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# Identifying Neurobiological Markers in Obsessive–Compulsive Disorder: A Study Protocol for a Cross-Sectional Study in Subgroups of Differing Phenotype

**Pasquale Paribello [1,2], Bernardo Carpiniello [1,2], Roberto Murgia [1,2], Antonio Andrea Porcheddu [1,2], Sabrina El-Kacemi [1,2], Marco Pinna [1], Martina Contu [1], Giulia Costa [3], Rossella Barbarossa [4], Egea Sanna [4], Sara Carucci [4,5], Alessandro Zuddas [4,5,†], Paola Fadda [3,6], Simona Dedoni [3], Carlotta Siddi [3], Patrizia Congiu [7,8], Michela Figorilli [7,8], Michela Fanzecco [7,8], Monica Puligheddu [7,8], Antonella Gagliano [9], Federica Pinna [1,2], Maria Scherma [3] and Mirko Manchia [1,2,10,*]**

1 Section of Psychiatry, Department of Medical Sciences and Public Health, University of Cagliari, 09121 Cagliari, Italy; p.paribello@studenti.unica.it (P.P.); bcarpini@iol.it (B.C.); murgiarob@gmail.com (R.M.); aaporcheddu93@gmail.com (A.A.P.); sabrinaelkacemi@gmail.com (S.E.-K.); marco87.pinna@gmail.com (M.P.); dssamcontu@gmail.com (M.C.); fedepinna@inwind.it (F.P.)
2 Unit of Clinical Psychiatry, University Hospital Agency of Cagliari, 09121 Cagliari, Italy
3 Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, 09042 Monserrato, Italy; gcosta@unica.it (G.C.); pfadda@unica.it (P.F.); dedoni@unica.it (S.D.); carlottasiddi@yahoo.it (C.S.); mscherma@unica.it (M.S.)
4 Child and Adolescent Neuropsychiatry Unit, Department of Biomedical Sciences, University of Cagliari, 09121 Cagliari, Italy; rossella.barbarossa@gmail.com (R.B.); egea.sanna@gmail.com (E.S.); sara.carucci@gmail.com (S.C.)
5 A Cao Paediatric Hospital, ASL, 09121 Cagliari, Italy
6 Neuroscience Institute, Section of Cagliari, National Research Council of Italy (CNR), 09042 Monserrato, Italy
7 Sleep Disorder Research Center, Department of Medical Sciences and Public Health, University of Cagliari, 09042 Monserrato, Italy; patcongiu@gmail.com (P.C.); michelafigorilli@gmail.com (M.F.); michelafanzecco@unica.it (M.F.); puligheddu@unica.it (M.P.)
8 Neurology Unit, University Hospital Agency of Cagliari, 09042 Monserrato, Italy
9 Neuropsychiatric Unit Department of Human and Pediatric Pathology "Gaetano Barresi", University of Messina, 98122 Messina, Italy; antonellagagliano.npi@gmail.com
10 Department of Pharmacology, Dalhousie University, Halifax, NS B3H 0A2, Canada
* Correspondence: mirko.manchia@unica.it
† Deceased.

**Abstract:** Obsessive–compulsive disorder (OCD) represents a frequent and highly disabling mental disorder. Past attempts to characterize different disease subgroups focused on the time of onset (late vs. early onset), presence of insight (poor insight), and post-infectious forms (pediatric acute-onset neuropsychiatric syndrome, PANS). Each subgroup may be associated with a differing impact on cognition, functioning, sleep quality, and treatment response profile. Certain lines of evidence suggest brain-derived neurotrophic factor (BDNF) levels may differ between individuals living with OCD as compared with controls, but there is a lack of evidence on the variation of BDNF levels in OCD subgroups. Lastly, the potential of assessing inflammatory states, electroencephalogram, and polysomnography to characterize these subtypes has been hardly explored. Estimates of drug-resistance rates indicate that 20% and up to 65% of affected adults and up to 35% of the pediatric population may not benefit from pharmacological treatments. At least part of the variability in treatment response could depend on the underlying biological heterogeneity. In the present project, we aim to increase the accuracy in characterizing the phenotypical and biological signature for the different OCD subtypes through clinical, cognitive, and sleep markers, along with other possible markers that may be biologically plausible.

