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Early Parenting Intervention – Biobehavioral Outcomes in infants with Neurodevelopmental Disabilities (EPI-BOND): study protocol for an Italian multicentre randomised controlled trial

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ABSTRACT

Introduction Neurodevelopmental disability (ND) represents an adverse condition for infants' socio-emotional and behavioural development as well as for caregiving (eg, parental sensitivity) and mother-infant interaction. Adverse exposures are associated with altered neuroendocrine hormones concentrations (eg, oxytocin and cortisol) and epigenetic regulation (eg, methylation of stress-related genes), which in turn may contribute to less-than-optimal mother-infant interaction. Parental sensitivity is a protective factor for childrens' development and early parental interventions (eg, video-feedback intervention) can promote parental caregiving and better developmental outcomes in children. The present multi-centric and longitudinal randomised controlled trial aims to assess if and to which extent early VFI could benefit both infants and mothers in terms of behavioural outcomes as well as neuroendocrine and epigenetic regulation.

Methods and analysis Dyads will be randomly assigned to the video-feedback Intervention Group or Control Group ('dummy' intervention: telephone calls). Infants with ND aged 3 to 18 months will be recruited from three major child neuropsychiatric units in northern Italy. A multi-layer approach to intervention effects will include videotapes of mother-infant interaction, maternal reports as well as saliva samples for hormones concentrations and target-gene methylation analysis (eg, *BDNF*, *NR3C1*, *OXTR and SCL6A4*) that will be obtained at each of the four assessment sessions: T_0, baseline; T_1, post-intervention; T_2, short-term follow-up (3 month); T_3, long-term follow-up (6 month). Primary effectiveness measures will be infant socio-emotional behaviour and maternal sensitivity. Neuroendocrine hormones concentrations and DNA methylation status of target genes will be secondary outcomes. Feasibility, moderation and confounding variables will be measured and controlled between the two groups.

Ethics and dissemination Ethics approval has been obtained in all three participating units. Results of the main trial and each of the secondary endpoints will be submitted for publication in peer-reviewed journals and international conferences.

Trial registration number NCT03853564; Pre-results.

Strengths and limitations of this study

- The *Early Parenting Intervention – Biobehavioral Outcomes in infants with Neurodevelopmental Disabilities* (EPI-BOND) is a multi-centre and longitudinal randomised clinical trial aimed at assessing the effectiveness of an early and brief family-centred intervention to promote parental caregiving with children diagnosed with neurodevelopmental disabilities.
- The EPI-BOND intervention consists of an eight-session video-feedback interactive support to parental caregiving that focus on four major thematic areas: Sensitivity-Responsiveness, Stimulation, Encouragement and Parental perspective.
- Mother-child interaction will be videotaped at four assessment points (before, after, 3 months and 6 months post-intervention) in order to obtain observational measures of maternal sensitivity (primary outcome) and child behavioural development (secondary outcome).
- According to a multi-layer assessment approach, saliva samples will be collected at each time assessment point to get information on both mothers and children's neuroendocrine regulation (cortisol and oxytocin) and the DNA methylation status of specific target genes related to behavioural development and stress regulation (exploratory outcome).
- Although a limitation of the study is the heterogeneity of the sample, which include children with different neurodevelopmental disabilities, the multi-centric and prospective nature of this study is warranted to improve our knowledge of the behavioural and biological markers of early parenting interventions.

INTRODUCTION

In 2016, about 53 million infants worldwide received a diagnosis of neurodevelopmental disability (ND), representing 13% of all health problems in childhood.[1] ND is a broad clinical label that includes diverse clinical conditions ranging from infant cerebral palsy

to genetic syndromes, from metabolic diseases to serious brain injuries related to severe preterm birth. Infants with ND share—at different degrees—high risk of impairment in several developmental domains,[2] including cognitive, behavioural and emotional areas, and altered capacity to cope with daily stress.[3–5] Furthermore, infants with ND have been described as having reduced interactive and attentional skills, using less vocal and affective signals, and exhibiting behavioural instability in day-to-day exchanges with parents.[6–8] These altered behavioural and socio-emotional patterns have been highlighted in infants with cerebral palsy,[9 10] autism spectrum disorder,[11 12] and in genetic diseases such as Williams syndrome[3] and Down syndrome.[13]

Such impaired behavioural and socio-emotional patterns dramatically impact parental psychological and emotional well-being as well as the quality of the early parent-child relationship. Crucially, parental sensitivity—defined as the ability of parents to appropriately read their infant's signals, to contingently respond, to provide stimulations of adequate intensity and to sustain the infant's attention—is challenged in the presence of ND conditions.[14 15] Parents may report critical emotional burden with heightened risk for chronic levels of distress, depression and anxiety.[16–19] It has been documented that parenting style of children diagnosed with ND may be less sensitive, either directive[20] or intrusive,[21 22] probably in an attempt to increase the opportunity of interpreting and responding to infants' unclear communicative signals. Notably, lower parental sensitivity is in turn associated with later socio-emotional and behavioural problems in children with ND.[23]

