of the family is registered within a table but it is also possible to upload the graphical tree. Moreover, in this form it is possible to account for any available information about both spontaneous and therapeutic miscarriages should be inserted.

Family member (*updatable)*

As advised by the red flag at the beginning of the form, here is where it should be reported which neurological disorder is present (the same of the proband or another one). Moreover, in this form it is possible to specify whether the family member has been enrolled in RENDER. This information must be collected if the compiler is interested in taking together or family members clinical information.

Pregnancy and delivery (*Updatable)*

In this form all information regarding pregnancy, delivery and birth parameters can be inserted (gestational age, pregnancy relevant event e.g. oligohydramnios ; prenatal imaging abnormalities e.g. increased nuchal translucency).

2.4.2 Neurological Data

Psychomotor development (*updatable*)

This form is highly relevant for the child neuropsychiatrist since traces the stages of development regarding motor, language and communication, and behavioral and social development. For each milestone it is possible to specify whether it has been acquired or whether that competence has been lost (regressed) and the timing of the event.

Evaluation date Today D-M-Y

MOTOR DEVELOPMENT

Indicate Yes/No even if age is not remembered

	Acquired	If yes, specify month	Regressed	If yes, specify month
Head control	NA		No	
Rolling over	NA		No	
Sitting	NA		No	
Crawling	NA		No	
Standing	NA		No	
Walking	NA		No	

General and neurological examination (*repeatable)*

This form can be considered as the standard objective neurological examination. It is divided into 2 sections:

- *Analytic neurological assessment*: It covers all the main neurological domains useful for the clinical assessment of a neurological disease

- *General assessment*: It allows to notify whether an extra-neurological involvement is present. Whether, for instance, an ocular system abnormality is present and thus 'yes' is selected, the flag '*compile the ocular system form'* will appear**.**

Behavioral assessment (*repeatable*)

This form is aimed at describing behavioral features of the patient. It is divided into 2 section:

- *Clinical assessment*: It allows to characterized clinical observations.
- *Neurodevelopmental disorders (according to DSM 5)*: It allows to report a diagnosis of neurodevelopment disorders according to the DSM 5.

Movement disorder (*repeatable)*

Whether a movement disorder has been diagnosed, this form is where details regarding its onset, features, exc according to the most recent international classification in use can be specified.
Whether a specific treatment has been implemented, the relative information can be inserted in the form 'therapy'.

Epilepsy (*repeatable)*

Whether a diagnosis of epilepsy is present, information regarding epilepsy onset and seizure features at onset 'epilepsy history' (Fig 14), and epileptological diagnosis according to the most recent ILAE classification (Scheffer 2017) and features at follow up 'epilepsy follow-up' (Fig 15) are to be inserted in this form. Whether more than one seizure type is present, according to the prevalence of the specific seizure type, primary seizure type and secondary seizure type can be inserted.
Information regarding EEG features and pharmacological and non-pharmacological interventions have to be inserted respectively in the 'electrophysiology' and 'therapy' forms.

2.4.3 Extra-neurological data

Auxological parameters and growth (*repeatable)*

In this form auxological parameters (weight, height , cranial circumference and Body Mass Index) for each follow up can be registered, alongside the presence of a disproportionate growth.

Head and neck (*repeatable)*

This form is where the compiler can notify the presence of any skull, facial and neck abnormalities or dysmorphism.

Limbs (*repeatable)*

This form is where the compiler can notify the presence of any upper and lower limbs abnormalities, including bones.

Axial (*repeatable)*

In this form any feature and dysmorphism or malformation regarding the axial body (thorax shape and bones and vertebral column).

Skin (*repeatable)*

In this form information regarding skin and hair abnormalities can be inserted (for instance presence of eczema or nevus exc).

Auditory system (*repeatable)*

In this form morphological and structural abnormalities of the middle and inner ear, alongsidehearing impairment can be detailed. Moreover, the result of specific audiometry test can be reported and being associated with execution date.

Ocular system (*repeatable)*

In this form anterior and posterior segment abnormalities and optic nerve abnormalities can be inserted. Moreover, results of instrumental organ specific tests such as visual acuity test, visual field test and Optical Coherent Tomography (OCT) can be placed.

Endocrine system (*repeatable)*

In this form endocrine system as well as homeostasis and metabolism information can be inserted. Whether data regarding biochemistry values (e.g. investigation of thyroid function though blood exam) have to be included, as reminded by the red flag, there is the specific form 'biochemistry'.

Cardiovascular and respiratory system (*repeatable)*

This form explores the cardiac system, the vascular system and the respiratory system in terms of malformations and other organ specific pathologies.

Gastrointestinal system (*repeatable)*

In this form any Gastrointestinal tract abnormality or pathology can be inserted.

Genitourinary system (*repeatable)*

In this form kidney and genital tract abnormalities or pathology can be inserted.

2.4.4 Instrumental data

Imaging (*repeatable)*

The main part of the form regards brain imaging abnormalities, which can be found according to localization: supratentorial, infratentorial, vascular and extra-parenchymal.

Whether two abnormalities regard the same main cerebral structure, at the end of the vertical compilation, a blue writing e.g. ' Check here if other cerebral cortex abnormalities are present' will indicate how to and where to put further information.

Example of specification of supratentorial abnormalities

Specify supratentorial abnormalities	☐ Basal ganglia, thalami and capsule ☑ Cerebral cortex ☐ Commissures ☐ CSF spaces ☐ Supratentorial ventricles ☐ White matter
Specify cerebral cortex abnormalities	☐ Atrophy ☑ Malformation of cortical development ☐ Oloprosencephaly ☐ Syntelencephaly ☐ Tumor or tumor-like ☐ Other
Specify Malformation of Cortical Development (MCD)	☐ Band heterotopia, double cortex ☐ Dysgiria, abnormal sulcation ☑ Focal cortical dysplasia ☐ Hemimegalencephaly spectrum ☐ Nodular heterotopia(s) ☐ Pachigyria, lyssencephaly ☐ Polymicrogyria ☐ Schizencephaly ☐ Other
Specify cerebral cortex localization for Malformation of cortical development	☐ Frontal ☐ Insula ☑ Occipital ☐ Parietal ☐ Temporal ☐ NA
Specify cerebral cortex pattern abnormalities	☐ Bilateral - asymmetric ☐ Bilateral - symmetric ☐ Diffuse ☐ Multiple lesions ☐ Single lesion

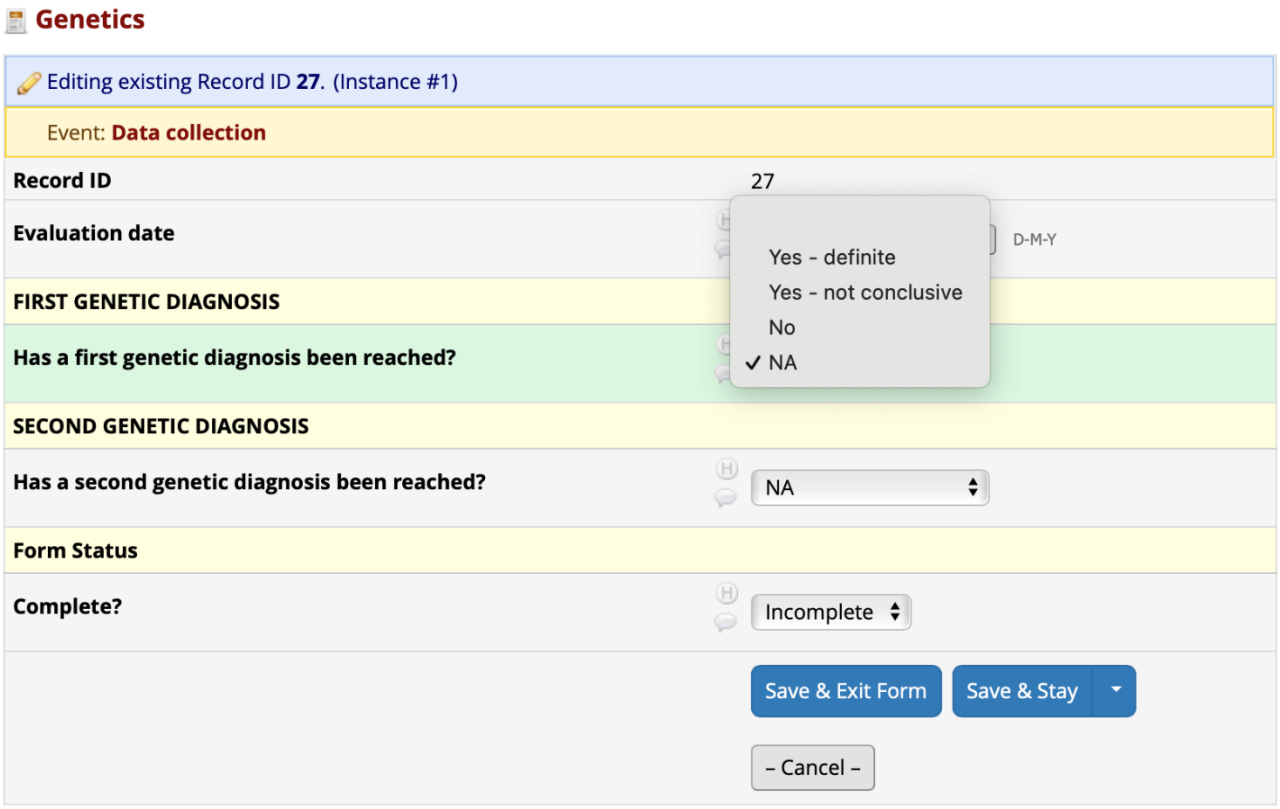
In the 'muscle' section related information regarding the imaging exam can be inserted.

Histopathology (*repeatable)*

In this form it is possible to report whether skeletal muscle/ peripheral nerve/ blood lymphocytes / bone marrow / liver / brain tissue have been analyzed, together with the methodology used and the histopathological result.

Genetics (*repeatable)*

This form allows to collect all genetic test results. It is structured into two main sections: *First genetic diagnosis* and *Second genetic diagnosis*.



For each diagnosis reached, it is possible to insert all the needed information to delineate up to three variants. Whether a single gene variant is present, in the field 'gene' the compiler can insert the gene name, searchable within a specific database.

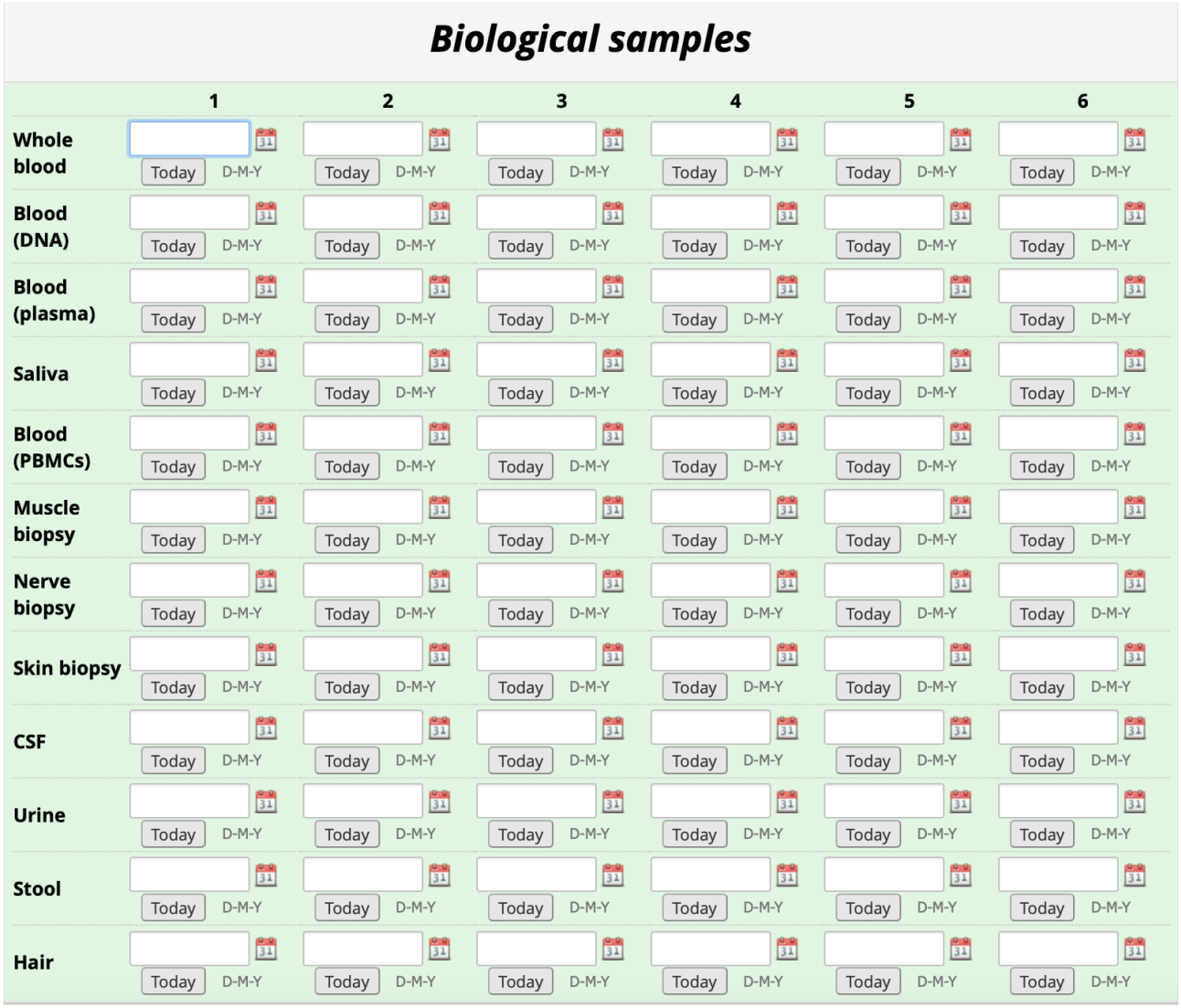
In order to harmonize data registration between different institute and to ensure that the data will be searchable, the compiler must follow the example in the transcription of the gene transcript as well as of the nomenclature at gDNA level, nomenclature at cDNA level and nomenclature at protein level.

Electrophysiology (*repeatable)*

In this form information regarding electrophysiological exams including electroencephalogram (EEG), electromyography (EMG), electroneurography (ENG), visual evoked potentials (VEP), somatosensory evoked potentials (SEP), brain auditory evoked potentials (BAEP) and electroretinography (ERG) are included. Compilation of the form should be done starting from the date of examination

Samples and biomarkers (Updatable**)**

Biological samples



In this form it is possible to trace which biological sample is stored and when has been collected.

Scales (Repeatable)

In this form quantitative results of specific standardized developmental or intelligence scales (e.g. Griffiths' developmental scale, Wechsler Intelligence Scale, exc) can be inserted.

Each domain i.e. neurodevelopmental profile (Figure 32a) and motor function (Figure 32b) will retain different scales according to age.

Testing (Updatable)

In this form it is possible to specify genetic, metabolic, imaging and electrophysiology exams performed, date of execution and the qualitative result (e.g. pathological vs non normal). This form should represent, beyond other, an efficient method to sum up the diagnostic workup of the single patient.

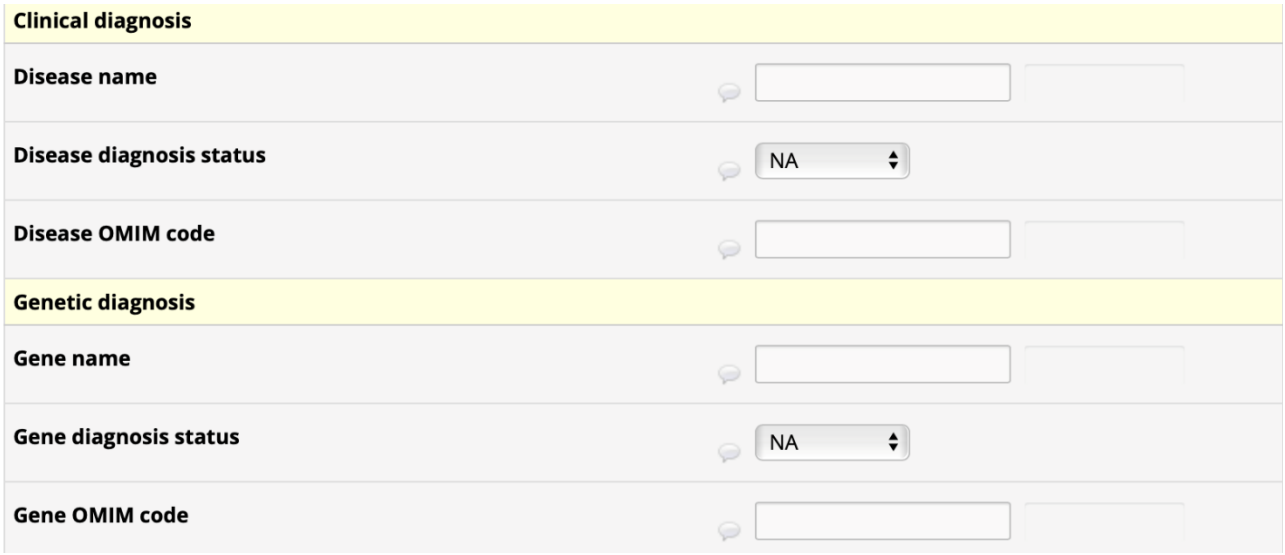
2.4.5 Diagnosis and therapy

Diagnosis (Updatable)

This form has been conceived to get a diagnostic summary. It is divided into 3 sections:

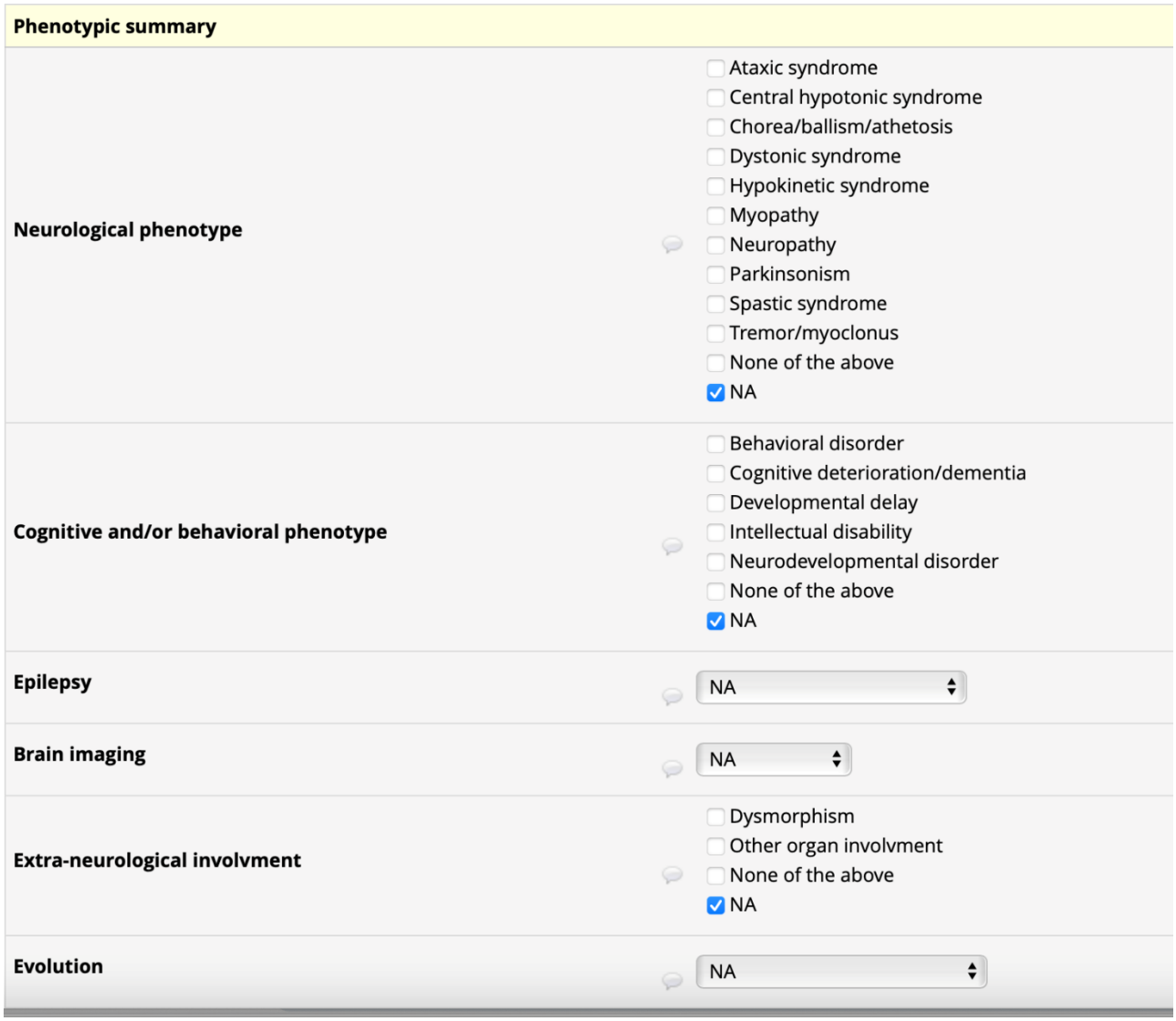
- *Clinical diagnosis:* The compiler can herein insert the disease name and disease OMIM code exploiting the presence of XX database (clinical diagnosis)
- *Genetic diagnosis:* The compiler can herein insert the gene name and OMIM code (genetic diagnosis).

Clinical and genetic diagnosis summary



- *Phenotypic summary:* The compiler can herein trace achieving a synthesis of the neurological, cognitive and behavioral phenotype, specifying the presence of epilepsy, brain imaging abnormality, extra-neurological involvement and disease evolution. This form **should not** be compiled in substitution of the other main neurologic forms.

Phenotypic summary



Medications and therapies (Updatable)

This form allows the registration of all medication (pharmacological and non-pharmacological, alongside experimental once) and therapies interventions, ongoing and suspended. For each medication and therapy selected, it is possible to specify duration, efficacy and side effects.

Biochemistry (Repeatable)

In this form quantitative values of specific blood, urine and cerebrospinal fluid examination can be inserted and associated to the relative data of execution. For each exam the qualitative range estimation, specific value, unit of measurement and normal range according to the referral lab can be added.

Metabolic (Repeatable)

In this form all metabolic investigations performed can be added, the biological sample utilized (blood/urine/cerebrospinal fluid) can be specified and then the quantitative information regarding a specific test abnormality can be fulfilled.

2.5 PROMs and PREMs inclusion in RENDER

In the context of rare pediatric neurological diseases, active involvement of people living with rare diseases and its representatives in guideline development and clinical studies is essential to achieve high-quality, as recognized by international best practice methodologies such Guidelines International Network (GIN).

Rare pediatric neurological diseases often present with complex clinical features and unmet needs that are difficult to quantify using traditional methods and medical assessments alone. PROMs and patient reported experience measurement (PREMs) provide a more holistic understanding of how these diseases affect patients and their families, offering insight into physical, emotional, and social well-being from the perspective of those who are most directly impacted.

PROMs in Rare Pediatric Neurological Diseases

Patient-reported outcome measures are instruments used to capture a patient's self-reported health status. For children with rare neurological diseases, PROMs are often adapted to suit the cognitive and developmental abilities of younger patients, when feasible. These measures can focus on various domains, including physical function, pain, mobility, cognitive abilities, and emotional well-being. PROMs are considered essential for understanding the day-to-day challenges in the routine life and in the system where the subject lives.

One of the significant challenges in using PROMs for pediatric neurological diseases is the need to ensure that the tools are appropriate and accurate according to specific and divergent phenotypes and pediatric age. In many cases, PROMs are supplemented by reports from parents or caregivers, who can provide valuable information about the child's health status and quality of life. PROMs can also be used to monitor disease progression and the effectiveness of interventions over time.

PREMs in Rare Pediatric Neurological Diseases

Parent-reported outcome measures (PREMs) are similar to PROMs but focus on the experiences and perceptions of the parents or caregivers of children with rare neurological diseases or subjects when adolescents and when an intellectual disability is not present. Since children with rare neurological diseases often may not have the cognitive abilities to report their symptoms or perceptions, PREMs provide a crucial perspective on how the disease impacts not only the child but also the family unit as a whole.

PREMs can assess a variety of factors, such as the emotional burden on parents, the challenges they face in accessing healthcare and support services, the consideration and decisional ability perceived in the care pathway, the financial impact of treatment, and their overall satisfaction with the care their child is receiving. These measures are invaluable in understanding the broader impact of rare diseases on families and can provide healthcare providers with insights into areas where additional support or resources may be needed.

In the case of rare neurological diseases, the role of the family is especially significant. PREMs allow healthcare providers and researchers to evaluate how patients and caregivers are coping with these challenges, identifying gaps in support services, and tailoring interventions that address both the child’s and the family's needs.

The Role of PROMs and PREMs in Research and Clinical Trials

Both PROMs and PREMs play an essential role in clinical trials and research in rare pediatric neurological diseases. These outcome measures can provide data on the impact of new treatments or interventions from the patient and family perspective, which is crucial for understanding the real-world effects of therapies. Moreover, under treatment supposed to change the natural disease history for conditions like spinal muscular atrophy, PROMs can help assess improvements or declines in functional abilities and new domains previously not covered, while PREMs can highlight how parents perceive the benefits and general effects of treatments. Moreover, since rare pediatric neurological diseases are often under-researched due to their low prevalence, integrating PROMs and PREMs into clinical studies helps fill important gaps in knowledge about how these diseases affect children's daily lives and their families. The insights gained from these measures can also drive policy changes and improve healthcare delivery, ensuring that treatments not only focus on core disease outcomes but also prioritize the quality of life of affected children and their families.

Challenges and Future Directions

Despite the value of PROMs and PREMs in rare pediatric neurological diseases, there are several challenges that need to be addressed to maximize their effectiveness. One major issue is the lack of standardized, validated tools for specific diseases. Moreover, in the new era of increasing availability of treatments that can change the disease natural history, considerations should be made taking into account continuously updatable disease trajectories and thus needs. While some PROMs and PREMs have been developed for different specific conditions like Duchenne muscular dystrophy, many rare diseases do not yet have disease-specific outcome measures. Developing and validating these tools will be critical for ensuring that outcome assessments accurately reflect the experiences of children with these conditions. Another challenge is ensuring the accessibility and usability of these measures for diverse patient populations, including those from different cultural, linguistic, and socioeconomic backgrounds. It is essential to ensure that PROMs and PREMs are not only available in multiple languages but also culturally sensitive and appropriate for various healthcare settings (Schoenmakers, 2024).

Integrating PROMs and PREMs assessment in the clinical practice should be a future direction both for clinical and research implications. In this direction, digital health technologies, such as mobile applications and wearables, into PROM and PREM assessments may enhance the accuracy and real-time monitoring of patients' symptoms and reports. These technologies could allow for continuous data collection, providing a more detailed and dynamic picture of how rare neurological diseases progress and how patients and families are managing them.

2.5.1 Methods:

The primary objective of this appendix study was the implementation of PROMs and PREMs in the assessment in rare neuropediatric diseases among the RENDER functions, believing that this effort would have conferred to RENDER an innovative appeal, in line with the actual interest in research approaches that are also patient-centered.

Secondary Objectives of the study will be the following:

1. To evaluate symptom burden and disease-specific challenges:
    - Measure of key symptoms and physical, emotional, and social functioning and quality of life
2. To explore the experiences of caregivers in managing neuropediatric rare diseases:
    - Assess caregiver stress, emotional burden, and quality of life through caregiver-specific PROMs and PREMs.
3. To capture children’s and caregivers’ experiences with healthcare services:
    - Evaluate expectations and satisfaction during the follow, with communication, care coordination, access to specialists, and support services using pediatric-focused PREMs.
4. To identify barriers and facilitators to care engagement:
    - Explore factors influencing the involvement of children and caregivers in healthcare decisions and adherence to therapeutic plans.

A review of the literature has been performed to identify an appropriate choice of standardized questionnaires available in Italian language to describe different Patient-Centered Outcomes (PCOs), PROMs and PREMs, considering the heterogeneity of pediatric neurological diseases that will be present in RENDER, the age of included subjects and the different degrees and typology of disabilities faced. Due to patient's age and functioning variability, different packages will be available according to the subject features and if the caregiver's form will always be available, the patient's form will be compilable only in specific occurrence (teenage and normal cognitive level).

The main domaines of PROMs and PREMs assessment will be the following:
-Pain
-Sleep
-Diet habits
-Quality of life
-Peer, family and social relationships
-Emotions and behavior
-Health engagement

As a first pilot project, the caregiver profiling was chosen to develop a specif dedicated form to be implemented in RENDER.

Cognitive validation of caregiver profiling 'package'

As a pioneering tool, the survey meant to achieve the caregiver profiling, whatever the proband age and diagnosis, was designed to be experimentally implemented in RENDER by undersigned, RENDER data manager and an academic psychologist with a relevant experience on PROMs and PREMs. A print version of the survey was offered to three couples of caregivers to assess its comprehensibility and compilation feasibility.

Validation by clinicians

Four clinicians with well recognized expertise on rare neuropediatric diseases research (2 belonging to RENDER project and 2 external of RENDER organization) revised the tool and provided suggestions and modifications that were subsequently welcomed with the design of a final version.

2.5.2 Results

The caregiver profiling form is now contained in the 'caregiver survey' form that can be compiled only from the caregiver through via a device connected to the platform offered by the clinician.

The survey is organized in 9 main sections:

1.The socio-demographic features

2. The care pahtway

2. Il percorso di cura

In questa sezione raccoglieremo la sua opinione sulla sua esperienza in merito al percorso di cura di suo/a figlio/a. Troverà domande a scelta multipla, per le quali sarà possibile selezionare più opzioni.

2.1 Quali sono le figure presenti nel percorso di cura di suo figlio/a?	☐ Pediatra curante ☐ Pediatra ospedaliero ☐ Neuropsichiatra ospedaliero ☐ Neuropsichiatra privato ☐ Neuropsichiatra presso l'ASST/ASL di riferimento ☐ Neuropsichiatra presso il Centro Riabilitativo ☐ Psicoterapeuta/Psicologo ☐ Ortopedico ☐ Cardiologo ☐ Endocrinologo ☐ Neuropsicomotricista ☐ Logopedista ☐ Fisioterapista ☐ Assistente sociale ☐ Altro
2.2 Pensando all'ultimo anno, con che frequenza ha incontrato il medico NPI del centro riabilitativo/della NPI territoriale che ha in carico suo figlio?	○ Circa una volta al mese ○ Circa una volta ogni tre mesi ○ Circa due volte all'anno ○ Una volta all'anno ○ Meno di una volta all'anno reset
2.3 Pensando all'ultimo anno, con che frequenza ha incontrato il medico NPI presso l'ospedale specializzato di riferimento (es. Mondino)?	○ Circa una volta al mese ○ Circa una volta ogni tre mesi ○ Circa due volte all'anno ○ Una volta all'anno ○ Meno di una volta all'anno reset

3. The participation to the care pathway

3. Partecipazione al percorso di cura

Qui di seguito troverà alcune affermazioni che descrivono possibili esperienze dei familiari durante il processo di cura del/della proprio/a figlio/a. Le chiediamo di leggere ciascuna affermazione e di esprimere il suo grado di accordo con ciascuna di esse su una scala da 0 a 6, dove 0= totalmente in disaccordo; 1= quasi completamente in disaccordo; 2= parzialmente in disaccordo; 3= né d'accordo né in disaccordo; 4= parzialmente d'accordo; 5= quasi completamente in accordo; 6=completamente d'accordo.

3.1a Partecipazione alle decisioni inerenti la cura di mio figlio/a

Mi sento coinvolto/a nelle decisioni riguardanti la cura di mio/a figlio/a

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6

reset

3.1b Partecipazione alle decisioni inerenti la cura di mio figlio/a

Posso esprimere la mia opinione quando si discutono le opzioni di trattamento di mio/a figlio/a

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6

reset

3.1c Partecipazione alle decisioni inerenti la cura di mio figlio/a

Le mie opinioni e suggerimenti sul percorso di cura e assistenza di mio/a figlio/a vengono presi in considerazione dai professionisti sanitari

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6

reset

3.1d Partecipazione alle decisioni inerenti la cura di mio figlio/a

Mi viene richiesto di partecipare attivamente nella scelta delle terapie o interventi per mio/a figlio/a

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6

reset

3.2a Comunicazione con i professionisti sanitari

I medici e gli operatori sanitari mi tengono informato/a sulle condizioni di salute di mio/a figlio/a

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6

reset

4.The services utilization

4. Uso dei Servizi

In questa sezione vogliamo approfondire le eventuali difficoltà che può incontrare nell'accesso ai Servizi di cura. Troverà una serie di domande a scelta multipla pensate per esplorare le possibili limitazioni o ostacoli che potrebbe affrontare, come aspetti logistici, economici, organizzativi o personali. La invitiamo a selezionare una sola risposta per ciascuna domanda, scegliendo quella che meglio rappresenta la sua esperienza.

4.1a Quanto frequentemente riscontra difficoltà legate ai seguenti aspetti?

Distanza dai centri specializzati

○ Mai ○ Raramente ○ Qualche volta ○ Spesso ○ Sempre

reset

4.1b Quanto frequentemente riscontra difficoltà legate ai seguenti aspetti?

Tempi di attesa troppo lunghi

○ Mai ○ Raramente ○ Qualche volta ○ Spesso ○ Sempre

reset

4.1c Quanto frequentemente riscontra difficoltà legate ai seguenti aspetti?

Costi finanziari eccessivi

○ Mai ○ Raramente ○ Qualche volta ○ Spesso ○ Sempre

reset

4.1d Quanto frequentemente riscontra difficoltà legate ai seguenti aspetti?

Mancanza di informazioni sui servizi disponibili

○ Mai ○ Raramente ○ Qualche volta ○ Spesso ○ Sempre

reset

4.1e Quanto frequentemente riscontra difficoltà legate ai seguenti aspetti?

Orari incompatibili con le esigenze familiari

○ Mai ○ Raramente ○ Qualche volta ○ Spesso ○ Sempre

reset

5. Economic and management impact of the care pathway

5. Impatto economico e gestionale del percorso di cura

Questa sezione è dedicata a comprendere come la patologia di suo/a figlio/a influisca sulle sue attività quotidiane, sia dal punto di vista economico che organizzativo. Troverà una serie di domande a scelta multipla, per le quali è possibile selezionare una sola risposta. La invitiamo a rispondere pensando alla sua esperienza personale, così da aiutarci a comprendere meglio le implicazioni che questa situazione può avere nella gestione della vita di tutti i giorni.