**Keywords:** biomarkers; obsessive–compulsive disorder; polysomnography; electroencephalography

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## 1. Introduction

### 1.1. Obsessive–Compulsive Disorder: Epidemiology and Disease Burden

Obsessive–compulsive disorder (OCD) is a frequent and highly disabling mental disorder. The prevalence of OCD reaches up to 3% of the general population [1], but subthreshold symptoms may be present in up to 28% of the general population [2]. The available data regarding OCD's impact on patient-reported quality of life indicates a significant role performed by the presence of the disease activity and that functional impairment may persist, albeit in a lessened form, even during remission phases [3]. Estimates of treatment resistance rates indicate that from 20% and up to 65% of affected adults, and up to 35% of the pediatric population may not benefit enough from pharmacological therapy [4]. On average, the age at onset is typically around 19 years old, albeit a significant heterogeneity has been described according to sex. Among males, up to a quarter of OCD symptoms will start before ten years of age [2]. These factors further underscore the importance of identifying targeted positive therapeutic strategies, especially considering the relevant impact this condition might have in the life trajectories of the affected individuals at such a critical time of personal growth and vocative development. OCD symptoms are typically heterogeneous, but despite this variability, our current nosological systems regularly enclose such heterogeneity under a single label [5]. The level of treatment resistance may indeed, at least in part, derive from heterogeneity in the underlying physiopathology, up to the point of making the first line of treatment inadequate for a sizeable portion of individuals.

### 1.2. OCD Subtypes

Historically, attempts at subcategorizing individuals living with OCD were made based on different patterns of symptoms at onset and on clustering of clinical features [6]. Others focused more on the presence or absence of comorbidity or on the longitudinal course of the disease [7–9]. The absence of insight ($OCD_{PoorInsight}$, $OCD_{PI;}$ [10]) and the age of onset have also been evaluated as possible elements to further subcategorize, with individuals having an earlier onset ($OCD_{EarlyOnset,}$ $OCD_{EO}$) typically presenting a higher level of tics comorbidity [9] and a higher level of concordant diagnosis among first-degree relatives [11]. Moreover, OCD may develop after an acute childhood infection, the so-called pediatric acute-onset neuropsychiatric syndrome, $OCD_{PANS}$, [12]). Numerous clinical, neuropsychological, and neurophysiological elements have been associated with different OCD subtypes, each with its distinctive patterns and leading to the definition of $OCD_{EO}$ ($OCD_{EARLYONSET}$, i.e., before puberty), $OCD_{LO}$ ($OCD_{LATEONSET}$, with an average age at onset of 23), $OCD_{PI}$, and $OCD_{PANS}$. For example, $OCD_{EO}$ tends to be associated with the most severe phenotype, presents a more concordant diagnosis among relatives, is more prevalent in males, has a higher degree of tic comorbidity, and is less responsive to treatment [13]. Moreover, different levels of cognitive functioning allow us to distinguish $OCD_{EO}$ from $OCD_{LO}$. $OCD_{EO}$ may present greater OCD symptoms burden as compared with $OCD_{LO}$ [14], with the former presenting worse visual recall as compared with the latter. In contrast, $OCD_{LO}$ has been reported to present more prominent deficits in cognitive flexibility [15]. $OCD_{PI}$ tends to be associated with a distinct clinical pattern, typically featuring a higher number of symptoms, disease severity, and a higher comorbidity level than other subtypes, especially with major depressive disorder [16]. $OCD_{PI}$ shares with schizophrenia a common pattern of neuropsychological and functioning impairment (also known as a schizo-obsessive disorder [10,17]). $OCD_{PANS}$ is featured by a heterogeneous constellation of symptoms, such as eating restriction, irritability, anxiety, cognitive impairment, tics, and choreiform movements [12]. In addition, from 14% up to 37% of $OCD_{PANS}$ patients experience psychotic symptoms, such as auditory and/or visual hallucinations, thought disorganization, and delusions [18]. Lastly, sleep disorders appear to represent a typical feature for $OCD_{PANS,}$ but they are not as frequent as in other subtypes [12,19]. Therefore, it is reasonable to assume that distinct neurobiological profiles may distinguish each OCD subtype.