Importantly, several studies suggested that improving parental sensitivity through early parenting support interventions is warranted to promote infants' developmental outcomes, even in the presence of ND conditions.[24–28] Specifically, beside social interaction and emotional support, parents also provide cognitive stimulation during exchanges with their infants, with long-term benefits for cognitive, language and socio-emotional outcomes up to preschool-age and school-age.[15 29 30] Parental sensitivity and teaching are associated with the developmental quotient of 23- to 47-month-old children with diverse ND conditions.[31] Notably, both paternal and maternal caregiving is associated with better cognitive and language development in preschoolers with ND.[32] As such, early supportive interventions directed at improving the quality of parental sensitivity and parent-infant interaction should be a priority in the population of children diagnosed with ND.[33 34]

The video-feedback intervention (VFI) is an early parent-child focussed intervention that aims to promote positive and sensitive parenting and, indirectly, support infants' behavioural and socio-emotional development.[35] By means of helping parents to watch 'from the outside' themselves interacting with their own child, VFI interventions support the parental ability of being attuned and sensitive in the interaction, provide child-sensitive teaching and encouraging support, provide stimulations that are attuned with infants' sensory profile and regulation, and develop a more appropriate representation of their child.[36 37] Different methodological VFI interventions have been developed to respond to the specific challenges of parent-child relationship under diverse at-risk developmental conditions.[38–43] The use of VFI has been also documented to be beneficial in dyads, in which the infant had sensory impairment,[44 45] cerebral palsy[46] and severe prematurity.[40] Beneficial effects include better socio-emotional regulation and behavioural development as well as increased parental sensitivity and responsiveness.[47]

Of note, recent research at the intersection between neuroscience, behavioural biology and infant research has revealed that precocious infant exposures to variations in caregiving may result in epigenetic modifications related to neuroendocrine regulation pathways[48] that are strongly involved in behavioural and socio-emotional developmental outcomes.[49] DNA methylation is an epigenetic mechanism which consists in a covalent modification, mostly occurring at the level of DNA dinucleotides rich in cytosine and guanine (ie, CpG sites), through the addition of a methyl group onto the cytosine ring. DNA methylation may directly contribute to inhibit transcriptional activity and gene silencing, but it also interacts with other epigenetic processes in remodelling the chromatin structure in which the DNA is wrapped. Studies on rodents have first documented that variations in the quality of maternal caregiving (ie, licking and grooming behaviours) are associated with differential methylation of genes involved in stress regulation, such as *NR3C1* (encoding for glucocorticoid receptors[50 51]), *SLC6A4* (coding for the serotonin transporter[52 53]), *BDNF* (coding for the brain-derived neurotrophic factor[54]) and *OXTR* (coding for the oxytocin receptor[55]). Notably, individual differences in maternal sensitivity, infant behaviour and socio-emotional regulation have been shown to be significantly linked with the concentration of a subset of the hormones which these genes encode—for example, salivary cortisol[56 57] and oxytocin.[58 59] Other studies have documented similar epigenetic regulation in human infants and children exposed to early stressful and adverse conditions (eg, trauma,[60 61] preterm birth,[62 63] maternal depression[64 65] and socio-economic disadvantage[66]) as well as protective parenting (eg, maternal sensitivity,[67] affectionate touch[68] and parenting style[69]).

Overall, these findings suggest a fascinating intervention perspective, that is, a developmentally supportive parenting behaviour could have an impact not only at the behavioural[70] and neuroendocrine levels,[71] but it may also contribute to the regulation of the epigenetic machinery.[48] For example, parenting intervention can have a significant impact on the whole genome DNA methylation in children with history of maltreatment;[72] while children displayed similar methylation patterns before parenting intervention, the status of DNA methylation significantly differed between children of the intervention group (IG)

and control group (CG) after the treatment. Nonetheless, the recent advances in human behavioural epigenetics have focused mainly on stressful and traumatic exposures, with very few attempts of investigating the effects of naturally occurring variations in caregiving on the offspring's epigenetic regulation.[67] Additionally, to the best of our knowledge, the potential epigenetic correlates of early interventions aimed at promoting developmentally supportive parenting behaviour in infants with ND remain unexplored.

AIMS

Primary aim and hypothesis

In the present randomised controlled trial (RCT), dyads of infants with ND will be randomly assigned according to computer-generated random sequential numbers to one of two groups: a video-feedback IG or a CG (ie, a 'dummy' intervention in which mothers will be contacted by phone). The primary objective will be to examine the effectiveness of VFI for mothers of infants with ND in terms of change in parental caregiving behaviour from before (T_0) to after (T_1) the intervention. We hypothesised that mothers of the IG would show a greater increase in T_0 to T_1 maternal sensitivity score change compared with mothers of the CG.

Secondary aim and hypothesis

A secondary objective will be to assess the effectiveness of VFI to increase infants' capacity for behavioural emotional regulation from before (T_0) to after (T_1) the intervention. We hypothesised that VFI-exposed dyads would exhibit better behavioural emotional regulation compared with non-exposed counterparts allocated to CG.

