ANNUAL REPORTING
2022

CALL:	**EEA grants Call for proposals 2018 - CRP**
Project code:	PROJECT: EEA-RO-NO-2018-0573
Project title:	Improving quality of life for Autism Spectrum Disorders patients by promoting strategies for early diagnosis and preventive measures
Project acronym:	IQUALASD
Duration (months):	57
Project signature date:	31.07.2019
Project eligibility end date:	30.04.2024
Total budget from the Programm (euro):	1,820,000
- Grant (85%):	1,275,000
- Co-financing (15%):	225,000
Own budget (euro):	320,000
Project Webpage:	http://www.eea-grant-autism.ro/ro/
Project Promoter organization:	Clinical Hospital of Psychiatry ”Prof. Dr. Alexandru Obregia” (OHP)
Principal Investigator:	Magdalena Budisteanu, MD, PhD, Senior Researcher
Project Partner organization (1):	University of Oslo (OU)
Project Partner organization (2):	“Victor Babes” National Institute of Pathology (IVB)

Iceland Liechtenstein Norway grants

Executive Agency for Higher Education, Research, Development and Innovation Funding

TECHNICAL REPORT (Part 1)

Explanation of the work carried out by the participants (max 10.000 characters, including spaces)

The **project focuses on autism spectrum disorders** (ASDs), neurodevelopmental conditions characterized by a specific combination of social relationships and communication deficiencies, repetitive behaviors, and restricted interests, with onset in early childhood. The comprehensive evaluation of a big cohort of patients with ASDs will contribute to the **establishment of the Romanian Registry for ASD**, and will offer data **for improving the early diagnosis and understanding of ASDs mechanisms**.

Phase IV: Integrative data analysis
Activities
A 4.1. Patient and control group enrollment
A 4.2. Genetic tests (Array-CGH studies, fragile X studies, genotyping, WES, other molecular genetic tests) of patients and control groups included in the study
A 4.3. Common analysis of data
A 4.4. Data management and database set up for Romanian National Registry for ASD patients
A 4.5. Elaboration of scientific papers / communications; training activities

PP performed A.4.1 and participated in A.4.3, A.4.4 and A.4.5.

Three new ASD patients were included in the study: two siblings (a 6-year-old girl and a 5-year-old boy) and a 5-year-old girl. Both siblings had speech delay, and the boy presented also mild intellectual disability, hyperkinetic behavior, and bilateral parietal white matter small lesions on brain MRI. The third case associated mild intellectual disability, epileptic seizures and brain malformation (left polymicrogyria and bilateral periventricular heterotopia). Blood samples from children and their parents were obtained and sent to partner P1 for genetic testing.

P1 performed A 4.2. and participated in A.4.3, A.4.4 and A.4.5.

P1 received 188 DNA samples from control individuals. P1 performed …….

P2 performed A 4.2. and participated in A.4.3, A.4.4 and A.4.5.

Genetic tests (Array-CGH studies, fragile X studies, genotyping, WES, other molecular genetic tests) of patients and control groups included in the study

Sample processing and DNA isolation continued as described in the previous years. Samples were received for control individuals (37 samples) and ASD individuals (three samples).
DNA genomic profiling
Array-based comparative genomic hybridization (**array-CGH**) was performed for …. individuals in the control group and for the patients enrolled in 2022. Agilent SurePrint G3 Human CGH Microarray Kit 180K, with a median spacing of 11 kb (in Refseq genes), was