### 1.3. Disentangling OCD Heterogeneity

However, despite a growing body of evidence suggesting the possibility of finding specific biomarkers that might support clinicians in distinguishing individuals living with OCD from healthy controls (HC), there is a dearth of research addressing this research question. Past reports suggest there might be lower levels of brain-derived neurotrophic factor (BDNF) among individuals living with OCD as compared with HC, but so far, this information is biased by the effect of pharmacological therapy in mediating the observed effect. Such lines of evidence might also point to a positive correlation between BDNF levels and treatment duration [20]. Currently, there are no data regarding BDNF levels in $OCD_{PANS}$ [21]. Nevertheless, in medication-free pediatric patients with obsessive–compulsive disorder, TNF-alpha and IL-12 levels have been found to be different from those of healthy children. Several lines of research have focused on the immune system response in OCD pathogenesis (involving both the innate and the adaptive response) [22]. In this setting, our proposal aims at increasing the accuracy in characterizing the phenotypical and biological signatures of the different OCD subtypes using clinical, cognitive, sleep, and plausible biological markers, including BDNF, S100β, pro-inflammatory markers (IL-1β, TNF-α), and C-reactive protein (CRP).

## 2. Materials and Methods

### 2.1. Study Hypothesis

This project aims to identify the bio-clinical signatures specific to each OCD subgroup. To this end, we will develop the study along three main objectives:

- To test whether individuals living with a classic OCD form (including $OCD_{EO}$ and $OCD_{LO}$) might have a distinct pattern of clinical, neurocognitive, neurophysiological (including sleep disorders, such as periodic limb movements [PLMD], anomalies in the sleep micro- or macrostructure, anomalies in EEG or in REM sleep), and biological markers that might allow distinguishing from $OCD_{PANS}$;
- To test whether it could be possible to differentiate such signatures between $OCD_{EO}$ and $OCD_{LO}$;
- To test whether $OCD_{PI}$ may be distinguished from classic OCD forms (i.e., $OCD_{EO}$ and $OCD_{LO}$) based on such signatures.

The classification of one of the study groups will be based on the following criteria:

- $OCD_{EO}$: defined on the basis of symptoms onset $\leq$ 17 years of age [15];
- $OCD_{LO}$: defined on the basis of symptoms onset after age 17 [15] with a total BABS score < 13 [23];
- $OCD_{PANS}$: defined according to the National Institute of Health 2010 pediatric acute-onset neuropsychiatric syndrome (PANS) criteria [24];
- $OCD_{PI}$: defined according to a total BABS score $\geq$ 13 points [23] with the age of onset > 17 years of age.

More specifically, our general hypothesis is that:

- Study subjects living with a classic form of OCD ($OCD_{EO}$ + $OCD_{LO}$) differ from $OCD_{PANS}$ in terms of (1) OCD symptoms severity and quality; (2) comorbidity for a sleep disorder, nonrestorative sleep, and specific sleep patterns; (3) cognitive functioning; and (4) biomarkers levels (the null hypothesis would be that $OCD_{EO}$ + $OCD_{LO}$ = $OCD_{PANS}$);
- $OCD_{EO}$ may differ from $OCD_{LO}$ in terms of (1) symptoms severity and quality; (2) sleep disorder comorbidity, nonrestorative sleep, and EEG patterns; (3) cognitive functioning; and (4) biomarker levels (the null hypothesis is that $OCD_{EO}$ = $OCD_{LO}$);
- $OCD_{PI}$ may differ from $OCD_{EO}$ + $OCD_{LO}$ and from $OCD_{PANS}$ in terms of (1) symptoms severity and quality; (2) comorbidity for sleep disorders, nonrestorative sleep, and EEG patterns; (3) cognitive functioning; and (4) biomarker levels (the null hypothesis is that $OCD_{PI}$ = $OCD_{EO}$ + $OCD_{LO}$ and $OCD_{PI}$ = $OCD_{PANS}$).