Exploratory aims

Additional exploratory objectives of the present study will include (a) the evaluation of long-term effects by assessing maternal sensitivity and infants' behavioural emotional regulation at 3 months (T_2) and 6 months (T_3) after the end of the intervention, as well as (b) the assessment of biochemical underpinnings of primary and secondary effects. More specifically, at each assessment session (T_0 to T_3), the two groups will be tested for differences in the methylation status of genes previously associated with behavioural emotional regulation (ie, *BDNF, NR3C1, OXTR* and *SLC6A4*) and secretion of hormones (ie, salivary cortisol and oxytocin) in response to socio-emotional stress. Given the nature of the exploratory objectives, no specific hypotheses were formulated.

METHODS

Study design

The *Early Parenting Intervention – Biobehavioral Outcomes in infants with Neurodevelopmental Disabilities* (EPI-BOND) is a RCT study funded by the Italian Ministry of Health that will be carried out in three child neuropsychiatric units in North Italy (Lombardia region) at: IRCCS Eugenio Medea, Bosisio Parini, (Lecco); IRCCS Casimiro Mondino, Pavia; and University of Brescia, Brescia. Multi-layer assessment will occur at each assessment session (see below, *Measures*) (figure 1). Layer 1 refers to the *behavioural outcomes* which represent the primary aim of the present study (ie, infant behaviour and socio-emotional regulation, Aim 1A; maternal caregiving behaviour and interactive engagement, Aim 1B). Layer 2 will include the assay of saliva samples for the assessment of maternal and infants' *neuroendocrine hormones concentrations* (ie, salivary cortisol and oxytocin). Layer 3 will include saliva samples for the estimation of target genes (ie, *BDNF*, *OXTR*, *NR3C1* and *SLC6A4*) *methylation status* in infants. Layer 4 will include mother-reported questionnaires to obtain measures of potential *confounders* such as maternal psychological and emotional state (ie, depressive and anxious symptoms), stress levels, mother-to-infant bonding and infants' temperament. Finally, Layer 5 will include an assessment of maternal *perceived efficacy* of the VFI programme through an ad hoc open-question tool.

Study population

Infants with a diagnosis of ND will be consecutively recruited with their mothers at the three participating facilities according to inclusion and exclusion criteria. Infants will be considered eligible to the study if they meet the following criteria: age between 3 and 18 months (corrected age in case of prematurity); up to 24 months chronological age; when available, development quotient (DQ) ≤70; presence of severe developmental risks (eg, extremely low birth weight, intrauterine growth restriction and very preterm birth); presence of cerebral lesions/malformations; hypoxic-ischaemic encephalopathies that require hypothermia treatment; any genetic syndromes with psychomotor retardation and suspected syndromic framework even if not yet identified. Infants will not be considered eligible in the presence of severe hearing (ie, deafness) or visual (ie, blindness) impairment. To minimise additional sources of variability within the sample, additional exclusion criteria will apply to mothers aged less than 18 years, with limited knowledge and mastery of the Italian language, with documented mental retardation or mental health conditions and related drug treatments, and not living with the father of the infant. No constraints will be adopted for socio-economic conditions, in order to maximise generalisability of the findings; nonetheless, socio-economic status (SES) will be assessed and will be checked as a potential confounder.

Patient and public involvement

Patients were not involved in the design of the present RCT. Patients' associations will be involved in dissemination and reach-out activities (see *Ethics and Dissemination* paragraph below).