5.1 Quanto il suo ruolo di caregiver ha influenzato la sua capacità di lavorare a tempo pieno o mantenere il suo lavoro?

- Per niente
- Solo in minima parte
- In parte
- In gran parte
- Completamente

reset

5.2 Quanto il suo ruolo di caregiver ha causato una riduzione del reddito familiare?

- Per niente
- Solo in minima parte
- In parte
- In gran parte
- Completamente

reset

5.3 Quanto frequentemente deve prendere permessi o ferie dal lavoro per assistere suo/a figlio/a? (solo se non è casalingo/disoccupato/pensionato)

- Mai
- Raramente
- Qualche volta
- Spesso
- Sempre

reset

5.4 Quanto il suo ruolo di caregiver la limita nel dedicare tempo ad attività personali? (Es. hobby, sport, socialità)

- Per niente
- Solo in minima parte
- In parte
- In gran parte
- Completamente

reset

5.5 Quanto il tempo richiesto per organizzare visite mediche, terapie o altre attività legate alla condizione di suo/a figlio/a influisce sulla gestione delle sue giornate?

- Per niente
- Solo in minima parte
- In parte
- In gran parte

6.Social network

6. Rete sociale

In questa sezione troverà alcune domande a scelta multipla, per cui è possibile selezionare una sola risposta, volte a descrivere il supporto per lei disponibile nella vita quotidiana.

6.1 Quali delle seguenti persone sono parte della sua rete di supporto sociale ? (Selezioni tutte le opzioni rilevanti)

- [ ] Partner o coniuge
- [ ] Familiari (es. genitori, fratelli, figli adulti)
- [ ] Amici
- [ ] Colleghi di lavoro
- [ ] Vicini di casa
- [ ] Membri di gruppi o associazioni (es. supporto caregiver)
- [ ] Operatori sanitari o sociali

6.2 Su quante persone fa affidamento regolarmente per il suo benessere personale? (Es. amici, familiari, vicini di casa)

- ( ) Nessuno
- ( ) 1 - 2 persone
- ( ) 3 - 5 persone
- ( ) Più di 5 persone

reset

6.3 Quante persone sente di poter contattare per ricevere supporto emotivo? (Es. parlare dei suoi sentimenti, preoccupazioni)

- ( ) Nessuno
- ( ) 1 - 2 persone
- ( ) 3 - 5 persone
- ( ) Più di 5 persone

reset

6.4 Quanto si sente supportato/a emotivamente dalla sua rete sociale?

- ( ) Per niente
- ( ) Solo in minima parte
- ( ) In parte
- ( ) In gran parte
- ( ) Completamente

reset

6.5 Quante persone sente di poter contattare per ricevere supporto pratico? (Es. aiuto con le attività quotidiane)

- ( ) Nessuno
- ( ) 1 - 2 persone
- ( ) 3 - 5 persone
- ( ) Più di 5 persone

reset

7.Lifestyle

7. Stile di vita

Lo stile di vita può influire sulla capacità di gestire situazioni stressanti. Di seguito troverà alcune domande a scelta multipla per esplorare il suo stile di vita. La invitiamo a leggerle con attenzione e a scegliere una sola risposta per ciascuna domanda.

7.1 Con che frequenza effettua attività fisica? (Es. attività all'aperto, sport)	○ Mai ○ Due volte al mese ○ Settimanalmente ○ Quotidianamente reset
7.2 Quante ore al giorno trascorre utilizzando dispositivi elettronici al di fuori dell'attività lavorativa? (Es. tablet, smartphone, computer)	○ Meno di 1 ora ○ 1 - 2 ore ○ 2 - 4 ore ○ Più di 4 ore reset
7.3 Partecipa regolarmente ad attività ricreative? (Es. hobbies, corsi artistici, attività di aggregazione)	○ Si almeno una volta alla settimana ○ Si occasionalmente ○ No reset

8.Psychological wellbeing

8. Benessere psicologico

Con il termine "Benessere psicologico" ci si riferisce alla capacità di un individuo di gestire situazioni stressanti, mantenere relazioni sane e perseguire obiettivi significativi. In questa sezione, troverà alcune domande a scelta multipla che voglio indagare la qualità del suo benessere psicologico. Le chiediamo di leggere ogni domanda con attenzione e di esprimere un'unica preferenza.

8.1 Si sente agitato e teso?	○ Quasi sempre ○ Spesso ○ Ogni tanto - a volte ○ Mai reset
8.2 Le cose che le piacevano una volta le piacciono ancora?	○ Come prima ○ Meno di prima ○ Molto poco ○ Quasi per nulla reset
8.3 Le capita di avvertire come un senso di paura, come se stesse accadendo qualcosa di terribile?	○ Si molto forte ○ A volte ma in modo non troppo oppressivo ○ Di rado ○ Mai reset
8.4 Riesce a ridere e ad accorgersi del lato comico delle cose?	○ Come ho sempre fatto ○ Un po' meno di prima ○ Indubbiamente molto meno di prima ○ No affatto reset
8.5 Le capita che le passino per la mente dei pensieri preoccupanti?	○ Molto spesso ○ Spesso ○ A volte - non troppo spesso ○ Solo ogni tanto reset
	○ Mai

9.Sleep quality

9. Qualità del sonno

La qualità del proprio riposo ha importanti riflessi anche sulla vita diurna. Dormire poco e male influenza negativamente l'andamento lavorativo, i rapporti sociali e la sfera psicologica dell'individuo, portando inevitabilmente con sè un peggioramento della qualità della vita. In questa sezione troverà alcune domande volte a descrivere la qualità del suo sonno. Le chiediamo di leggerle con attenzione e di selezionare un'unica risposta per ogni domanda.

9.1 Durante il mese passato, a che ora è andato a letto di solito? Now H:M

9.2 Durante il mese passato, quanto tempo in minuti le è occorso in generale per prendere sonno?

9.3 Durante il mese passato, a che ora si è alzato di solito al mattino? Now H:M

9.4 Durante il mese passato, quante ore di sonno effettivo ha dormito la notte? (Questo numero può essere diverso dal numero di ore trascorse a letto)

9.5 Durante il mese passato, con quale frequenza ha avuto difficoltà di sonno poichè si svegliava nel mezzo della notte o presto al mattino?

- Mai durante il mese passato
- Meno di una volta alla settimana
- Una o due volte alla settimana
- Tre o più volte alla settimana

reset

9.6 Come giudica globalmente la qualità del suo sonno durante il mese scorso?

- Molto buona
- Abbastanza buona
- Abbastanza cattiva
- Molto cattiva

reset

Form Status

It is currently in progress the administration of this survey and data collection with a multipurpose: establishing whether the present form is adequate to track PREMs and caregiver mental health profiling, and search for domains related to care engagement perception, lifestyle and social network that can be relevant for the caregiver of patients with rare disease wellbeing.

2.6 RENDER governance and financing

Governance

Access to RENDER is center-specific. Each center remains controller of its own patients' data and each center is able to see only data of its own patients. Pseudonymization creates a univocal code for each patient, through an algorithm to encrypt social security number allowing to check for duplicates. The ideal path to utilize RENDER includes the approval from local Ethics Committee and the subscription of an agreement with Mariani Foundation. The responsible for data treatment is CBIM (external data host).Through an informed consent patients or legal representative authorize loading of pseudonymized data onto the platform, analysis of data for research purposes and sharing of data with other centers based on additional agreements among centers.
RENDER will be likely entrusted to the Mariani Foundation, which is committed to establishing an ad hoc Scientific Committee for RENDER management, to be composed of the founders of RENDER, that is, those who made its creation possible.

Financing

It is envisaged that the ordinary management and maintenance of the RENDER platform (through the agreements with the body hosting the platform and with the group of bioengineers guaranteeing its proper functioning and implementation) will have fixed costs.
The Scientific Committee will be able to contribute with its own research funds to the implementation of RENDER (e.g. the development of additional functionalities such as the link to a space for neuroimaging) but will not bear the costs of ordinary operation and maintenance.

2.7 RENDER promotion and diffusion

Several initiatives to raise awareness about RENDER have been implemented, from a dedicated Mariani's course entitled 'Corso teorico-pratico di aggiornamento sulle malattie neuropediatriche rare' meant for younger child neurospychiatrists and geneticists, that took place in Pavia on October 2024, to several interventions at scientific congresses and patients' associations initiatives meant to promote RENDER as a potential tool to be embraced in light of unmet needs in the current research field of rare neurological diseases.

RENDER platform will hopefully represent a 'ready to use tool' at the disposal of researchers and Centres dealing with rare diseases, with a specific regulation policy. To help collect and share data to improve diagnosis, treatment, and research related to complex and often overlooked conditions, RENDER should become widespread and well-integrated in the research practice. The goal is to make the registry a central resource for researchers, healthcare professionals, patients, and families, fostering collaboration among all involved parties. Until now academic and clinical researchers, patient advocacy organizations, policymakers, and health agencies concerned with rare diseases and pediatric health have been involved to share RENDER philosophy.

RENDER PIs are preparing training materials to assist researchers in its utilization and align compilation, including a dedicated detailed manual and a video-tutorial.

For patients and families, the message will focus on security, privacy, and the importance of participating in the registry to facilitate access to innovative treatments and contribute to research.

Among patients' association the PIs already had contacts with patients' associations interested in the tool: COL4A1/A2 association, Kleefstra syndrome association, UNIAMO.

Thanks to the recent funding obtained through the PNRR in collaboration with a group of researchers from TIGEM / University Federico II of Naples, the development of new capabilities for RENDER is planned. In particular, the related PNRR project will focus on optimizing data sharing using cutting-edge technologies, developing a biorepository linked to the platform, implementing innovative computational pipelines for querying and extracting data.

Among the planned novelties, there is the development of a dedicated website to gain access to RENDER, to share ongoing projects and novel research calls involving RENDER utilization and to keep track of the researchers, Institutions and patients' association entering the RENDER consortium. By sharing data about RENDER diffusion and implementation and the population numbers included in RENDER, the list of pathologies and genetic diagnoses populating the platform, together with the scientific publications, RENDER users and families might perceive a higher degree of engagement becoming more participating and compliant in taking part to research.

2.8 RENDER philosophy and future perspectives

At least in Italy, most studies on rare diseases are still based on local databases, which require great effort to compile and maintain, being difficult to consult and share, and often not GDPR compliant. This represents an enormous limitation for scientific research especially for rare and ultra-rare genetic diseases, for the understanding of which the extensive knowledge of every single patient is precious. RENDER project stands from the ambitious goal to implement a single and thus economic registry dedicated to rare and ultra-rare neuropediatric diseases -excluding those already covered by specific active registries- to collect in a systematic and harmonic way clinical, genetic and instrumental data of patients. The platform has thus been designed to be advantageous since equal, with same access rules for every participant, and economical, because being singular it definitely carries lower development and maintenance costs. Importantly, RENDER is flexible and ready to welcome undiagnosed patients and complex clinical cases as it was realized to be able to embrace heterogenous and deep phenotyping. Patients' associations and representatives will be furtherly actively involved in the validation process and the design of future studies, in the effort to promote research which will also be patient-centered outcome. RENDER should become as widespread as possible, and easily accessible, in respect of its regimentation, by patients' associations that are orphan of a disease registry, and by researchers willing to participate to competitive calls. A specific thought should be devoted on how to practically make RENDER well-known in the field, and easily accessible both by researchers and patient's' representatives, taking advantage of scientific societies and organisations.

III. Deep phenotyping of cerebral malformations

Brain development, whether typically or following an insult, is determined through a dynamic interplay between plastic and homeostatic processes, as well as environmental influences (Piaget, 1954; Johnson, 1997). In the context of ongoing neural maturation, the mechanisms that drive developmental change play a crucial role in both the heterogeneity of phenotypic outcomes and the increased vulnerability of the immature brain to adverse consequences. Thus, the effects of any insult to the developing brain must be understood not only from isolated time points but as part of the brain's evolutionary trajectory, considering its continuous and progressive development.

In this context, the outcomes of congenital brain malformations—whose manifestations vary greatly depending on the type, extent, and severity of the malformation, as well as the genetic pathways involved—are influenced by several factors. Among these, the remarkable plasticity of the brain's connectome during development stands out as a key contributor. For instance, in the case of callosal dysgenesis, the abnormal brain circuitry is driven by long-distance plasticity mechanisms that reroute growing axons, establishing alternative neural pathways. These pathways offer partial compensatory effects, ensuring some degree of interhemispheric communication despite the malformation. Recent research has demonstrated that such conditions may lead to extensive rewiring of the brain, with new circuits emerging to enable functional compensatory interhemispheric integration (Tovar-Moll et al., 2014). This example highlights the profound adaptive potential of the developing brain, despite its structural anomalies.

The genetic etiology of brain malformations, coupled with mechanisms of functional compensation, may partly explain the variability seen in developmental trajectories. Advances in genetic research could provide deeper insights into how specific genetic variants contribute to the diverse outcomes observed in individuals with these conditions. Understanding these mechanisms will be essential for identifying phenotype-genotype correlations, which can, in turn, guide clinical decision-making and intervention strategies.

Frequently, studies on the neuropsychological functioning of individuals with specific malformations rely on caregiver questionnaires, which may not provide a complete or accurate representation of the patient's abilities (Summers et al., 2017). Even when more comprehensive reports are available, the diversity of conditions and individual factors makes it difficult to identify clear and consistent phenotypes (Siffredi et al., 2018).

Ultimately, a more effective understanding of the genetic underpinnings, structural features, extensive behavioral and neuropsychological outcome description of brain malformations can help clinicians provide more accurate prognoses and devise personalized treatment and rehabilitation strategies. Achieving these goals will require concerted efforts in data sharing, multicenter collaborations, and the establishment of a systematic, patient-centered approach.

In this second part of my PhD thesis, I will focus on specific brain malformations of the midline and of the posterior cranial fossa, that I had the chance of studying in depth during my clinical work. In particular, I focused on septo-optic dysplasia etiopathogenesis, on sleep features of subjects with midline malformation (septo-optic dysplasia and corpus callosum agenesis), cerebellar heterotopia and the fetal finding of vermian malrotation as a risk for postnatal brain malformations.

3.1 SOD: a heterogeneous condition

Septo-optic dysplasia (SOD), also known as de Morsier syndrome, is a congenital disorder belonging to the mid-line brain malformations group (De Morsier, 1956). Starting from a clinical case description where septo-optic dysplasia was associated for the first time to SON gene, considering its clinical heterogeneity and debated etiology, SOD was deemed to represent a good model to search for new biochemical and etiopathological pathways at the basis of the variability of phenotypes. Moreover, the results of the literature review were retained to be used to retrospectively and prospectively approach genetic analysis of out cohort of patients with SOD.

The study of SOD has resulted in the following three publications:
-Pasca L, Politano D, Morelli F, Garau J, Signorini S, Valente EM, Borgatti R, Romaniello R. Biological pathways leading to septo-optic dysplasia: a review. Orphanet J Rare Dis. 2025 Apr 3;20(1):157. doi: 10.1186/s13023-025-03541-6.

- Pasca L, Politano D, Cavallini A, Panzeri E, Vigone MC, Baldoli C, Abbate M, Kullmann G, Marelli S, Pozzobon G, Vincenzi G, Nacinovich R, Bassi MT, Romaniello R. A Novel De novo Heterozygous Mutation in the SON Gene Associated with Septo-optic Dysplasia: A New Phenotype. Neuropediatrics. 2024 Jun;55(3):191-195. doi: 10.1055/a-2114-4387.

Prevalence of SOD has been estimated to be 1 in 10.000 live births (Webb, 2010). Traditionally, SOD has been characterized by the association of a classic clinical-neuroradiological triad consisting of midline brain defects, hypoplasia of the optic nerves and/or chiasm, and hypothalamic-pituitary axis dysfunction (Ganau,2019). Currently, at least two out of three of such findings are required for a clinical diagnosis of SOD (Signorini, 2011). If only one element of the triad is documented, it should be referred to as a distinct entity.
The typical midline malformation is represented by the absence or disruption of septum pellucidum, a thin transparent membrane located in the brain between the body and anterior horns of the lateral ventricles (Sarwar, 1989). Other midline brain abnormalities described in SOD include: thinning or agenesis of corpus callosum and structural abnormalities of the hypothalamic-pituitary (HP) axis, namely hypoplasia of the pituitary infundibulum and/or gland, and ectopic location of the posterior pituitary (Ganau, 2019). When a malformation of cortical development (MCD) is associated to SOD, the term “SOD-plus” should be adopted, while the term “SOD-spectrum” should be used in cases presenting with wider range of congenital anomalies, with an increasing gradient of severity.

Septo-optic dysplasia (SOD) typically has a low rate of genetic diagnosis, however recent data on animal models and clinical reports show interesting insights into alterations in new and sometimes overlapping pathways. Not surprisingly, SOD phenotype might derive from alterations in transcriptional pathways that intersect during brain development.

SOD etiology

A combination of genetic predisposition and prenatal exposure to environmental factors is believed to come into play as the etiology of SOD (Ganau, 2019). Being SOD such a heterogeneous condition, disruption of many developmental steps from early patterning to neuronal specification and guidance of commissural axons might come into play. Indeed, involved structures in SOD do have different embryonic origin: pituitary gland, hypothalamus, optic nerves, and forebrain all develop from the anterior neural plate, with the neurulation process starting at the third week of gestation. Pituitary gland and optic nerves originate around the 4^{th}-7^{th} week of gestation, while the structure of corpus callosum differentiates as a commissural plate within the lamina terminalis during the 4th-5th week of gestation, with earliest callosal axons appearing at around 10 weeks of gestation and the achievement of the complete morphology at around 15 weeks of gestation (Achiron, 2001) and septum pellucidum formation directly related to corpus callosum development.

Vascular hypothesis

One of the most studied theories regarding the etiology of SOD portrays this complex neuro-ophthalmological syndrome as a vascular disruption sequence (Lubinsky, 1997), resulting from a defect in blood circulation of the uterine-placental unit, the placental-fetal unit and/or the fetus itself (Van gelder, 2010). Young maternal age has been considered one of the most prominent factors associated to SOD. Later on, young maternal age and SOD have been extensively found to be associated in numerous studies (Atapattu, 2012). Similarly, a probable vascular etiology has been hypothesized for other congenital disorders, some of which co-occur with SOD, such as gastroschisis and amniotic band syndrome. A possible explanation is that young maternal age correlates with a higher rate of binge alcohol consumption, cigarette smoking and use of illicit drugs during pregnancy, well-known risk factors for fetal vascular disturbances. Another significant association with SOD is the use of some drugs with a vascular effect during pregnancy such as valproic acid (Fisher, 2001), phencyclidine, phenylpropanolamine and cocaine .

In 1995 Lubinsky defined SOD as a developmental anomaly supporting the hypothesis of a vascular disruption sequence affecting the proximal trunk of the anterior cerebral artery as the possible cause, without taking into account the upcoming acquisitions in the field of genetics.

Genetic hypothesis

At present, in the great majority of cases, a unique cause of SOD cannot be identified. The majority of SOD diagnoses seems to be sporadic. Only rare familial cases associated to autosomal recessive inheritance have been described (Garau, 2019). Generally, less than 1% of all cases have been associated with mutations in the few known SOD genes: *HESX1 (MIM# 182230)*, identified in 1998 (Dattani, 1998), and *SOX3*, *SOX2*, and *OTX2* being recognized subsequently, involved in different stages of eyes and midline structures embryonic development (McCabe, 2011).

HESX1 belongs to the family of homeobox genes, essential for early differentiation of the forebrain and adenohypophysis . *SOX2 (MIM#206900), SOX3 (MIM#300123),* and *OTX2 (MIM#610125)* genes encode for transcription factors involved in regulation of other DNA regions that are crucial for early formation of different tissues. More specifically, *OTX2* and *SOX2* both play intricate roles in the embryonic development of the optic nerve . *SOX3* gene encodes a member of the SOX family transcription factors involved in the regulation of embryonic development and in the determination of the cell fate; the encoded protein may act as transcriptional regulator after forming protein complexes. With recent advances of next generation sequencing (NGS) techniques and their implementation in the clinical practice, many genes and genetic pathways have been studied extensively and have been associated with different SOD clinical phenotypes; in some cases, a genetic predisposition to vascular disturbances has been found as with *COL4A1 (MIM#180000)* and *COL4A2 (MIM#614519)*, whose alteration leads to vascular disruption sequences, particularly in the central nervous system (CNS), leading to CNS development perturbation ranging from slight white matter alterations to porencephaly, with rarer cases expressing a SOD-like phenotype.

SOD clinical features A wide clinical heterogeneity ranging from asymptomatic to very severe neurological and endocrinological involvement has been associated with SOD. The earliest clinical manifestations usually include neonatal signs of hypoglycemia and hyperbilirubinemia with the evidence of visual impairment of heterogeneous degree. The onset of endocrine disorders is highly variable and central hypothyroidism (70%) is considered the most frequent, followed by growth hormone deficiency (55%), adrenal insufficiency (50%) and central diabetes insipidus (30%) (Ganau, 2019).. Many different neuro-ophthalmological presentations are documented, with the most frequent clinical finding of abnormal eye movements, which usually can be appreciated by the first three months of life, especially in cases of SOD with bilateral optic nerve hypoplasia, and deficit of visual fixation and smooth pursuit (Hellstrom, 2000). The cognitive profile can range from normal intellectual abilities associated to neuropsychological fragilities to profound intellectual disability. Other neurodevelopmental disorders, beyond intellectual disability, have been described in SOD, such as autism spectrum disorder, and other less complex behavioral problems. Almost 30% of patients with SOD are known to have epilepsy, presenting either with infantile spasms, generalized tonic-clonic seizures, or myoclonic seizures. Seizures secondary to metabolic disorder (hypoglycemia or hyponatremia) are also common in the first period of life. Furthermore, drug resistant focal epilepsy is frequently observed in SOD-plus conditions (Alkhateeb, 2017; Karatas, 2009). Finally, sleep disorders with different severities may be part of the clinical picture and may be ascribed both to midline defects and to visual impairment (Yates, 2018).

SOD plus syndrome

As already stated, SOD might be associated with other brain malformations and, in the presence of Malformations of cortical development (MCDs), the term SOD plus syndrome has been adopted (Barkovich, 2012). Among MCDs, schizencephaly, polymicrogyria, focal cortical dysplasia and nodular heterotopia are recurrent findings in SOD-plus cases. According to Barkovich classification, both unilateral and bilateral schizencephaly are reported in SOD-plus cases available in literature. When SOD is associated to other brain abnormalities, a more complex and severe phenotype with poorer prognosis is to be expected. See *table 1* for a summary of reported neuroradiological patterns of SOD-plus.

REFERENCE	NUMBER OF PATIENTS	ASSOCIATED BRAIN MALFORMATION	ADOPTED SOD NEURORADIOLOGICAL CRITERIA
MILLER ET AL 2000	3	a. Right perisylvian polymicrogyria b. Right open-lip schizencephaly c. Left parietal polymicrogyria	a. Septum pellucidum agenesis Optic chiasm hypoplasia b. Septum pellucidum agenesis Optic chiasm hypoplasia c. Septum pellucidum agenesis Optic chiasm hypoplasia
CAMINO ET AL 2003	1	Right frontal subependymal nodular heterotopia	Bilateral optic nerve hypoplasia Septum pellucidum agenesis
KWAK ET AL 2008	1	Thickening of bilateral insular cortex	Septum pellucidum agenesis Optic nerve hypoplasia
KARATAS ET AL 2009	2	A) tetraventricular communicating hydrocephalus, atrophy of the left hemisphere and brain stem B) Porencephalic area in the right hemisphere	-

MATUSHITA ET AL 2010	1	Polymicrogyria, involving insula, frontal and temporal lobes	-
TRABACCA ET AL 2012	1	Right occipital cortical dysplasia	-
SIGNORINI ET AL	7	Polymicrogyria; Schizencephaly; aspecific abnormal cortical development	Olfactory bulb agenesis; cerebellar vermis hypoplasia
LABATE ET AL 2013	1	Bilateral perisylvian polymicrogyria	Septum pellucidum agenesis
ZORIC ET AL 2014	1	Left temporal lobe polymicrogyria	-
CALLIE ET AL 2017	13	Polymicrogyria (isolated/bilateral/ perysilvian/frontal)(47%) Left open-lip schizencephaly (29%) Schizencephaly with polymicrogyria at a distant site (18%) Grey matter heterotopia (35%) Transmantle cortical dysplasia (6%)	-
VALENZUELA ET AL 2017	1	Frontal cortical dysplasia and agyria	-

GUTIERREZ ET AL 2018	1	Right fronto-temporal closed-lip schizencephaly Left fronto-parietal polymicrogyria	Septum pellucidum agenesis Corpus callosum hypoplasia Bilateral optic nerve, optic chiasm and pituitary stalk hypoplasia
WANG ET AL 2020	1	Right open-lip schizencephaly Right midbrain hypoplasia Right oculomotor nerve hypoplasia	Absence of the septum pellucidum Bilateral optic nerve hypoplasia
OUAZZANI ET AL 2022	1	Closed-lip schizencephaly	-

Table 1. Literature review of SOD-plus neuroradiological pattern

The association of SOD with polymicrogyria and nodular heterotopia supports the idea that SOD etiology might come from alterations of different stages at diverse timing in fetal neurodevelopment and cannot be explained by one isolated event, whether vascular or not.

3.1.1 A novel de novo heterozygous mutation in the *SON* gene associated to Septo-optic dysplasia

The SON DNA-Binding Protein gene (*SON*), located on chromosome region 21q22.11, encodes an RNA and DNA binding protein, which functions in efficient RNA splicing of transcripts as well as in gene repression, and it is ubiquitously expressed. Thus, a wide variety of genes are under its control and SON has been reported to be involved in cell cycle regulation and stem cell maintenance. Although the functional significance of SON in neural development is largely unknown, Ueda et al have revealed, through *Son* knockdown in the developing mouse brain, that SON insufficiency can cause neuronal migration abnormalities and reduced spine density on mature cortical neurons. *SON* pathogenic variants have been recognized as causative of Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome (OMIM #617140), a multisystemic neurodevelopmental disorder with a broad phenotypical spectrum, mainly characterized by intellectual disability (ID), hypotonia, facial dysmorphisms, visual abnormalities, brain, musculoskeletal and visceral malformations (Kushary, 2021). Brain imaging findings reported to date include corpus callosum defects, ventriculomegaly, abnormal cerebral cortical gyration, white matter abnormalities, cerebellar defects, and in one case septum pellucidum thinning has been described. In this study, the authors describe for the first time a novel *SON* pathogenic variant in a patient showing SOD diagnostic triad, suggesting a possible relationship between septo-optic dysplasia and *SON* gene alterations.

Case Report The patient is the second child of unrelated parents. He was born at term after a pregnancy characterized by a decrease in fetal growth since the 32nd gestational week. At birth, the patient presented with adequate length and head circumference (respectively 48 cm = -1.18 SDS and 33.5 cm = - 1.3 SDS) but was small for gestational age for weight (2,680 kg = -2.25 SDS); the APGAR score was within normal limits (9/10). The newborn screening resulted slightly positive for congenital hypothyroidism; in consideration of the persistent mild neonatal hyperthyreotropinemia (TSH 10-12 mU/L) with FT4 values within the normal range; at 30 days of life, levothyroxine therapy was started at a dosage of 5.7 µg/kg/day. Starting from the first month of life, he showed psychomotor delay and nystagmus. When he was one-year-old, his weight and length were respectively 7 kg (-3.72 SD) and 72 cm (-3.27 SD); blood tests showed: undetectable IGF1; normal values of prolactin (16 ng/ml, nv 2.1-17.7), ACTH (27 pg/ml, nv 7.2-52 pg/ml) and cortisol (137 ng/ml, nv 48-195 ng/ml); undetectable LH and FSH; AMH and inhibin B within normal limits for sex and age. Facial dysmorphisms became evident and consisted in low set ears and broad and depressed bridge (shown in Fig. 1). At the same age, the study of fundus oculi and array-CGH were performed with normal results. At 20 months, a brain MRI was performed showing a complex midline malformation as partial segmental agenesis-dysgenesis of the corpus callosum, partial agenesis of the septum pellucidum, mild optic nerve and chiasm hypoplasia and a slightly small pituitary gland with slightly thin pituitary stalk. Cardiological examination, as well as abdomen ultrasound and electroencephalogram were performed with normal results. In consideration of the growth delay and the neuroradiological findings, therapy with growth hormone was started at a dose of 0.15 mg/kg/week. A Whole Exome Analysis detected a novel heterozygous variant c.1069_1070delAG (p.Arg357Thrfs*8) in *SON* gene classified as "likely pathogenic" according to the American College of Human Society guidelines. Segregation analysis failed to detect the variant in both healthy parents, supporting a *de novo* origin. It is a frameshift variant deriving from a 2bp deletion in the first part of *SON* gene exon 3, resulting in a very premature stop codon inserted in the resulting mRNA. At the age of 24 months neurological assessment is characterized by nystagmus, esotropia, hypotonia and mild psychomotor delay.

Discussion The Zhu-Tokita-Takenouchi-Kim syndrome is a rare condition belonging to the group of developmental disorders, mainly characterized by intellectual disability, epilepsy, autism spectrum disorders, facial dysmorphisms, visual abnormalities, brain malformations, musculoskeletal abnormalities and visceral malformations (Kushary, 2021). ZTTK syndrome is thought to be caused by *SON* heterozygous variants. *SON* is a highly conserved and ubiquitously expressed gene located on chromosome 21, preferentially expressed in undifferentiated stem cells, composed of 12 exons and encoding the SON DNA and RNA-binding protein which plays an important role in cell cycle progression and RNA splicing regulation during development.Furthermore, recent studies have shown that *SON* haploinsufficiency in neuronal progenitors results in reduced mRNA expression and abnormal RNA splicing of multiple genes critical for neuronal migration, organization, brain development (e.g., *FLNA*, *TUBG1*, *PNKP*, *WDR62*, *PSMD3*, *HDAC6)*, and metabolism (eg. *PCK2*, *PFKL*, *IDH2*, *ACY1*, *ADA*) causing neuronal migration defects and dendritic spine abnormalities (Ueda, 2020). This evidence highlights the potential role of *SON* as a main regulator of essential genes. Nevertheless, the molecular mechanisms underlying the neural abnormalities caused by *SON* alterations remain unclear because the roles of the multifunctional nuclear protein SON are diverse and not fully understood. Beyond the RNA splicing functions indeed, other functions, such as transcriptional regulation, are more relevant to neural pathology, since SON interacts with more than a thousand genes via its DNA-binding region and is involved in the transcriptional repression of many target genes. Considering the many different functions of *SON* gene, it is to be expected that pathogenic variants in this gene can cause defects of multi-organ development. The likely pathogenic *SON* gene frameshift variant c.1069_1070delAG (p. Arg357Thrfs*8) carried by the patient herein described, absent from Genome Aggregation Database, derives from a 2bp deletion in the first part of *SON* exon 3, resulting in a very premature stop codon inserted in the resulting mRNA. This loss-of-function variant would lead to a truncated version of the protein missing all its functional domains (an arginine/serine-rich domain (RS domain), a G-patch domain and a double-stranded RNA-binding domain (DSRM)). Indeed, as demonstrated by Kim and colleagues, transcripts bearing a premature stop codon undergo nonsense-mediated mRNA decay with a reduced dosage of *SON* transcript (Kim, 2016). *SON* is significantly depleted of LoF variants with a probability of Loss-of-function Intolerance (pLI) of 1.00. Effects of *SON* haploinsufficiency on embryonic development are documented and result in several neurodevelopmental disorders associated with ID and developmental delay associated with severe brain and eye malformations. The clinical phenotype of the patient can be considered to overlap with ZTTK and SOD syndrome, while the neuroradiological findings strictly fit with the classic features associated with SOD. To date, *SON* pathogenic variants have never been associated with SOD syndrome, although in ZTTK syndrome a recurring

involvement of midline structures can be observed; in the study of Kushary et al, a thin septum pellucidum at brain MRI has been reported. Given *SON* direct and indirect involvement in the regulation of brain and other tissues' development, the authors suggest a possible relationship between septo-optic dysplasia and *SON* alterations, assuming that SON might have a regulatory function of the genes involved in SOD syndrome. However, further patients with *SON* variants and SOD phenotype are necessary to strengthen this hypothesis.

3.1.2 Biological pathways associated to SOD

A literature review was conducted on published studies reporting biological and genetic findings that might be responsible for determining SOD in order to highlight possible dysregulated genes and altered functional pathways leading to SOD. By searching for a better understanding of underlying biological and genetic pathways, it might be feasible to improve the diagnostic yield of the syndrome and shed light into new areas of research.

Literature search was conducted and updated in November 2023, using PubMed and Google Scholar to identify primary research articles or case reports with available full text using the following search string "case reports," "humans," "septo-optic dysplasia," "optic nerve hypoplasia," with a recognized genetic diagnosis. Moreover, a review of genetic pathways with an involvement in SOD etiology was conducted. This review represents the authors' perspective based on selected literature. Restrictions about the publication period were not set, and only documents published in peer-reviewed English journals were selected.

Study selection

Included primary research articles or case report studies responded to the following inclusion criteria: presence of a clinical-radiological diagnosis according to the most recent SOD diagnostic criteria and a confirmed genetic diagnosis with alteration in genes other than the already well-recognized ones (*HESX1, SOX2, SOX3,* and *OTX2)*. Articles reporting on genetic and biological pathways implicated in SOD etiology were also included.

Figure 1 resumes genetic pathways and related genes potentially implied in SOD pathogenesis as well as well-known SOD associated genes. As appreciable from the figure, the presented pathways often may converge and thus, the implicated genes may function as cascading regulators at multiple levels.

Genes associated with Septo-optic dysplasia

Twelve articles with genetic findings associated to SOD or SOD plus other than *HESX1, SOX2, SOX3*, and *OTX2* genes were considered. Whole exome sequencing (WES) was performed in nine out of twelve cases. CGH array was performed in two cases, karyotype was performed in two cases. Pathogenic variants and a genetic rearrangement were found in nine and three patients respectively. SOD-plus patients carried complex genetic rearrangements identifiable at CGH array. Seven out of twelve patients also showed clinical features other than SOD diagnostic triad.

A detailed list of studies and genes is reported below. All available information about described variants are reported. See table 2.

Reis et al (Reis, 2022) described a single case carrying a *de novo ARID1A (MIM# 614607)* variant, *ARID1A*:c.6625C>T(p.Gln2209*), with SOD according to the presence of two out of the three diagnostic criteria (absence of septum pellucidum and corpus callosum, optic nerve hypoplasia). Systemic anomalies such as a hypoplastic big toe-nail, cleft palate, choanal atresia, sparse hair, and heart defects (ventricular septal defect and a patent foramen ovalis) were found in the described patient. *ARID1A* gene encodes a member of the SWItch/Sucrose Non Fermenting (SWI/SNF) complex. Variants in *ARID1A* are known to be responsible for Coffin-Siris syndrome, which is characterized by intellectual disability associated to agenesis or hypoplasia of the corpus callosum. The identification of a role for *ARID1A* in SOD proposes the involvement of this gene and related pathway in this disorder, which was never reported before.

The study of Reinstein and colleagues (Reinstein,2015) describes a family (two patients) carrying a homozygous missense variant in *TAX1BP3* gene, presenting agenesis of corpus callosum and absence of septum pellucidum, hypogonadotropic hypogonadism, bilateral optic disc hypoplasia in one of the two members, microcephaly, facial dysmorphisms, and severe dilated cardioyompthy. TAX1BP3 is highly expressed in developing heart and brain, encoding a small PDZ-containing protein implicated in the regulation of the Wnt/β-catenin pathway. Variants in the genes encoding Wnt/β-catenin pathway proteins (*TAX1BP3* e *TCF7L1*) have been hypothesized as causative of hypopituitary axis developmental defects with available studies on animal models (Osmundsen, 2017).