Considering the exploratory nature of the present study, it is difficult to hypothesize the direction of the observed difference in terms of either neuropsychological tests, plasma

biomarkers, or EEG/polysomnographic markers or indeed, the required sample size to detect a difference between study groups. Notwithstanding these limitations, we anticipate the following:

- Higher symptoms severity for $OCD_{EO}$ vs. $OCD_{LO}$;
- Worse cognitive performances for $OCD_{PANS}$ vs. $OCD_{EO}$ and $OCD_{LO}$;
- Greater impact on sleep architecture for $OCD_{PANS}$ as compared with the remaining subtypes.

### 2.2. Study Design

The study is organized into two different work packages dedicated to clinical assessment and phenotypic delineation and biological analysis of blood samples, respectively.

### 2.3. Clinical Assessment

OCD diagnosis in both pediatric and adult patients will be established according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Neuropsychological tests will be performed with the use of the MATRICS Consensus Cognitive Battery [25], as it has been employed in children, adolescents, and young adults.

- Adult population: OCD symptom severity will be assessed with the Italian version of the Yale–Brown Obsessive–Compulsive Scale–Second Edition (Y-BOCS-II) [26]. The Brown Assessment of Belief Scale (BABS), a semi-structured interview assessing seven different items related to the level of insight on a particular belief, will be employed to assess the level of insight surrounding the eventual obsessive thoughts [27].
- Pediatric population: OCD symptoms severity will be assessed with the use of the Italian version of the Children Y-BOCS scale [28]. Behavioral and affective symptoms will be assessed through the use of the Italian version of the Child Behavior Checklist (CBCL 6-18) [29]. To assess the level of insight surrounding obsessional beliefs, the BABS-Adolescents will be employed [27]. The Yale Global Tic Severity Scale will be employed to assess the severity of motor and vocal tics, such as the number, frequency, intensity, complexity, and interference with daily functioning [30].
- Pittsburg Sleep Quality Index assessing (1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) usual sleep efficiency, (5) sleep disorders, (6) use of hypnoinducent, and (7) diurnal dysfunction.

Whole sample: sociodemographic, personal, and family history comprising: (1) patient's ethnicity, (2) psychiatric comorbidities, (3) medical comorbidities, (4) father's age at birth, (5) mother's age at birth, (6) socio-economic status (self-assessed on three levels), (7) education level, (8) father ethnicity, (9) mother ethnicity, (10) family history of mental disorders, (11) family history of suicide behaviors, and (12) lifetime history of substance use disorders.

### 2.4. Sleep Study and Polysomnography

All study participants will be asked to wear a wrist actigraph for fourteen days to assess circadian rhythm regularity. By the end of the monitoring period, complete nocturnal polysomnography will be carried out and registered (digital video polysomnography, vPSG) within the laboratory of sleep medicine. The analyses of the resulting vPSG data will be carried out by a researcher with specific training in the field and certified by the AIMS (Italian Sleep Medicine Academy: MP, PC, and MF) and according to the American Academy of Sleep Medicine guidelines.

The following elements will be assessed to evaluate sleep macrostructure:

- Total Bed Time (TBT);
- Total Sleep Time (TST);
- Sleep Efficiency (SE);
- Wake After Sleep-Onset (WASO);

- The proportion of TST spent in the various sleep REM and NREM stages (N1, N2, N3, and REM);
- REM sleep duration, Period Limb Movement Index (PLMI), Apnea Hypopnea Index (AHI);
- Frequent position changes.

Moreover, the following elements of sleep microstructure will be assessed through the analysis of the Cyclic Alternating Pattern (CAP)

- CAP total time in NREM;
- CAP rate;
- Number of A phases and prevalence of each subtype of A phase (A1, A2, and A3).

### 2.5. EEG Recording

EEG will be recorded with the use of High-density EEG caps employing 64 surface electrodes non-polarizable Ag–AgCl electrode-cap using the BQS 98 System Micromed. Both power spectrum and synchronization data will be calculated for non-overlapping epochs (1 s at 256 samples). The obtained data will be spatially arranged following the EEG montage.