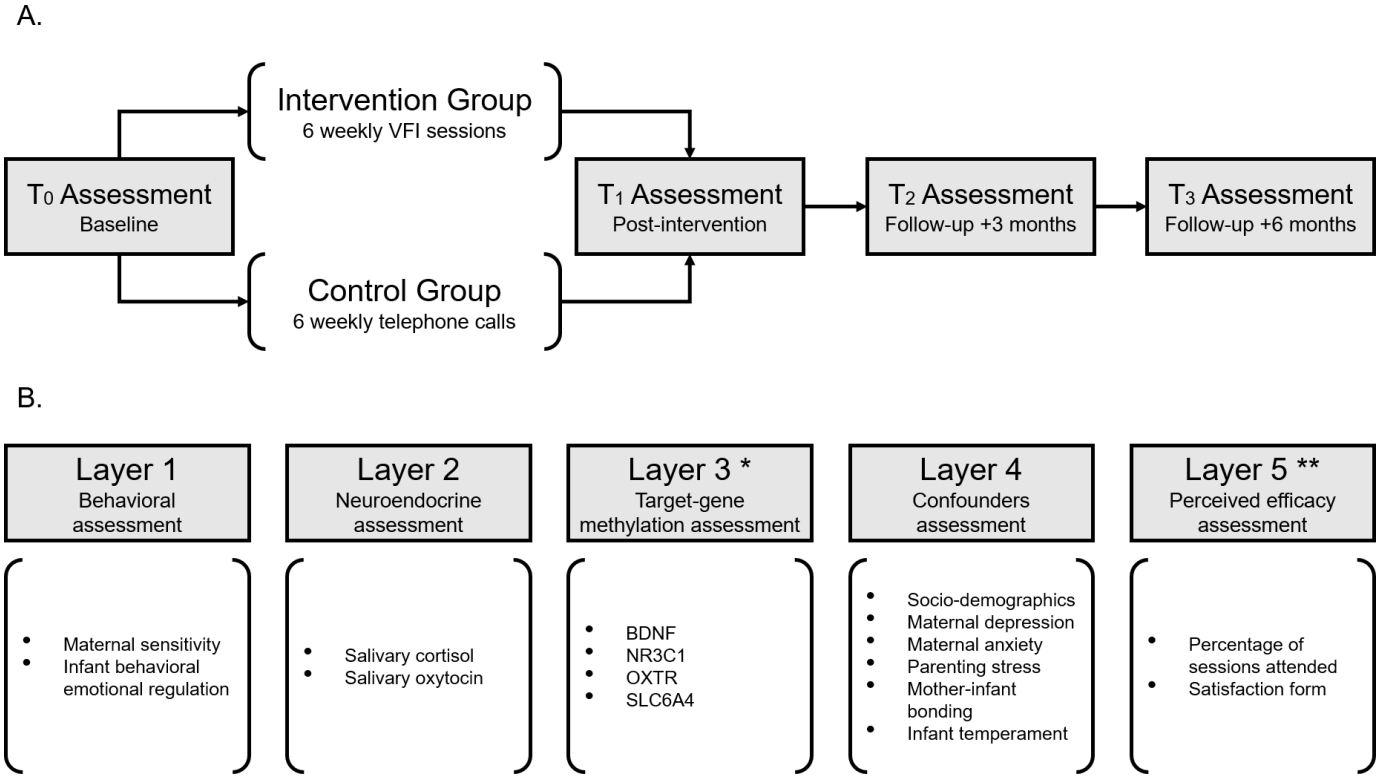

Figure 1 Overview of the study design (A) and description of the multi-layer nature of each assessment session (B). Note: *Only infants; **Not included in session T_0. VFI, video-feedback intervention.

Procedures

Enrolment and allocation

At the time of recruitment, informed consent (online supplementary file S1) will be obtained from both mothers and fathers. Randomised allocation to each of the RCT arms will be done after the recruitment according to a computerised 0/1 sequence generator. One sequence will be generated for each participating unit. The assignment to the RCT arms (ie, IG or CG) will be decided before recruitment, to reduce selection and allocation bias.[73]

Assessment sessions

The EPI-BOND study has four prospective assessment sessions: T_0, baseline; T_1, post-intervention; T_2, short-term follow-up (3 months); and T_3, long-term follow-up (6 months) (see figure 1). The four assessment sessions will take place in an ambulatory setting at the three different units involved in the project. At each assessment session, the researcher assistant will welcome the dyad by providing information on the procedures and mother-infant interaction will be videotaped. First, a 10 min unstructured mother-infant interaction with a standard set of toys (ie, ball, rattle and building blocks) will be used for coding maternal sensitivity and infant behavioural regulation. Second, the 10 min Face-to-Face Still-Face (FFSF) double-exposure paradigm[74] will be used for coding behavioural[74] and neuroendocrine[75] responses of socio-emotional stress in infants. During the FFSF procedure, age-appropriate socio-emotional stress is elicited by manipulating maternal interactive availability by asking the mother to maintain a still, poker face while staring at the infant without talking, touching or smiling to him/her. The double FFSF paradigm is to be preferred when salivary cortisol is also assessed alongside behavioural response.[75]

At the end of the lab session, socio-demographic information will be obtained (T_0) or updated ($T_{1\text{-}3}$) through maternal reports and mothers will complete a set of questionnaires (see below). The recorded interaction will be coded by trained research assistants according to well-validated observational tools for the assessment of mother-infant interaction, including the Parenting Interactions with Children: Checklist of Observations Linked to Outcomes (PICCOLO[15]) for maternal sensitivity and the Global Rating Scales (GRS[76]) for infants' socio-emotional behaviour.

Biological data collection, storage and analysis

Neuroendocrine samples. At each assessment session, research assistants will collect from both the mother and the infant one salivary sample for cortisol and oxytocin concentration measurement before the FFSF procedure (ie, baseline); moreover, only for infant, three additional samples will be obtained for salivary cortisol concentration assays related to neuroendocrine post-stress regulation at 15 (ie, reactivity) and 30 (ie, recovery) minutes after the end of the FFSF procedure.[77] Due to the circadian rhythm of the neuroendocrine systems, all interactions will occur in the morning, between 09:00 and 12:00 a.m. Nonetheless, the actual time of saliva sampling will be included in

the analyses as a potential confounder if between-group differences will arise at baseline for any of the assessment sessions. Oral stimulants will be avoided for both mothers and infants. Salivary samples will be stored at −80°C until assayed in biology lab. Intra-assay and inter-assay variation coefficients will be computed separately for infants' and mothers' samples. Infants' feeding will not be allowed during the hour preceding the lab session.