A decreased expression of the NR2F1 protein has been described in association to SOD by Gazdgah et (Gazdgah, 2022) in a patient with initiation codon de novo missense variant in *NR2F1* (MIM#615722) showing absence of septum pellucidum, truncation of the rostrum of corpus callosum and slender infundibulum, Chiari I malformation, developmental delay, seizures, optic atrophy and coloboma. NR2F1 protein is a nuclear hormone receptor and transcriptional regulator belonging to chicken ovalbumin upstream promoter transcription factors (COUP-TFs), which are orphan receptors of the steroid/thyroid hormone receptor superfamily (Takamoto, 2005). Murine studies showed that *COUP-TFI* and *COUP-TFII* (*Nr2f1* and *Nr2f2*) genes are essential for early neural development and organogenesis. Moreover, Tang and colleagues (Tang, 2010) revealed that COUP-TFs are crucial for dorsalization of the eye and that *PAX6* and *OTX2*, described in SOD cases, are directly regulated by COUP-TFs.

A novel hemizygous out-of-frame deletion in *FLNA* (MIM#300321), c.6355 + 4_6355 + 5delAG, located in intron 38 of the gene, was found in a patient with neonatal hypoglycemia, optic nerve hypoplasia and dysmorphisms of corpus callosum described by Fernandez-Marmiesse and colleagues, thus presenting with two out of three SOD diagnostic criteria (Fernández-Marmiesse, 2016). The patient also presented with interventricular septum hypertrophy and limb anomalies, well known findings in *FLNA*-associated syndrome. DNA studies showed that this variant results in the production of three aberrant *FLNA* transcripts, the most abundant of which results in the retention of intron 38. FLNA is implicated in signaling pathways that mediate organogenesis in multiple systems, involving the central nervous system during embryonic development (Zhang, 2013). The clinical picture of the reported patient potentially expands the phenotypic variability associated to *FLNA*.

A maternally inherited pathogenic *ENG* variant was found in a patient Hereditary hemorragic telangiectasia and optic nerve hypoplasia, pituitary gland hypoplasia and dysfunction, thus showing two out of three SOD diagnostic criteria (Kawano-Matsuda, 2020). *ENG* (MIM#187300) encodes for endoglin, which is a 180-kD glycoprotein expressed on endothelial cells, acting as an ancillary receptor for several transforming growth factors (TGF)-β superfamily ligands and modulating TGF-β1 and TGF-β3 responses (Toporsian, 2005). This nonsynonymous variant was estimated to be pathogenic since previously reported in a patient with HHT (McDonald, 2011) and functional prediction algorithms suggested that this variant might cause change of splice site. Kawano-Matsuda and colleagues (Kawano, Matsuda, 2020) hypothesized that latent vascular insults during the fetal development might represent the common pathogenesis of congenital malformations both in the extremities and in midbrain that are found in SOD, and that the underlying microvascular abnormality of HHT during the development of cerebral midline may lead to SOD.

A heterozygous *TUBA1A* likely pathogenic variant, c.715A>C, was found by Reyes-Capò and colleagues (Reyes, 2018) in a patient with corpus callosum agenesis, severe optic nerve hypoplasia, band heterotopia and cerebellar hypoplasia. Mutations in *TUBA1A gene* (MIM#611603), which encodes the microtubule-related protein α-tubulin, have been associated with a wide range of brain malformations including abnormalities of cortical development, hippocampi, basal ganglia, corpus callosum, cerebellum and brainstem. *TUBA1A* alterations have been described in two cases of optic nerve hypoplasia but variants in *TUBA8* (MIM#619840), also coding for an alpha tubulin as *TUBA1A*, have been linked to optic nerve hypoplasia (Hebebrand, 2019). Therefore, alpha tubulin components seem to be involved in both midline structures and optic nerve development.

Pasca and co-authors (Pasca, 2023) recently reported an overlapping phenotype of Zhu-Tokita-Takenouchi-Kim syndrome (ZTTK) and SOD in a patient carrying a novel de novo *SON* gene (MIM#617140) heterozygous frameshift variant, c.1069_1070delAG, (p.Arg357Thrfs*8), and showing congenital hypothyroidism, psychomotor delay, dysmorphisms, growth delay, partial agenesis of septum pellucidum and corpus callosum, mild optic nerve, chiasm hypoplasia, and a small pituitary gland. The authors hypothesized that *SON* gene might have a regulatory function on the genes involved in SOD based on recent studies showing that *SON* gene haploinsufficiency in neuronal progenitors results in reduced mRNA expression and abnormal RNA splicing of multiple genes critical for neuronal migration, organization, brain development (e.g., *FLNA*, *TUBG1*, *PNKP*, *WDR62*, *PSMD3*, *HDAC6)*, and metabolism (eg. *PCK2*, *PFKL*, *IDH2*, *ACY1*, *ADA*) causing neuronal migration defects and dendritic spine abnormalities (Ueda, 2020). Effects of *SON* haploinsufficiency on embryonic development are documented and result in several neurodevelopmental disorders associated with severe brain and eye malformations (Kim, 2016) .

SMCHD1 (MIM# 603457) encodes an epigenetic regulator that controls DNA methylation of multiple genomic loci (Kinjo, 2020). Heterozygous *SMCHD1* mutations were identified in patients with Bosma arhinia microphthalmia syndrome (BAMS), an extremely rare syndrome whose clinical triad is represented by the absence of the nose, microphthalmia, and hypogonadotropic hypogonadism (HH). Kninjo and colleagues (Kinjo, 2020) described a patient with p.Asp398Asn variant in *SMCHD1* showing combined pituitary hormone deficiency (CPHD), optic nerve hypoplasia and thin retinal nerve fiber layer, therefore satisfying the criteria for SOD. Whole exome sequencing excluded additional variants in other HH/CPHD-causative genes. In vitro assays confirmed functional impairment of the described variant. These results suggest that the clinical consequences of *SMCHD1* mutations are broader than currently recognized, including septum pellucidum/corpus callosum hypoplasia, hearing loss, and cleft palate; HH, and eye anomalies have been documented in both conditions.

Slavotinek and colleagues described a patient bearing a homozygous VAX1 variant (p.Arg152Ser), predicted to be of LOF nature, in a proband of Egyptian origin with microphthalmia, small optic nerves, cleft lip/palate and corpus callosum agenesis, hence with a SOD phenotype (Slavotinek, 1012). VAX1 is essential for basal forebrain development, indeed it has been shown that Vax proteins function as activators of a dominant negative isoform of the Wnt signaling mediator TCF7L2, which is expressed throughout the developing forebrain (Hallonet, 1999). The functional study conducted by the authors suggest that one mechanism whereby the mutation exerts its phenotypic effects is through the hyperactivation of Wnt signaling.

Table 2: Review of SOD patients' neuroradiological, clinical and genetic findings with a genetic diagnosis other than *HESX1, SOX2, SOX3*, and *OTX2* genes were considered from the literature

First author	Year	Country	n of patients	Performed genetic testing	genetic diagnosis	ONH	midline anomalies
Al-Salihi	2023	Qatar	1	exome	TUBB mutation	yes	Septum pellucidum agenesis, stretched and thin CC, hypoplastic splenium
Bravo	2012	USA	1	karyotype	interstitial deletion of the proximal portion of the long arm of chromosome 14	yes	CC agenesis
Dhamija	2013	USA	1	CGH-array	unbalanced 5;12 translocation	yes	Septum pellucidum agenesis
Fernández-Marmiesse	2019	Spain	1	NGS (brain morphogenesis defects) negative, mRNA expression studies	rare intronic variant c.6355 + 4_6355 + 5delAG in hemizygous state in the FLNA gene (reference sequence NM_001456.3)	yes	CC hypoplasia
Gazdagh	2022	UK	1	Exome	NR2F1 initiation codon de novo missense variant	yes	Absence of septum pellucidum, truncation of the rostrum of CC
Kawano-Matsuda	2020	Japan	1	NGS	ENG de novo variant	yes (left)	No
Kinjo	2020	Japan	1	exome	SMCHD1:p.Asp398Asn	yes	No
Pasca	2023	Italy	1	exome	SON:c.1069_1070delAG, (p.Arg357Thrfs*8)	yes	Partial agenesis of CC and septum pellucidum
Reinstein	2015	Israel	1 family	karyotype, exome	TAX1BP3 homozygous missense variant	yes	CC and septum pellucidum agenesis
Reis	2022	USA	1	exome	ARID1A:c.6625C>T(p.Gln2209*)	yes	CC and septum pellucidum agenesis

Reyes-Capó	2018	USA	1	Exome	TUBA1A(NM_006009.4):c.715A>C(p.Thr239Pro)	yes	Absence of septum pellucidum, truncation of the rostrum of CC
Singh	2004	USA	1	CGH-array	8q deletion/3p trisomy	dentato-olivary dysplasia	deficient pituitary stalk, hypoplastic piyuitary gland, small optic nerves, absence of left olfactory bulb, absence of posterior half of CC
Slavotinek	2012	USA	1	exome	VAX1:p.Arg152Ser	yes	Corpus callosum agenesis

First author	hypothalamic/pituitary axis involvement	Malformation of cortical development	other MRI abnormalities	Associated clinical features	ACMG classification
Al-Salihi	No	No	No	Facial dysmorphisms	NA
Bravo	hypopituitarism	No	Ventriculomegaly, inferior vermis hypoplasia	Facial dysmorphisms, microcephaly, tracheomalacia, bronchopulmunary dysplasia, hypospadia	-
Dhamija	hypopituitarism	No	No	Pre-axial polydactyly; patent ductus arteriosus, atrial and ventricular septal defects; facial dysmorphisms; hyperreflexia	-
Fernández-Marmiesse	pituitary hypoplasia, growth rate drop (normal tests)	No	Delayed myelination	Interventricular septum hypertrophy	VUS (described as pathogenic PMID : 31234783)
Gazdagh	Growth hormone deficiency	No	Arnold-Chiari malformation	Iris coloboma, 2-3 toe syndactyly	Pathogenic (PMID: 26986877; 30945278; 34787370
Kawano-Matsuda	hypothalamic pituitary dysfunction and hypoplasia	No	No	Strabismus, pulmunary AV fistulas	Pathogenic
Kinjo	Hypogonadotropic hypogonadism	No	No	No	Uncertain significance (parents testing NA)
Pasca	Congenital hypothyroidism	No	No	Dysmorphisms, growth delay	Likely Pathogenic
Reinstein	hypogonadotropic hypogonadism	No	No	Cardiomyopathy, facial dysmorphisms, macrocephaly	VUS, described as Pathogenic PMID:25645515

Reis	No	No	No	Hypoplastic big toe-nail, cleft palate, choanal atresia, sparse hair, and heart defects	Likely Pathogenic
Reyes-Capó	no	No	Band heterotopia and cerebellar hypoplasia	No	Likely Pathogenic
Singh	hypopituitarism	cortical dysplasia	Abnormal myelination, dento-olivry dysplasia	Cardiac malformation	-
Slavotinek	No	No	No	Microphthalmia and cleft lip/palate	Likely pathogenic

Genetic pathways associated to Septo-Optic Dysplasia

- **Ras-RAF-EMK-ERK/mitogen-activated protein kinase signaling**

The Ras-RAF-EMK-ERK/mitogen-activated protein kinase signaling pathway (ERK/MAPK pathway) finds its first actor in receptor-linked tyrosine kinases, which then triggers an intracellular phosphorylation cascade leading to phosphorylation and activation of ERK1/2-MAPK. The above results in different cellular events from proliferation, changes in cell differentiation, apoptosis and senescence (Zhang, 2002). *HRAS, KRAD* and *BRAF* are genes involved in ERK/MAPK pathway and participate in different steps of neurodevelopment processes including neural stem cell proliferation, neurogenesis, gliogenesis, and oligodendrocyte differentiation and myelination (Gimenez, 2019). Germline mutations in components of ERK/MAPK pathway are known for being responsible of a set of syndromes defined as RASopathies (Kim, 2019). Gualtieri and colleagues reported the association of SOD and RASopathies in the presence of *BRAF* gene mutations leading to a gain-of-function activation of MAPK pathway (Gualtieri, 2021). Activation of the MAPK pathway in the progenitors of the pituitary gland leads to abnormal terminal differentiation of hormone-producing cells, transient expansion of the pituitary stem cell pool followed by cell growth arrest and apoptosis leading to postnatal hypopituitarism. The authors also analyzed the expression pattern of BRAF during human embryonic development, and BRAF mRNA transcripts were localized throughout the neural tube, the retina, dorsal root ganglia, cranial nerves, and in the developing endocrine hypotalamo-pituitary axis, with prevalent expression in the ventral diencephalon and the Rathke's pouch (Gualtieri, 2021).

- **Wnt/β-catenin signaling**

- Wnt/β- catenin signaling pathways are recognized to have a major role in embryonic development, body axis patterning, and cell migration (Komiya, 2008). Specifically, the Wnt/β-catenin pathway plays critical roles in the proper patterning of the central nervous system from the earliest stages of neural development, driving neurodevelopmental processes such as CNS regionalization, neural progenitor differentiation, neuronal migration, dendrite development, synaptogenesis and adult neurogenesis (Mulligan, 2017). The stability of β-catenin, which is a strong transcriptional activator, is critical for normal WNT/β-catenin signaling function (Liu, 2022). In the absence of WNT ligands, β-catenin is phosphorylated and degraded, rendering the pathway inactive. β-Catenin can then translocate into the nucleus and interact with members of the T-cell factor/lymphoid enhancer factor family to activate the expression of target genes. In mammals, transcription factors like TCF7L1, TCF7L2*,* and LEF1, have a β- catenin–interacting domain at the N terminus. It is recognized that, in the absence of stable β-catenin, TCF/LEF factors can repress target genes of the pathway by the involvement of corepressors. It has been observed that alterations of the Wnt/β-catenin signaling pathway disrupt midbrain and hindbrain regionalization, and cause neural tube defects including conditions such as anencephaly, spina bifida, and craniorachischisis. *TCF7L1,* for instance, is crucial to maintain normal expression of the hypothalamic signals involved in the induction and subsequent expansion of Rathke's pouch progenitors, through its repressing activity of Wnt pathway (Gaston massuet, 2016). As mentioned above, Vax proteins are activators of the canonical Wnt signaling mediator TCF7L2, having as an effect regulation of TCF7L2 target genes and Wnt signaling.

Moreover, *VAX1* apparently interacts with a downstream target of Wnt pathway that is PAX6 (MIM# 607108) (Hallonet, 1999). Compound heterozygous mutations in PAX6 has been detected in two patients with complex brain and ocular malformations classifiable as SOD plus (Glaser, 1994). Heterozygous PAX6 mutation have been detected in patients presenting with various brain midline defects among which corpus callosum hypoplasia (Sisodiya, 2001) but also ONH (Azuma, 2003). PAX6 is a transcription factor important for ocular development, by orchestrating the differentiation of different cell lines into the tissues constituting the eye, and for central nervous system embryonic development, through the government of cortical progenitor cell proliferation, neurogenesis, and neuronal layer formation. PAX6 also plays a crucial role in establishing dorso-ventral patterns, differentiating diverse CNS cell types, and defining boundaries along the anterior-posterior axis. Mutations of genes involved in Wnt/β-catenin signaling are the most represented in SOD diagnoses (*SOX2, SOX3, OTX2, TAX1BP3* and *TCF7L1).*

FGFR1 and FGF8 players

In humans, mutations in *FGF8* and *FGFR1* genes are known to cause congenital hypogonadotropic hypogonadism (CHH) without or with anosmia (Young, 2019), Kallman syndrome, Hartsfield syndrome (Palumbo, 2019), holoprosencephaly and split hand/foot malformation (Villanueva, 2015). Raivio and colleagues (Raivio, 2012) have described a genetic overlap in patients with combined pituitary hormone deficiency CPHD/SOD carrying heterozygous mutations in *FGFR1* and *FGF8*, hypothesizing that mutations in genes generally associated with CHH/Kallman syndrome may also be associated with CPHD/SOD. Kallman syndrome is a developmental disease showing hypogonadism with anosmia but also absent or incomplete puberty, sexual immaturity, infertility, and midline defects. In addition, *FGF8* mutations have been found to be associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction (McCabe, 2011).

During formation of the olfactory bulb and GnRH neurons, FGF8 acts mainly via FGFR1, i.e. one of the four FGF receptors (Linscott, 2019) and its three isoforms (FGFR1-IIIa, FGFR1-IIIb and FGFR1-IIIc). Studies on mice carrying null mutations in FGFR1 revealed its fundamental role in early embryonic development, which reflects its involvement in neuralization and precursor proliferation (Klimaschewski, 2021). Nuclear FGFR1 is required for neuronal differentiation and is expressed in Rathke's pouch but also in the neuroepithelium where it regulates anterior-posterior patterning of telencephalon, being responsible for producing most of the frontal cortex (Borello, 2008). FGF8 has two isoforms with distinct activity during brain development: FGF8a which exerts mainly a neural activity inducing the midbrain proliferation, and FGF8b, which is involved in mesoderm induction and differentiation [93]. Moreover, murine transcriptome data have identified members of the FGF8 signaling network during pituitary development (Kumar, 2021). Thus, FGF8 and FGFR1 might be early involved in processes leading to SOD.

- **PKB-AKT pathway**

The phosphoinositide-3-kinase-protein kinase B/Akt (PI3K-PKB/Akt) pathway activation is controlled via a multistep process. Fully active PKB/Akt mediates numerous cellular functions including angiogenesis, metabolism, growth, proliferation, survival, protein synthesis, transcription, and apoptosis (Hemmings, 2021)

PI3K activates protein kinase B, also known as AKT, which represents a central node, being a positive regulator of several signaling pathways modulating cell proliferation, growth and survival, such as mTOR pathway. Particularly, in neurons located in the hippocampus, cerebral cortex and cerebellum, activation of the AKT/mTOR pathway seems to be essential for neuronal development and synapse formation. The important function of PI3K in neurons has been demonstrated for its involvement in severe brain pathologies, such as developmentally-associated brain malformations, namely megalencephaly and focal cortical dysplasia (Jansen, 2015) Overall, studies on animal models and humans, indicate that PI3K/AKT is a central pathway for the integration of developmental signals that are necessary for brain development (Sanchez -Alegria, 2018).

- **PROK2/PROKR2 players**

PROK2 and its receptor PROKR2 are primarily expressed in the CNS, where they influence the olfactory bulb development and GnRH neural migration, but are produced in many other organs and tissues (Matin, 2011). PROKR2 activation leads to mobilization of calcium, stimulation of phosphoinositide turnover and activation of p44/p42 mitogen-activated protein kinase .

Alterations of the PROK2/PROKR2 signaling pathway have been identified as causes of human Kallman syndrome. Specifically, PROK2/PROKR2 signaling has been recently demonstrated to be crucial for the tangential and radial migration of olfactory bulb interneurons (Valdes-Socin, 2014). Prok2 and prokr2 gene knockout mice both present abnormal GnRH neuron migration, agenesis, or hypoplasia of the olfactory bulbs, in association with hypogonadotropic hypogonadism. Raivio and colleagues (Raivio, 2012) searched for mutations in the PROK2/PROKR2 genes in patients with CPHD/SOD, identifying loss-of-function mutations in PROKR2 in unrelated CPHD/SOD probands but found the same variant (PROKR2 R268C variant) in heterozygous state in HH/Kallman syndrome patients, healthy first-degree relatives of Kallman syndrome probands, and in one of 250 healthy controls. With these findings, Raivio and colleagues hypothesize that such involvement of PROK2/PROKR2 signaling pathway do not cause major midline defects per se, though it may act as a genes' modifier.

- **SHH pathway**

Sonic Hedgehog signaling (HSS) pathway is one fundamental network regulating key events of developmental processes. The pathway modulates the Shh protein, which constitutes one important signaling molecule implicated in the control of neurogenesis and neural patterning during CNS development (Yang, 2021). Shh signaling pathways is divided into canonical and non-canonical signaling, meaning a direct or indirect mediation of other pathways. Recent studies show that Shh regulates the development of the CNS through synergistic effect with temporal regulation appearing indispensable in determining the phenotype (Yang, 2021)

In humans, loss of function mutations in SHH are known to result in a variable clinical expression of holoprosencephaly phenotype (Monteagudo, 2020), which results from imperfect separation of the cerebral hemispheres and craniofacial structures due to a reduction in SHH signaling from the prechordal plate. According to the timing and location of SHH signal disruption, a different phenotype might come out, possibly including SOD presentation.

GLI2 is an obligatory mediator of SHH signal transduction and is recognized among genes essential in pituitary formation. Loss-of-function mutations in the human *GLI2* gene (MIM# 610829) are associated with phenotypes belonging to holoprosencephaly (HPE) spectrum, whose primary features include defective anterior pituitary formation and pan-hypopituitarism, with or without overt forebrain cleavage abnormalities, and HPE-like mid-facial hypoplasia (Roesller, 2003). In the study of Soares Paulo and colleagues (Paulo, 2015) a single heterozygous nonsense *SHH mutation* (p.Tyr175Ter) was found in a patient presenting with hypopituitarism and alobar HPE but its contribution to phenotype is uncertain as the *in silico* analysis did not predict it to be pathogenic. In the same study, a novel heterozygous missense variant in GLI2 (p.Leu761Phe) was found in a patient with SOD and CPHD; the same variant was found in the unaffected mother, with a possible explanation of incomplete penetrance. The resulting affected leucine residue is well conserved and lies in the GLI2 acetylation domain, which has been showed to be a key transcriptional checkpoint of Hedgehog signaling; in silico analysis predicted this variant to be damaging. Functional studies of the genetic variants described are needed to confirm genotype-phenotype correlation

Finally, SHH signaling pathway resulted to be a key target of prenatal ethanol exposure and animal models with mutations in the Shh pathway genes showed a profound increase in the penetrance and severity of HPE when exposed to sub-teratogenic doses of ethanol (Hong, 2012)

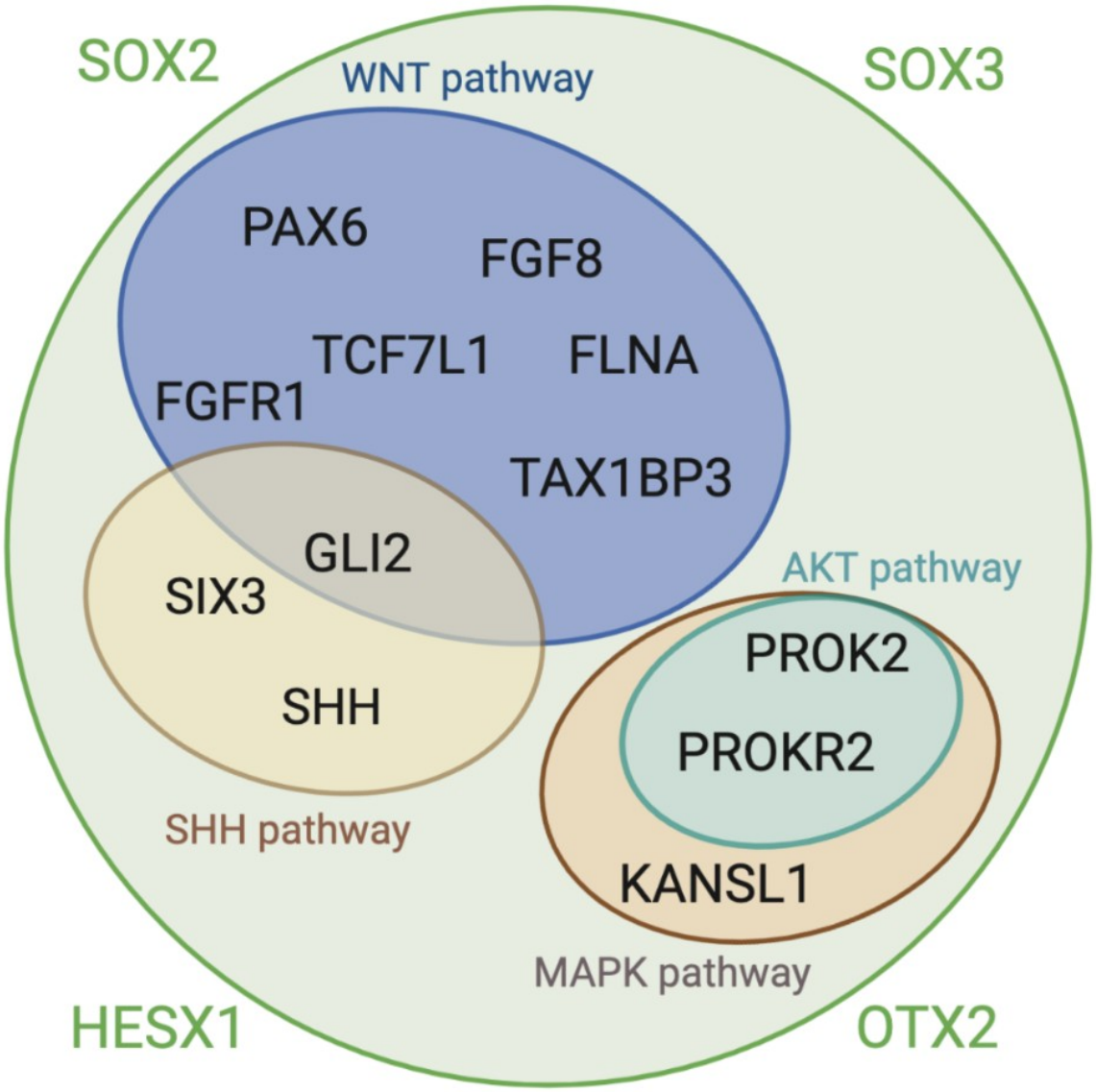


Figure 1. Genes and Genetic pathways associated to SOD

SOD typically has a low rate of genetic diagnosis, however recent data on animal models and clinical reports show interesting insights into alterations in new and sometimes overlapping pathways. Not surprisingly, SOD phenotype might derive from alterations in transcriptional pathways that intersect during brain development. Moreover, those pathways might be already associated to other disease phenotypes and interplay with genes and pathways known to have a role in SOD determination. Concurrent alterations in brain structures with different timing in development of SOD corroborates the hypothesis that the cause for this syndrome is related to an alteration in different stages of neurodevelopment and cannot be explained by one isolated event, whether vascular or not. When considering new plausible genes as responsible for SOD phenotype, other neurological and extra-neurological findings are usually found in addition to standard SOD diagnostic clues. The present data suggest that investigation for a genetic etiology should be warranted in individuals with a clinical diagnosis of SOD corresponding to the presence of at least two diagnostic criteria, particularly in the presence of additional syndromic anomalies. Moreover, as suggested in previous literature. multi patients born from older, multigravida mothers should also represent good candidate for genetic testing.

3.1.3 Sleep profile of patients with SOD: study protocol and preliminary results

This study protocol has resulted in a publication:

Pasca L, Morelli F, Catalano G, Quaranta CA, Vitali H, Ballante E, Dattrino F, Crema F, Rota P, Varesio C, De Giorgis V, Romaniello R, Signorini S, Franco V. Sleep profile in patients with septo-optic-pituitary dysplasia: protocol for a prospective cohort study. BMJ Open. 2025 Jan 15;15(1):e090675. doi: 10.1136/bmjopen-2024-090675.

Among the potential health challenges that subjects with cerebral malformations may face, sleep concerns are frequent and may include chronic insomnia, sleep-related breathing disorders, and circadian rhythm disorders. The wide variety and severity of sleep disorders found in children with congenital malformations sometimes reflects the underlying heterogeneity of disease processes and spectrum of severity.

Children with SOD may experience a variety of sleep difficulties. To date, only a limited number of studies have examined sleep characteristics in patients with SOD, primarily focusing on individual cases or small case series (ebb, 2010). Among the most reported findings are sleep fragmentation, disrupted circadian rhythms, and reduced sleep efficiency. All of the structural and functional defects of SOD are potential mechanisms accounting for the lack of circadian rhythm. Both midline brain abnormalities and reduced visual input may contribute to alter melatonin secretion. However, a specific melatonin profile in patients with SOD has not been identified, and the single factors contributing to sleep disturbances in SOD have not been thoroughly investigated. A range of sleep abnormalities has been reported in patients with isolated agenesis of corpus callosum (ACC) alone, including greater sleep onset delay, less sleep duration, greater bedtime resistance, sleep anxiety, night awakenings, parasomnias, sleep-disordered breathing, and daytime sleepiness (Yates, 2018). Interestingly, polysomnographic studies of individuals with ACC have found increased slow-wave sleep but reduced spindles-slow waves coupling, decreased REM sleep, and decreased interhemispheric coherence (Nielsen, 1993; Ingam 2017). Since light plays a crucial role in circadian entrainment, individuals with reduced or absent light perception, such as those with severe visual impairment or blindness, are at increased risk of experiencing sleep difficulties. The absence of vision is often associated with both increases in reported sleep disturbances and incidence of free-running circadian rhythms (Aubin, 2012). Individuals who are completely blind experience greater sleep disturbances compared to those with residual vision. An alteration of the sleep architecture and its microstructure was sometime reported in blind adults, as well as spindles development (Vitali, 2024).

The protocol of a study meant to evaluate sleep quality, sleep characteristics, and melatonin profiles in pediatric patients diagnosed with SOD and compare these findings with children who have isolated visual impairment (i.e. related to disorders involving pre-geniculate structures) and those with agenesis of the corpus callosum was developed. Considering the heterogeneity of SOD features and phenotype, investigating possible determinants and major structural contributors to sleep outcomes could shed a light on sleep development and potentially on sleep control strategies.

Materials and methods

This is an observational study. Participants will be enrolled from the Child Neuropsychiatric Unit of the IRCCS Mondino Foundation (Pavia, Italy). The study has been approved by the local Ethics Committee (N°0049187/23) and registered at https://clinicaltrials.gov (NCT06262152). The multidisciplinary team has more than 10 years of experience in the clinical management of patients with neurodevelopmental disorders, cerebral malformations and bilateral visual impairment. The estimated duration of the study is two years.

Participants

Three groups of participants will be enrolled in the study, with participants screened consecutively according to the relevant inclusion and exclusion criteria as outlined below.

Patients with SOD (Group A). Inclusion criteria are: 1) clinical diagnosis of SOD with or without a defined genetic diagnosis, according to current diagnostic criteria, in the presence of optic nerve involvement; 2) absence of malformation of cortical development ; 2) age 3-18 years; 3) best corrected grating or visual acuity ≥0.5logMAR 4) availability of at least 2 serial sleep EEGs performed during clinical follow-up; 5) absence of epilepsy; 6) stable drug therapy, if present, during the previous three months.

Patients with visual impairment (Group B). Inclusion criteria are: 1) diagnosis of congenital or early acquired isolated bilateral Visual Impairment (VI) due to the involvement of the so called 'anterior' visual pathway (i.e the pre-geniculate component of the *retino-geniculate pathway*) with or without a known genetic diagnosis (e.g., isolated eye and/or optic nerve maldevelopment, inherited retinal dystrophies,). 2) age 3-18 years; 3) best corrected grating or visual acuity ≥0.5logMAR without significant variations at the previous clinical follow up; 4) availability of serial sleep EEGs performed during clinical follow-up; 5) absence of epilepsy; 6) stable drug therapy, if present, during the previous three months.

Patients with agenesis of corpus callosum (Group C). Inclusion criteria are: 1) presence of isolated corpus callosum complete agenesis on brain MRI; 2) age 3-18 years; 3) best corrected grating or visual acuity ≥0.5logMAR; 4) availability of at least 2 serial sleep EEGs performed during clinical follow-up; 5) absence of epilepsy; 6) stable drug therapy, if present, during the previous three months.

In all three groups exclusion criteria are: 1) absence of informed consent; 2) presence of severe developmental delay, intellectual disability and/or severe motor impairment; 3) melatonin consumption; 4) presence of cortical visual impairment. In group B, patients are also excluded for the presence of cerebral malformation/lesion.

Patient and public involvement

Patients and family associations will be acknowledged. We will share the results of this study in the community to highlight the importance of sleep outcome and influence on the overall disease.

Outcome measures

Primary Outcome

The main aim of this study is to describe sleep features (circadian rhythm, sleep quality and efficiency) of patients with SOD compared to patients with an isolated bilateral visual impairment and patients with corpus callosum agenesis.

Secondary Outcomes

Secondary outcomes include: i) investigating whether structural and clinical features of SOD syndrome (such as visual acuity, epileptic abnormalities on EEG, midline abnormalities, presence and localization of other brain malformations) might influence sleep outcome and how; ii) description of melatonin profile in patients with SOD compared to those with PVI and those with ACC; iii) comparison of sleep EEG patterns between patients diagnosed with SOD and those in the other two groups.

A Sleep EEG recording will be scheduled at the time of study inclusion and previously performed EEG will be reviewed. EEG assessment will include the analysis of background activity, sleep macro and microstructure with the evaluation of presence/absence/distribution of neurophysiological elements, and eventually interictal discharges.

Data collection and planned evaluations

Study data will be collected in case report forms (CRFs) designed ad hoc and entered into a dedicated database. Preliminary patient framing involves review of the medical history (clinical and possibly genetic diagnosis, brain MRI findings, remote and recent history, physiological history) according to available clinical records. At the time of study inclusion, the referring clinician will perform sleep screening, evaluation of comorbidities and neurological examination. Importantly, patients' history of recurrent otitis media, snoring, tonsils enlargement and any other clue for conditions that might impair sleep will be ruled out.

Sleep quality will be assessed through the completion of standardized sleep questionnaires by patients and their caregivers, as well as participation in an interview to examine sleep habits and hypnic profiles. Sleep questionnaires employed will include: i) Pittsburgh Sleep Quality Index (a self-completed questionnaire assessing sleep quality over a 1-month time interval); ii) Epworth Sleepiness Scale (a self-completed questionnaire assessing daytime sleepiness); iii) children sleep habits questionnaire assessing any nature of sleep disturbance.

Sleep quality and sleep–wake cycle will be evaluated, based on child's inability to go to bed, delay in falling asleep, sleep duration, overnight awakenings, anxiety related to sleep, parasomnia, respiratory disorders, and daytime sleepiness.

Patients will be asked to wear an actigraph (Philips Respironics Actiwatch Spectrum) on the non-dominant hand for 7 to 14 days at T0 and for the same interval at T1 served as a quantitative measure of movement to test sleep efficiency (the ratio of total sleep time to sleep period), total nighttime sleep duration, rest activity and number of awakenings. During that period patients will be asked to compile a sleep diary.

Melatonin levels

For each subject, one 4 mL blood and one 2 mL saliva samples will be collected simultaneously during each assessment, always in the morning. Patients will be instructed to abstain from eating for a at least 30 minutes before collecting saliva. Blood samples will undergo centrifugation within 12 hours (1500 g for 15 minutes), and the resulting serum will be stored at -80°C until measurement. Saliva will be collected via passive drooling, aspirated with a syringe, and transferred into 2 mL polypropylene tubes. Melatonin will be measured in both serum and saliva samples using a validated in-house LC-MS/MS method.