### 2.6. Blood Sampling Procedure and Preparation

Furthermore, we will collect and analyze peripheral blood samples to study the peripheral levels of a series of candidate biomarkers. Blood samples for each patient will be taken at the same time of the day at recruitment. After blood sampling, the plasma will be collected using EDTA or heparin as an anticoagulant. After that, it will be centrifuged at approximately $1000\times$ *g* for 15 min. Supernatant plasma samples will be collected in small aliquots and stored immediately at $-20$ °C for future analysis. IL-1β, TNF-α, and CRP plasma levels will be assessed using human AlphaLISA Immunoassay Kits. The detection will be separately performed in 96-well plates (PerkinElmer, cat. no. 6002350) following the kit instructions (PerkinElmer, cat. no. AL3160HV for IL-1β, detection range 0.37–30,000 pg/mL; no. AL325HV for TNF-α, detection range 0.013–30 ng/mL; and no. AL233C for CRP, detection range 7.3–1,000,000 pg/mL). Luminescence will be read with an EnSight Multimode Plate Reader with AlphaLISA setting (excitation at 680 nm and emission at 615 nm). Obtained data will be analyzed using the MyAssays® Desktop. BDNF and S100β plasma levels will be assessed using a commercial human enzyme-linked immunoassay (ELISA) kit (Booster Immunoleader Biological Technology Co., Ltd., Pleasanton, CA, USA, cat. no. EK0307 for BDNF, sensitivity <2 pg/mL; MyBioSource, cat. no. MBS2503148 for S100β, sensitivity: 18.75 pg/mL). The optical density absorbance of each sample will be measured using a microplate reader (Thermo Scientific Multiskan FC, Waltham, MA, USA) set at 450 nm. Obtained data will be analyzed using the Thermo Scientific SkanIt Software 3.0 for Multiskan FC. All samples will be assayed in duplicate.

### 2.7. Statistical Analysis

Comparison of socio-demographic and clinical features, and of biomarker serum levels between the four subgroups, will be made using parametric (ANOVA) or nonparametric (Kruskal–Wallis) test, according to the data distribution and variance homogeneity. Similarly, a group-by-group comparison will be conducted with Tukey's Test or nonparametric Dunn's Post hoc test, adjusting for multiple comparisons, as appropriate. Receiver operating characteristic (ROC) curve analysis will be performed to test the discriminating power of clinical and biological markers panels between OCD subgroups. For sleep and EEG recordings, ANOVA for repeated measures will be used for comparisons of power spectra and synchronization within and between groups. Coherence data will be subjected to Fisher's transformation, yielding z-coherences with an approximately normal distribution. The relation between the modification of percentage changes in seizure frequency and in power spectra and synchronization distribution will be assessed from a bivariate scattergram plot and Fisher's R to Z two-tailed test. α will be set at 0.05. All these analyses will be performed

using specific toolboxes in MATLAB or packages in R. Considering previous estimates [16,20] of effect size in comparisons between (1) OCD subgroups and (2) OCD cases versus controls for clinical and biological markers, our OCD sample size (N = 30) will have more than 80% of statistical power to detect a statistically significant difference with an $\alpha$ of 0.05.

## 3. Project Development, Ethics, and Relevance of Anticipated Results

### *3.1. Relevance of Anticipated Results*

We expect that our study will better delineate clinical and biological elements associated with distinct OCD subtypes, with the objective of lessening the impact of heterogeneity in understanding the underlying biology. With a proof-of-concept approach, the identified elements will be tested for their capacity to discriminate efficiently between the different OCD subtypes. Lastly, the association between different OCD subtypes and specific sleep disorders, such as PLMD and REM sleep anomalies, may further contribute to identifying specific neurobiological patterns instrumental in better understanding OCD pathogenesis.