Methylation samples. A separate saliva sample will be collected from both interactive partners at the very end of the session for epigenetic analysis of target genes DNA *methylation status*. The saliva will be collected from infants using the ORAcollect OC-175 kits (DNA Genotek, Ottawa, Canada) and stored at +4°C. Genomic DNA will be extracted following manufacturer's protocols and its quality will be assessed using a Qubit fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The methylation status of specific portions of the *NR3C1, SLC6A4, BDNF and OXTR* genes will be assessed by polymerase chain reaction amplification of bisulfite-treated DNA followed by next-generation sequencing on a NEXTSeq-500 (Illumina Inc, San Diego, California, USA).[78 79] We will analyse one region of *SLC6A4* (chr17:28562750–28562958, 20 CpGs); one region of *NR3C1* (chr142763694-142764254, 44 CpGs); one region of *BDNF* (chr11:27723017–27723244, 14 CpGs); two regions of *OXTR*: promoter (chr3:8811489–8811838, 9 CpGs) and intron 1 (chr3:8810654–8810919, 13 CpGs).

Questionnaires

Socio-demographic data. Demographics and socio-economic family situation will be collected by means of ad hoc questionnaires filled by mothers at each assessment session. Demographic variables will include: age and gender of the infant, specific diagnosis, developmental quotient (when available), rehabilitative programmes, use of medications, presence of siblings, health status of siblings, parental age, educational level, occupational status, psychological and physical health, and family SES.

Maternal emotional state. Beck Depression Inventory (BDI[80]) is a 21-item self-report instrument widely used to assess the presence of depressive symptoms in mothers. Each item is rated on a 4-point Likert scale. The State-Trait Anxiety Inventory (STAI-Y[81]) is a 40-item self-report tool that allows to obtain self-reported measures of transient (state; items 1 to 20) and stable (trait; items 21 to 40) anxiety symptoms. Each item is rated on a 4-point Likert scale. The Parenting Stress Index (PSI[82]) is a 36-item self-report questionnaire to measure the overall stress experienced in parenting on three sub-scales: parental distress, dysfunctional parent-child interaction and difficult child. Each item is rated on a 5-point Likert scale.

Mother-to-infant bonding. The Maternal Postnatal Attachment Scale (MPAS[83]) is a 19-item self-report instrument to evaluate post-natal maternal attachment. Each item is rated on a 5-point Likert scale.

Infant-related assessments. The Infant Behaviour Questionnaire-Revised (IBQ-R[84]) is a 37-item self-report tool used to evaluate temperamental traits of infants between 3 and 12 months and is divided in three broad scales: Negative emotionality (NEG), which represents a disposition to display negative emotions; Positive affectivity/Surgency (PAS), which is a disposition toward positive emotionality and activity; and Orienting/Regulatory Capacity (ORC), which is a disposition to show attentional, inhibitory, and activational control. Each item is rated on a 7-point Likert scale. The Griffiths Mental Development Scales–Third Edition (GMDS-III[85]) provides the DQ of the infants on five areas: (1) Foundations of learning, that assesses critical aspects of learning during the early childhood years; (2) Language and communication, which measures overall language development, including expressive language, receptive language and use of language to communicate socially with others; (3) Eye and hand coordination, that considers fine motor skills, manual dexterity and visual perception skills; (4) Personal–social–emotional, a construct relating to the child's developing sense of self and growing independence, interactions with others, plus many aspects of emotional development; and (5) Gross motor, that assesses postural control, balance and gross body coordination, among other abilities.

Maternal perceived efficacy. The Caregiver Health Engagement Scale (CHE-S[86]) is a 7-item self-report tool providing an assessment of the maternal emotional experience in managing the ND of the child and of the caregiving engagement with the intervention. Each item is rated on a 7-point Likert scale. The satisfaction questionnaire is created ad hoc for IG arm's mothers to assess their emotional experience, the degree of satisfaction and utility, and their opinions on the VFI. The questionnaire is composed by two open questions and 5-point Likert items. Each item is rated on a 5-point Likert scale (1, totally disagree; 5, totally agree).

Coding systems

Maternal behaviour. The PICCOLO coding system[87] provides an observational measure of developmentally supportive parental behaviour which has been used also with children diagnosed with ND. It is composed of a list of 29 parenting behaviours observable in the interaction with their children, divided into the following four domains: *affection*, that involves the physical or verbal expression of affection, positive emotions, positive evaluation and positive regard; *responsiveness*, which includes reacting sensitively to a child's cues and expressions of needs or interests and reacting positively to the child's behaviour; *encouragement*, that includes parents' support of children's efforts, exploration, independence, play, choices, creativity and initiative; *teaching*, which incorporates cognitive stimulation, explanations, conversation, joint attention and shared play. Each item is rated on a 3-point Likert scale (0=never observed, 1=rarely observed and 2=often observed). Independent coders will be trained to gold-standard 85%.

Infant behaviour. The GRS coding system[76] is a macro-analytic tool to examine mother-child interaction behaviours. For the aims of the present study, the seven infant-related scales will be rated by trained coders on 5-point items grouped into two dimensions: good-poor regulation (items: attentive (5) vs avoidant (1); active positive communications (5) vs no communications (1); and positive vocalisations (5) vs no vocalisations (1)) and inert-fretful (items: engaged with environment (5) vs self-absorbed (1); lively (5) vs inert (1); happy (5) vs distressed (1); and calm (5) vs fretful (1). A global Good-Poor score for infant behaviours is obtained by averaging the scores obtained at each item (range: 1 to 5). Independent coders will be trained to gold standard 85%.