Ethical Considerations

Written informed consent will be obtained from caregivers and participants above 14 years old. Parents will be exhaustively informed about the study during a counseling session.

Sample size and statistical methods

The three groups will be compared using three different parameters, namely the PSQI score, the ESS score and the mean activity score detected by the actigraph.

We estimated the sample size for conducting a MANOVA test with three outcome variables (PSQI score, ESS, and mean activity score of the actigraph) across three groups. A sample of 45 participants (15 per group) would allow us to detect an effect size of 0.16 (measured by Pillai's trace) with a type I error rate of 0.05 and a power of 0.8.

The study will analyse three groups based on their PSQI score values, ESS questionnaire results, and mean activity score values from actigraph data through a MANOVA test. If significance (threshold at 0.05) is detected, post-hoc comparisons will be conducted with appropriate corrections for significance level, such as Bonferroni adjustments.

Categorical variables will be presented as counts and percentages, compared across groups via chi-squared or Fisher’s exact test. For continuous variables, we will report mean and standard deviation or median and interquartile range if appropriate, with between-group comparisons made using ANOVA for normally distributed variables or Kruskal Wallis test. If significance is detected, post-hoc comparisons will be conducted through t test or Dunn test with Bonferroni adjustments.

. Multivariate analysis, if necessary, will involve regression models. A *p*-value of 0.05 or lower will denote statistical significance.

The statistical analysis will be carried out by the BioData Science Unit at the Mondino Foundation (Pavia, Italy), utilizing the R Statistical Software (v4.2.0; R Core Team 2022).

Discussion

The aims of this study are to describe the sleep features of SOD patients and investigate the potential role of the core clinical features of SOD spectrum in determining the sleep outcome. Indeed, sleep problems are frequent in this population and represent a substantial cause of distress to family. Moreover, these disturbances can exacerbate disease symptoms, cognitive and behavioral issues, and even seizures when they occur. Neurodevelopmental disorders often manifest with alterations of sleep that might result from both clinical and structural features. The mechanisms underlying neurodevelopmental impairments in children with SOD are complex and involve multiple factors, which may also extend to sleep patterns. In a recent review, Mann et al suggest that the prevalence of neurodevelopmental impairments in children within the SOD spectrum might be considerable. The high heterogeneity among the samples in relation to their neuroanatomical and clinical features prevented an assessment of their specific contributions to the phenotype. However, the authors suggest to consider standardized assessment of neurodevelopmental impairments alongside routine care, underlining the need of future research aimed at identifying the causal mechanisms.

Previous research showed that patients with SOD with abnormal sleep-wake rhythmicity presented a higher prevalence of developmental delay compared to those with normal rhythmicity (100% vs 15% respectively). Additionally, those with sleep-wake rhythm disturbances showed a greater incidence of corpus callosum hypoplasia or absence (66%) compared to those with normal sleep- wake rhythmicity (30%). Similarly, Garcia-Filion et al. (Mann, 2023) found that corpus callosum hypoplasia, but not absent septum pellucidum or pituitary gland malformations, was associated with increased risk of developmental delay (Garcia-Fillon, 2017). At this purpose, Alt et al. and Signorini et al. reported a high proportion of normal cognitive development in their participants diagnosed as SOD-plus (additional cortical malformations) but no data are available concerning sleep features in SOD-plus condition compared to SOD (Alt, 2017). Cognitive-developmental impairment has been the primary focus in research assessing children with SOD spectrum conditions, with the majority of studies assessing intellectual disability and developmental delay, rather than behavioral, emotional and sleep outcomes .

Evidence on sleep profiles of patients with SOD is scarce. Rivkees and colleagues (Rivkees, 2001) described a young child with SOD who showed arrhythmicity in sleep patterns with random sleep distributed throughout the day and night. Webb and colleagues (Webb, 2010) characterized sleep-wake cycles in a cohort of six children with SOD, analyzing actigraphy and 24-h melatonin profile. All six children were found to have sleep fragmentation with poor sleep efficiency due to frequent and prolonged night awakenings. Moreover, melatonin profiles showed a substantial variation in the timing and amount of melatonin produced, although no consistent pattern among all children could be found.

Given the known importance of light in circadian entrainment, visually deprived individuals may have an increased risk of sleep difficulties also due to abnormal melatonin secretion. Children with early-onset visual impairment may present altered sensorimotor processing, a developmental competence related to sleep spindles, possibly reflecting a disrupted sleep pattern (Campus, 2021). Moreover, a different pattern of maturation of cortical activity in visually impaired toddlers and children compared to typically developing peers has been reported. Such differences involved the development of sleep patterns as well and may be related to the atypical development of competencies such as sensorimotor process frequently (Vitali, 2024). Aubin and colleagues examined 30-day actigraphy recordings in 11 blind individuals compared to sighted individuals(Aubin, 2016). Although they did not find group differences, they did find greater variability in sleep efficiency and time of sleep onset in blind individuals. Further analyses demonstrated abnormal timing of melatonin consistent with abnormal circadian rhythm, but preservation of cortisol secretion profile (Ingram, 2017).

Ingram and Churchill performed a survey of 66 children with ACC and found that overall 78% had clinically significant sleep problems. Sleep problems were significantly correlated with overall quality of life regardless of age. Considering the poor sleep quality of patients with ACC, corpus callosum involvement and role in sleep outcome should be considered when studying sleep features in patients with SOD.

Among the strengths of this study, it represents the first attempt to comprehensively investigate sleep outcomes in patients with SOD, and at identifying different structural and clinical factors that influence sleep features in the heterogeneous spectrum of SOD phenotype. Moreover, the study includes both objective and subjective sleep outcome combining the use of actigraphy, sleep EEG and standardized sleep questionnaires. The study findings may guide the clinician in describing the sleep profile detectable in SOD patients and could potentially facilitate targeted intervention that should improve the quality of life of patients and their caregivers.

The present study has several limitations. First, the estimated number of participants is a small number, nevertheless, in rare diseases, the sample size is usually modest. Another limitation of the study is the absence of a nocturnal polysomnography as objective sleep evaluation, which will not be undertaken in this exploratory study. Moreover, the single detection of melatonin could be framed as a limitation of the study but a continuous serial monitoring would have necessarily implied hospitalization of the patient, thus also possibly negatively influencing the sleep quality.

Preliminary results

Figure 1. Distribution of clinical diagnoses in the included population

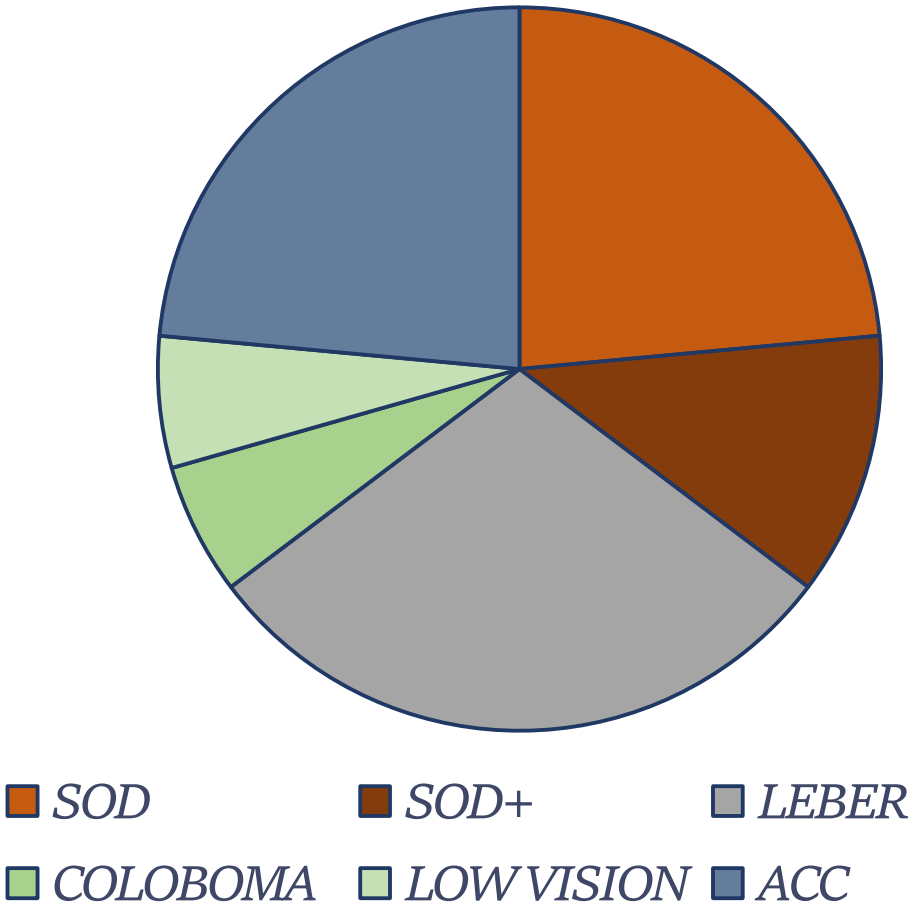

SOD
SOD+
LEBER
COLOBOMA
LOW VISION
ACC

Figure 2 .Clinical features of included population

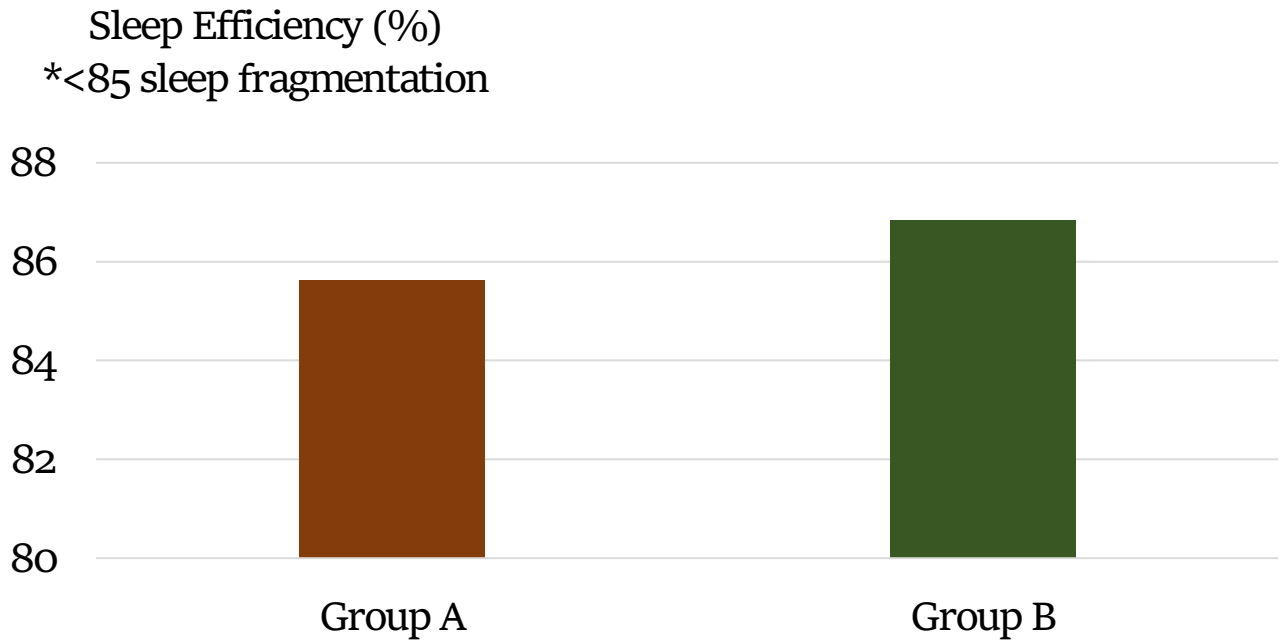
ID	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	***V01***	***V02***	***V03***	***V04***	***V05***	***V06***	***V07***
Diagnosis	SOD	SOD	SOD	SOD+	SOD	SOD+	Leber	Leber	Leber	coloboma	Low vision of unknown origin	Leber	Leber
Age (y)	7	17	16	6	7	5	17	11	9	13	17	8	10
Sex	M	F	M	F	M	F	M	F	F	F	M	M	F
BCVA	0,2/10	1/10	4/10	Light percepetion	No response	4/10	Light response	3/10	Light response	0,5/10	2,5/10	0,2/10	0,2/10
Endocrine status	GHD	no	no	no	no	no	//	//	//	//	//	//	//
MRI	Chiasm, nerve and optic tracts hypoplasia	Optic nerve, pituitary and chiasm hypoplasia	Optic nerve, pituitary and corpus callosum hypoplasIa	Frontopolar polymicrogyria, dysmorphic corpus callosum, thinned anterior commissure, optic tracts and chiasm hypoplasia	Optic nerve, chiasm, corpus callosum, pituitary gland hypoplasia and absence of septum pellucidum	Frontopolar polymicrogyria, absence of septum pellucidum, optic nerve hypoplasia	Moderate enlargement of the lateral ventricles	Optic nerve and chiasma hypoplasia	Thin corpus callosum	Normal	Superior vermian hypoplasia	Normal	Normal
Neuropsychological assessment	within normal range	within normal range	moderate intellectual disability	Moderate intellectual disability	within normal range	within normal range	within normal range	within normal range	within normal range	within normal range	within normal range	within normal range	within normal range

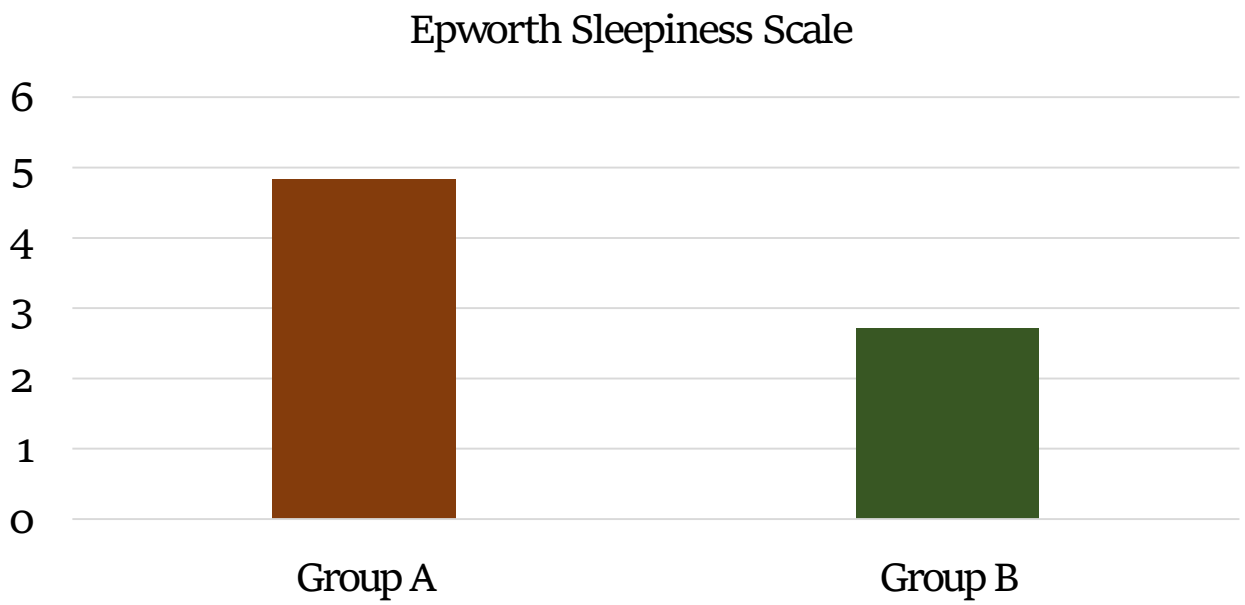
Figure 3. Scores of utilized sleep questionnaires

ID	*S01*	*S02*	*S03*	*S04*	*S05*	*S06*	*Average*
Age	7	17	16	6	7	5	9,66
PSQ	4	4	7	//	7		5,5
ESS	4	4	4	7	7	3	**4,83**
SDSC	52	//	//	40	//	37	43
Total Sleep Time median (TST) (minutes)	396	329	451	//	455	468	**418**
Sleep Efficiency median (SE) (%)	84,09	81,95	89,13	//	87,62	84,39	**85,63**
Vector Index of daily sedentariness (VIDS) (minutes)	52,25	12,73	39,71	//	44	64,68	42,67
Sleep Onset Latency (SOL) (minutes)	7,96	34,91	13	//	9,46	5,68	14,20
N° Awakenings (n)	33,43	15,73	29,86	//	36,38	43,93	31,86

Figure 4. Actigraphic data

ID	*V01*	*V02*	*V03*	*V04*	*V05*	*V06*	*V07*	*Average*
Age	17	11	9	13	17	8	10	12,14
PSQ	5	4	2	2	4	2	10	4,14
ESS	1	2	3	2	7	2	2	2,71
SDSC	//	//	//	//	//	//	//	//
Total Sleep Time median (TST) (minutes)	398	461	514	437	422	501	503	**462**
Sleep Efficiency median (SE) (%)	86,46	82,29	90,32	86,88	86,82	87,69	87,41	86,84
Vector Index of daily sedentariness (VIDS) (minutes)	29	52,29	37,86	31,46	34,39	41,46	48,54	39,29
Sleep Onset Latency (SOL) (minutes)	16,67	18,27	7	16,54	11,57	8,79	15,04	**13,41**
N° Awakenings (n)	31,5	50,62	33,14	35,07	42,14	44,71	40,92	39,73


Sleep Efficiency (%)
*<85 sleep fragmentation
88
86
84
82
80
Group A
Group B


Epworth Sleepiness Scale
6
5
4
3
2
1
0
Group A
Group B

Comment on the preliminary results

As a preliminary result, included subjects have a limited insight of subjective sleep quality and this datum is in favor of a strong need for clinical screening investigations. The group of patients with a diagnosis of SOD seems to have an increased incidence of fragmented sleep, regardless of neuroradiological findings, and elevated daytime sleepiness compared to group B (low vision). Patients with low vision included in group B are instead characterized by a more regular sleep profile but reduced total sleep time and increased sleep onset latency. The latter could highly rely on dysregulated melatonin secretion and circadian process. Future directions will include the achievement of a detailed analysis of sleep profile features across the different groups, correlation of sleep profile with clinical and structural characteristics of SOD and correlation of sleep profile and melatonin levels detected in the single individual.

3.2 Sleep in patients with corpus callosum agenesis

The present review has resulted in a manuscript currently under submission.

Agenesis of corpus callosum (ACC) [OMIM 217990] is one of the most common congenital cerebral malformations and occurs in approximately one in 4000 live births (Paul LK 2007). Thanks to the advent of highly performing prenatal diagnostic techniques, ACC diagnosis is easier to achieve during pregnancy, raising relevant prognostic questions (Edwards 2014).

Individuals with ACC may have a wide range of intellectual, academic, and behavioral impairments, as well as altered ability to process socially complex emotions (Siffredi, 2018). While syndromic ACC patients show more severe outcomes (Romaniello 2017), patients with isolated ACC have 70-80% normal neurodevelopmental outcomes (D'antonio 2016). However, impairments in their higher cognitive domains, emotional functioning, and basic vital functions such as feeding and sleep have been observed even in children with normal neurodevelopmental outcomes (Doherty 2006).

Sleep is a vital neuroendocrine function that regulates body homeostasis and neuronal organization during early brain development (Krueger 2016).
Different brain structures participate to sleep, that is rather a local than a whole-brain phenomenon (Jobst 2017, Avvenuti 2022). Corpus callosum, even if not primarily involved in sleep generation, can be considered a structure involved in sleep determination as regulating the synchronization of neural activity between homologous regions across hemispheres. Studies on healthy adults' samples show that CC integrity is associated to better sleep efficiency, and REM sleep preservation (Altendhal 2020). An animal mouse study has recently highlighted that the loss of an important mammalian CLOCK gene (BMAL1) (Guan 2021) during the embryonic phase impairs not only circadian rhythm but also sleep architecture, resulting in reduction of corpus callosum volumes. Hence, it has been speculated that CC might contribute to sleep function by mediating interhemispheric coherence and synchronization (Bernardi 2021).

Sleep is an overlooked domain in people carrying ACC. Studies in children' samples carrying ACC have reported anecdotally increased difficulty getting asleep, nighttime awakenings, parasomnias, daytime sleepiness, sleep anxiety, and enuresis (Yates JF, 2018). Instead, there are data on sleep and acallostomized subjects that inform us what the differences may be in terms of structure and connectivity. The present narrative review aims to determine the coverage of the body of the literature about sleep and ACC and identify available evidence on possible pathophysiology of sleep outcomes influenced by ACC. This review attempted to intercept information on sleep features in subjects with isolated ACC, both partial and complete, across different ages. Since literature on subjects with ACC is poor, knowledge on acallostomized patients has been included.

Sleep architecture in individuals with ACC

Only one study investigated sleep architecture (Nielsen et al. 1992) in 4 adults with ACC, showing a more significant percentage of deep sleep and a lower percentage of NREM 2 than controls. Resembling results were found in a previous study of a patient who underwent posterior partial callosotomy (Montplaisir et al. 1990), highlighting that the absence of CC might affect sleep similarly in both acallosal and partial callosotomy patients.

Nielsen and colleagues also reported that ACC patients showed more REM sleep periods and shorter REM cycle lengths than controls. The authors hypothesized two possible roles of the corpus callosum in sleep: regulation the timing of ultradian rhythm, and generating a higher frequency rhythm, and support of the neural synchronization between homologous regions in the two hemispheres. However, Nielsen and colleagues hypothesized that CC might interfere with synchronization within each hemisphere explaining the increased deep sleep and decreased NREM 2 sleep documented. Indeed, its absence can intensify the slow-wave activity due to the disruption of the desynchronizing effect of contralateral callosal fibers, which, in normal conditions, leads to a reduction in slow-wave activity. Conversely, callosotomy or ACC could cause a decrease in interhemispheric synchrony between homologous regions typically promoted by callosal fibers. The hypothesis of the role of the corpus callosum in the synchronization of neuronal populations was then confirmed in recent studies, as shown in the following paragraphs.

The cortical synchronization activity during sleep in individuals with ACC

One of the first studies that investigated the interhemispheric EEG coherence during sleep in 4 complete ACC adults showed that this population manifests lower values than controls for all sleep stages and all bands but not during wakefulness (Nielsen et al., 1993). Nielsen et al. also evidenced a region specificity involving both the frontal and central regions in all sleep stages, and the parietal region during light and deep sleep, but not during REM (Nielsen et al., 1993). The occipital cortex seemed least affected, maybe reflecting the activity of the posterior commissure, which can be an alternative pathway for interhemispheric and compensatory activity. Some MRI data supported this hypothesis by showing changes in white matter connectivity in the anterior and posterior commissures of individuals with ACC, suggesting these structures as possible alternative pathways for interhemispheric information transfer (Tovar-Moll et al., 2014).

A general reduction of EEG coherence agrees with the previous findings in partial callosotomy patients (Montplaisir et al. 1990) and newborns (Kuks et al., 1987). In the latter, the coherence reduction relative to controls was specific for the delta band (0-3Hz). However, in both groups, the coherence was higher in active sleep than in quiet sleep and increased with age.

Although no studies have provided further details on EEG coherence during sleep in ACCs, two studies have considered slow waves activity in adults with a complete resection of CC (Avvenuti et al., 2020; Bernardi et al., 2021). In agreement with connectivity results, slow waves manifested a lower probability of cross-hemispheric spreading and a higher inter-hemispheric asymmetry in the acallostomized patients (Avvenuti et al., 2020). From another point of view, a complete callosotomy caused an enhanced prevalence of unihemispheric slow waves aligned to previous results.

Finally, Bernardi et al (2021) demonstrated acallostomized patients changes in slow waves-spindles coupling. Evidence regarding coupling measures showed that split-brain individuals exhibited a diminished spatial association between spindles and slow waves. More recently, interhemispheric coherence of background and sleep spindle activity was measured in children with corpus callosum dysgenesis, evidencing the role of corpus callosum in the correct maturation of both the waking and sleeping brain (Guillou et al., 2024).

Sleep quality in individuals with ACC

The few literature data available on ACC individuals and their sleep quality, showed sleep difficulties across all ages in children with ACC, with different nature of sleep concerns according to age. For instance, children in pre-scholar age tend to have more bedtime resistance and sleep anxiety compared to older children with ACC and healthy children (Doherty et al; Ingram et al). The clinical associated features and their contribution to sleep outcomes in ACC participants remain unexplored. Zhan et al. (Zhan et al. 2021) utilized Brief infant Sleep Questionnaire to assess sleep habits in a cohort of preschooler carrying ACC , showing that patients' sleep settling time was positively correlated with the internalizing and dysregulation dimensions, indicating that patients who needed more settling time might have more internalizing and dysregulation problems that might also be associated with attention and social skills problems (Zhan 2021). More recently, Kwon et al. reported that children (of average age of 5 years) with ACC have poor sleep quality (Kwon et al 2024).
However, since sleep quality is rarely assessed in individuals with ACC, often the available information on sleep outcome does not rely on extensive anamnestic or standardized tool utilization.

Sleep disturbances in individuals with ACC

Doherty et al. examined a sample of almost 200 children (above the age of five) with ACC and compared their health issues with those of their siblings and sleep emerged as a major concern (Doherty). The results showed that siblings were more likely to have regular sleep patterns with fewer difficulties compared to the children with ACC. Specifically, children with ACC had more trouble falling asleep, experienced more fragmented nighttime sleep, and had a higher incidence of enuresis (D. Doherty et al., 2006).

A subsequent study, through questions about sleep in children of five years of age and older, found significantly greater difficulties with falling asleep and enuresis in children with ACC compared to their typically developing siblings (Moes P, et al). Considering individuals with simple ACC, further comparisons were made between individuals with complete and partial ACC. While no significant differences were found, it was observed that individuals with partial ACC were more likely to have sleep disturbances than those with complete ACC. Sleep problems were also found in a sample of ACC children aged 2-11, as examined by Badaruddin et al (Badaruddin, 2017). The aim of the study was to summarize parent observations of relatively high-functioning children with ACC, using the Child Behavior Checklist for 61 children aged 2-11 years. The survey found a high rate of sleep problems among younger children with ACC (ages 2-5) (Badaruddin et al., *Child Psychiatry Hum Dev*, 2007).

In their study, Ingram and Churchill (Ingram and Churchill, *Pediatric Neurology,* 2016) examined sleep in children aged 5-18 using the Children's Sleep Habits Questionnaire: they found that 78% of the children had clinically significant sleep problems. Children with ACC exhibited significantly more sleep difficulties across all measured subdomains compared to a previously reported sample of typically developing children, with sleep difficulties significantly related to daytime quality of life. Moreover, children with a diagnosis of autism and developmental delay showed more severe sleep issues. The study found no specific association between the type of corpus callosum dysgenesis (partial or complete), the presence of seizures, partial blindness, and sleep problems in different domains. Finally, children with ACC had similar bedtimes to typically developing children but woke up earlier and had shorter total sleep durations.

Additionally, there is a report of an individual with complete ACC who developed narcolepsy with cataplexy (S.-H. Pelidou et al., *Sleep Medicine*, 2009), a disorder characterized by REM sleep intruding into wakefulness. The lack of the CC and the resulting enlargement of the third ventricle has been hypothesized to impair the excitatory function of the hypothalamic orexin neurons, ultimately leading to the development of narcolepsy. Therefore, the ACC may downregulate the excitatory activity of orexin neurons to the limbic system and brainstem, resulting in excessive daytime sleepiness and cataplexy.

Kwon et al. performed a retrospective study of 20 patients with ACC aged around 5 years who had polysomnography and identified obstructive sleep apnea (OSA) as a prevalent sleep disorder in the analyzed cohort, highlighting the need for screening for sleep-related disorders in patients with ACC. There was no significant difference in sleep quality or the presence of OSA between those with complete and partial ACC in the cohort, although OSA was more common in younger children (Kwon A et al., *J Clin Sleep Med*. 2024).

Dreaming in individuals with ACC

It has been hypothesized that the corpus callosum may be involved in dreaming and dream recall, although it does not represent an essential anatomical structure for the production and verbal reporting of dream experiences (Nielsen et al 1993). Anecdotal reports of adults with complete ACC showed a possible tendency to have more contentless dreams, shorter dreams, and more distressful dreams (Nielsen,1993), and dreams less verbally elaborated dreams than control ; these data have been interpreted in light of the lower verbal-intellectual abilities in the acallosal group (Martinetti 1985, Butler and Watson 1985; Greenwood et al., 1977). The less successful dream recall in the laboratory and the more frequent contentless dream experience (Montplaisir 1985; Nielsen 1993) have been interpreted as due to the deficit in transferring right to left hemispheres activity as well as to impaired social experience of ACC subjects compared to control. Regarding the dream content, difference in ACC subjects compared to control were noted in terms of a greater representation of known persons compared to unknown persons; and a lower representation of animal characters in dreams of ACC subjects, again justified as a reflection of different level of social experience (Nielsen 1993).



		Acallostomized adults	ACC adults	ACC children
	NREM	• ↑ deep sleep • ↓ NREM2	• ↑ deep sleep • ↓ NREM2	-
	REM	-	• ↑ REM periods • shorter REM cycles	-
	EEG coherence during sleep	• ↓general interhemispheric coherence	• ↓ general interhemispheric coherence	• ↓ interhemispheric coherence in delta bend in newborns • ↓ spindle interhemispheric coherence in children (0-15y)
	EEG coherence during wakefulness	-	• Similar to control	• Similar to control
	Slow Waves	• ↓ probability of cross-hemispheric spreading • ↑ inter-hemispheric asymmetry • ↓ spatial association between spindles and slow waves.	-	-

Sum up of sleep architecture and neurophysiological features in acallostomized and individuals with Agenesis of the corpus callosum (ACC).

Discussion

Corpus callosum dysgenesis has been linked to sleep disturbances especially in the first years of life, but the contribution of CC to sleep abnormalities is yet to be defined. Unfortunately, neurophysiological studies have been conducted almost exclusively in participants with acquired CC loss, namely acallostomized individuals (Bernardi 2020) showing modifications of sleep ultrastructure and microstructure. Patients with ACC have not been systematically involved in sleep structure and connectivity studies.

The studies aimed at describing acallostomized subjects connectivity, increased our knowledge about the physiological mechanisms of sleep in the disruption of the CC. Namely, CC seems to play a role in facilitating slow-waves transmission across hemispheres, which through their propagation should mirror experience-dependent plasticity (Massimini et al., 2004; Murphy et al., 2009). The residual propagation across hemispheres in acallostomized subjects is due to alternative pathways, including anterior and posterior commissures or cortical-subcortical-cortical loops. Considering the role of slow waves in modulating the local spindles activity to favor the organization of information processing and brain plasticity (Cox et al., 2014), it is reasonable to expect changes also in the slow waves-spindles coupling mechanisms in split-brain patients (Bernardi et al., 2021). Specifically, it has been proposed that the slow waves generated by cortico-cortical circuits can regulate the expression of spindles within particular networks to finely tune sleep-dependent brain plasticity (Bernardi et al ,2021). Evidence regarding coupling measures showed that split-brain individuals exhibited a diminished spatial association between spindles and slow waves suggesting that, despite their temporal synchronization being preserved, slow waves and spindles are regulated independently in terms of their topographic expression (Bernardi et al., 2021). Therefore, it remains unclear if this is partially an effect of experience-dependent brain plasticity or not. When sleep is considered, available literature outlines a decreased sleep efficiency and the presence of sleep disturbances in a relevant percentage of pediatric subjects with ACC in the first years of life. The dimension of sleep disturbance reported mainly regards sleep-wake transition, difficulty in initiating and maintaining sleep, and sleep breathing disorders. Beyond a longitudinal and more extensive objective characterization of sleep throughout age, what is still missing is the analysis of clinical features that influence sleep outcome, such as the presence of epilepsy, visual deficit, hearing deficit. Moreover, the anatomy has not been related to different sleep outcomes even though Ingram and colleagues outlined a possible worse sleep outcome in individuals with partial agenesis compared to patients with a complete agenesis of CC (Ingram et al). This finding may suggest that the presence of a portion of the CC is not sufficient to reduce sleep problems (Moes P, et al).This insight is quite interesting in light of the knowledge of the recurrence of a more complex clinical picture in patients with partial compared to complete ACC, and this might in part be searched in the genetic substrate. Indeed, Romaniello et al showed that partial agenesis of CC is found more often in syndromic agenesis of corpus callosum and agenesis of corpus callosum plus patients, suggesting the involvement of genes correlated with whole brain development rather than with CC fibers guidance/proliferation only (Romaniello 2017) justifying the most severe clinical features in this condition. However, we cannot completely exclude that the anatomy itself has significance. Indeed, some MRI data reveal changes in white matter connectivity in the anterior and posterior commissures of individuals with ACC, suggesting they could be alternative pathways for

interhemispheric information transfer during wakefulness (Severino 2017, Tovar-Moll et al., 2014; Siffredi et al., 2019). Moreover, this is associated with increased local connectivity (Owen et al., 2013; Meoded et al., 2015), evidencing a plastic readaptation of neural structures in the acallosal brain (Chiarello, 1980; Dennis, 1976). Although the presence of these plastic mechanisms during sleep was not evident, they likely occur and characterize the brain of fully ACC patients rather than partial ones. Instead of developing compensatory mechanisms, we can hypothesize that the latter try to maintain those already existing with worse outcomes. In this scenario, literature has completely overseen the role of circadianity in sustaining developmental adverse outcomes in both syndromic and non-syndromic ACC conditions.

Another path to be pursued in future research should be the microstructure, namely an index of sleep stability in NREM (Bruni et al., 2010) that reflects the processes of sleep maintenance and arousability. Among the various sleep parameters, a key role is thought to be played by sleep continuity/fragmentation, and the sleep of ACC patients might present an unbalanced instability. Finally, future research should consider more broadly ACC and midline defects and syndromic conditions including ACC, for instance ACC to understand the role of circadianity in sustaining developmental and sleep adverse outcomes in both syndromic and non-syndromic ACC conditions.

3.2.1 Sleep features in subjects with corpus callosum agenesis: results from a pilot study

Thanks to the meeting and collaboration with multidisciplinary researchers composing the International consortium of Corpus callosum (IRC5), I had the chance to introduce the interest on studying corpus callosum relationship with sleep. Considering the preliminary research conducted on sleep features in subjects with ACC, preliminary analyses were implemented to describe subjective sleep features of very young patients (6-12 months) with ACC, present in a shared database. Available information included demographic and clinical data and responses of a standardized age- oriented sleep habits questionnaire, namely BRIEF Infant Sleep Questionnaire (BRIEF).

The **BRIEF Infant Sleep Questionnaire** is an assessment tool designed to collect information about sleep patterns and behaviors in infants and very young children to better understand sleep-related issues in young children and track factors that may influence the quality of their sleep.

The questionnaire consists of a series of closed-ended questions or Likert scale responses (e.g., from 1 to 5) that explore aspects such as sleep duration, difficulty falling asleep, night awakenings, and consistency of sleep-wake patterns. The primary goal of BRIEF is to identify sleep-related problems in infants and children, enabling specialists to monitor any disorders or difficulties and provide targeted interventions.

