EARLY DIAGNOSIS IN AUTISM SPECTRUM DISORDERS: SUGGESTIONS FROM ANIMAL MODELS

S. Orlandi[1,2], C. Manfredi[1], A. Guzzetta[3] and M.L. Scattoni[4]

[1] Dept. of Information Engineering, University of Firenze, Firenze, Italy, silvia.orlandi@unifi.it
[2] Dept. Electronic and Information Engineering "Guglielmo Marconi", University of Bologna, Bologna, Italy
[3] Dept. of Developmental Neuroscience, Stella Maris Scientific Institute, Pisa, Italy, a.guzzetta@inpe.unipi.it
[4] Dept. of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Roma, Italy, marialuisa.scattoni@iss.it

Abstract: **Autism Spectrum Disorders (ASDs) have an estimated prevalence rate of 1/88 suggesting that ASD represents a significant public health problem. Although causes of ASD are still unknown, the strongest evidence appears to be genetic. In this regard, studies on mouse models bearing mutations identified in ASD candidate genes could be extremely helpful to identify early behavioral traits that cannot be studied in human infants since at present ASD cannot be diagnosed reliably before two years of age.**
Our team recently characterized early phases of neurobehavioral development in several animal models of autism detecting quantitative and qualitative abnormalities in their vocal and motor repertoires. These results suggested us to assess early vocal and motor repertoires in high-risk infants (siblings of ASD children) and in control infants. An approach linking experimental findings obtained in animals to human infants might be successful in order to identify early markers of ASD. Aim of our project is to develop an automatic system to record newborn cry and movements during the first six months of life with a specific protocol. Our acoustic analysis is focused on fundamental frequency (F0), number of cry-episodes and F0 shapes. Preliminary results showed some differences concerning fundamental frequency between normal and high-risk cases. Moreover high-risk subjects emitted a lower number of cry-episodes than control subjects. Following the mouse methodological approach, we are also investigating melody in high risk infants searching for early indicators of ASD.

Keywords : **Infant's cry, Autism Spectrum Disorders, Autism Animal model, acoustic analysis**

I. INTRODUCTION

Autism Spectrum Disorders (ASDs) are a group of complex disorders of brain development characterized at different levels. by difficulties in social interaction, verbal and nonverbal communication and repetitive behaviors. They include autistic disorder, childhood disintegrative disorder, pervasive developmental disorders not otherwise specified and Asperger syndrome [1].

In most cases, ASDs signs appear in the first year of life, when it is not yet possible to carry out a reliable diagnosis. This is because the diagnostic tests currently used and considered gold-standard for ASD are based on the identification of behavioral symptoms which are more evident after the 24th month of life. Etiology of ASDs is still unknown, although several studies identified genes mutations, copy number variants and abnormal neuro-transmission in specific brain areas such as limbic system, amygdala, cerebellum and sub cortical areas that mediate motor control [2].

Preclinical research on ASD currently represents an emerging field in translational neuroscience. In fact, animal models of ASD can provide translational tools to identify neurochemical markers and behavioral patterns that cannot be studied in human infants since at present ASD cannot be diagnosed reliably before two years of age. In this regard, mouse models bearing mutations identified in ASD candidate genes and that exhibit a clear autistic-like phenotype would be most promising. An approach linking experimental findings obtained in animals to human infants might be successful in order to identify as early as possible vulnerable behavioral patterns associated with alteration in selected genetic and biochemical markers.

Our team recently characterized early phases of neurobehavioral development in several animal models of autism [3-5]. In particular, to address communication deficits, we investigated ultrasonic vocalizations detecting qualitative abnormalities in their vocal repertoire that may resemble the atypical vocalizations and the monotonic tone found in some autistic infants [6,7]. These data collected on animal models suggested us to assess early vocal and motor repertoire in infants siblings of ASD children and in a population of about 200 healthy infants. Previous studies showed that some age-specific motor and vocal traits are altered in ASD children [8-10]. In most of these studies general movements (GMs) and infant crying analyses were assessed by home-videos of children's first birthday party, but this method presents some limits. First of all, existing data refer to the assessment of a restricted number of infants (e.g. 10-12) [10]. Moreover, the method may be not reliably standardized because of methodological differences in the quality of recordings and in the setup of the observations.

Claudia Manfredi (edited by), *Models and analysis of vocal emissions for biomedical applications : 8 th international workshop : December 16-18, 2013*
ISBN 978-88-6655-469-1 (print) ISBN 978-88-6655-470-7 (online)

Finding links between GMs and cry analysis is desirable and of great relevance since they both reflect the development and the integrity of the central nervous system and can be exploited for early clinical diagnosis enabling a more effective intervention of several pathologies since they are easy to perform, cheap and marker-less. Although perceptual analysis of movements and crying is carried out in specialized clinics, the lack of automatic tools requires to clinicians a great deal of time with a large margin of error. It is therefore important to develop semi-automatic qualitative methods to support the clinical diagnosis allowing for feature extraction and perceptual analysis with marker-less techniques.