Blinding

For practical reasons, the psychologists who will carry on the intervention will not be blind to the random arm allocation. However, the recorded mother-infant interactions will be coded by research assistants who will be blind to the session and the RCT arm.

Interventions

VFI protocol (IG arm)

In each of the three units involved in the EPI-BOND study, the intervention will be conducted by a trained psychologist. The VFI protocol is inspired by the dialogic principles of the approach to collaborative consultation[88] to build a positive alliance of work with the family. Dyads randomised to the IG will receive six weekly 90 min VFI sessions organised in two subsequent phases: *Sharing the focus* (four sessions) and *Interactive integration* (two sessions) (table 1). During the *Sharing the focus* sessions, the psychologist will review with parents segments of the videotapes obtained at T_0 and focus on four different relational themes (ie, sensitivity-responsiveness, physical stimulation, teaching-encouragement and parenting perspective). During the *Interactive integration* phase, the insights developed from *Sharing the focus* will be applied to the real-time interaction between the mother and her infant under the guidance of the psychologist. During these sessions, the psychologist will make use of different psychological techniques, which are aimed at maximising the integration between what has been discussed 'behind the screen' and what is happening in the here-and-now of the dyadic interaction.

Table 2 Description of sessions in each telephone call sessions

Phase	Sessions	Definition
1	Approach	Child's reaction to novel situations (eg, stranger adults or places)
2	Mood	Prevailing tone of child's emotional regulation
3	Rhythm	Routines that the child has both at food level and at rest level
4	Activity level	Proportion between active and inactive physical activity
5	Attention	Child's ability to be attentive, the duration of attention and his distractibility
6	Touch	Physical contact between the parent and the infant

'Dummy' intervention (CG arm)

According to previous VFI RCTs,[89] the mothers of infants randomised to the CG arm will receive six weekly 15 min telephone calls according to previous studies.[89] The telephone calls will be carried out by a trained psychologist. The six telephone call sessions will focus on gathering information about the development of the infant by referring to six specific thematic areas (table 2) taken by adapting the main scales of the Revised Infant Temperament Questionnaire.[90] As such, contrary to the IG arm, mothers of infants randomised to telephone calls will not receive a tailored support through the VFI, but rather followed-up and monitored for the behavioural development of their infants during the same 6-week period and will receive general information about how to support their infants' behaviour and regulation capacities.

Training

The assistant psychologists responsible for carrying on the VFI and the dummy intervention will be adequately trained through theoretical, methodological and role playing sessions. The training will take place in the first

Table 1 Description of phases and sessions in the video feedback intervention

Phases	Sessions	Content
Sharing the focus		
1	Sensitivity-responsiveness	Relational aspect of the mother-infant interaction
2	Stimulation	Somato-sensorial component of mother-infant relationship
3	Encouragement	Cognitive aspect of mother-child interaction
4	Parental perspective	Mother's inner experience in the interaction with the infant
Interactive integration		
5 and 6	Real-time mother-infant interaction	Integration of insights obtained in sessions 1 to 4 during the live occurrence of mother-infant interaction in presence of the psychologist

3 months of the study. They will learn to select videotape segments according to the four relational themes and gold standard comparison will occur with referral to coding by a senior researcher (RM).

OUTCOMES

Primary outcome measures

The primary aims of the EPI-BOND study are (Aim 1A) to improve infants' behavioural and socio-emotional regulation and (Aim 1B) to support parental caregiving behaviour. Infants' socio-emotional regulation will be measured at each assessment session by coding, respectively,the unstructured mother-infant interaction and the FFSF procedure with the infant-related scales of the GRS.[76] Developmentally supportive parental behaviour will be assessed by means of the PICCOLO scales.[87] The score of each scale is obtained by summing the scores of the variables from which it is made.

Secondary outcome measures

The secondary goals of the EPI-BOND study are to exploratory assess whether the VFI interventions in IG mothers and infants—compared with CG counterparts—associate with differential (Aim 2A) methylation regulation of specific genes (ie, *BDNF, NR3C1, OXTR and SLC6A4*) and (Aim 2B) neuroendocrine regulation during face-to-face interaction (ie, salivary oxytocin) and in response to socio-emotional stress (ie, salivary cortisol).

Neuroendocrine regulation. Both salivary oxytocin and cortisol values will be checked for normal distribution and log-transformed if needed. The salivary oxytocin concentrations ($oxtrT_{0\text{-}3}$) will be assessed for differences between and within group by means of a generalised linear model. The hypothalamic-pituitary-adrenal (HPA) axis stress regulation will be assessed in terms of *direction* and *magnitude*.[91] *Direction* will be assessed by computing the difference between baseline and the highest post-FFSF sample salivary concentration: negative values will mean an increase, whereas positive values will mean a decrease of salivary cortisol concentration from before to after the exposure to socio-emotional stress.[76] To detect a significant change in any direction, the increase or decrease will have to be equal or higher than one SD. *Magnitude* of HPA axis response to stress will be measured by means of a validated stress regulation index, namely the area under the curve (AUC) with respect to increase (AUC-I) at each assessment session.[92] The AUC is estimated through the trapezoidal rule and computed as the integral defined by plotting baseline and post-stress samples.[93] More specifically, the AUC-I is adjusted for inter-individual baseline variations excluding the area defined by fluctuating baseline levels between participants.[94] The two indexes will be assessed independently as secondary outcomes for salivary cortisol regulation; moreover, an interactive model testing *magnitude* as the outcome and *direction* as a moderator will also be tested.