It focuses on various aspects of infant sleep, including:

- The time it takes for the infant to fall asleep
- Frequency and duration of nighttime awakenings
- Behavior during the night (e.g., crying or difficulty falling back asleep)
- Sleep routine (e.g., presence of a regular bedtime routine)
- Sleep quality (e.g., whether the infant appears rested upon waking)

In the specific subset of patients with ACC in the first year of life is extremely interesting to study sleep patterns since compensation mechanisms and certain disease manifestations might be still latent.

Methods

For each included subject information including age at caregivers' questionnaire compilation, type of ACC (partial or complete; isolated or plus), presence of epilepsy, visual or auditory deficit have been analyzed. A descriptive statistic has been performed regarding BISQ questionnaire different items' responses at 6 months and 12 months and clinical characteristics of included patients.

-A comparison of BISQ data of ACC patients has been performed with BISQ data reported in the literature. Here two approaches were used: a t test that directly compares the mean (and sd) values of the two groups, and a non-parametric test that compares the data of the patients with a simulated dataset of controls that have the mean and sd values as the ones reported in literature.

-To determine what sleep feature/ problem influences the most Parents' perception of sleep quality (last question of BISQ) univariate non parametric statistical tests were applied.

-Influence of the following clinical variables on sleep features: vision deficit, hearing deficit, epilepsy, corpus callosum anatomy, presence of additional brain structural anomalies were considered in a logistic regression analysis with a stepwise feature selection method to obtain the best subset of variables based on AIC.

- Univariate non parametric paired statistical tests were applied to evaluate sleep features from 6 to 12 months within subjects.

Preliminary Results

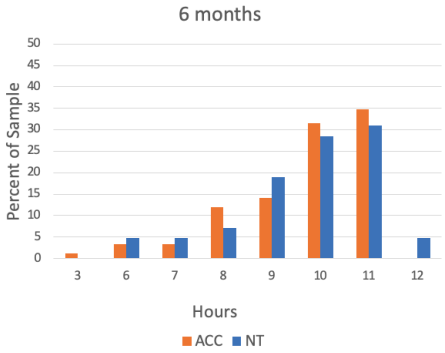
Clinical features

(% OF Sample w/data)	%Isolated ACC (101)	%ACC Plus (37)	%cACC (99)	%not cACC (40)
Epilepsy (73%)	3	23	7	13
Vision impairment (79%)	17	59	31	28
Hearing impairment (73%)	6	17	9	10

Corpus Callosum Dysgenesis	Community Comparison
N = 139 (71 w/data at 6 & 12m)	N = 116 (29 w/data at 6 & 12m)
52 females; 87 males	NA

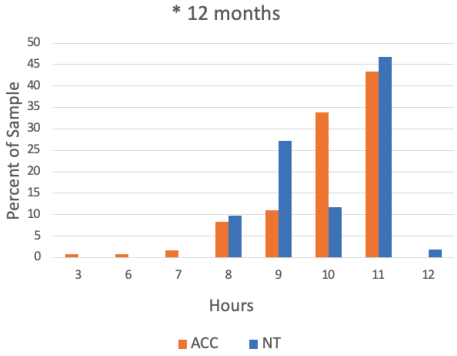
	Isolated	Plus
cACC	74 (40)	24 (14)
pACC	16 (8)	3 (1)
hypoplasia	11 (5)	5 (2)
dysgenesis	0	5 (1)

Hours spent asleep at night



ACC mean= 9.77 (1.52)
NT mean = 9.89 (1.36)

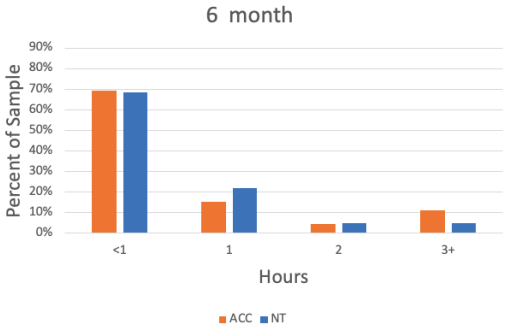
t(132) = -.43, p = .665, d = .81, CI [-.45, .28]

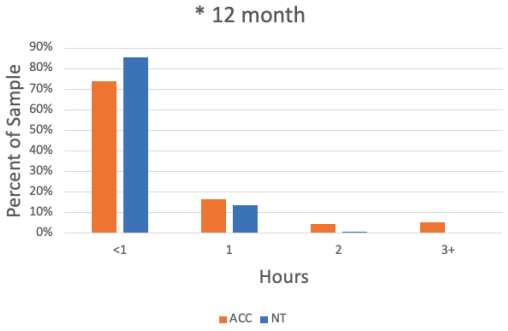


ACC mean= 10.13 (1.35)
NT mean = 10.58 (0.87)

t(203) = -2.96, p = .003, d = .389, CI [-.66, -.12]

Time spent awake during night





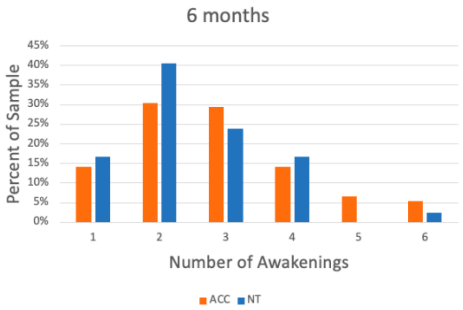
ACC mean= 0.86 (1.57)
NT mean = 0.61 (0.91)

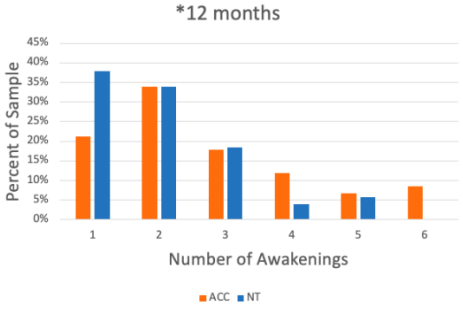
t(130) = .961, p = .338, d = .18, CI [-.19, .55]

ACC mean= 0.60 (1.31)
NT mean = 0.31 (0.47)

t(145) = 2.28, p = .024, d = .30, CI [.03, .56]

Number of awakenings during night





ACC mean= 2.91 (1.57)
NT mean = 2.52 (1.19)

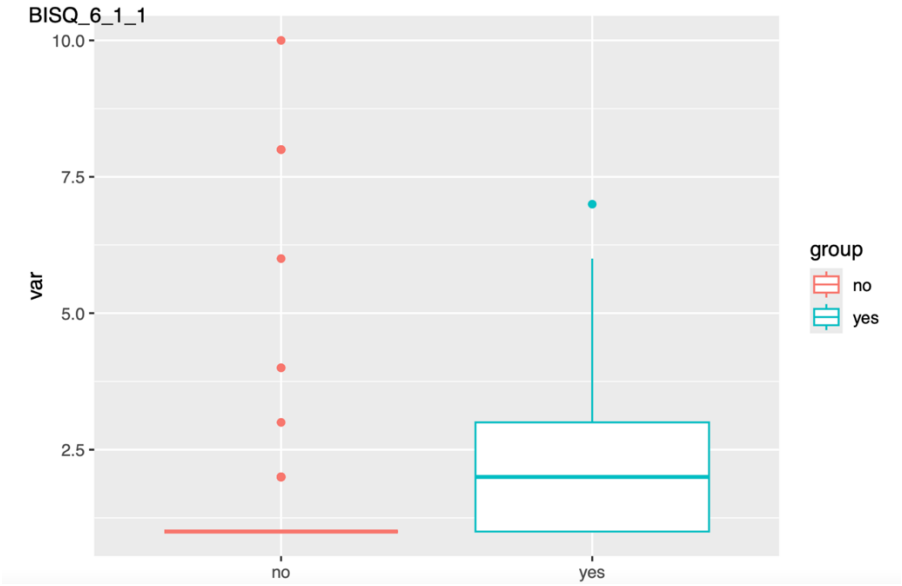
t(132) = 1.43, p = .155, d = .27, CI [-.10, .63]

ACC mean= 2.90 (1.97)
NT mean = 2.06 (1.12)

t(190) = 3.96, p < .001, d = .52, CI [.25, .78]

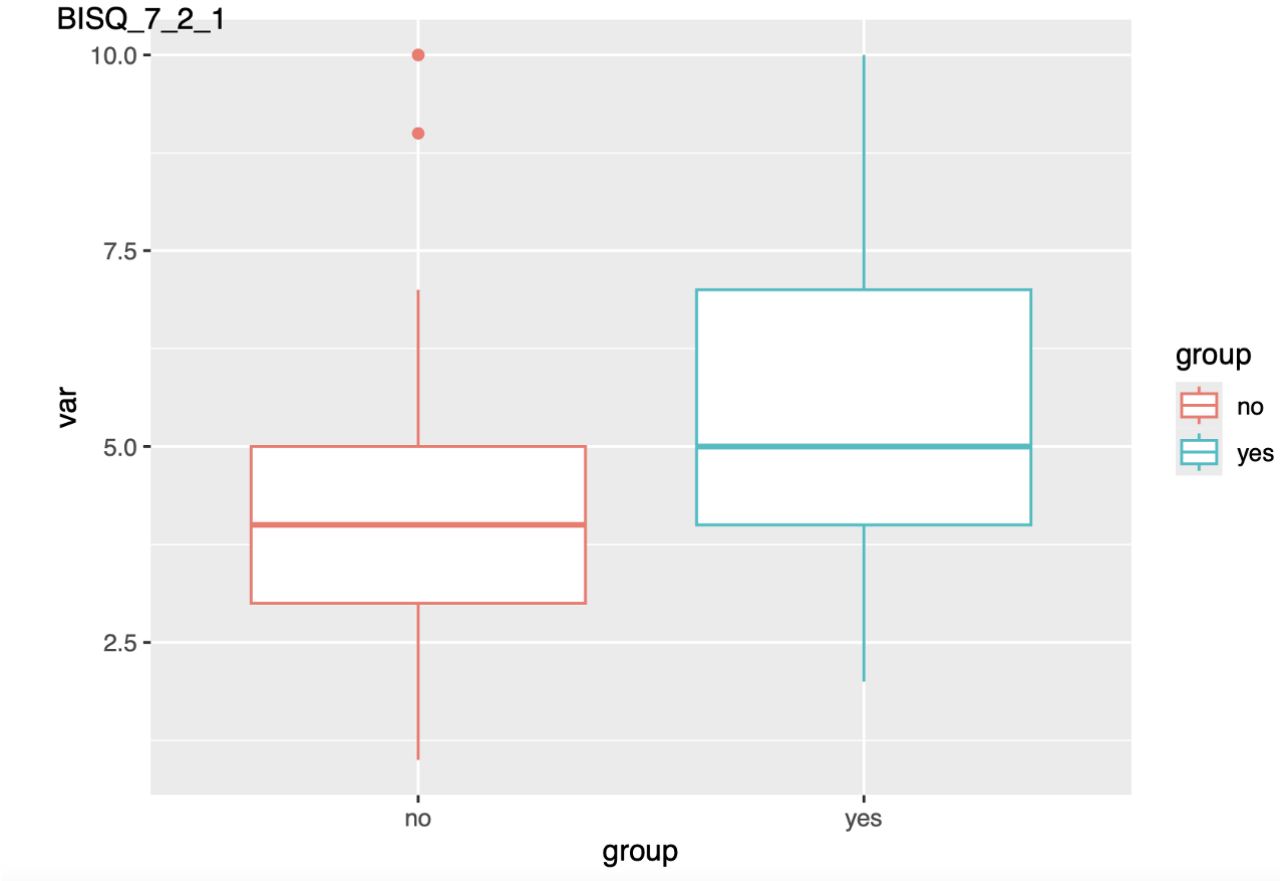
What influences the parents' perception of the child's sleep?

- Time during night spent in wakefulness



p-value: 0.0012

- Sleep latency



p-value 0.0036

Comment on preliminary results

The data suggest that there are no significant differences in sleep patterns between 6 and 12 months of age, indicating a relative stability in sleep-wake rhythms during this developmental period. However, a worsening of sleep outcomes appears to be present at 12 months when compared to typically developing controls. Notably, while sleep efficiency (the proportion of time spent asleep during the night) generally improves with age in typical development, this trend seems to reverse in patients with partial corpus callosum dysgenesis, who exhibit poorer sleep outcomes compared to those with complete agenesis. These findings raise important questions about the neurophysiological differences between CC subtypes and their specific impact on sleep development. Broadening the collection of population-level data is essential to identify the key contributors to sleep profiles, including potential genotype-phenotype correlations. Given the established link between cognitive development and sleep quality in infancy, unraveling the physiological underpinnings of these altered patterns may support the design of targeted interventions aimed at improving sleep and, consequently, the quality of life in patients with CC malformations.

4. Posterior cranial fossa malformations

In this chapter the neuroimaging pattern will be highlighted as relevant in the diagnostic pathway and in the genetic counselling presentation. The studies herein presented resulted in the following publications:

-Carrara A, Mangiarotti C, Pasca L, Politano D, Abrusco FD', Barbero VC, Carpani A, Borgatti R, Pichiecchio A, Valente EM, Romaniello R. Cerebellar Heterotopia: Broadening the Neuroradiological Spectrum of KBG Syndrome. Cerebellum. 2024 Aug;23(4):1736-1740. doi: 10.1007/s12311-024-01661-6.

- Pasca L, Arrigoni F, Romaniello R, Severino MS, Politano D, D'Abrusco F, Garau J, Giorgis V, Carpani A, Signorini S, Orcesi S, D'Arco F, Alfei E, Cattaneo E, Rognone E, Uccella S, Divizia MT, Infantino P, Valente EM, Borgatti R, Pichiecchio A. Neuroradiologic, Clinical, and Genetic Characterization of Cerebellar Heterotopia: A Pediatric Multicentric Study. AJNR Am J Neuroradiol. 2025 Jan 8;46(1):170-177. doi: 10.3174/ajnr.A8450.

- *Politano D, D'Abrusco F, Pasca L, Ferraro F, Gana S, Garau J, Zanaboni MP, Rognone E, Pichiecchio A, Borgatti R, Valente EM, De Giorgis V, Romaniello R. Cerebellar heterotopia in an 11-year-old child with KDM6B-related neurodevelopmental disorder: A case report and review of the literature. Am J Med Genet A. 2024 Jun;194(6):e63555. doi: 10.1002/ajmg.a.63555.*

-*Romaniello R, Pasca L, Panzeri E, D'Abrusco F, Travaglini L, Serpieri V, Signorini S, Aiello C, Bertini E, Bassi MT, Valente EM, Zanni G, Borgatti R, Arrigoni F. Superior Cerebellar Atrophy: An Imaging Clue to Diagnose ITPR1-Related Disorders. Int J Mol Sci. 2022 Jun 16;23(12):6723. doi: 10.3390/ijms23126723.*

4.1 Insights on ITPR1-related disorders: MRI findings as a hallmark for diagnosis

Heterozygous pathogenic variants of *ITPR1* have been associated with a broad clinical spectrum, ranging from adult-onset Spinocerebellar Ataxia type 15 (SCA15) to early-onset Spinocerebellar Ataxia type 29 (SCA29) and Gillespie syndrome, while homozygous variants have been described only in the early onset form (Klar, 2017). Both familial and sporadic cases with early-onset cerebellar ataxia associated with *ITPR1* gene mutations have been reported to date (Sasaki, 2015). According to available studies, brain magnetic resonance imaging of *ITPR1*-related disorders is characterized by cerebellar atrophy as the main finding. Extra-cerebellar findings have been rarely reported, involving both supratentorial regions and pons (Novak, 2010). Most of the studies describe a pattern of cerebellar atrophy which is more severe in the vermis than in the cerebellar hemispheres, while only one patient with a pattern consistent with Ponto Cerebellar Hypoplasia (i.e. "dragonfly" cerebellum) has been reported so far (Van Dijk, 2017). In some cases, a progression of cerebellar atrophy over time is observed. More recently, the pattern of atrophy was noted to be more severe in the superior part of the hemispheres (and vermis) than in the inferior part (McEntagart, 2016). According to these observations, we reviewed a cohort of *ITPR1*-mutated patients to better define the characteristics of cerebellar alterations. In addition, genetic analysis of the *ITPR1* gene was performed on patients with superior cerebellar atrophy at MRI to establish the value of this sign as a predictive clue for the diagnosis of *ITPR1* gene-related disorders.

Materials and Methods

Three different italian centers participated to this retrospective study: Scientific Institute E. Medea, Bosisio Parini (LC), Bambino Gesù Children's Hospital, Romeand Neurological Scientific Institute C. Mondino, Pavia. Two different groups of subjects were recruited. *Group a* included patients already diagnosed with a pathogenetic *ITPR1* gene variant through a clinical diagnostic test, while *Group b* included subjects without a genetic diagnosis, who presented a brain MRI pattern of isolated superior hemispheric and vermian cerebellar atrophy, identified through an extensive review of our neuroradiological databases. All the available images of *Group a* patients were collected and reviewed independently by two experts in pediatric neuroradiology to assess the cerebellar and cerebral findings. In particular, sagittal and coronal slices were used to evaluate the presence, distribution and severity of cerebellar atrophy in the upper and lower cerebellum. Signal alterations of cerebellar cortex and white matter as well as supratentorial findings were also investigated. When multiple studies were available, the progression of atrophy was recorded. Patients of *Group b* were first identified by querying the neuroimaging databases, selecting those patients affected by predominantly or exclusively superior cerebellar atrophy with no history of acquired conditions (e.g. infective, ischemic etc.). Images were then reviewed to confirm the pattern of cerebellar atrophy predominantly involving the superior part of the hemisphere and vermis and selected subjects underwent genetic analysis of the *ITPR1* gene. Only selected patients for whom DNA material was available were finally included in *Group b*.

For genetic studies, genomic DNA was extracted from peripheral blood of the patients and their parents. Next Generation Sequencing (NGS) analysis was performed either on targeted panels of genes causative of various forms of cerebellar ataxias (16 patients), clinical exome (1 patient) or whole exome (2 patients). The targeted gene panels were sequenced using either Nextera (Illumina) or SureSelect (Agilent Technologies) enrichment protocols and run on MiSeq or NextSeq sequencing platforms (Illumina, San Diego, California), with an expected coverage of >99% of targeted genomic regions. For clinical exome and whole exome, DNA libraries were amplified using the SureSelectXT Focused Exome (Agilent Technologies) and Twist Human Core Kit (Twist Bioscience) respectively, and sequenced on a NextSeq platform (Illumina). Bioinformatic analysis was carried out by aligning sequences to the human reference genome (GRCh37) using Bowtie2 or BWA v0.7.5. ANNOVAR and GATK Unified Genotyper were used to call variants, which were annotated through the eVANT v1.3 software (enGenome). Subsequent filtering steps allowed to exclude intronic variants, synonymous variants not affecting splicing, and variants with frequency > 1% in human variation databases. We used several *in silico* tools to predict pathogenicity of identified variants, including Deleterious Annotation of genetic variants using Neural Networks (DANN), Combined Annotation-Dependent Depletion (CADD), Polymorphism Phenotyping v2 (PolyPhen-2) and Sorting Intolerant from Tolerant (SIFT). Variants were classified according to the American College of Human Society (ACMG) guidelines. Segregation was verified by Sanger sequencing in the families. Accession numbers are the following: human *ITPR1* mRNA: NM_001168272.1; human ITPR1 protein: NP_001161744.1.

Results

Demographic data

Group a included fourteen patients (8 females and 6 males) from ten unrelated families, with average age at the last follow-up of 18 years (min 2 years; max 56 years). One patient received a diagnosis of SCA15, 11 had SCA29, while the last 2 had Gillespie syndrome.

Six patients (5 females and 1 male, all sporadic) with a superior cerebellar atrophy were initially identified but due to lack of DNA from one patient, *Group b* finally included five patients. The average age at last follow-up was 7 years (min 6 months; max 14 years). All patients had a SCA29 phenotype except patient 4 (see Table I).

Table I. Clinical, neuroradiological and genetic features of enrolled patients

Group A									
Family	**Patient**	**Gender; Age at last evaluation**	**Clinical features**	**Superior cerebellar atrophy**	**Diffuse atrophy**	**Progression over time**	**Genetics**	**ACMG Classification**	**CADD; DANN**
I	1. Proband	M; 9 years	DD, ID, hypotonia, ataxia, facial dysmorphisms, cryptorchidism	+	-	No	c2084G>A; p.S695N	Uncertain significance	20.7; 0.9919
	2. Sister	F; 7 years	DD, ID, hypotonia, ataxia	+	-	NA			
II	3. Proband*	M; 28 years	Severe motor delay, normal cognitive level, hypotonia, ataxia, postural tremor, slurred speech, nystagmus, OMA	+	-	NA	c.805C>T; p.R269W	Pathogenic (ClinVar: Pathogenic) [1]	26.399; 0.9992
	4. Mother*	F; 56 years	Motor delay, normal cognitive level, hypotonia, ataxia, slurred speech, OMA	+	-	NA			
	5. Brother*	M; 23 years	Severe motor delay, mild ID, hypotonia, ataxia, slurred speech, nystagmus	+	-	NA			
III	6. Proband	F; 2 years	Ambulation not achieved, mild ID, hypotonia, ataxia, nystagmus, bilateral iris hypoplasia	+	-	NA	c.7786-7788delAAG p.K2596del *de novo*	Pathogenic [9]	//

IV	7. Proband	M; 13 years	DD, mild ID, hypotonia, ataxia, tremor, nystagmus, OMA	-	+	Yes	c.800C>T p.T267M *de novo*	Pathogenic (ClinVar: Pathogenic) [10]	26.2; 0.9993
V	8. Proband*	F; 19 years	Severe motor delay, normal cognitive level, hypotonia, ataxia, postural tremor, slurred speech, nystagmus, OMA	-	+	NA	c.722G>A p.R241K	Pathogenic (ClinVar: Pathogenic) [1]	28.1; 0.9954
	9. Mother*	F; 42 years	Slurred speech, normal cognitive level	+	-	NA			
VI	10. Proband*	F; 29 years	Severe motor delay, moderate ID, hypotonia, ataxia, slurred speech, nystagmus, bilateral iris hypoplasia, ptosis	-	+	NA	c.7727A>T p.N2576I *de novo*	Likely pathogenic [11]	28.5; 0.9913
VII	11. Proband	M; 12 years	Hypotonia, ataxia, dysarthria, nystagmus, OMA	+	-	NA	c.805C>T; p.R269W *de novo*	Pathogenic (ClinVar: Pathogenic) [1]	26.399; 0.9992
VIII	12. Proband	F; 3 years	DD, hypotonia, ataxia, nystagmus, dysarthria, OMA	+	-	Yes	c.805C>T; p.R269W *de novo*	Pathogenic (ClinVar: Pathogenic) [1]	26.399; 0.9992
IX	13. Proband*	M; 6 years	Moderate motor delay, normal cognitive level, hypotonia, ataxia, postural tremor, slurred speech, nystagmus	+	-	NA	c.839C>A p.A280D *de novo*	Pathogenic [1]	28.2; 0.9976

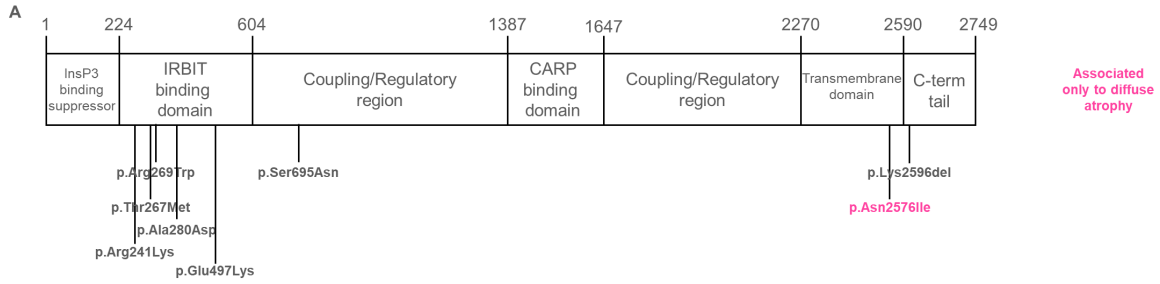
X	14. Proband*	F; 7 years	Severe motor delay, normal cognitive level, hypotonia, ataxia, postural tremor, slurred speech, nystagmus	+	-	NA	c.1488G>A p.E497K *de novo*	Likely pathogenic (ClinVar: Likely Pathogenic) [1]	29.2; 0.9994
Group B									
XI	15. Proband	F; 6 years	Severe motor delay, ataxia, hypotonia, nystagmus	+	-	NA	c.805C>T p.R269W *de novo*	Pathogenic (ClinVar: Pathogenic) [1]	26.399; 0.9992
XII	16. Proband	F; 18 months	Severe motor delay, hypotonia, ataxia, OMA	+	-	NA	c.800C>T p.T267M *de novo*	Pathogenic (ClinVar: Pathogenic) [10]	26.2; 0.9993
XIII	17. Proband	F, 14 years	progressive spastic paraparesis	+	-	NA	negative	//	//
XIV	18. Proband	F, 7 years	Moderate motor delay, moderate ID, hypotonia, ataxia, slurred speech, nystagmus	+	-	NA	c.1489G>A p.E497K *de novo*	Likely pathogenic (ClinVar: Likely Pathogenic) [1]	29.2; 0.9994
XV	19. Proband	M, 12 years	Ambulation not achieved, moderate ID hypotonia , ataxia, slurred speech, nystagmus, OMA	+	-	No	c.800C>T p.T267M *de novo*	Pathogenic (ClinVar: Pathogenic) [10]	26.2; 0.9993

*Previously published patients [1,11]; +: present; -: absent; NA : not available; DD: developmental delay; ID: intellectual disability; OMA: ocular-motor apraxia.

Genetic data

Pathogenic or likely pathogenic variants in the *ITPR1* gene were overall detected in 16 patients, while 2 siblings carried a novel variant of unknown significance (VUS) (p.S695N; CADD 20.7), which was absent from our *in house* database of over 2000 WES, as well as from the gnomAD population database (Figure 1, Table I).

A



B

MUTATION	DOMAIN	N. OF PATIENTS	RADIOLOGICAL FINDINGS
p.Arg241Lys	IRBIT binding domain	2 (familial)	Superior cerebellar atrophy (1) Diffuse atrophy (1)
p.Thr267Met	IRBIT binding domain	3 (de novo)	Superior cerebellar atrophy (2) Diffuse atrophy (1)
p.Arg269Trp	IRBIT binding domain	6 (3 familial, 3 de novo)	Superior cerebellar atrophy (5) ? (1) pt 5
p.Ala280Asp	IRBIT binding domain	1 (de novo)	Superior cerebellar atrophy (1)
p.Glu497Lys	IRBIT binding domain	2 (de novo)	Superior cerebellar atrophy (2)
p.Ser695Asn	Coupling/Regulatory region	2 (familial)	Superior cerebellar atrophy (2)
p.Asn2576Ile	Transmembrane domain	1 (de novo)	Diffuse atrophy (1)
p.Lys2596del	C-term tail	1 (de novo)	Superior cerebellar atrophy (1)

Figure 1. Genetic findings in enrolled patients. **A.** schematic representation of the position of variants within functional domains of the ITPR1 protein. Most variants are located in the IRBIT domain, suggesting that loss of the channel function impairs the IP3-induced Ca2+ release. **B.** summary of variants and radiological features found in our cohort.

In *Group a,* only the p.S695N missense variant was novel, while all other missense variants (p.R269W, p.T267M, p.R241K, p.N2576I, p.A280D, p.E497K) as well as a one-amino acid deletion (p.K2596del) had already been reported.

In *Group b*, five patients were genetically tested, of whom 4 were found to carry the following *ITPR1* previously reported missense variants: p.R269W; p.T267M (2 unrelated patients); p.E497K (Barresi, 2017).

Neuroradiological data

In Group a, 11/14 patients showed a pattern of predominant superior cerebellar atrophy (very mild to severe) while 3/14 patients showed diffuse atrophy (Figure 2).

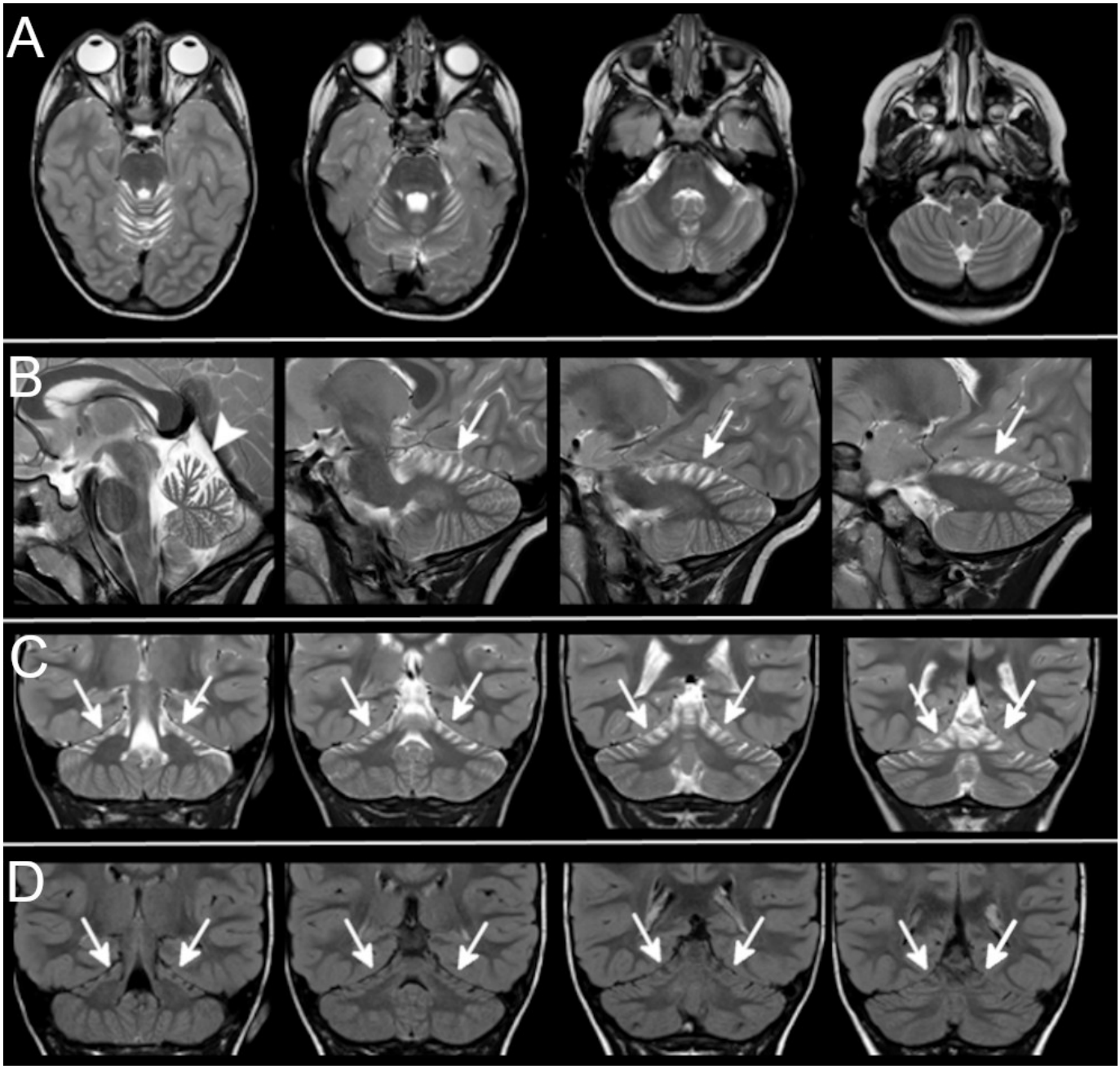


Figure 2. Superior cerebellar atrophy. Axial (A), sagittal (B), coronal (C) T2-weighted and coronal FLAIR (D) sections show the typical pattern of superior cerebellar atrophy (*Patient 17- table I*). The superior part of cerebellar hemispheres (arrows) and vermis (arrowhead) show marked atrophy with enlarged cortical CSF spaces. No cerebellar signal alterations can be detected on T2-weighted and FLAIR sections. The inferior part of the cerebellum is not atrophic and looks normal.

In cases of superior atrophy, both the upper part of the vermis and hemispheres were affected. Follow-up studies were available in three cases: in one case, diffuse cerebellar atrophy became evident between 5 months and 6 years of age (Figure 3A); in the second case, superior atrophy was not present at 8 months of age (Figure 3B) and it became evident at 3 years of age; in the last one, very mild superior cerebellar atrophy remained stable over a 4 years' period (Figure 3D). Regarding signal alterations, a very mild hyperintensity of the superior cerebellar cortex close to the vermis was noticed in three cases on FLAIR but not on T2-weighted images. The significance of this finding remains unclear as it could be partially related to artifacts at the interface between cortex and enlarged CSF spaces. Supratentorial findings were mostly normal; in two patients from the same family, a dysmorphic corpus callosum with enlarged lateral ventricles was observed.

Patients of *Group b* were retrospectively selected according to MRI reports and images. They showed in 4/5 cases a clear pattern of superior cerebellar atrophy with almost normal inferior cerebellum, and in 1/5 a diffuse atrophy, more severe in the upper cerebellum (Figure 4). One patient had a follow-up scan that did not document any progression over a 2 years' period between 12 and 14 years of age (Figure 3C).