This paper presents a new tool for the management of patient data, tests and reports, acquisition of audio and video data, their editing and analysis to support clinicians with perceptual diagnosis.

II. METHODS

This work is linked to the Italian grant project "Young Researcher 2008: Non-invasive tools for early detection of autism spectrum disorders", aiming to detect early markers of Autism Spectrum Disorders (ASDs) through the study of infant crying and GMs during the first six months of infant's life. This project aims to identify normative ranges for acoustical and motor parameters in a population of about 200 healthy newborn/infants, both male and female (control group). The control group will be compared with 15-20 "high-risk" newborn/infants, i.e siblings of children already diagnosed with ASD [11, 12].

Infants were audio and video recorded at home five times during the first six months of life according to a specific protocol [12]: at 10 days, 6, 12, 18 and 24 weeks of life. The protocol also includes clinical assessment performed using a set of questionnaires (Italian Questionnaire of Temperament; Bayley Scales of Infant Development; the first child vocabulary, MacArthur - Bates Communicative Development Inventory and the Modified Checklist for Autism in Toddlers (*M-CHAT*)). Informed consent was obtained from parents. The protocol was approved by the local ethical committee (Istituto Superiore di Sanità, Roma, Stella Maris Hospital, Pisa and Bambin Gesù Hospital, Roma, Italy).

Cry analysis was carried out by the estimation of acoustic parameters such as fundamental frequency (F0), intensity, resonance frequencies of the vocal tract and length of each cry episode [13].

GMs analysis was performed with a perceptual technique that is often used by clinicians to diagnose motors problems associated with impairment of the central nervous system such as the early diagnosis of cerebral palsy [14].

A. ARAD System

According to the project [11, 12] and in co-operation with ISER tech srl (Prato, Italy), we developed a new tool for audio/video acquisition and analysis for contact-less diagnosis, in particular in neonatology area, named ARAD (Acquisition, Reporting and Analysis for Diagnosis), shown in Fig. 1. ARAD is designed for home use to minimize the discomfort for the involved subjects and the impact of the external environment on children habits. Hence, the basic requirement is the ease of transport and assembly of the system. It includes a laptop connected to a high-speed USB webcam (Logitech HD pro webcam C910) able to provide a 1280x1024 pixel video stream, a sound board (Tascam US-144-MK2) and a professional microphone (Shure SM58).

ARAD allows the management of personal data and medical history of the patient, data acquisition, personalized test editing, audio/video editing and reporting in a single software tool. The storage of data patient is managed through a centralized database structured to guarantee privacy and personal data protection. Personal data management is integrated with the centralized database that allows the user viewing reports of patients.

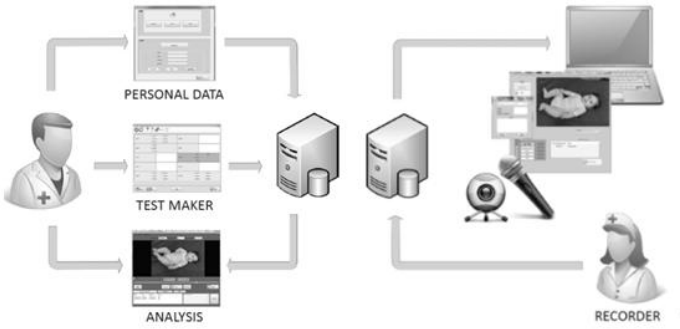


Fig. 1-ARAD System

The system consists of four parts:

a) Personal Data Management System

an integrated management system to share data and medical history with dedicated interface and for patient's clinical storyboard. It allows importing and exporting signals, images and tests from external sources.

b) Test maker

an interactive environment for the creation and run of specific clinical tests with modular customizable structure. It is possible to insert multimedia contents, images, audio / video screenshots and monitor the results.

c) Recorder

an integrated environment for capturing multichannel audio/video with the possibility to enter notes and contextualized information through the wizard and flexible setup of the system.

d) Analyzer

tool devoted to the perceptual analysis and audio/video editing. Provides the ability to easily cut /copy/evaluate sequences of interest and enter clinical assessments without the need to resort to the use of other software.