DNA methylation. The DNA methylation of target genes will be assessed at specific CpG sites (figure 2), previously explored for associations with infants' behaviour and/or stress regulation as well as with exposure to early environmental variations in caregiving. The analyses will be carried out on a CpG-specific level as well as on clusters of CpG sites obtained by means of Principal Component Analysis. All the analyses will be adjusted for multiple comparisons through the Benjamini-Hochberg correction.

Feasibility outcome measures

Feasibility will be assessed by means of *acceptance of the intervention* and *maternal experience* with the VFI. These measures will be obtained at the end of the eighth session (namely T_1). As for *acceptance of the intervention*, an ad hoc satisfaction form will be delivered to mothers randomised to the IG arm who will be asked to respond to both open questions and 5-point Likert items (1, totally disagree; 5, totally agree) meant to depict the emotional experiences of parents during the intervention, their perception of the usefulness of VFI intervention and an evaluation of what was most helpful and most challenging for them. Additionally, mothers will be asked if they would suggest the intervention to other parents and which changes they would propose to the procedure. As for the *maternal experience* with the VFI, two self-report tools measuring caregiving engagement with the intervention (CHE-S[82]) and with their child (MPAS[83]) will be administered at the end of session 8.

Mediation measures

As reported above, we aim to explore the potential mediation role played by neuroendocrine and epigenetic regulation in affecting the association between exposure to the VFI intervention and primary outcomes. In the absence of previous research assessing the association between methylation of target genes and neuroendocrine regulation mediating the effect of early parental support intervention—especially with infants diagnosed with ND—we do not expect a specific direction for the mediation effect.

Confounding variables

Demographics variables, diagnosis and DQ will be measured and used as confounding variables between IG and CG.

Demographic variables

After pre-test, parents will complete a self-design demographic and socio-economic questionnaire (see *questionnaires* for more information).

Diagnosis

The diagnoses and degree of severity of children's pathologies will be taken into consideration to better balance the intervention and control groups. Infants with specific pictures of neurodevelopmental risk will be included in the study (see *inclusion criteria* for more information).

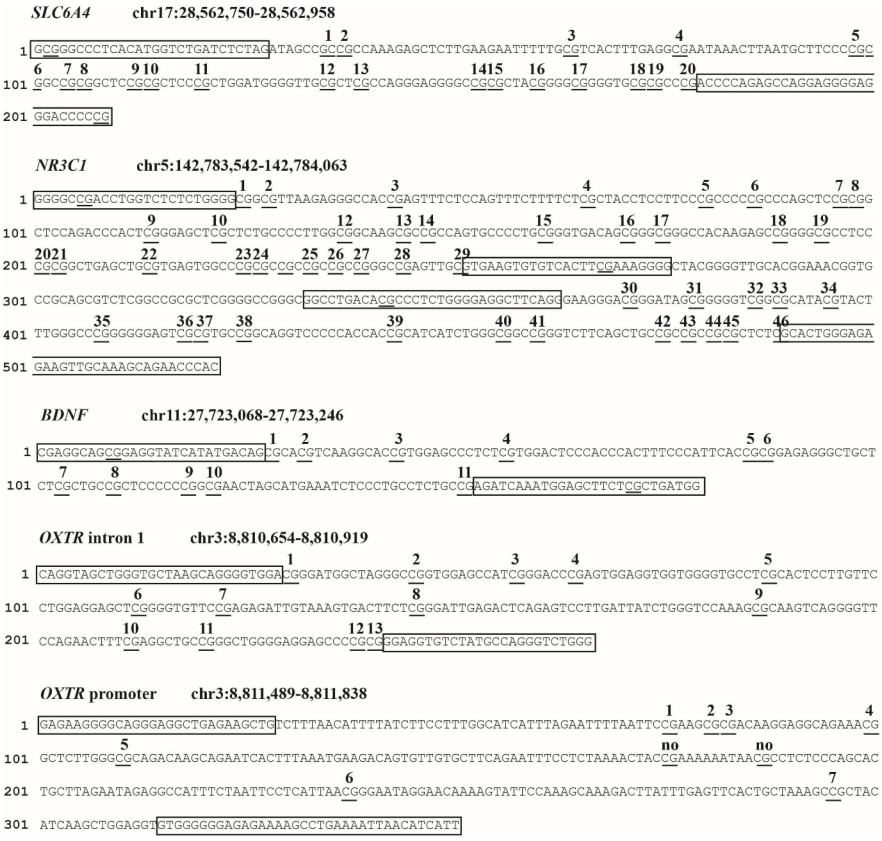

Figure 2 Graphic representation of target genes (*SLC6A4; NR3C1; BDNF; and OXTR*) CpG sites. CpG, cytosine and guanine

Development quotient

The DQ of the infants will be available before the enrolment of the mother-child dyad and will be taken into consideration to balance the two groups (control and intervention) during the research. Infants with a DQ ≤70 on the Griffiths Mental Development Scales, third edition,[85] will be included in the research.