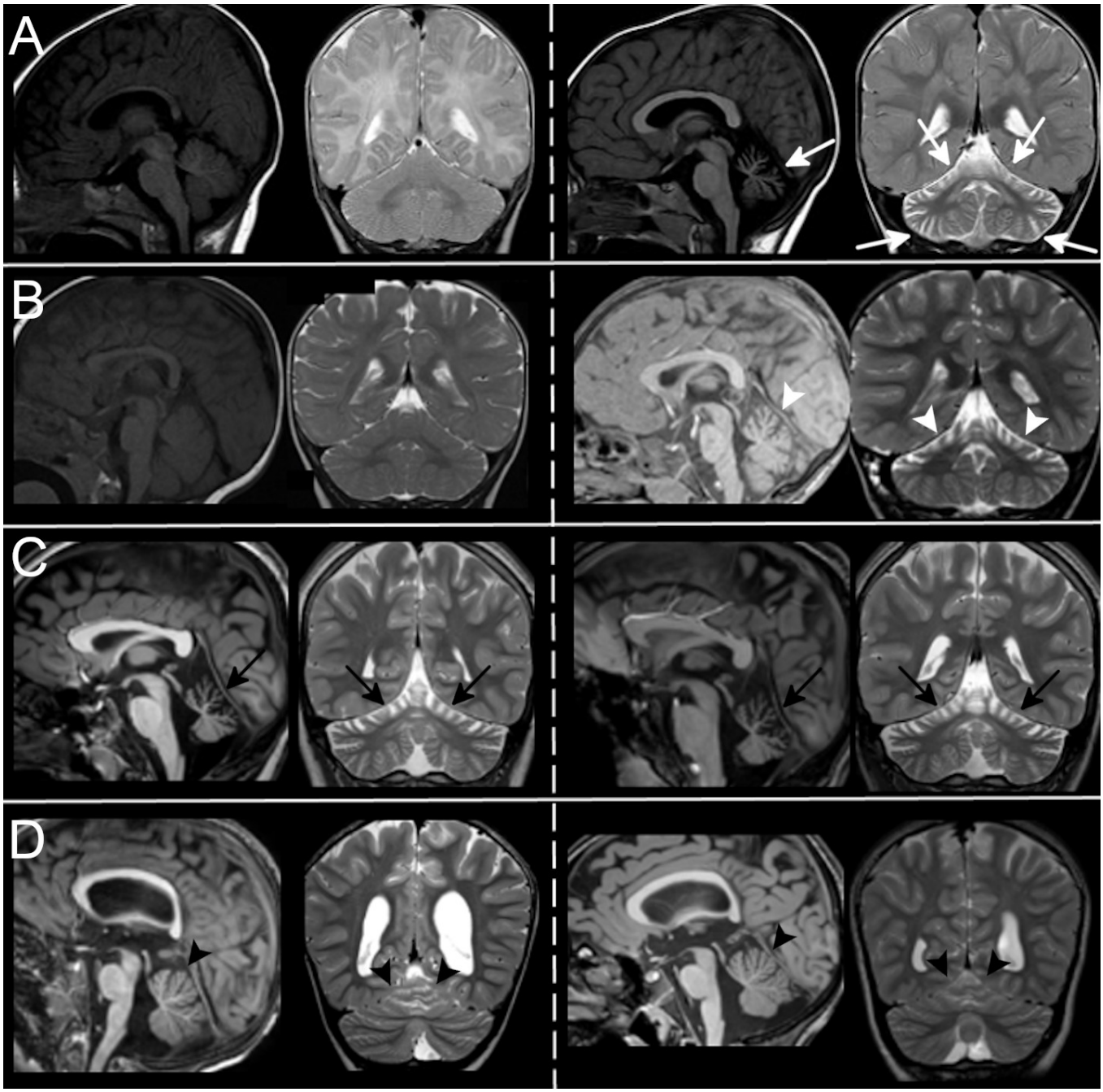


Figure 3. MRI evolution over time. In A, *Patient7- table I* has a normal cerebellum at 5 months of age (left) while he shows a diffuse cerebellar atrophy (white arrows) at 6 years of age (atrophy was also present at 2 years of age. Not shown here). In B, *Patient 12- table I* develops superior cerebellar atrophy (white arrowheads) between 8 months of age (left, normal cerebellum) and 3 years (right, cerebellar atrophy). No progression of atrophy is seen in *Patients 1 and 18- table I*, with moderate (C, black arrows) and very mild (D, black arrowheads) superior cerebellar atrophy within a 2-years and 4-years period respectively.

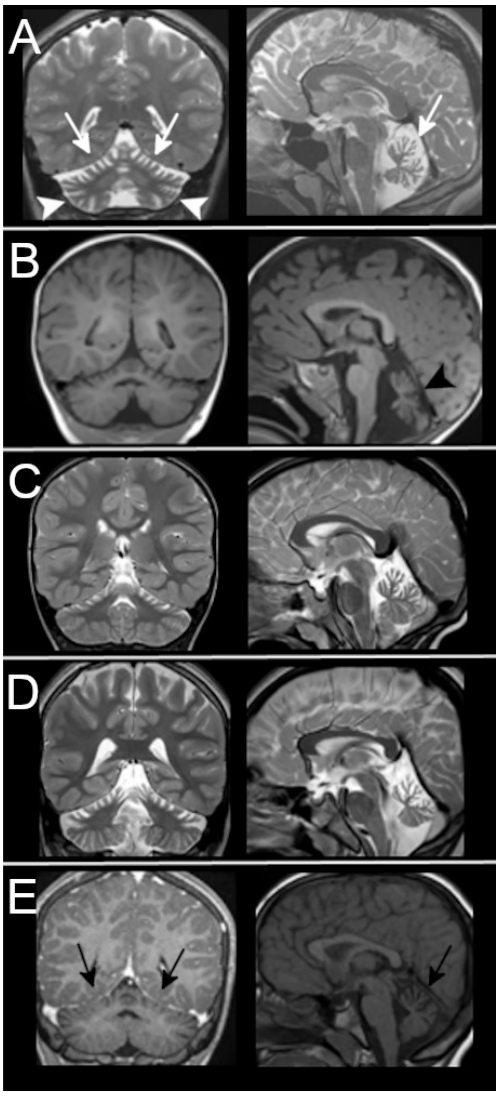


Figure 4. MRI findings in patients from group B. **MRIs of the five patients retrospectively selected according to the imaging pattern are shown here.** All of them have superior cerebellar atrophy. *Patient 14- table I* in A shows diffuse cerebellar atrophy that involves also the inferior cerebellum (arrowheads) but that is more severe in the upper part of vermis and hemispheres (white arrows). The patient with mild superior atrophy (black arrows) in E tested negative for *ITPR1* gene defects.

Supratentorial findings were unremarkable in all cases.

Neuroradiological, genetic and clinical data of all patients with *ITPR1* variants reported in the literature are summarized in Table II.

Table 1. gene mutations described up to date.

Study /Number of patients Journal	Age range	Phenotype	Infratentorial Imaging	Associated neuroradiological findings	Progression of cerebellar atrophy
Hara et al. 2008 2 families (10 affected members) ***Neurology***	NA	SCA15	Cerebellar atrophy	-	NA
Di Gregorio et al. 2010 2 families (4 affected members) ***Cerebellum***	44-81 years	SCA15 (buccolingual dyskinesias, facial myokymias, pyramidal signs)	Cerebellar vermis atrophy with a mild involvement of the hemispheres in some individuals	-	NA
Novak et al. 2010 1 family (3 affected members) ***Mov Disord***	38-56 years	SCA15	Moderate cerebellar atrophy, which preferentially involves the superior vermis	Cortical parietal and temporal atrophy	NA
Huang et al. 2012 1 family (3 affected members) ***Orphanet J Rare Dis***	5-45 years	SCA29	Mild cerebellar vermis atrophy	-	Yes
Sasaki et al. 2015 4 patients ***J Neurol***	6-12 years	SCA15, SCA29	Superior cerebellar hemispheres atrophy, vermian diffuse atrophy	Atrophy of the pontine tegmentum	Yes

Gerber et al. 2016 *Am J Hum Genet*	5 patients	1.5-18 years	Gillespie syndrome	Cerebellar atrophy	Thin CC	Yes
Mc Entagart et al. 2016 [*Am J Hum Genet*	13 patients	13-55 years	Gillespie syndrome	Atrophy mainly affecting the superior vermis, involving superior cerebellar hemispheres more than the inferior	Abnormal periventricular T2/FLAIR white matter signal adjacent to the frontal horns	Yes
Shadrina et al. 2016 *Cerebellum Ataxias*	1 family (2 members)	affected	54 years	SCA15	Mild cerebellar atrophy	- NA
Barresi et al. 2017 *Clin Genet*	4 families (6 members)	affected	7-28 years	SCA29, SCA15	Cerebellar and/or vermis atrophy	- Yes
Dentici et al. 2017 *Gene*	2 patients	2-29 years	Gillespie syndrome	Cerebellar atrophy, predominantly in the vermis	-	NA
Klar et al. 2017 *Eur J Hum Genet*	Family (6 members)	affected	17-45 years	SCA29	Cerebellar atrophy most pronounced in the vermis	- NA

				The vermis inferior is almost absent and the vermis superior showed hypoplasia with superimposed atrophy		
Van Dijk et al. 2017	1 patient	6 years	SCA29	The vermis inferior is almost absent and the vermis superior showed hypoplasia with superimposed atrophy	Hyperintensities in medulla oblungata	No
Am J Med Genet				Almost absent inferior vermis, hypoplasia with atrophy of the superior vermis		
Zambonin et al. 2017	21 patients	28m-49 years	SCA29	Cerebellar atrophy, often with superior cerebellar hemispheres and vermis	Pontine atrophy	Yes
Orphanet J Rare Dis						
Paganini et al 2018	One family (2 affected members)	6-9 years	Gillespie syndrome	Generalized atrophy, mainly-vermis atrophy		Yes
Am J Med Genet						

Synofzik et al. 2018 ***Eur J Hum Genet***	5 families (1033-80 affected members)	years	SCA15	Cerebellar atrophy with a major involvement of vermis	- NA
Wang et al. 2018 ***Cerebellum***	4 patients	6-51 years	SCA29	Cerebellar hemisphere atrophy	- NA
Stendel et al. 2019 ***Neuropediatrics***	1 patient	NA	Gillespie syndrome	Atrophy of the anterior cerebellar-vermis	NA

CC: Corpus Callosum; NA: not available; SCA: Spinocerebellar Ataxia.

Discussion

Imaging findings in *ITPR1*-mutated patients are sparsely reported in the literature, and not systematically addressed. In the available studies, the most frequently described neuroradiological feature is a diffuse cerebellar atrophy, sometimes reported as predominantly involving the vermis, and just in a minority of cases involving the superior vermis and hemispheres .

In our study population, all three *ITPR1*-related phenotypes were represented. We included both patients previously diagnosed with a pathogenic or likely pathogenic *ITPR1* variant as well as patients who were directed to *ITPR1* genetic testing due to the presence of isolated superior vermian and hemispheric cerebellar atrophy. We also included two affected siblings carrying a novel *ITPR1* variant classified as VUS (p.S695N), since the clinical and neuroradiological phenotype in both patients were highly suggestive of an *ITPR1*-related defect. Unfortunately, the family is from Morocco and parents were not available for clinical examination and segregation analysis.

Considering all *ITPR1* mutated patients, a characteristic pattern of superior vermian and cerebellar atrophy was present in 83%, while in the remaining cases (3/18, 17%) a less peculiar diffuse cerebellar atrophy was noted. We searched for specific clinical features or a different severity manifestation in patients with diffuse cerebellar atrophy, but we could not identify differences compared to patients who showed the more typical superior cerebellar involvement.

Of note, we observed a variable expression of the neuroradiological pattern among patients carrying the same *ITPR1* variant, with the only exception of carriers of the recurrent p.R269W variant, who showed a fully concordant imaging phenotype of superior cerebellar atrophy. Overall, we failed to detect reliable correlates between the protein domain harboring the mutation and the pattern of cerebellar atrophy observed (diffused vs predominantly superior). We speculate that other regulatory factors might influence the pattern of expression of the protein in the cerebellum, as already suggested by Kerkhofs et al)Kerkhofs, 2018).

Aside from the characterization of neuroimaging pattern, the analysis of *Group b* patients highlights the importance of recognizing superior cerebellar atrophy as a diagnostic clue for *ITPR1*-related disorders, since four out of five subjects presenting this peculiar imaging trait, retrospectively selected from two large imaging databases, tested positive for pathogenic variants in the *ITPR1* gene. Predominant atrophy of the upper parts of the cerebellum has never been previously associated to proven genetic conditions (Poretti, 2008), while superior vermian atrophy has been described in neonates suffering from hypoxic-ischemia. This represents a novel and key element for improving the diagnosis of children with either static or progressive cerebellar atrophy.

Based on our observations, an MRI pattern of mild to severe atrophy involving the superior part of cerebellar hemispheres and vermis (typically without any signal alterations within the cortex), with normal supratentorial brain and without history and cerebral signs of hypoxic-ischemic injury, might represent a very important insight and should prompt to suspect a possible *ITPR1* gene-related disorder and genetic testing is highly recommended.

The retrospective nature of this study represents a limit for an even more extensive definition of the imaging spectrum of *ITPR1*-mutated patients. For instance, due to the lack of seriate MRIs in all patients, we could not establish the presence and severity of atrophy at symptoms' onset, neither we could assess its progression over time. The few cases with follow-up imaging suggest that atrophy is not evident in the very first months of life, but it becomes evident during early childhood and may remain stable afterward. However, further confirmation is needed for this preliminary observation.

In conclusion, through a careful review of MRI images, we demonstrated a peculiar pattern of cerebellar atrophy in patients with *ITPR1* gene defects and we propose it as a diagnostic clue that might orient the choice of genetic testing.

4.2 Cerebellar heterotopia: a multicentric study for deep phenotyping

Cerebellar heterotopia (CH) is a neuroradiological finding characterized by the presence of abnormal areas resembling cerebellar cortex within the cerebellar white matter. In 2002, for the first time, Patel and colleagues classified CH as a subtype of focal cerebellar dysplasia (Patel, 2002). Cerebellum development is a complex process which can be summarized into four steps, namely organization of the cerebellar territory, establishment of cerebellar progenitors, migration of granule cells, and formation of cerebellar nuclei and circuitry. The pathogenesis of CH is still debated and complex, but it may be described as the result of under migration of Purkinje cells, over-migration of granule cells, impairment of programmed cell death, or a combination of these developmental mechanisms. While genetic causes have not been clearly elucidated so far, CH may result from disruptions in normal cerebellar development. In essence, CH may arise from alterations in protein function and genetic pathways that affect cerebellar migration or cell death/survival programming. For example, dysfunction of intercellular matrix proteins or vascular endothelial growth factors implicated in granule cell migration might play a role in CH determination (Almodovar, 2010). Additionally, mice with pathogenic mutations in genes encoding cellular guidance proteins may exhibit phenotypes with CH (Kuramoto, 2004). To date, no single gene has been conclusively associated with CH.

Microscopic cerebellar cell rests have commonly been observed in fetuses and newborns autopsies of infants with isolated visceral and skeletal malformations, infants with trisomy defects and infants with no obvious malformation, as reported by Rorke and colleagues (Rorke, 1968). Four histologic subtypes of cerebellar cell rests have been described and appear to be related to cerebellar localization in infants, with the majority found in children without somatic or cerebral malformations. These four types of cell rests include compact groups of mature neurons, focal and perivascular immature granular cell collections, well-organized mixed cell rests composed of all components of a cerebellar folium arranged in normal relationships (heterotopias), and poorly-organized mixed cell rests. Rorke and colleagues found that heterotopias were the least common malformation in all groups, with a higher percentage of trisomic infants. On the other hand, the discovery of macroscopic cerebellar heterotopia, detectable by magnetic resonance imaging (MRI), has been rarely reported to date, thus its prevalence is unknown. Although it can be observed as an isolated finding, cerebellar heterotopia has been mainly reported in the context of cerebellar dysgenesis and in syndromic conditions, such as trisomy 13, trisomy 18, and CHARGE syndrome (Wtight, 2019).

It is worth noting that the presence of ectopic neurons within cerebellar white matter does not appear to cause cerebellar-specific symptoms (. Therefore, a clear clinical correlation of CH is not expected.

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Aim of the study: Given the absence of a standardized description of CH, here we aim at providing a comprehensive neuroradiological, clinical, and genetic characterization of a cohort of pediatric patients with CH detectable on MRI

Methods

Recruitment and inclusion criteria

This is a retrospective multicenter study involving four Italian Centers, namely Mondino Foundation (Pavia), Medea (Bosisio Parini Lecco), Gaslini Children's Hospital (Genova) and Ospedale dei Bambini Vittore Buzzi (Milano). Written informed consent was obtained from the parents or legal representatives of all involved patients. The study complied with institutional regulations for anonymized retrospective studies and was approved by the local ethics committee of National Neurological Institute C. Mondino (N° 0099934/21).
Patients with a diagnosis of CH were selected from the neuroimaging databases of the four Centers from 2013 to 2023. CH was defined as the presence of one or more nodules with signal intensity identical to the cerebellar cortex in all sequences within cerebellar white matter. MRI exams of all patients were reviewed by three neuroradiologists (PA , MS and FA) with 15 to 25 years of experience in pediatric neurororadiology, who confirmed the presence of CH and assessed other relevant imaging findings.
Patients were subdivided into two groups according to MRI findings:
-Group A: patients with CH either isolated or associated with cerebral dysmorphisms or with malformations involving only the cerebellum
-Group B: patients with CH associated with other brain malformations (namely malformations of the cortex, posterior cranial fossa, midline)

Information regarding demographic, clinical, neuroradiological, and genetic data were collected for each patient.

Clinical examination

A standardized evaluation of clinical presentation was performed using clinical records.
The clinical data collection included: developmental history, neurological and general examination, dysmorphological evaluation, screening for extra-neurological involvement, cognitive/developmental assessment, electroencephalogram.

Genetic testing

Genetic testing i.e. karyotype, CGH array, whole exome sequencing (WES) have been variably performed in the patients, as part of their diagnostic workout. Only (likely) pathogenic variants according to American College of Medical Genetics and Genomics (ACMG) Classification guidelines were considered.

Imaging studies

Brain MRI studies were performed using both 1.5 T (7 patients) and 3T (25 patients) scanners with at least T1 and T2-weighted sections with a slice thickness of 3 mm or less. The presence of CH was evaluated , on all available image weightings. DTI weigthed sequences with reconstructions on 3 planes were available in 31/32 patients. All subjects had T2-weighted sections on at least 2 planes (axial and coronal) with a slice thickness of 1.5/3 mm. FLAIR were acquired in 31/32 patients, with 3D sequences in 11 cases. SWI or T2*-FFE sequences were available in 21 subjects while DWI/DTI data were available in 31/32 patients.
The number of nodules, their symmetry or asymmetry, and location (peripheral subcortical or in the deep white matter; in the superior or inferior cerebellum with respect to a plane passing through the horizontal fissure) were recorded in all patients.
Associated cerebral dysmorphisms or malformations were defined, also according to biometric reference measures[11,12]. The anomalies of the corpus callosum were evaluated based on the paper by Garel et al. [11]; a corpus callosum was considered thin or thick if its measurements were beyond 2 standard deviations from the normal values for age provided in the paper. A band-like corpus callosum was defined as having a uniform thickness along its entire course and lacking an isthmic indentation.

Statistics

Quantitative data were presented as mean and standard deviation or median and interquartile range, and categorical data, as frequencies and percentages.

Results

The recruited cohort included 32 patients, of whom 9 females (28%) and 23 males (72%). Mean age was 9,2 years (range 1-18 years). Demographic data and summarized clinical, genetic and imaging features are shown in table 1, while detailed clinical findings for each patient are reported in supplementary table 1.

Considering the entire cohort, heterotopic nodules were mostly located in the peripheral subcortical white matter (n=28/32; 87,5%) and typically in the inferior part of the cerebellar hemispheres (n=27/32; 84%) *Figure 1*. In one case, nodules were detected in the deep white matter and in three patients in both deep and peripheral subcortical regions. Superior location was found in four patients and superior plus inferior distribution in only one. Nodules were more frequently bilateral (n=20/32; 62,5%) and usually had a lentiform or ovoid shape, with overall length of a few millimeters. In very few cases they were lobulated with a diameter up to 10 mm. The lobulated appearance of larger lesions may be due to the presence of closely situated small nodules. In other cases, the nodules were generally limited to a maximum of 1 or 2 per patient.

According to the associated imaging phenotype, eighteen patients were assigned to group A and fourteen to group B.

Group A (n=18)

Clinical findings:

In this group, 14/18 (78%) patients had a history of developmental delay of different extent. In particular, 12/18 (67%) reported language delay. Seven (39%) patients received a diagnosis of neurodevelopmental disorder other than global developmental delay and language disorder, including specific learning disorder, autism spectrum disorder (ASD), intellectual disability, developmental coordination disorder. Abnormal behavior, embracing social problems, emotional and dysregulation difficulties, was present in the majority of the patients (12/18; 67%). Five (27%) patients presented clumsiness/ coordination problems. Specific cerebellar signs, namely oculomotor apraxia and dysmetria, and ataxic gait were observed only in two patients.

Electroencephalogram recording showed non specific, non epileptiform generalized abnormalities (n=3) and epileptiform abnormalities, associated with either focal or generalized epilepsy (n=2).

Extra-neurological signs and symptoms were rarely (n=5) detected and included not specific facial dysmorphisms, growth deficit/retardation, cardiovascular and appendicular malformations.

Genetic results:

Fourteen (78%) out of 18 patients performed genetic testing (either karyotype, search for Fragile X expansion, array CGH and/or WES) and overall a genetic diagnosis was reached in 5. Of these, four carried pathogenic *de novo* heterozygous variants in autosomal dominant genes: *ANKRD11* (c.2404_2407del; p.Leu802LysfsTer60), *ANKRD11* (c.2398_2401del; p.(Glu800Asnfs*62), *KDM6B* (c.2705del; p.Leu902HisfsTer13); and *PAK1* (c.A427G: p.Met143Val); and the remaining one had a 1,5Mb *de novo* deletion of chromosome 1p35-1p34.3.

The patients carrying a pathogenic variant of *PAK1* gene, *KDM6B* and *ANKRD11* have been previously published[13].

Imaging findings:

Nodules of cerebellar heterotopia were either monolateral (n=8; 44%) or bilateral with symmetric distribution (n=10; 55%). Localization was peripheral subcortical and in the inferior portion of cerebellar hemispheres in all patients. *See table 2*

Eight patients presented with single or multiple brain dysmorphisms / minor malformative findings associated with CH. Minor dysmorphisms of corpus callosum were observed in seven (39%) patients, including thin (n=2), thick (n=3), dysmorphic (n=1) or band-like (n=1) shape. Five (28%) patients had mild vermian hypoplasia, mainly involving the inferior vermis in three *Figure 1, Supplementary material*. Additional observed findings were a small posterior fossa (n=1), a small area of periventricular white matter damage with cavitation (n= 1), and platybasia (n=1). *No signs of cerebellar hemorrhage were identified.*

Group B (n=14)

Clinical findings:

Ten patients had a clinical diagnosis of CHARGE syndrome. The remaining four patients had the following clinical diagnosis: syndromic intellectual disability (n=2); epileptic encephalopathy (n=1) and Down syndrome (n=1). All patients had a history of developmental delay. Specific cerebellar signs were present in only one patient. An extra-neurological involvement was found in all patients.

Genetic results:

In the 10 patients with CHARGE syndrome, genetic testing confirmed the presence of pathogenic variants in the *CHD7* gene. Of the remaining four cases, three received a genetic diagnosis, including a pathogenic heterozygous variant in the *DYNC1H1* gene (c.10247_10279dup;p.Leu3416_Asn3426dup), a complex chromosomal rearrangement (4q34-qter monosomy and 13q231-qter trisomy), and trisomy 21.

Imaging findings:

In the ten patients with CHARGE syndrome, nodules of CH were mainly but not exclusively bilateral, symmetrical, and mainly located iuxtacortically and in the inferior portion of the cerebellar hemispheres.

In four patients, CH was associated with complex brain malformations: one showed dysgyric cortex, vermian hypoplasia, ectopic neuro-hypophisis, small adenohypophysis, thin optic nerves, thick lamina quadrigemina, and abnormal inner ear structures; the second one had cerebellar dysplasia, periventricular nodular heterotopia and dysmorphic temporal horns; a third presented polymicrogyria, heterotopic subependymal nodules, malrotated hippocampi and dysmorphic basal ganglia; finally, the fourth patient had pons hypoplasia. *Figure 2 and Figure 3.*

In these cases, CH was found to be either bilateral or unilateral, located in both peripheral subcortical and deep white matter and in the superior and inferior portions of cerebellum**.** No signs of cerebellar hemorrhage were identifie***d.***

Discussion

CH has received poor attention in previous hindbrain malformation classifications . Lack of awareness of this easily overlooked imaging finding may be the primary driver behind lack of identification.

This retrospective multicenter study provides a description of CH neuroradiological patterns in a cohort of pediatric patients, clinically and genetically characterized. Such comprehensive evaluation of CH imaging features and associated clinical characteristics and genotype represents a first attempt to highlight the importance of this finding in the diagnostic pathway. In fact, neurodevelopmental and functional outcome of several cerebellar malformations is far from being defined and the phenotypic spectrum is often broad, ranging from normal or near-normal functioning to profound disability for a given malformation (Bolduc, 2009). Like in other brain regions, cerebellar neuronal migration relies on appropriate spatiotemporal patterns (Rahimi, Balaei, 2018). The migration process takes place both prenatally and postnatally and is controlled by several molecules, leading to the establishment of elaborate compartments and circuitry.

Wright and colleagues in 2019 described CH as a recurrent finding in a cohort of 35 patients with CHARGE syndrome, with a prevalence of 77%. Moreover, CH has been previously reported in patients with trisomy 21 or trisomy 18 and rarely associated with other genetic conditions such as Turner syndrome, ornithine carbamoyltransferase deficiency , MKS3-related Meckel syndrome, occipital horn syndrome, OPHN1-related syndrome and Fryns syndrome in single patients. Importantly, the presence of cerebellar hemorrhages, not uncommon in subjects born prematurely, should be ruled out since could eventually be mistaken for HC; in the presented cohort no history of cerebellar hemorrhage was encountered and no signs of hemorrhage were observed on MRIs; only one patient was born preterm.

In our cohort, clinical findings of patients with CHARGE syndrome are in line with the literature, showing a pattern of CH characterized by recurrent appearance and location. More precisely, the distribution pattern mainly resulted in a bilateral, symmetrical and peripheral subcortical disposition, typically located in the inferior portion of cerebellar hemispheres. CHARGE syndrome is part of the CHD7- related disorder spectrum, caused by point mutations or deletions of the *CHD7* gene (*MIM *608892*) , which is known to be involved in embryonic development. Indeed, Reddy and colleagues demonstrated a critical role for CHD7 in the formation, differentiation and migration of neural crests . In 2013 Yu et al (Yu, 2014) showed that reduced FGF8 expression, which is a critical signal for early cerebellar development, results from CHD7 haploinsufficiency and is responsible for cerebellar vermis hypoplasia, a common finding in CHARGE. Moreover, during earlier stages of cerebellar development, CHD7 regulates the accessibility, histone acetylation and RNA Polymerase II binding at gene enhancers implicated in cerebellar morphogenesis (Reddy, 2021). Collectively, these data suggest that CHD7 governs multiple phases of cerebellar development through the accurate regulation of gene transcription; folding and migration anomalies may arise from these deregulated cellular processes that consequently reorganize themselves. Thus, unsurprisingly, CH is well represented in this genetic condition, together with vermian hypoplasia and cerebellar dysgenesis which are other common findings.

In the present study, when CH was associated with other complex brain malformations outside the CHARGE spectrum, it was coarse, localized in the deep white matter, and distributed both in the superior and inferior portions of cerebellum. Associated malformations involved both infra- and supra-tentorial brain.

A relevant contribution of the present study is the description of patients with isolated CH or CH combined with minor malformative/dysmorphic findings. In these cases, CH consistently showed peripheral subcortical localization in the inferior portion of cerebellar hemispheres, with either unilateral or bilateral distribution. Up to 35% of these patients had corpus callosum dysmorphisms, and a similar percentage showed vermian hypoplasia, mainly involving the inferior vermis. Corpus callosum dysgenesis or dysmorphisms can often be found in association with other minor malformations or brain dysmorphisms such as periventricular heterotopia (Hung, 2016) in patients with neurodevelopmental disorders and variable clinical presentation.

Inferior vermian hypoplasia (IVH) is characterized by a volumetric reduction of the inferior portion of the cerebellar vermis and patients with isolated IVH have been reported to show delayed development, gross and fine motor disabilities, as well as social-communication deficits, and behavioral problems.

In our cohort, patients with isolated CH showed high prevalence of developmental delay, with an even greater occurrence of language development delay. Unlike isolated cerebral heterotopias, which can be completely devoid of clinical correlates, cerebellar heterotopias are thus likely to be associated with at least some degree of developmental delay.

Neurodevelopmental disorders were the most represented clinical diagnoses, including intellectual disability, autism spectrum disorder, and specific learning disorder. Recurring features in the cohort were also behavioral problems including social skills impairment, and motor difficulties. Of note, specific cerebellar signs were observed only in two patients and only one patient showed extra neurological malformations. Indeed, specific cerebellar signs were not expected to be determined by the presence of CH; conversely, the imaging finding of CH can provide further insight to comprehensively assess neurodevelopmental disorders, considering the impairment of specific cerebro-cerebellar circuits that may be relevant, for instance, to the development of ASD, language and behavioral issues. And, according to the presented results, cerebellar heterotopias are most often associated with at least some degree of developmental delay

A direct comparison of clinical features of group A and group B was not carried out for a two-fold reason. Firstly, considering the complexity, the localization, and extent variability of associated brain malformations of group B as opposed to the recurrent finding of almost isolated CH in group A, we assume that in the former major events of disruption are involved in the developmental process, thus unsurprisingly leading to composite and heterogeneous syndromic phenotypes. Moreover, group B was mainly represented by the group of CHARGE patients, who do have a well-known spectrum of clinical features and the remaining 4 patients were then few and with such a heterogeneous neuroimaging finding that a mere comparison with group A was deemed as trivial.

The genetic etiology of many brain malformations remains poorly understood and the yield of genetic testing has been widely reported to be low in patients with minor cerebellar malformative findings. Nevertheless, access to standardized extensive genetic testing is not always available and this could negatively bias this outcome. The same is applicable to our cohort, in which 76% patients with isolated CH underwent some genetic testing, but a detailed assessment inclusive of whole exome sequencing was performed only in 35% representing one considerable limitation of this study, being it retrospective. Overall, a genetic diagnosis was reached in 23% of patients.

A novel *de novo* frameshift variant in the *KDM6B* gene was found in a patient with peripheral cortical monolateral inferior CH and neurodevelopmental disorder. *KDM6B (MIM *611577)* pathogenic variants have been recently described as associated with a rare "neurodevelopmental disorder with coarse facies and mild distal skeletal abnormalities' ' syndrome. Neuroradiological features in only three single patients have been reported, showing mild cerebellar cortical and subcortical atrophy and/or paracerebellar ventricular enlargement (Rots, 2023). *KDM6B* is highly expressed in cerebellar neurons, where it plays an important role in neuronal migration during development, as well as in non-neuronal cells such as Bergmann glia, which constitute the scaffold for neuronal migration (Buffo, 2013); moreover, the clinical features associated to this syndrome (e.g. hypotonia, ASD and ADHD traits) represent a link to possible cerebellar circuits dysfunction. A *de novo* pathogenic variant in the *ANKRD11* gene was found in two patients showing peripheral cortical, bilateral symmetric, inferior CH, associated with inferior vermian hypoplasia and thick CC in one case and dysmorphic CC in the other. Pathogenic variants in *ANKRD11* are responsible for KBG syndrome (*MIM *611192*), typically characterized by developmental delay, short stature, and characteristic dysmorphic findings. To date, only unspecific neuroradiological defects have been reported in KBG patients, such as white matter abnormalities, corpus callosum defects, cerebellar vermis hypoplasia (Cavuelas, 2022), cortical abnormalities including periventricular nodular heterotopia and, only recently, CH (Carrara, 2024). *ANKRD11* encodes for a protein mainly expressed in neurons and glial cells of the developing brain, playing a crucial role in proliferative processes of cortical neural precursor cells. Moreover, *ANKRD11* contributes to the global regulation of transcription, possibly modulating the expression of other genes playing a role in regulation of cortical development. Among these genes, *NCOR2 (MIM *600848)* was reported to be co-expressed in Purkinje cells with *ANKRD11* and *CHD7*. As for *CHD7, ANKRD11* might also be implicated in potential molecular and cellular mechanisms by which chromatin remodelers contribute to brain morphogenesis during development and disease.

A third patient with peripheral cortical, bilateral, inferior CH, who featured intellectual disability and epilepsy, was found to carry a *de novo* pathogenic variant in the *PAK1* gene (*MIM *618158*) gene, which encodes a member of serine/threonine p21-activating kinase family that regulates cell motility and morphology and is expressed in the cerebellum as well . Previous reports on associated brain MRI findings included single descriptions of periventricular and subcortical white matter abnormalities. All these observations, along with the emerging literature linking cerebellar functions to neurodevelopmental disorders such as ASD (Bruchage, 2018), prompt further studies to better understand the role of the above-mentioned genes in cerebellar development.

Three of the four non-CHARGE patients belonging to group B received a genetic diagnosis. One patient carried a d*e novo* variant in the *DYNC1H1* gene (*MIM *614563*), which encodes for a cytoplasmic dynein ubiquitously expressed in the brain and with functions in intracellular motility. Neuroradiological findings in patients with *DYNC1H1* mutations include cerebellar hypoplasia and dysplasia (Becker, 2020). A second patient had a complex clinical phenotype associated with trisomy 21. Cerebellar abnormalities are frequently observed in association with Down syndrome; trisomy 21 is linked with a delay in ciliogenesis, recognized as a cause of dysregulation of neuron outgrowth and cell migration (Jewett, 2023). The third patient had a complex chromosomal rearrangement involving chromosomes 4 and 13, leading to both neurological and extra-neurological multiple malformations. A clear male predominance (78%) has been observed, nevertheless up to date no X-linked mutations have been found. Even though speculative, a possible explanation could be the presence of unknown regulatory genes on X chromosomes linked to cerebellar development. In the overall cohort CH showed a higher prevalence of inferior cerebellum localization, with a clear predominance in group A. Superior CH has been described in four patients, three of whom belong to group B. The paucity of patients may reflect the relative inferior prevalence of such condition although a precise mechanism for such an occurrence remains unknown. Patients with superior CH show a more severe neurodevelopmental phenotype although genotype-phenotype correlations cannot be drawn given the reduced number of patients and the frequent association with other structural brain anomalies. One possible explanation of this finding is that in light of the different origin of superior and inferior cerebellum, distinct cell types arising from diverse subregions of a primordium can be affected by a developmental disruption, thus leading to a different contribution to the assembly of a complex three-dimensional structure, according to long fate genetic mapping of cell movements (Sagier, 2005) .Clinical phenotype may be predominantly dependent on the genetic cause of the condition although superior cerebellum, being relevant to higher cognitive function, as opposed to inferior cerebellum, mainly controlling motor function, may be one of the reasons for such clinical severity. Further studies investigating functional consequences of CH on brain function are needed. The genetic diagnostic yield was predictably higher in patients presenting CH and other major brain malformations compared to isolated CH with or without associated brain dysmorphisms. Nevertheless, overall, considering the limited access to complete genetic testing for patients with isolated CH, who underwent WES in 35% of cases, the diagnostic yield registered in the latter group of patients appears to be relevant. Given the presented results, the presence of CH in association with developmental delay and or syndromic features, might thus represent a negative prognostic sign. Moreover, the presence of a superiorly located CH seems to correlate with a more severe clinical picture: this element might be relevant for the counseling. Regarding the imaging protocol for

detecting CH, we believe that T1-weighted sequences in the coronal plane often raise the initial suspicion of heterotopia, which should then be confirmed in at least one other plane. Therefore, the use of 3D T1-weighted sequences with a voxel size of at least 1 mm is highly beneficial for diagnosis. Additionally, assessing signal intensity on other weighted sequences (particularly T2-weighted and, if available, FLAIR) is essential to determine the isointensity of the anomalies relative to the cerebellar cortex. Diffusion-weighted imaging (DWI) sequences are especially helpful in differentiating CH from small gliotic lesions that may affect the cerebellum. While the morphology and signal characteristics of heterotopias on standard morphological sequences are often highly indicative of CH, DWI can provide additional specificity, for instance, in cases of gliosis where T1 and T2 signal characteristics might overlap with those expected for gray matter. It is important to emphasize that due to the often millimetric size of cerebellar heterotopias, evaluation in multiple planes is essential to avoid missing these anomalies.