B. Analysis tool

ARAD is equipped with a devoted tool for the analysis of audio and video recordings. It provides objective cry parameters and simplifies GMs perceptual analysis. Specifically:

a) Cry analysis

On the selected crying frames time-frequency analysis is carried out according to the methods used in [11, 13, 15]. Extracted parameters are: fundamental frequency of cry excerpts (F0), vocal tract resonance frequencies, number of cry-episodes, vocal percentage, number and length of voice breaks.The recorded sound is band-pass filtered by a Butterworth filter of order 5 and a cut-off frequency of 50–1000 Hz.

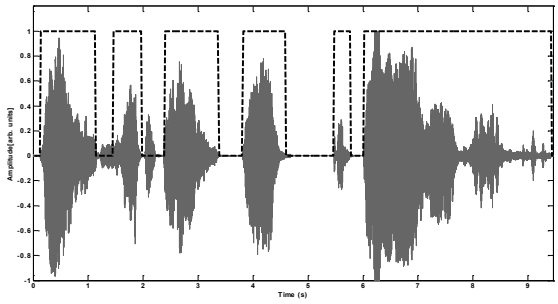


Fig. 2-An example of V/UV detection

Detection of crying episodes (voiced frames lasting at least 150ms) is performed using a robust newly developed Voiced/Unvoiced (V/UV) detection procedure [15]. An accurate detection of starting and ending points of the events allows avoiding incorrect splitting of a single event into several intervals that may occur in the case of irregular and quasi-stationary signals as newborn infant cries are, disregarding noise and silence while retaining those irregularities that may be a diagnostic index of possible pathologies or malfunctioning. An example of V/UV selection is shown in Fig. 2. On selected voiced frames F0 is estimated with a two-step procedure already found successful when analyzing newborn cries [14].

In each cry episode, the fundamental frequency presents a well-defined trend (melody). Based on mouse data [3, 6]) and previous human studies [16], four typical patterns are detectable in the infant crying: symmetrical (frequency rising and falling around a central peak), rising (frequency peak appears near the end of the episode), falling (frequency peak appears at the beginning), and plateau (with an almost constant frequency). The automatic extraction of these patterns is under study.

b) General movements analysis

On selected video frames that contains the GMs the clinician can built, compile and export specific tests. It is also possible to enter number of motion sequences and of abnormal movements, as well as the duration and the time instant at which they occurred.

. III. RESULTS

At present we have collected data from 75 children, namely 66 control (CC) and 9 high-risk cases (HRC). Acoustic analysis was performed only on 3 high-risk cases that were found positive to the GMs analysis qualitatively carried out by clinicians. We analyzed 30 seconds of cry for each infant at 10 days, 6, 12, 18and 24 weeks of life (10000 CC and 474 HRC hunger cry-episodes). Preliminary results show that, in high-risk subjects:

1. the number of cry-episodes is 37% lower than in control subjects;
2. the number of vocalic zones is 20% lower than in control subjects;
3. F0 is about 50 Hz lower than in control subjects and shows less variability.

The ontogenetic profile of F0 in the first 6 months of life is shown in fig. 3.

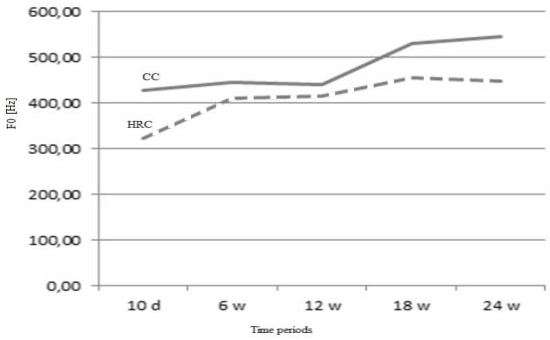
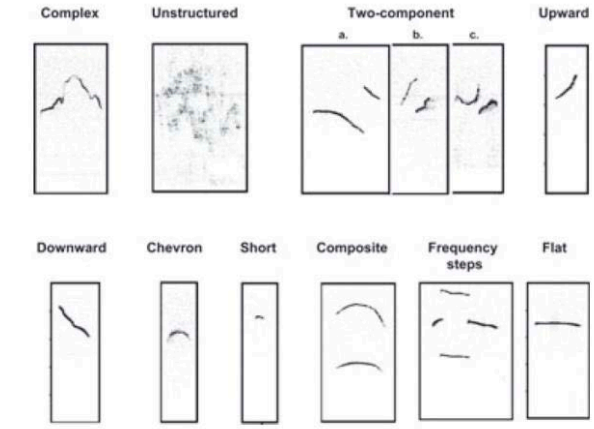