Maternal well-being

Additional measures will be obtained at each assessment session for maternal well-being using the PSI, BDI and STAI-Y to control for group differences in the presence and severity of perceived parenting stress, depressive and anxious symptomatology.

Statistics

Sample size

In the absence of previous clinical trials of VFI with mothers of infants with ND, sample size estimation has been estimated using G*Power software according to previous meta-analytic evidence of VFIs for parental sensitivity (Fukkink *et al*, 2008). Setting d=0.47, alpha=0.05, and beta=0.20 resulted in n=73 participants per group. Moreover, due to the longitudinal nature of the study, a 20% drop-out risk was estimated from T_0 to T_1, leading us to estimate a starting sample size of 91 infants for each group. Consistently, each unit will have to enrol 24 dyads per group.

Drop-out estimation

All families who accepted to participate in the study will be followed from baseline assessment to follow-up assessment 11 months later. In the case of family drop-out, the reasons will be documented and analysed to possibly improve the implementation of intervention. Intention-to-treat analyses will be conducted to account for drop-out.

Plan of data analysis

Research assistants who will be blind to allocation will do data entry. The principal investigator (PI) will be responsible of checking data quality through cross-check validation. All statistical analyses will be done using the SPSS programme (V.21.0 for Windows). Baseline characteristics will be obtained by means of descriptive analysis. All outcome measures will be scaled as continuous. Differences in baseline characteristics between experimental and control groups will be checked. Differences between IG and CG groups in the T_0 to T_1 change in primary endpoint will be evaluated by means of mixed-model analysis of variance, including group as the between-subject variable, time as the within-subject variable and the interaction term group*time. A similar model will be used for the secondary endpoint. As the neuroendocrine and epigenetic outcomes correspond to exploratory aims, the two groups will be assessed for the presence of significant differences in neuroendocrine salivary concentrations (cortisol and oxytocin) and DNA methylation of selected

target genes separately at each time point by means of independent sample t-tests. Moreover, multiple Pearson's bivariate correlations will be used to explore the presence of significant associations between primary and secondary endpoint measures, salivary concentrations of oxytocin and cortisol as well as the CpG site DNA methylation status of selected target genes. Benjamini-Hochberg (q<0.10[78]) algorithm will be used to check for multiple comparison bias.

Data management, monitoring and protection

After informed consent for participation in the study is obtained, all participating families will be assigned a number. The list of numbers—personal data combinations—will be stored separately from the data. Only the PI will have the opportunity to check interim analysis and to initiate procedures for trial termination in case of any risk for a given participant. The data will be analysed so that no conclusions can be drawn about individual participants. All data will be stored on a password-protected server, and if applicable, in lockable cabinets in lockable rooms. Data access will be established to assistant researchers nominated by the PI and according to ethical procedures that adhere to the European laws for privacy 2.0 and that are actually in use at the hospital facilities. All assistant researchers with access to the data will sign a non-disclosure statement, which states they will not disclose any information about research participants to a third party. The Italian Ministry of Health carries out regular monitoring and supervision for the adherence to the study protocol and any change to the study plan will be adequately communicated and discussed with this entity.

ETHICS AND DISSEMINATION

Publication and dissemination plan

Results of this study will be published in a peer-reviewed, international journal and will be presented at international and national scientific conferences within the field of neurodevelopmental disabilities. Additionally, activities will be promoted to create support guidelines aimed at specific groups, such as parents' associations, to better support and promote the parenting of families of children with NDs. We also plan to publish a paper about the intervention protocol and another to bring the research results to interested practitioners. Generally accepted criteria related to the actual and documented involvement of researchers in data planning, collection, analysis and/or drafting will be used to determine authorship. The future use of biological specimens for future ancillary studies is not established. The use of professional writers is not planned.

Ethical considerations

This study was carried out in accordance with the recommendations of ethics committees of every single unit with written informed consent from all participants. All participants gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the IRCCS Eugenio Medea, Bosisio Parini, (Lecco); IRCCS Casimiro Mondino, Pavia; and University of Brescia, Brescia.

Actual status of the trial

The study protocol was approved by ethics committees in December 2018. After being granted permission by the ethics committees of the IRCCS Eugenio Medea, Bosisio Parini (Lecco); IRCCS Casimiro Mondino, Pavia; and University of Brescia, Brescia, to start the enrolment, the first dyad was enrolled in September 2019.

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Contributors RM, LP, RG, EF, AC and SO wrote the funding proposal for this study. All authors contributed to the final design of the present study. ER coordinates recruitment of participants and data collection. RG coordinates biological analyses. RM and LP supervise the progress of the study. LP and ER wrote the first version of this manuscript draft. All authors provided suggestions for the improvement of the manuscript. All authors read and approved the final submission.

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