One of the main limitations of this study is indeed the incomplete availability of genetic testing in the overall cohort. Other limitations of this study are its retrospective design which precludes detailed correlation of CH with neurological deficits which does not imply causation, the relatively small population and the lack of advanced MRI techniques that could help explore the impact of CH on the overall architecture of the brain. The acquisition of high-resolution DWI data and the use of advanced modeling methods (like Constrained Spherical deconvolution) have provided interesting information on the reorganization of white matter bundles in many supratentorial malformations and could potentially be applied to this cohort to unveil structural modifications of the white matter which were not detected by standard anatomical sequences.

In conclusion, we present the most extensive sample of CH to date. Not only we do confirm CH as a recurrent feature of CHARGE syndrome, highlighting its specific distribution pattern, but we also show that CH can occur both in an isolated form, with a mainly peripheral subcortical inferiorly located pattern, or associated to minor malformative findings (mainly corpus callosum dysmorphisms and vermis hypoplasia) in patients with different phenotypes of neurodevelopmental disorders. These results confirm a possible correlation between cerebellar morphological and functional developmental disruption, underlining the relevance of taking into account the presence of CH both in the diagnostic process and in the genetic counseling.

Future studies on larger cohorts, and, possibly, a more extensive and homogeneous genetic assessment will likely provide further elements to better classify and comprehend the pathogenesis and clinical correlations to this intriguing malformation.

TABLE 1. Clinical, neuroradiological and genetic features of the cohort

	Group A (18 patients)	Group B- Non CHARGE (4 patients)	Group B- CHARGE (10 patients)
Clinical features			
Developmental delay	14/18 (78%)	4 /4 (*100%*)	10/10 (*100%*)
Language delay	12/18 (*67%*)	4/4 (*100%*)	10/10 (*100%*)
Intellectual disability	7/18 (*39%*)	3 / 4 (*75%*)	10/10 (*100%*)
<u>Neurological findings</u>			
Hypotonia	5/18 (*28%*)	2/4 (*50%*)	4/10 (*40%*)
Clumsiness/ coordination deficit	5/18 (*28%*)	2/4 (*50%*)	2/10 (*20%*)
Specific cerebellar signs	*2/18 (11%)* *Oculomotor apraxia and dysmetria, ataxic gait*	1 /4 (*25%*)	-
<u>Behavioral and psychiatric comorbidities</u>			
Social problems	4/18 (*22%*)	-	-
Externalizing problems	6/18 (*33%*)	-	1/10 (*10%*)
Internalizing problems	2/18 (*11%*)	-	-
Neurodevelopmental disorder	10/18 (*55,5%*) *Specific learning disorder (n=1); Autism spectrum disorder (ASD) (n=2); language disorder and developmental coordination disorder (n=1); language disorder (n=2); intellectual disability (n=3); global developmental delay (n=1)*	-	-

<u>Extra neurological findings</u>			
Facial dysmorphisms	2/18 (*11%)*	1 / 4 (*25%*)	-
Growth deficit/ retardation	3/18 (*17%)*	-	2/10 (*20%*)
Skeletal malformations	2/18 (*11%)*	2/4 (*50%*)	4/10 (*40%*)
Cardiovascular malformations	2/18 (*11%)*	3 /4 (*75%*)	5/10 (*50%*)
Neuroradiological features			

CH pattern	Monolateral peripheral subcortical inferior (n=8) (*44%*) Bilateral peripheral subcortical inferior (n= 10) (*55%*)	Peripheral subcortical and deep WM unilateral/bilateral superior / inferior (n=3) (*75%*) Bilateral peripheral subcortical Superior (n=1) (*25%*)	Deep WM bilateral superior (n=1) (*10%*) Bilateral peripheral subcortical inferior (n=7) (*70%*) Monolateral Peripheral Subcortical inferior (n=2) (*20%*)
Associated cerebral findings			
Vermian hypoplasia	5/18 (*28%*)	3 /4 (*75%*)	
Corpus Callosum dysmorphisms	7/18 (*39%*)	4/4 (*100%*)	10/10 (*100%*)
Other	-	-Dysgiric cortex, vermian hypoplasia, ectopic neuro-hypophisis, small adenohypophysis, thin optic nerves, thick lamina quadrigemina, and abnormal inner ear structures; -cerebellar dysplasia, periventricular nodular heterotopia and dysmorphic temporal horns; -polymicrogyria, heterotopic subependymal nodules, malrotated hippocampi and dysmorphic basal ganglia; -pons hypoplasia	4/10 (*40%*) Inner ear malformation; olfactory nerve hypoplasia; coloboma; brainstem hypoplasia; Cerebellar dysplasia
Genetic features			

Pathogenic variants	5/18 (*28%*)	3 /4 (*75%*)	10/10 (*100%*)

ADHD= Attention Defict Hyperactivity disorder ; ASD= Autism spectrum disorder; CH= Cerebellar Heterotopia; WM= White matter

TABLE 2. Neuroradiological findings of the cohort

Patient	Group	CH Pattern	Corpus Callosum	Vermis	Other findings
1	A	Peripheral subcortical monolateral, inferior	-	Inferior vermian hypoplasia	-
2	A	Peripheral subcortical bilateral symmetric, inferior	-	-	-
3	A	Peripheral subcortical bilateral symmetric, inferior	Thick	Vermian hypoplasia	Platibasia
4	A	Peripheral subcortical bilateral symmetric, inferior	Thin	Inferior vermian hypoplasia	-
5	A	Peripheral subcortical , monolateral, inferior	-	-	-
6	A	Peripheral subcortical l, monolateral, inferior	band-like shape	-	-

7	A	Peripheral subcortical bilateral superior	-	-	Small periventricular cavitation
8	A	Peripheral subcortical bilateral inferior	-	-	-
9	A	Peripheral subcortical monolateral (right) inferior	-	-	-
10	A	Peripheral subcortical monolateral (right) inferior	-	-	-
11	A	Peripheral subcortical monolateral (left) inferior	-	-	-
12	A	Peripheral subcortical monolateral (left) inferior	Thin	Mild vermian hypoplasia	Small posterior fossa
13	A	Peripheral subcortical bilateral inferior	-	-	-

14	A	peripheral subcortical monolateral (left) inferior	-	-	-
15	A	peripheral subcortical bilateral inferior	-	-	-
16	A	Iuxtacortical bilateral inferior	Thick	-	-
17	A	peripheral subcortical bilateral inferior	Thick	Inferior vermis hypoplasia	-
18	A	peripheral subcortical bilateral inferior	Dysmorphic	-	-
19	B - CHARGE	Deep WM bilateral superior	Dysmorphic	Vermis hypoplasia	Inner ear malformation Brainstem hypoplasia
20	B – CHARGE	Peripheral subcortical bilateral inferior	Dysmorphic	Vermis hypoplasia	Inner ear malformation Olfactory nerve hypoplasia Brainstem hypoplasia
21	B- CHARGE	Peripheral subcortical monolateral (right) inferior	-	Vermis hypoplasia	Inner ear malformation Brainstem hypoplasia

22	B – CHARGE	Peripheral subcortical bilateral inferior	-	Vermis hypoplasia	Inner ear malformation Olfactory nerve hypoplasia Dysmorphic hippocampi Brainstem hypoplasia
23	B-CHARGE	Peripheral subcortical bilateral inferior	-	Vermis hypoplasia	Inner ear malformation Olfactory nerve hypoplasia Ventricular dilatation
24	B - CHARGE	Peripheral subcortical monolateral (right) superior	-	Mild vermis hypoplasia	Inner ear malformation Ventricular dilatation
25	B - CHARGE	Peripheral subcortical bilateral inferior	-	Vermis hypoplasia	Inner ear malformation Olfactory nerve hypoplasia

26	B-CHARGE	Peripheral subcortical bilateral inferior	Thin CC	Vermis hypoplasia	Pontine hyoplasia Cerebellar dysplasia Hypoplastic clivus Inner ear malformations Ventricular dilatatation Persistent trigemina artery Dysmorphism of hippocampi Olfactory nerve hypoplasia
27	B – CHARGE	Peripheral subcortical bilateral inferior	Mild thinning CC	Vermian hypoplasia	Pons hypoplasia Right carotid severe hypoplasia Inner ear malformation Olfactory nerves agenesis

28	B – CHARGE	Peripheral subcortical bilateral inferior		Vermian hypoplasia	Pons hypoplasia Skull base malformation Malformed hypothalamus Inner ear malformation Olfactory nerves agenesis
29	B	Peripheral subcortical and deep unilateral inferior	Thin	Inferior vermian hypoplasia	Dysgiric cortex ectopic neurohypophisis and small adenohypophysis thin optic nerves Thick lamina quadrigemina abnormal inner ear structures
30	B	Peripheral subcortical and deep white matter / unilateral, superior and inferior	Thin	-	Cerebellar dysplasia Periventricular nodular heterotopia Dysmorphic temporal horns
31	B	Peripheral subcortical bilateral superior	dysplastic CC and small anterior commissure	mild vermian hypoplasia	Bilateral polymicrogyira; heterotopic subependymal nodules, malrotated hippocampi, dysmorphic basal ganglia

32	B	Peripheral and subcortical deep WM bilateral inferior	Thin	Vermian hypoplasia	Pons hypoplasia cerebral atrophy

Group A : isolated CH; Group B : CH associated with other brain malformations

CC: corpus callosum; CH: cerebellar hypoplasia; WM= white matter

FIGURES

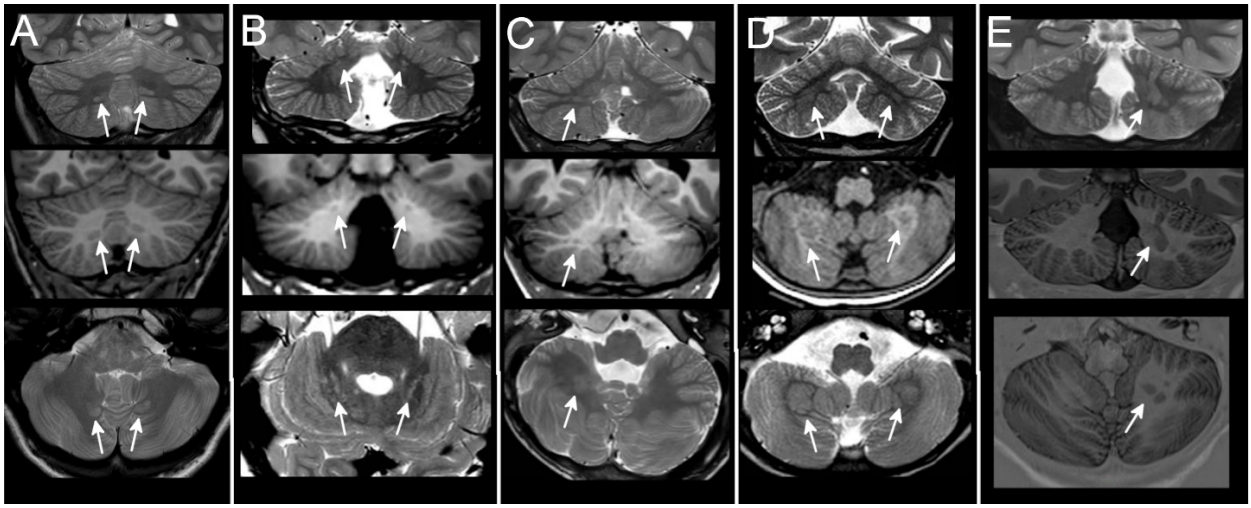
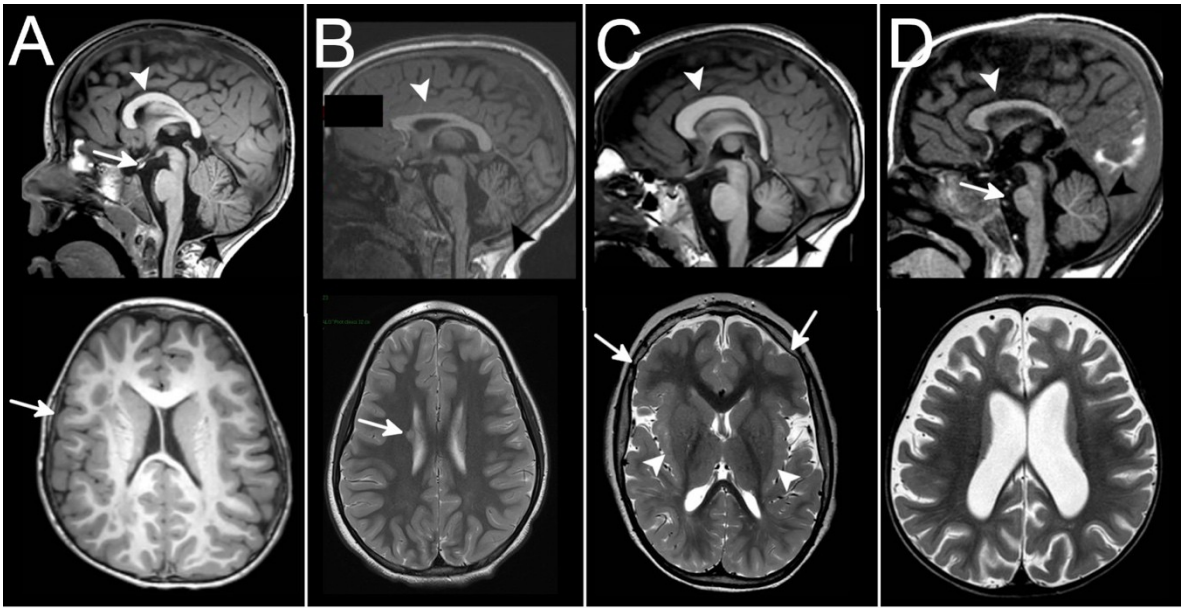


FIGURE 1. Pattern of cerebellar heterotopias

In the top row, coronal T2 sections are shown; in the middle row, coronal and axial T1 sections are presented; while in the bottom row, axial T2 sections are illustrated, except for the patient in E, where an axial IR section is shown.

Inferior bilateral (A), and superior bilateral (B) small nodules in the peripheral subcortical white matter are shown. Bigger nodules could be unilateral (C) or bilateral (D) in the deep white matter of the inferior cerebellum or they could be located in both the inferior and superior part of the hemisphere (E). In all sequences, the cerebellar heterotopia nodules exhibit signal intensity isointense to the cortex and show no signs of edema.

Figure 2. Associated brain findings in group B, other than CHARGE



T1-weighted sagittal and T1 and T2-weighted axial sections are shown

The 4 patients of group B not affected by CHARGE showed in all cases complex dysmorphisms of the corpus callosum (white arrowheads in A-D) and hypoplasia or dysplasia of the vermis and cerebellum (black arrowheads in A-C).

Patient A also had an ectopy of the neurohypophysis (white arrow in the upper panel) and a pattern of dysgiric cortex (white arrow in the lower panel). Patient B had an heterotopic periventricular nodule (white arrow) while patient C showed polymicrogyria (white arrows) and abnormal basal ganglia (white arrowheads in the lower panel). Patient D had severe pons hypoplasia and diffuse cerebral atrophy (related to a cardiovascular event)

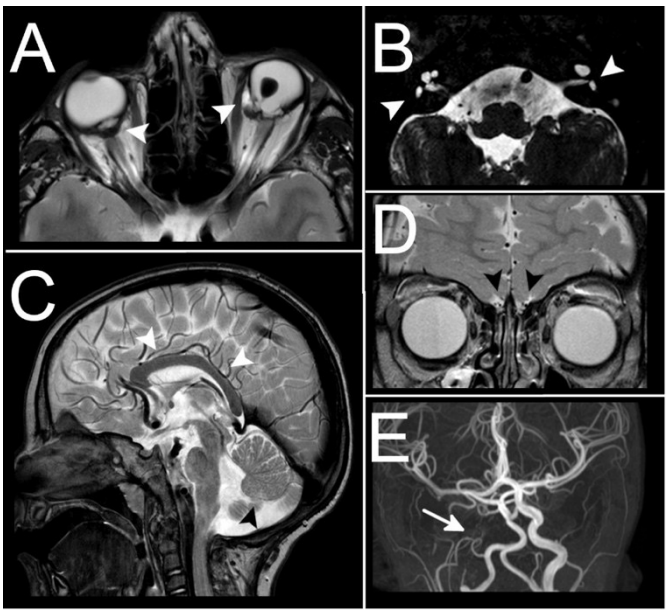


Figure 3. Common findings in CHARGE patients

As part of the spectrum, CHARGE patients could show colobomas (white arrowheads in A), malformation of the inner ear and semicircular canals (white arrowheads in B), vermian hypoplasia (black arrowhead in C), corpus callosum dysmorphisms and thinning (white arrowhead in C) and thinning/agenesia of the olfactory tracts (black arrowheads in D). One patient also had an agenesis of the right carotid artery (white arrow in E)

Examples of clinical case report of CH as a phenotypic clue driving genetic examination

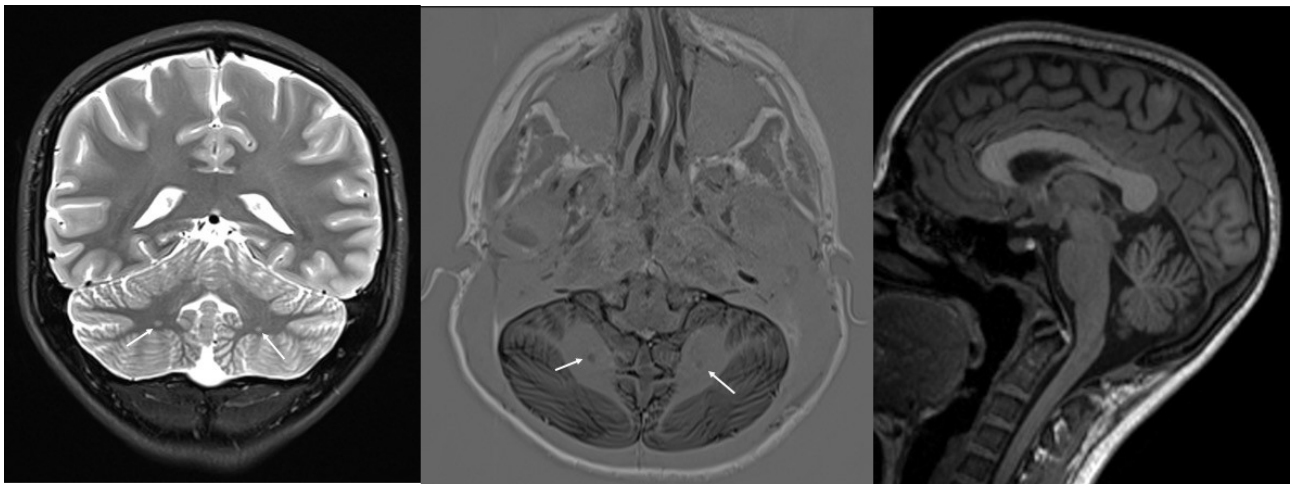
Case report 1

The patient is a girl aged 12 years and 11 months, first child of unrelated parents, born at term after a physiological pregnancy. She presented a length of 49 cm (37th percentile), a weight of 2,780 kg (13th percentile) and a head circumference of 32 cm (5th percentile); Apgar score was within normal range. Feeding problems were detected from the second year of life. She later showed growth failure with length and weight parameters settling below the third percentile. Motor developmental milestones were referred as normally attained. A mild language delay became evident with two-word phrases composition after two years of age. During school age, some difficulties in visuo-spatial, fine motor coordination and school learning were observed. Wechsler Intelligence Scale for Children-IV (WISC-IV) was performed at 12 years, showing a cognitive level at the lower normal range (FIQ=89), with a relative working memory and executive functions impairment. Speech and psychomotor rehabilitation associated to special education were implemented. Adaptive functions were characterized by poor daily living skills and socialization. In addition, emotional dysregulation and externalizing behavior were observed. At the same age, a dysmorphological and neurological examination detected facial dysmorphisms characterized by bushy eyebrows and micrognathia, and bilateral nystagmus in extreme laterality of gaze. At brain MRI, two nodules of cerebellar heterotopia, mild vermian cerebellar hypoplasia and thick corpus callosum were observed (see Figure 1). Moreover, skull platybasic appearance, hypoplasia of the occipital condyles and axis dysmorphism were documented. WES detected a pathogenic *de novo* deletion c.2404_2407del (p.Leu802LysfsTer60) in exon 9 of *ANKRD11*, leading to a diagnosis of KBG syndrome (see Figure 2).

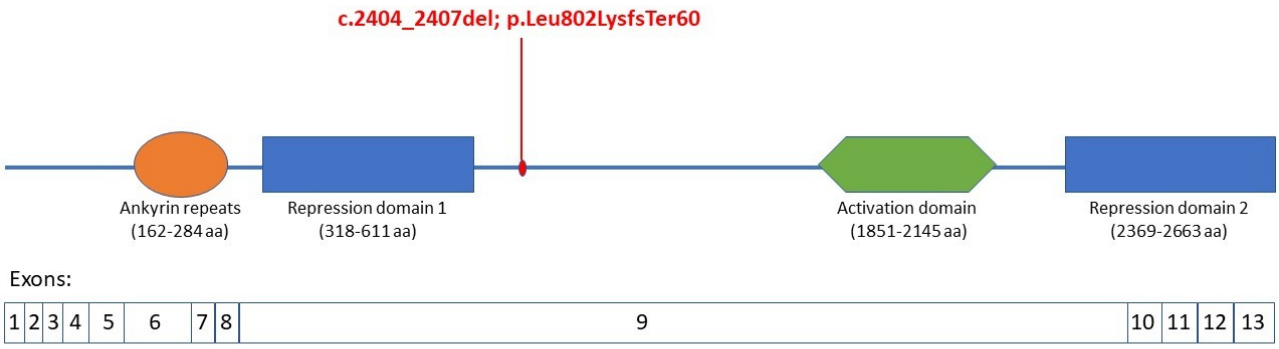
Discussion

KBG syndrome is caused by haploinsufficiency of *ANKRD11* (Cayuelas et al., 2022; de Boer et al., 2022; Goldenberg et al., 2016; Swols & Tekin, 2022). A limited number of subjects with KBG syndrome underwent neuroradiological examination, showing unspecific and inconstant supratentorial findings such as cortical malformations (e.g. periventricular nodular heterotopia and focal cortical dysplasia), hippocampal malrotation, corpus callosum hypoplasia and unspecific white matter defects; in addition, posterior fossa anomalies including Dandy-Walker malformation and cerebellar vermis hypoplasia have also been occasionally reported (Cayuelas et al., 2022; Goldenberg et al., 2016; Lo-castro et al., 2012; Low et al., 2016; Oegema et al., 2010; Scarano & Graziano, 2019; Zollino et al., 1994). Brain imaging in our patient showed corpus callosum dysmorphisms and cerebellar vermis hypoplasia which, for the first time in a KBG patient, were associated to a neuroradiological pattern consistent with cerebellar heterotopia. This is a congenital malformation characterized by the presence of clusters of neurons within the white matter of cerebellar hemispheres, and it is classified among disorders of cerebellar foliation, more precisely in the subgroup of rhombomere 1 anomalies (Barkovich et al., 2009). Cerebellar heterotopia can involve all cortical layers, sometimes an entire folium adjacent to normal folia. (Rorke et al., 1968). Despite its malformative nature suggests a genetic origin leading to a faulty programming of cerebellar development, no causative genes responsible for CH have been identified to date (Rorke, 1994).

ANKRD11 has a unique protein structure containing two repression domains and one activation domain (Bestetti et al., 2022; Zhang et al., 2004) and it is mainly expressed in neurons and glial cells of the developing brain, playing a crucial role in proliferative processes of cortical neural precursor cell (Gallagher et al., 2015; Ka & Kim, 2019; Zhang et al., 2004). It was shown that ANKRD11 regulates pyramidal neuron migration and dendritic differentiation in the developing mouse cerebral cortex, and indeed its knockdown resulted in delayed radial migration of cortical neurons, significantly reduced branching and dendrite growth and abnormal dendritic spine morphology (Ka & Kim, 2019). *ANKRD11* contributes to the global regulation of transcriptional process in neural precursors, possibly modulating the expression of other genes playing a role in regulation of cortical development (Gallagher et al., 2015). Among these genes, *NCOR2* (Nuclear Receptor Corepressor 2) was reported to be co-expressed in Purkinje cells with *ANKRD11* and *CHD7* (Chromodomain Helicase Dna-Binding Protein 7) mutated in CHARGE syndrome (Coloboma, Heart, choanal Atresia, Retardation, Genital and Ear anomalies), which has also been recently associated with symmetrical heterotopia in subcortical white matter of bilateral inferior cerebellar hemispheres (Persson et al., 2021; Vissers et al., 2004; Wright et al., 2019). All these observations, along with the emerging literature linking cerebellar functions to neurodevelopmental disorders such as ASD (Bruchhage et al., 2018), prompt further studies to better understand the role of *ANKRD11* gene in cerebellar development.



Brain MRI of the proband



The ANKRD11 variant of the proband

Case report 2

The patient, a 11-year-old boy, is the first child of unrelated healthy parents, born via cesarean section at the 38th week of gestation after a regular pregnancy. Auxological parameters and Apgar score at birth were normal. Neonatal period was characterized by gastroesophageal reflux persisting in the first months of life. Developmental milestones were reached regularly except for independent walking, acquired at 18 months of age. At 4 years, he experienced pediatric obstructive sleep apnea during sleep due to adenotonsillar hypertrophy. He underwent surgery for right inguinal hernia at 5 years; an alopecia areata episode occurred during infancy and spontaneously resolved; mild tricuspid insufficiency was also described. At the age of 9 years and 5 months, the patient experienced one isolated epileptic seizure lasting 15-20 seconds characterized by loss of consciousness and generalized hypertonia. At neurological examination, only clumsiness was observed. A prominent forehead and mild synophrys, broad fingertips, ligamentous laxity, prominent spine lordosis, bilateral genu valgum and pronated feet were also evident. Neuropsychological profile was characterized by normal cognitive level (full intelligence quotient at WISC-IV was 88), executive functions deficits (impulsivity, shorter attention span) and dysgraphia according to age related neuropsychological tests. Low frustration tolerance, irritability, oppositionality and social-relational abnormalities (poor eye contact and difficulties in making new friends) and attention deficit hyperactivity disorder (ADHD) traits were also noted. Electroencephalogram showed diphasic wave-and-spike abnormalities during sleep as well as a generalized slowing and punctate activity affecting the left center-temporal leads. Brain MRI revealed a single subcortical cerebral heterotopia nodule in the right inferior cerebellar hemisphere (Figure 1). WES analysis revealed the novel heterozygous *de novo* c.2705del (p.Leu902HisfsTer13) variant in exon 11 of *KDM6B* classified as pathogenic according to ACMG criteria (PVS1, PM2, PS2) (Richards et al., 2015) (Figure 1S).

The main genetic, clinical and neuroradiological findings associated to *KDM6B* mutated patients described in literature to date are summarized in Tables 1 and 1S where only patients bearing (likely) pathogenic *KDM6B* variants were included.

Discussion

"Neurodevelopmental disorder with coarse facies and mild distal skeletal abnormalities" caused by heterozygous pathogenic variants in *KDM6B*, is a recently described syndrome characterized by a set of clinical features such as gastroesophageal reflux disease, non-specific facial dysmorphisms, ligamentous laxity, hypotonia, language delay, motor delay, cognitive impairment, sleeping problems. ADHD and ADHD traits, autism spectrum disorder (ASD) and ASD traits, psychosis, epilepsy and behavioral problems are also described (Insa Pineda & Gómez González, 2022; Rots et al., 2023; Stolerman et al., 2019).

In humans, *KDM6B* is a gene located on the long arm of chromosome 17 (17q21.32). It contains 22 exons with the start codon located at the level of exon 4 (Stolerman et al., 2019), encoding for a histone demethylase containing the Jumonji C (JmjC) domain. The JmjC domain is the protein catalytic domain that specifically demethylates di- or tri-methylated lysine 27 on histone H3 (H3K27) leading to the activation of gene expression together with a zinc motif domain (Jones et al., 2018; Patel et al., 2002). *KDM6B* is known to be involved in the mechanisms of differentiation of multiple cell types during development such as endodermal (Kartikasari et al., 2013) and T-cell differentiation (Li et al., 2014), mesodermal and cardiovascular differentiation from embryonic stem cells (Ohtani et al., 2013), osteogenic programming of embryonic cells (Yang et al., 2013; Ye et al., 2012) and neuronal cells fate specification (Wijayatunge et al., 2014, 2018). The effect of *KDM6B* haploinsufficiency on neurodevelopment has also been studied in animal models and led to ADHD/ASD traits (Gao et al., 2022).

In line with the reported *KDM6B* phenotype, our patient presented with mild motor delay, but he didn't show language impairment or intellectual disability (ID), common clinical presentations of the disease. He also showed ligamentous laxity, hypotonia, ASD and ADHD traits, reported in about half of the patients(Insa Pineda & Gómez González, 2022; Rots et al., 2023; Stolerman et al., 2019). In addition, our patient's medical history was positive for gastroesophageal reflux disease and a single episode of generalized-onset seizure, findings already described in a small subset of patients(5). Dysgraphia has never been described in association with *KDM6B* variants, while a specific learning disorder, dyslexia, was already described once in a proband's affected father (Rots et al., 2023).

Only non-specific neuroradiological features in single patients have been reported in this condition, while normal brain MRI was reported in most patients (Rots et al., 2023; Stolerman et al., 2019). Our patient's brain MRI revealed the presence of a single isolated cerebellar nodular heterotopia never described to date. Cerebellar involvement was described at brain MRI in three single patients respectively showing: cerebellar mild cortical atrophy, subcortical cerebellar atrophy and paracerebellar ventricular enlargement (Rots et al., 2023). Cerebellar heterotopias, categorized by Patel and Barkovich among isolated cerebellar hemispheric dysplasia (Patel & Barkovich, 2002), are nodules of neurons abnormally located in the cerebellar white matter due to an alteration in neuronal migration during cerebellar development (Laure-Kamionowska & Maślińska, 2011). To date, cerebellar heterotopias have been reported as recurrent findings in CHARGE syndrome (Wright et al., 2019) and trisomy 21 (Battin, 1986; Laure-Kamionowska & Maślińska, 2011), they have sometimes been described in trisomy 18 (Battin, 1986) and rarely associated, almost always in single patients, to other genetic conditions such as Turner syndrome (Laure-Kamionowska & Maślińska, 2011), ornithine carbamoyl transferase deficiency (Harding et al., 1984), MKS3-related Meckel syndrome (Adams et al., 2012), occipital horn syndrome (Palmer & Percy, 2001), OPHN1-related syndrome (Rocas et al., 2013) and clinically diagnosed Fryns syndrome (Clark & Fenner-Gonzales, 1989; Slavotinek, 2004). Of note, *KDM6B* is highly expressed in cerebellar neurons, where it plays an important role in the main genetic pathways involved in mechanisms of cerebellar neurons migration during development such as SDF-1/CXCR4 (Huang et al., 2014), BDNF/TrkB (Borghesani et al., 2002) and SHH signaling (Lewis et al., 2004; Liu et al., 2022; Shi et al., 2014), as well as in non-neuronal cells such as Bergmann glia (Wijayatunge et al., 2018), which constitutes the scaffold for neuronal migration (Buffo & Rossi, 2013).

The patient herein reported carries a novel *de novo* frameshift pathogenic variant in exon 11 of *KDM6B*; truncating variants represent the prevalent type of genetic alteration, and appear to be scattered throughout the gene, while missense and inframe indel variants were found in the protein critical sites of activity. No specific genotype-phenotype correlations have emerged from the analysis of the patients' clinical features in the literature (Rots et al., 2023).

Pathogenic variants in other genes encoding proteins constituting the histone modification machinery, particularly demethylases and methylases, have been associated with various NDDs (Kim et al., 2017). Lavery et al. described the phenotypic similarities among an emerging class of congenital regulopathies, in particular Kleefstra syndrome type 1 and 2, Kabuki syndrome type 1 and 2 and Rubinstein-taybi syndrome type 1 and 2, arising from alteration in *KMT2C, KMT2D, EHMT1, KDM6A, CRBBP,* and *EP300* genes respectively, through the physical and functional association of these proteins in the KMT2C/D COMPASS complex which combines to another complex, the WRAD complex (composed of RbBP5, ASH2L, DPY30 and WDR5) to fulfil its role. (Lavery et al., 2020). The lysine demethylase KDM6B has been found to co-precipitate with the WRAD-composing proteins (De Santa et al., 2007) and to be highly homologous and functionally relatable to KDM6A, which is part of the KMT2C/D COMPASS complex (Agger et al., 2007). Even though this hypothesis needs further proof, given the well-established regulatory activity of the protein on gene expression via histone post-translational modification, it can be speculated that *KDM6B*-related NDD may represent another congenital regulopathy.

In conclusion, our patient findings strengthen but also broaden the phenotypic and genotypic spectrum for this condition since already described clinical features intermingle with yet undescribed findings such as dysgraphia, and cerebellar heterotopia.

Figure 1. Coronal Inversion Recovery (IR) and T2-weighted images showing a thin elongated signal alteration, likely of gray matter nature in all sequences shown, located in the right inferior cerebellar hemisphere (white arrow), compatible with an isolated cerebellar heterotopic nodule.

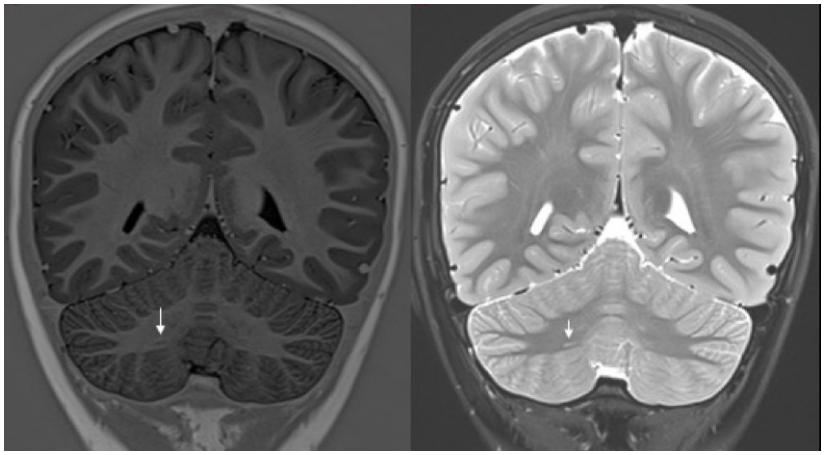


Table 1. Most relevant clinical features of reported patients and our case

Characteristics of literature patients	Number of patients, %	Our patient
De novo inheritance	64 /73, 88%	+
Language delay	72 /78, 92%	-
Motor delay	67 /75, 89%	+
Aspecific facial dysmorphisms	61 /70, 87%	+
ID/learning problems	42 /65, 65%	-
ASD/ASD traits	51 /79, 65%	+
Hypotonia	40 /72, 56%	-
Neonatal feeding difficulties	25 /63, 40%	-
Ligamentous laxity	27 /63, 43%	+
ADHD/ADHD traits	31 /73, 42%	+
Nonspecific MRI abnormalities	15 /46, 33%	+
Sleeping problems	21 /68, 31%	+
Gastroesophageal reflux disease	18 /64, 28%	+
Broad fingers/fingertips/hands/toes/feet	18 /67, 27%	+
Constipation	12 /63, 19%	+
Epilepsy/Seizures	11 /70, 16%	+
Cerebellar MRI abnormalities	4 /46, 9%	+
Psychosis in patients more than 12ys	4 /61, 7%	-
Elbow hypertrichosis	Reported once	-

Abbreviations: ADHD, Attention Deficit Hyperactivity Disorder; ASD, Autistic Spectrum Disorder; ID, intellectual disability; MRI, Magnetic Risonance Imaging; ys, years.