### *3.2. Project Development*

Our project will develop over two years. In the clinical arm, the units will recruit a total of 60 individuals living with OCD in adulthood, further characterized as follows: N = 30 $OCD_{LO}$ and N = 30 $OCD_{PI}$. The child psychiatry unit will also recruit 60 individuals living with OCD in childhood, further characterized as follows: N = 30 $OCD_{PANS}$ and N = 30 $OCD_{EO}$. Polysomnography and EEG for the entire sample (N = 120) will be performed. At the moment of recruitment, we will collect blood samples that will be used for peripheral biomarker analyses (i.e., BDNF, S100β, IL-1β, TNF-α, and CRP). Four months will be dedicated to this phase, requiring a research assistant to implement the required methodology. Lastly, data analysis will be performed to test the a priori hypothesis. EEG and sleep recordings will be performed at the sleep disorder unit. Figure 1 summarizes the flowchart of recruitment for the present project.

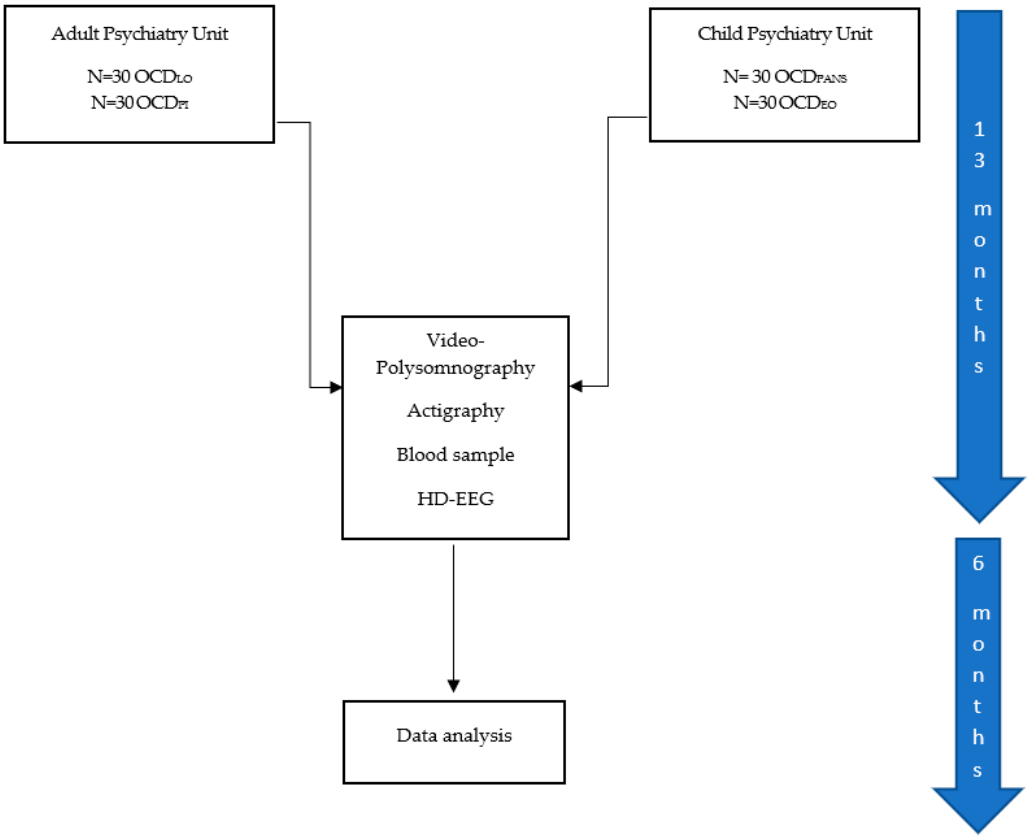


**Figure 1.** Timeline of the research project.

### 3.3. Identification of Biological Samples and Instrumental Examination Reports

Data gathered from clinical, laboratory, and instrumental examination assessments will be identified through a pseudonymization procedure with the use of progressive alphanumeric code. All study participants will sign an informed consent form upon recruitment. Study participation is completely voluntary and will have no implication in terms of clinical assistance received for the underlying disorder. This study will not result in the administration of any intervention that would otherwise influence decisions surrounding care.