Fig. 3-F0 in first 6 months of infants'life

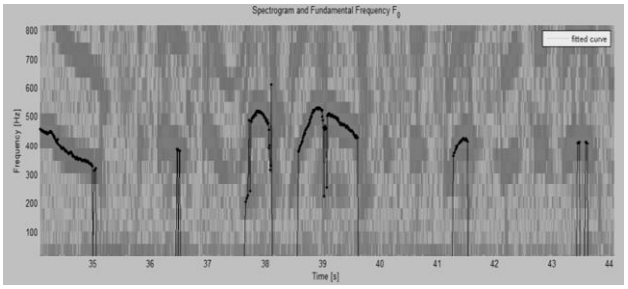
IV. DISCUSSION

Mice and humans both vocalize through the vocal folds and the ultrasounds and crying emitted by their pups/infants are structurally similar and have the same communication value. Thus future studies will be devoted to correlate infant crying of high-risk infants with the ultrasonic vocalizations emitted by animal models of ASD in terms of sound patterns. Our studies on animal models of ASD revealed that pups have a restricted vocal repertoire when tested at postnatal day 8 (10 waveform patterns and an example of typical newborn cry are

illustrated in Fig. 4). We are also investigating melody in high risk infants searching for early indicators of ASD.



(a)



(b)

Fig. 4, (a) typical sonograms of ultrasonic vocalizations [6] and (b) an example of baby's cry melody at 10 days

ACKNOWLEDGMENT

Supported by the Italian Ministry of Health Grant (GR3), Young Researcher 2008, "Non-invasive tools for early detection of Autism Spectrum Disorders". The authors thank ISER Tech srl for its contribution to this work.

REFERENCES

[1] Diagnostic and statistical manual of mental disorders (4th ed., text rev.). Washington, DC: APA, 2000

[2] Bauman ML, KemperTL, Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci, 2005, 23(2,3) pp 183-187

[3] Romano E, Michetti C, Caruso A, Laviola G, Scattoni ML. Characterization of neonatal vocal and motor repertoire of reelin mutant mice. PLoS One. 2013 May 21;8(5):e64407

[4] Michetti C, Ricceri L, Scattoni ML. Modeling social communication deficits in mouse models of autism. Special Interest Section .Animal Models in Autism, Autism-Open Access, 2012

[5] Scattoni ML, Ricceri L, Crawley JN. Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. Genes Brain Behav. 2011 Feb;10(1) pp 44-56

[6] Scattoni ML, Gandhy SU, Ricceri L, Crawley JN. Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. PLoS One. 2008 Aug 27;3(8):e3067

[7] Scattoni ML, McFarlane HG, Zhodzishsky V, Caldwell HK, Young WS, Ricceri L, Crawley JN. Reduced ultrasonic vocalizations in vasopressin 1b knockout mice. Behav Brain Res. 2008 Mar 5;187(2), pp 371-8

[8] Phagava H, Muratori F, Einspieler C, Maestro S, Apicella F, Guzzetta A, Prechtl HF, Cioni G General movements in infants with autism spectrum disorders. Georgian medical news, 2008, 156, pp. 100-105

[9] Esposito, G., Venuti, P., Developmental changes in the fundamental frequency (f0) of infants' cries: A study of children with Autism Spectrum Disorder, Early Child Development and Care, 2010, 180 (8), pp. 1093-1102

[10] Baranek GT, Autism During Infancy: Retrospective Video Analysis of Sensory-Motor and Social Behaviors at 9–12 Months of Age, J Autism Dev Disord, 1999, 29(3), pp. 213-224

[11] Orlandi S, Manfredi C, Bocchi L, Scattoni ML, Automatic newborn cry analysis: a non-invasive tool to help autism early diagnosis, ConfProc IEEE Eng Med BiolSoc, 2012, pp. 2953-2956

[12] Bocchi L, Orlandi S, Manfredi C, Puopolo M, Guzzetta A, Vicari S, Scattoni ML, Early Diagnosis of Autism Spectrum Disorders – Design of the Data Acquisition and Management System, 5th European Conf. of the Int.Fed. Med. Biol.Eng, Budapest, Hungary, IFMBE 2011, Proc.37, pp. 187-190

[13] Manfredi C, Bocchi L, Orlandi S, Donzelli GP, High-resolution cry analysis in preterm newborn infants, Med. Eng. &Phys, 2009, 31(5), pp. 528-532

[14] Einspieler C, Prechtl HFR, Bos AF, Ferrari F, Cioni G, Prechtl's Method on the Qualitative Assessment of General Movements in Preterm, Term and Young Infants. Cambridge University Press, 2004

[15] Orlandi S, Dejonckere PH, J. Schoentgen J, Lebacq J, Rruqja N, Manfredi C, Effective pre-processing of long term noisy audio recordings: An aid to clinical monitoring, Biomedical Signal Processing and Control, 2013, 8(6), pp. 799-810

[16] Wermke K, Mende W, "Musical elements in human infants' cries: in the beginning is the melody", Musicae Scientiae, 2009, 13, pp. 151-173