4.3. Long term clinical and neuropsychological outcome of prenatal vermis malrotation

4.3.1 Study protocol and preliminary results

Cerebellum develops very slowly according to a process that begins at the 3rd week of gestation until at about 20 months of postnatal life. This results in a high vulnerability since various agents, genetically based and/or acquired, can impair its development. Cerebellar malformations may be the result of early destruction related to acquired factors or from altered programming of development secondary to genetic alterations. A correct distinction between these two etiopathogenetic categories is of great importance for proper genetic counseling. In parallel, an increasingly detailed study of cognitive and neuropsychological abilities as well as the description of different behavioral phenotypes, has led to a better understanding of the relationship existing between the lesions found on neuroradiological investigation and cognitive-behavioral profiles. Indeed, the cerebellum contains numerous afferences and efferences involved in various functional systems, including affective, cognitive and motor processing (Dovjak, 2019).

Hindbrain malformations comprise a group of genetically and developmentally diverse disorders the clinical manifestations of which range from normal neurocognitive development to severe psychomotor delay (Parisi and Dobyns, 2003; Barkovich et al, 2009). The most crucial time window, in terms of outcome, for cerebellar disruption of any etiology is the perinatal and early postnatal period (Sathyanesan et al., 2019). During this time, the cerebellum undergoes a structural refinement, involving granule cell migration, Purkinje cell (PC) dendritic arborization, and synaptogenesis; any event perturbing this critical period can potentially affect cerebro-cerebellar circuits with long-term effects on neurodevelopmental trajectories (Badura et al., 2018; Stoodley, 2016). When the disruption occurs early during development, cerebellar damage associates with cognitive deficits and behavioral disorders. Neurodevelopmental and functional outcome of several cerebellar malformations are far from being defined and the phenotypic spectrum is often broad, ranging from normal or near-normal to profound disability for a given malformation. It is now well recognized that cerebellar damages could not only hesitate in sensorimotor impairments but also in a broader spectrum of non-motor deficits, including cognitive, linguistic, and socio-affective behavioral changes. Indeed, through the cerebellar peduncles, the cerebellum is connected to other cortical and subcortical brain structures giving rise to an integrative brain system that supports execution of complex behavioral sequences.

With an increasingly recurrent diagnosis of cerebellar and posterior cranial fossa malformations performed prenatally, often the imaging data cannot be associated to a defined postnatal prognosis and thus an effective counselling (Boltshauser, 2004; Triulzi et al, 2006; Chapman et al, 2015; Poretti et al, 2015; Conte et al, 2016; Tee et al 2016; van Doorn et al, 2016; Manganaro et al, 2017; Limperopoulos et al, 2006; Long et al, 2006; Forzano et al, 2007; Bolduc et al, 2009; Poretti et al, 2009; Patek et al, 2012; Vasudevan et al, 2012; Tarui et al, 2014). Systematic clinical and genetics follow-up studies a large population of subjects identified in the fetal (and thus pre-clinical) stage, could largely help filling this gap.

Fetal magnetic resonance imaging or in utero MRI (iuMRI) is an important diagnostic tool in the field of prenatal diagnosis, and its use has widely spread during the last two decades. In utero MRI is not routinely performed before the 18th week of gestational age due to the small size of the fetus and because movements usually do not allow to add any significant diagnostic information to US examination. Moreover, some structures such as corpus callosum or the cerebellar vermis are not fully developed before the 18 weeks. Despite MRI provides better information in late second and third trimesters, in several countries the deadline for pregnancy termination is within the 24th–25thweeks. Therefore, iuMRI is frequently performed before that age so to play a crucial role in terms of parent counselling and pregnancy management.

Fetal posterior fossa anomalies are numerous, extremely heterogeneous, and include both malformative and clastic, disruptive lesions. Unfortunately, posterior fossa examination can be difficult at ultrasound, especially in the third trimester, due to the progressive ossification of the skull base (Moltoni, 2021). Conversely, MRI allows direct visualization of the cerebellar hemispheres, vermis, and brainstem in three orthogonal planes. Furthermore, it simultaneously provides information about associated supratentorial and even extracranial anomalies that can influence prognosis. A universally accepted classification scheme for posterior fossa malformations is still lacking, and the range of midbrain-hindbrain abnormalities that can be diagnosed with fetal MRI is vast (rhombencephalosynapsis, diencephalic-mesencephalic junction dysplasia, pontocerebellar hypoplasia etc.) (Jissendi, 2015)

A subset of posterior cranial fossa anomalies, the so-called “cystic malformations of the posterior fossa”, constitute one of the most frequent indications for fetal brain MRI. These are Mega Cisterna Magna (MCM), Dandy-Walker malformation (DWM), Persistent Blake’s Pouch (PBP) and Inferior Vermian Hypoplasia (IVH). They constitute a range of conditions that often share imaging features, but very different clinical outcomes.

Blake pouch is a physiological embryologic structure that arises from the tele choroidea along the roof of the developing rhombencephalon which is seen in the first trimester, and is typically not visible by imaging by the second trimester (Cornips 2010). Blake pouch fenestrate to varying degrees in the late first trimester and beginning of second trimester and can persist in the fetal and postnatal period, either in isolation or coexisting with vermian hypoplasia. The persistence of Blake pouch is a frequent cause of referrals to many fetal imaging and, rarely, requires special consideration since it can result in obstructive hydrocephalus (Bontognali, 2017).

Importantly, a common "early" second trimester marker and recurrent precursor finding of posterior fossa anomalies is an upward (anticlockwise) rotation of the cerebellar vermis: certain e features of delayed cerebellar vermis de-rotation have been described and could be helpful in pregnancy management of questionable cases (Conte 2020), although no definite predictive assessment to guide genetic counselling and exhaustively inform future parents is available.

The specific objective of the study is to provide more detailed data about the finding of fetal isolated upward rotation of the cerebellar vermis and the consequent neuroradiological and clinical outcome in the postnatal life in the long term, in terms of clinical and neuropsychological outcome. By achieving clinical and neuroradiological outcome data in the postnatal period and in the long term, this research will hopefully serve in stratifying the prognosis and thus provide referral information for the prenatal counselling in front of specific imaging findings.
The project has been organized into consecutive phases that are modular to each other (recruitment phase and clinical study phase), which have been implemented in relation to the degree of adherence and collaboration expressed by the research participants.

Methods

Patients recruitment was held in collaboration with the Department of Neuroradiology of Buzzi Hospital in Milan, where iuMRI conducted for the study of cerebellar and posterior cranial fossa abnormalities identified by fetal ultrasound took place. By having access to the database of at about 80 patients who underwent fetal MRI due to the presence of cranial fossa abnormalities at US from 2003 to 2019, a phone call to all patients was attempted for the study proposal, distinguishing 4 steps of collaboration:

- Phone interview to assess medical and family history, psychomotor development milestones, academic performance, presence of behavioral issues, results of any clinical / neuropsychological/ instrumental assessment

- In person clinical evaluation: neurological examination and clinical interview

- Neuropsychological evaluation both in presence or in telemedicine with a standardized battery including:

Behavioral assessment:

CBCL (6 to 18 years - by parent)

CONNERS 3 (6 to 18 years - by parent and teacher; 8 to 18 years - self-assessment)

Cognitive assessment:

WISC IV (from 6 to 16 years and 11 months)

Leiter 3 (from 3 years)

Assessment of motor skills and visuomotor integration:

Movement ABC-2 (from 3 to 16 years)

VMI - Developmental Test of Visual-Motor Integration (from 3 to 18 years)

Figure of Rey A-B

Visual perception assessment

TVPS 4 -Test Of Visual Perceptual Skills (4 to 18 years old)

Memory Assessment:

MBT

Verbal: forward and backward digit memory (BVN 5-11/12-18)

Visual-Spatial: Courses

Courses (BVN forward only for 1st-2nd grade)

Courses (BVS forward and backward from 3rd grade)

MLT

Immediate and delayed selective word recall (BVN 5-11/12-18)

Attention Assessment:

Nepsy II (3 to 16 years old) - Visual attention and auditory attention

CPT - Continuous Performance Test - visual sustained attention

Executive function assessment:

Modified Wisconsin: cards/ Computerized comprehensive Wisconsin

Tower of London (ages 4 to 13)

Language and pragmatic assessment 6 to 12 years:

BVL 4-12 (repetition of nonwords, sentences, naming, lexical comprehension, grammatical comprehension)

12 years old:

BVN (12-18): auditory discrimination, lexical naming, sentence generation, phonemic and categorical fluency

- Blood sample collection and genetic investigation (analysis of the trios)

- Brain MRI 3T execution (whether a brain MRI has not been performed postnatally and after the age of 2 years)

Intra uterine fetal Brain MRI exams were reviewed independently by two expert neuroradiologists experienced in the field, who coded the imaging finding, and, according to the literature, focused their measurement and assessment on the following parameters: vermian area, anteroposterior pontine diameter, vermian angle rotation.

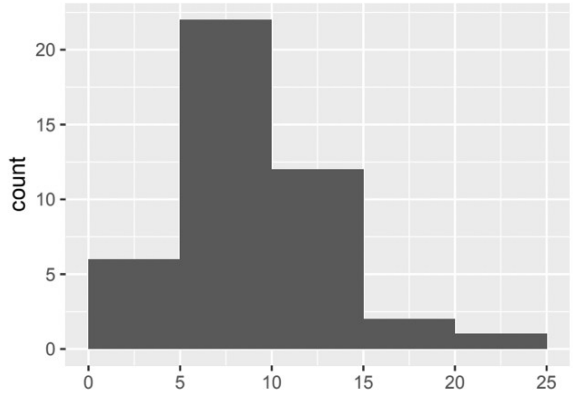
Statistical analyses

Both descriptive statistics of the overall cohort and correlation analyses were performed. In particular, Wilcoxon Rank Sum Test, a non-parametric statistical test used to compare two samples or groups and logistic regression analysis have been utilized to estimate the capabilities to predict postnatal clinical and neuroradiological outcome by the prenatal MRI findings (vermian area, AP diameter, vermian angle rotation).

Preliminary Results

Demographic data

The clinical interview has been performed with the caregivers of 43 patients (21 males and 22 females).



Median	9
1st and 3rd quartiles	7; 12.5
Min. and max.	2; 22
Mean	9.6
Standard dev	4.08

Age at the time of the interview.

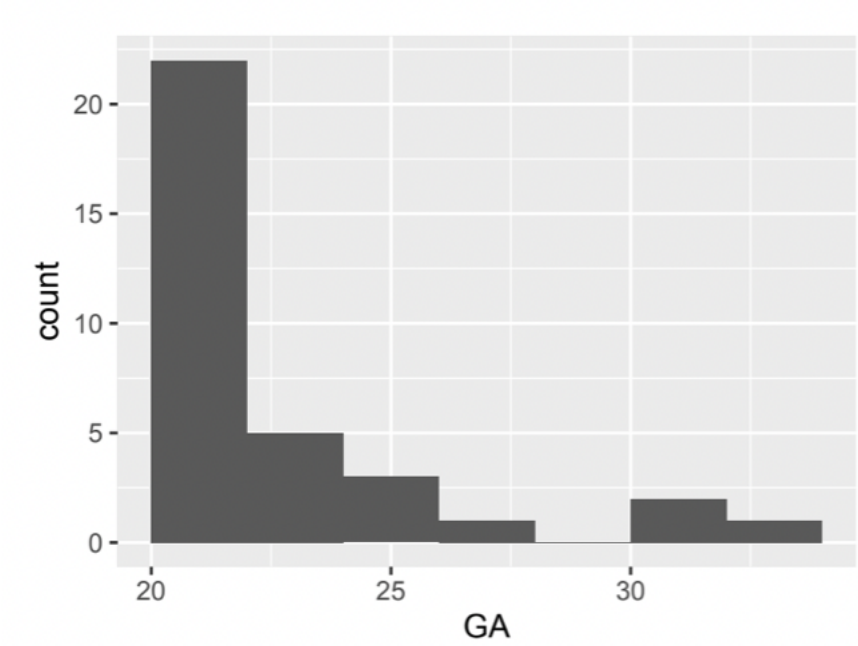
Interview outcome

The conducted phone interview, that was partially executed in presence for 10 patients who accepted to adhere to the in person clinical evaluation, showed that out of 43 patients, 3 patients had coordination problems, 4 patients had intellectual disability and syndromic conditions, 7 patients had minor neuropsychological or learning fragilities.

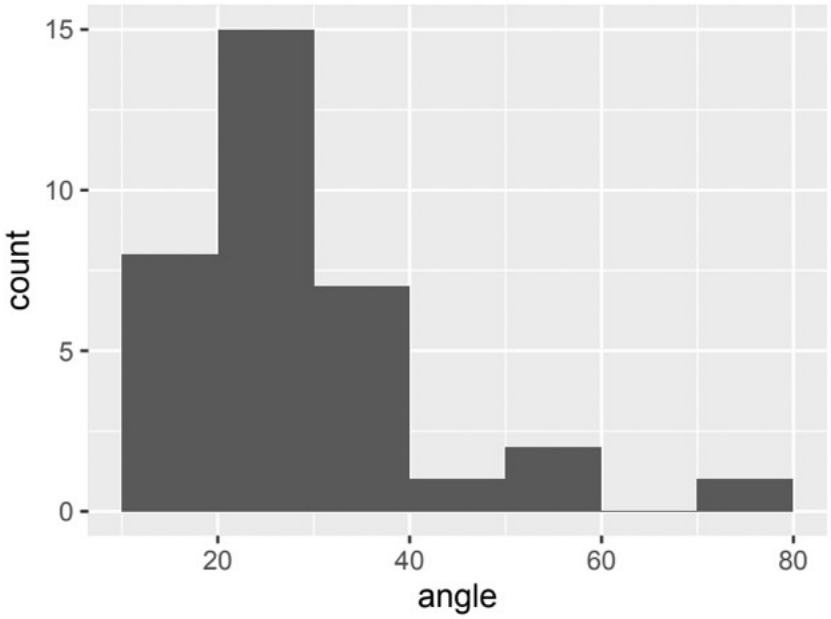
Relevant features of the cohort:

Active child neuropsychiatry take-over	28%
Normal motor development	84%
Normal language development	74%
Ongoing or previously performed rehabilitation	28%
School problems	20%
Behavioral problems	28%
Previous abortion	37%

Intra uterine brain MRI parameters:

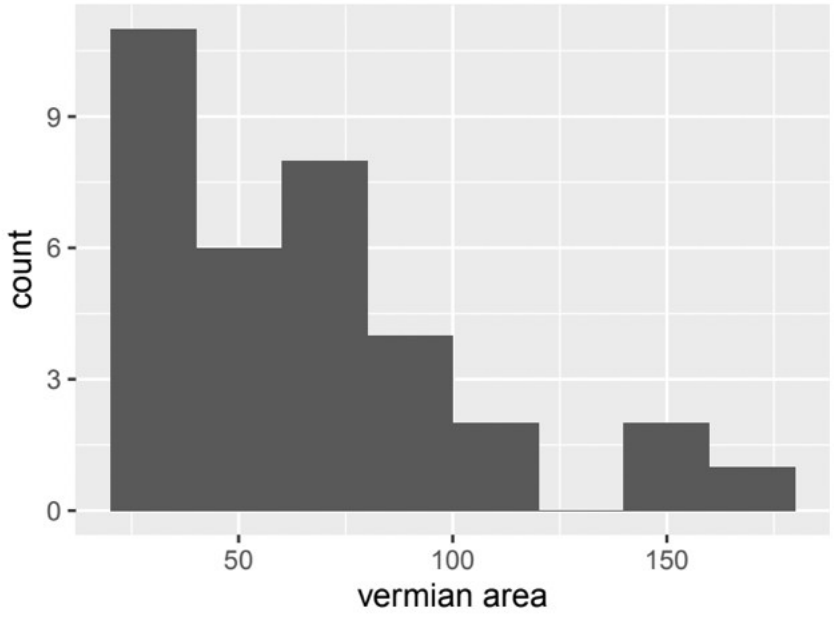


Gestational age at iuMRI



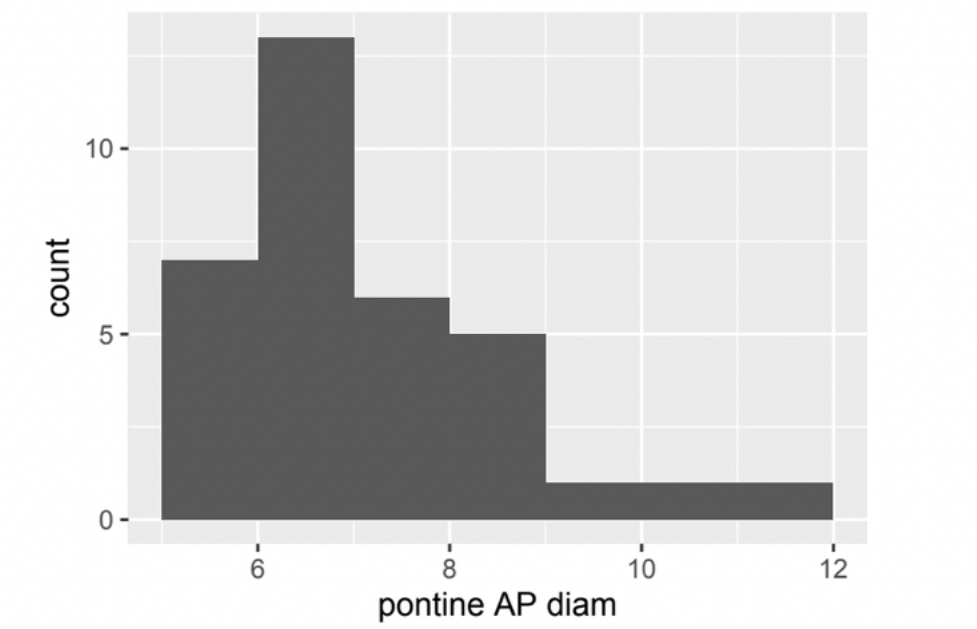
Feature	Result
Variable type	numeric
Number of missing obs.	0 (0 %)
Number of unique values	20
Median	25
1st and 3rd quartiles	21; 32
Min. and max.	13; 79
Mean	28.76
Standard dev	13.82

Vermian angle rotation at iuMRI.



Feature	Result
Median	57.5
1st and 3rd quartiles	40; 80.5
Min. and max.	22; 176
Mean	67.03
Standard dev	37.25

Calculated vermian area at iuMRI



Feature	Result
1st and 3rd quartiles	6.5; 7.88
Min. and max.	5.5; 11.5
Mean	7.41
Standard dev	1.41

Pontine anteroposterior diameter at iuMRI.

Neuropsychological outcome

	Total (n=14)	In presence (n=9)	Remote (n=5)
Age (y) M (DS)	12 (4,11)	10 (3,60)	14 (4,18)
Gender (m/f) %	50/50	44,44/55,55	60/40

Total sample data by age and gender. Sample description of presence vs remote conditions.

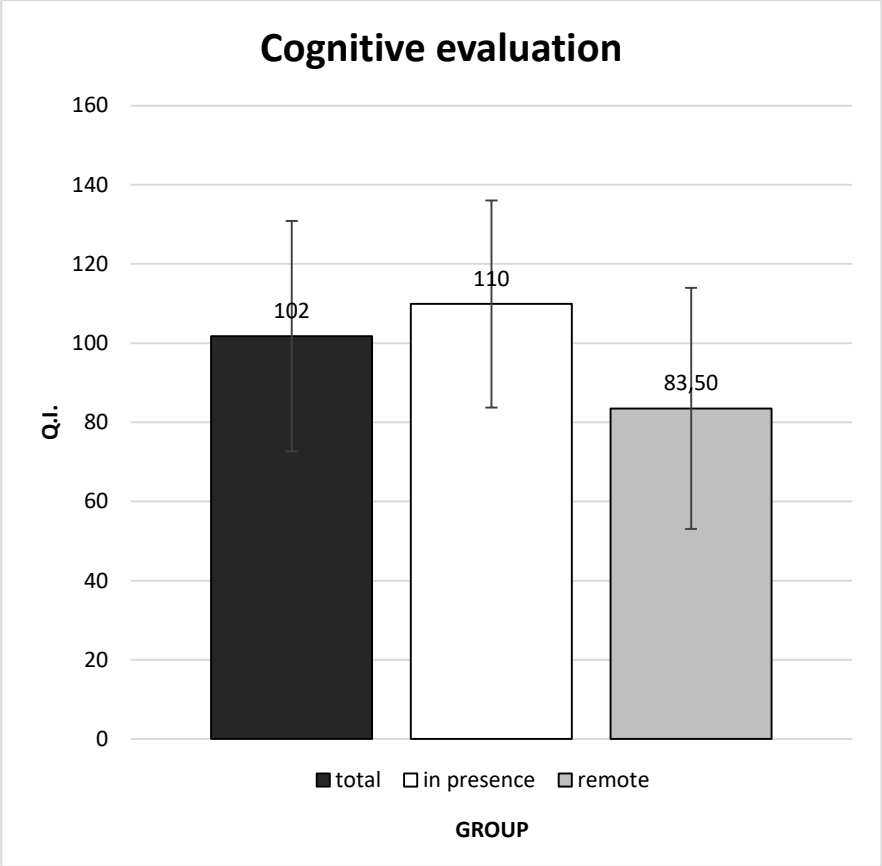


Figure 1. IQs (M) obtained administering WISC IV and RAVEN Matrices, discriminating the whole sample vs patients in the presence condition vs remote conditions. Total sample IQ 102 (DS 29,1). Remote condition IQ 83,50 (DS 30,45). In presence condition IQ 110 (DS 26,14).

	Below average (%)	Sample numerosity
Cognitive Evaluation (IQ)		
WISC IV	11	9
RAVEN Matrices	25	4
Attention and Executive Function		
Auditory Attention (NEPSY-II)	10	14
Visual Attention (NEPSY-II)	11,1	9
Tower of London (TOL)	12,5	8
Memory		
Forward Verbal Digit Span (BVN 5-11, 12-18)	14,28	14
Backward Verbal Digit Span (BVN 5-11, 12-18)	14,28	14
	0	9
Corsi Block-Tapping Test	16,66	5
Backward Corsi task	7,14	14
Rey-Osterrieth Complex Figure Recall	14,28	14
Word Immediate Recall (BVN 5-11, 12-18)	14,28	14
Word Delayed Recall (BVN 5-11, 12-18)		
Language		
BVL 4-12	0	5
Denomination	20	5
Lexical Comprehension	20	5
Grammar Comprehension	16,66	6
Non-Words Repetition	100	6
Sentence Repetition	0	8
BVN 12-18 Speech	12,5	8
Discrimination	0	8
Sentence Generation	12,5	8
Lexical Denomination	12,5	8
Fonemic Fluency		
Categorial Fluency		
Motor abilities		
Rey-Osterrieth Complex Figure Copy Task	14,28	14
Visual-Motor Integration test (VMI)	14,28	14

Emotional and Behavioural Functioning		
CBCL Internalizing Problems	28,57	14
CBCL Externalizing Problems	0	14
CBCL Total Problems	0	14
CBCL Affective Problems	7,14	14
CBCL Anxiety Problems	14,28	14
CBCL Somatic Problems	14,28	14
CBCL Attention Deficit/Hyperactivity	0	14
Problems	0	14
CBCL Oppositional Defiant Problems	0	14
CBCL Conduct Problems	14,28	13
Conner's 3 Inattention	7,14	13
Conner's 3 Hyperactivity/Impulsivity	14,28	13
Conner's 3 Learning Problems	7,14	13
Conner's 3 Executive Function	0	13
Conner's 3 Aggression	7,14	13
Conner's 3 Peer Relationship	21,42	13
Conner's 3 ADHD Index	7,14	13
Conner's 3 Global Index	14,28	13
Conner's 3 Restlessness-Impulsivity	0	13
Conner's 3 Emotional Instability		

Table 2. Below average scores (%) found by administrating neuropsychological evaluation to the sample.

Postnatal Brain MRI outcome

Twenty out of 43 patients did not perform a postnatal brain MRI. In the majority of cases caregivers stated that since their offspring was healthy, they wanted to skip an instrumental exam that was not retained mandatory. In the subpopulation that performed a postnatal brain MRI, 13 subjects had a brain MRI abnormality: in detail, 8 patients had a cisterna magna/ Dandy Walker malformation which was already diagnosed at the time of study inclusion, 4 patients had a vermian hypoplasia, and in four cases an extracerebellar abnormality was present: pons hypoplasia (1), nodular heterotopia (1), thinning of corpus callosum (2).

Validity of iuMRI parameters in postnatal outcome prediction

The interview data showed good predictive power when all variables were included in the model, particularly when gestational age was used as a correction factor. In contrast, for MRI-based predictions, the inclusion or exclusion of gestational age appeared to have less impact; however, the overall predictive power was higher, especially for the most recent scans. In general, a greater vermian angle rotation was more likely associated to a negative outcome in terms of both neuroradiological postnatal finding and clinical outcome. Regarding the predictive value of the angle measure, this was more pronounced in the outcome of postnatal MRI data, though improvements were also observed in the interview outcomes when the latest MRI scans were considered and gestational age was included—both in binary outcome models and those with multiple outcome categories.

Discussion

In presence of posterior fossa suspected abnormalities during fetal development, differential diagnosis is challenging, ranging from benign asymptomatic conditions to severe malformations associated with neurological impairment. The four most common anomalies, Dandy–Walker malformation (DWM), vermian hypoplasia (VH), Blake's pouch cyst (BPC), and mega cisterna magna (MCM), might show similar US findings but are associated with very different prognoses. The advent of iuMRI, which is anyhow not available even in tertiary centers, is certainly helpful in giving more information but still, radiologists do not have at their disposal reference charts and parameters to achieve accurate categorization that can be informative for genetic counselling and pregnancy decisions.

Upward rotation of the vermis has been suggested as a key critical finding in the differential diagnosis. Unfortunately, both US and MRI evaluation of vermis rotation finding are still subjective, and no definition of normal and abnormal cases has been provided to date, leading to categorization of fetal upward rotation of the vermis a challenge. In the last decade new methods to assess the normal position of the cerebellar vermis over the brainstem have been attempted, for instance measuring the angle between the cerebellar vermis and the internal occipital crest (vermian–crest angle) with intrauterine MRI (Spinelli, 2019).

Salsi et al published results of one of the most relevant cohort (111 patients) with a prenatal diagnosis of isolated upward vermis rotation made at a median gestational age of 21 weeks +3 days, and a postnatal follow-up available in 102 infants (mean 7 months, range 0–10 years of age) (Salsi , 2021). In 37.9% of the cases, a regression of the finding with restoration of normal anatomy was noted at a follow-up and a rotation angle of 25° or less was found to predict regression with a probability in excess of 90%.

In this paper, Salsi and colleagues sustain that vermian upward rotation is most likely a normal variant of fetal anatomy without clinical consequences, at least at an early follow-up in terms of neurological evaluation and suggest that an angle of 25° or less predicts intrauterine regression of the finding. Nevertheless, they did not achieve postnatal brain MRI findings in the long term.

Thus, there is gap in the literature regarding availability of solid data about clinical and neuroradiological follow-up in the long term of patients with upward vermis rotation. Considering the delicacy and complexity of the cascade that can result in terms of decisional and affective implications for the medical team and the parents, our study, availing itself of the conspicuous case history and clinical expertise of Buzzi Hospital, aimed to provide referral data for research and clinical purposes in the field.

Our data showed better predictive power when considering not only the vermian angle but also vermian area and anteroposterior pontine diameter in the model, and as long as gestational age was higher. In general, a greater vermian angle rotation was more likely associated to a negative outcome in terms of both neuroradiological postnatal finding and clinical outcome. These results are partly in line with the report of Salsi et al, giving power to the entity of vermian angle rotation but also throwing light to the importance of including in the evaluation model other parameters, in consideration of the timing of findings acquisition. The strength of this study is the availability of the highest median long term follow-up compared to previous literature, together with quantitative measurements in the prenatal imaging through a solid methodological procedure.

The limitations of this study are primarily the partial adhesion to the in presence evaluations and to the imaging exam in the overall cohort. Moreover, the data achieved with the phone interview, when the subsequent steps envisaged by the research protocol were not feasible, remain subjective.

We noticed that the contacted caregivers were for the most part very scarred and “traumatized” by the prenatal journey, which they remember as being characterized either by a lot of uncertainty or by communications of high risk in the face of an excellent subsequent course. For this reason, many did not want to consider hospital admission. In any case, we believe that the bias should be considered that in the absence of obvious problems parents were more reluctant to carry out evaluations.

IV. General discussion

4.1 The importance of disposal of adequate tools for innovative research in rare neuropediatric diseases

Each subject with a rare neurodevelopmental disorder (RND) may present with distinct and unique clinical features and levels of functioning. This variability necessitates harmonized diagnostic approaches, along with appropriate and targeted tools and techniques to accurately describe and understand phenotypes. Furthermore, advanced research methodologies are crucial to uncover the underlying mechanisms and identify disease biomarkers.

Comprehensive patient monitoring, as well as active engagement of patients and caregivers in defining outcomes, are essential for patient-centered care, accurate natural history studies, and the development of targeted therapies. Data-sharing frameworks are vital for fostering innovative and integrated research. In this context, the availability of regulated and easily accessible biorepositories and patient registries is fundamental for collecting extensive data.

The RENDER project represents a concrete resource supporting multistakeholder approaches to address unmet needs in the field of neuropediatric diseases. Looking ahead, future directions should consider enabling access to registries—and to RENDER—via artificial intelligence, to process aggregated data. This advancement could significantly enhance the utility of registries by providing aggregated data from multiple international sources, resulting in more robust datasets, the identification of common patterns, more accurate diagnoses, and more powerful statistical analyses.

However, the mindset of many researchers is not yet fully aligned with recognizing the universal value and advantages of pursuing and analyzing aggregated data. Nevertheless, such data lay the foundation for developing personalized or precision medicine, where treatments are tailored to the genetic, environmental, phenotypic, and clinical characteristics of individual patients.

In the field of rare neuropediatric diseases, a collaborative approach—such as "swarm working"—is essential to pool expertise across disciplines and tackle complex research challenges on a global scale. Cross-border collaboration ensures access to diverse patient populations and expands the data pool, potentially overcoming current limitations related to data protection and privacy constraints.

Once again, the RENDER project—through its design and philosophy, developed over time by a dedicated group of researchers—is well-positioned to meet these emerging needs.

4.2 Sleep: an important building block in the deep phenotyping

Over the last few decades, there has been a much-needed increase in the study of sleep problems in children with rare neuropediatric diseases and neurodevelopmental disorders. Sleep plays a critical role as a fundamental component of deep phenotyping and should primarily be considered a behavioral state. In many rare conditions, sleep disturbances are not only common but may also serve as key symptoms or diagnostic criteria.

Given that parents of individuals with rare diseases often cite sleep as an area in which more information and support are needed, it is crucial to delineate the prevalence and specific profiles of sleep disorders within these populations. The meta-analysis by Agar and colleagues (Agar, 2021) underscores the need for a more detailed, syndrome-specific sleep profile to be incorporated into clinical and diagnostic criteria and considered in both assessment and intervention strategies.

Since sleep provides a unique window into brain physiology and post-disruption adaptations, monitoring sleep patterns and quality allows researchers to gain deeper insights into how disorders impact the brain across different stages of development. A particularly relevant example is the role of sleep in individuals with agenesis of the corpus callosum (ACC): we know that connectivity changes and post-disruption adaptations occur and influence developmental trajectories and functional outcomes. However, further research is needed to understand what sleep biomarkers can reveal about brain maturation and their implications for neurodevelopment.

When combined with clinical, genetic, and neuroimaging data, sleep information may contribute to building a comprehensive phenotype—essential for identifying disease mechanisms, monitoring disease progression, and evaluating treatment effects. In light of this, clinicians who actively screen for and remain vigilant about sleep issues in patients with rare neuropediatric and neurodevelopmental disorders are more likely to identify opportunities to address behavioral and neuropsychological challenges, improve overall patient well-being, and reduce caregiver stress.

4.3 The importance of 'minor' malformative findings in the diagnostic yield and the need of long term outcome description

Given the rapid advancements in neuroimaging technologies and their increasing application even at the prenatal stage, there is a growing capacity to detect brain malformations early. This enhanced diagnostic capability enables the development of more comprehensive and alternative clinical profiles, as well as a deeper understanding of neuropsychological functioning. Moreover, studying these malformations provides invaluable insights into both typical brain development and its disruptions.

The long-term clinical outcome evaluation of such conditions—such as in studies examining the evolution of individuals with prenatal findings of vermian malrotation—is of dual importance. Firstly, it plays a crucial role in guiding counseling by providing prognostic stratification to be shared with prospective parents. Secondly, it offers valuable insights into normal neurodevelopment and cerebellar involvement.

The genetic etiology of many brain malformations remains poorly understood, and the diagnostic yield of genetic testing is often reported to be low in patients with distinct malformative features. However, this outcome may be negatively biased by the fact that standardized, comprehensive genetic testing is not always pursued or accessible.

Notably, findings from studies on pediatric patients with cerebellar heterotopia (CH) suggest that even isolated CH—when associated with developmental delays or syndromic features—can lead to a significant genetic diagnostic yield. Therefore, these seemingly 'minor' imaging findings should be emphasized, as they may reflect underlying disruption events or broader connectivity changes. These should be carefully considered during both the diagnostic process and in efforts to better understand disease mechanisms and phenotypes.

V. List of publications

Pertaining with PhD project:

- Pasca L, Arrigoni F, Romaniello R, Severino MS, Politano D, D'Abrusco F, Garau J, Giorgis V, Carpani A, Signorini S, Orcesi S, D'Arco F, Alfei E, Cattaneo E, Rognone E, Uccella S, Divizia MT, Infantino P, Valente EM, Borgatti R, Pichiecchio A. Neuroradiologic, Clinical, and Genetic Characterization of Cerebellar Heterotopia: A Pediatric Multicentric Study. AJNR Am J Neuroradiol. 2025 Jan 8;46(1):170-177. doi: 10.3174/ajnr.A8450. PMID: 39406511; PMCID: PMC11735438.

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