### 3.4. Ethic-Regulatory Considerations

This study will be conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice protocol and approved by the responsible Ethical Committee of the Cagliari University Hospital Agency for the adult and childhood arms of the project (see below). If, during the study will be necessary to amend the study protocol, the informed consent form or other relevant documents will have to be approved by the responsible Ethical Committee. In case of amendments, the local Ethical Committee will have to approve the revised protocol before recruiting further subjects. The Principal Investigator will ensure that all researchers involved in this project have the required training and are familiar with the study protocol.

### 3.5. Privacy Protection and Biological Sample Handling

Biological samples will be identified with the use of an alphanumeric code through a pseudonymization procedure and stored in a dedicated facility.

## 4. Conclusions

### 4.1. Potential Impact of Study Results

OCD is a complex psychiatric disorder with a multifactorial etiology and a relatively heterogeneous clinical manifestation [31]. This heterogeneity is evident cross-sectionally (between individuals) and longitudinally (intra-individually) in the course of the disorder. Despite the vast literature on the presence of clusters of symptoms or severity levels that identify distinct subgroups of individuals living with OCD, we are still unable to positively treat all patients and to reach an adequate level of prediction of treatment response and/or illness trajectories. Past attempts at developing diagnostic biomarkers for OCD have also proven unproductive [32]. However, other than a diagnostic aid capable of distinguishing OCD patients from HC, the identification of biomarkers capable of informing treatment decisions still represents a valuable objective to pursue. Indeed, the potential impact of diagnostic tools capable of predicting treatment response and, consequently, improving existing treatment algorithms and lessening the duration of untreated illness for individuals living with OCD would be substantial [33]. In this setting, one possible benefit of studying different OCD subtypes with a clearer biological correlation is the prospect of increasing clinicians' capacity to match the right treatment to the right patient and advancing the research in the field in the pursuit of a better understanding of its biological underpinnings [33,34]. With the present project, we aim to test the possible interaction between inflammation, sleep architecture, BDNF levels, and clinical phenotype in modulating specific clinical presentation of OCD, possibly helping to develop precision approaches in this complex psychiatric disorder. Another possible additional benefit of this research could entail the development of alternative nosological classifications. Indeed, considering its inception in a deeply rooted translational and multidisciplinary approach, studies, such as the present project, may be instrumental in promoting the development of a more informative diagnostic system in terms of treatment selection compared to the current nosological classification. At this stage, the available evidence surrounding the significance of any of these elements taken singularly is far too scarce to draw any firm conclusion on the matter and justify the development of adequately powered prospective studies assessing the accuracy of panels for predictive purposes. The project is meant not

only to study the hypothesis of an immune-inflammatory cause for some OCD subtypes but also to clarify potentially pathogenetic pathways, at least in part, shared between the different OCD subtypes. Therefore, our project, with its developmental perspective, may represent a particularly valuable addition to the field by integrating clinical and biological data in different OCD subtypes, at different phases of life, with different comorbidity and different treatment history (i.e., duration, type, doses). Moreover, depending on the results of the present project, we might gain additional insight into closely related conditions, such as body dysmorphic disorders or eating disorders. Arguably, a sizeable portion of early-onset eating disorders may instead be more correctly classified as a pediatric acute-onset neuropsychiatric syndrome. Therefore, gaining additional insight into potential biological, neuropsychological EEG/polysomnographic differences between different OCD subgroups may further inform diagnostic algorithms for related disorders.

### *4.2. Recruitment Status*

Recruitment is actively being carried out in the Adult Psychiatry and Child Psychiatry Unit at the moment of writing the present protocol. The Child and Adolescent Unit is currently working on the prescreening of young subjects.

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**Institutional Review Board Statement:** The study will be conducted in accordance with the Declaration of Helsinki and has approval by the Ethics Committee of Hospital Agency University of Cagliari for the adult arm (protocol number NP/2022/1379 on 30 March 2023) and developmental arm (protocol number PG/2023/1669 on 1 February 2023).

**Informed Consent Statement:** Written informed consent will be obtained from all study participants upon recruitment.

**Data Availability Statement:** Data will be available upon request to the Principal Investigator (M.M.) when study procedures will be completed.

**Conflicts of Interest:** The authors declare no conflict of interest.

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