used for array-CGH, following the manufacturer's protocol as described previously. Microarray slides were scanned with SureScan Agilent Scanner with the generation of a tiff image file for each slide. Data extraction and analysis was performed with Agilent Cytogenomic Software v.5.1.2.1 with incorporated Feature Extraction software. The raw data from all the hybridized grids were extracted maintaining the same parameters as in the previous analyses; quality metrics were evaluated for each sample. The variation calling threshold was set at a log2 ratio value 0.30 for at least three consecutive probes. The genomic profiles were also manually evaluated for probe uniformity in the called intervals. CNVs classification was performed according to ACMG guidelines. CNVs annotation was performed for ASD group using GRCh 37 (hg 19) build. Public resources / databases (UCSC genome browser, DGV, OMIM, DECIPHER, ClinVar, ClinGen Genome Dosage Map, GnomAD SV etc) and scientific papers were used for CNVs annotation and interpretation. Data analysis was completed for the entire patient group. The genomic profiles of ASD individuals were correlated with the phenotypic data in order to assess the clinical significance of CNVs. Thirteen clinically relevant CNVs were detected in this stage, in addition to those previously described. These CNVs, classified according to ACMG guidelines as pathogenic and likely pathogenic, consisted of nine deletions, three duplications and one extra chromosome Y in an individual with XYY genotype. The distribution of the clinically relevant CNVs across the genome was the following: four variants on chromosome 2, four variants on chromosome 15, and one variant on each of the chromosomes 3, 5, 11, X and Y. With the exception of the additional chromosome Y, the median size of these CNVs was 493.5 Kb, encompassing over 400 genes among which 170 are included in the OMIM database.

FMR1 gene investigation for trinucleotide repeat expansion associated with Fragile X syndrome. Methylation specific MLPA - MS-MLPA – was performed for 21 male patients, following the protocols described in the previous intermediate reports. Triplet primed PCR (TP-PCR) was performed with the protocol optimized and described previously in our project report. All male patients were investigated; the prevalence of full mutation was 4 out of 228 (1.75%), similar with current literature data.

Common analysis of data and data management and database set up for Romanian National Registry for ASD patients were performed with the contribution of all partners.

In the entire patient group, clinically relevant genomic defects CNVs were detected in ~ 10% of the ASD individuals. The genomic defects encompassed chromosomal aneuploidies, extra Y chromosome in 2 male patients, as well as deletions and duplications. The size of CNVs ranged from small anomalies (28 kb) to large variants (16.8 Mb), with a median of 1.6 Mb. Recurrently involved chromosomes were chromosome 15 (6 patients), chromosome 2 (4 patients), and chromosomes 11. Relevant CNVs were mainly deletions (19 out of 28 CNVs). Although deletions size varied widely, the median size of deletions was lower compared to duplications (1.5 Mb vs 2.3 Mb).

Most of these patients had complex phenotypes which included global developmental delay / moderate or severe intellectual disability, dysmorphic features, epileptic seizures, and congenital heart malformations.

Genotype – phenotype correlations

Correlations between the genotype and the phenotype of the patients were performed for all cases with pathogenic variants. Thus, 13 children – 12 boys, 1 girl - presented pathogenic variants:

1. Duplication of 2q11.1q11.2 in a 4-year-old boy with mild developmental delay,

dysmorphic facial features, muscle hypotonia, frequent respiratory infections.
2. Deletion of 6q12 in a 5-year-old boy with mild motor delay, moderate cognitive and speech delay, dysmorphic facial features.
3. Deletion of 2q24.3 in a 7-year-old boy with mild motor delay, severe cognitive and speech delay, dysmorphic facial features
4. Deletion of 15q24.1q24.2 in a 8-year-old boy with mild motor delay, mild cognitive delay, hypotonia; dysmorphic facial and limb features, with family history of speech delay.
5. Deletion of 5q11.2 in a 4-year-old boy with speech delay, dysmorphic facial features, and a family history of speech delay.
6. Deletion of 15q11.2 in a 3-year-old boy with speech delay, dysmorphic facial features, congenital heart malformation, and febrile seizures.
7. Duplication of Xq28 in a 6-year-old boy with global developmental delay, epilepsy, dysmorphic features, feeding difficulties with failure to thrive in first year of life, Leiner-Moussous syndrome.
8. Duplication of Y in a 15-year-old boy with Asperger syndrome, motor tics, tall stature, and macrocephaly.
9. Deletion of 2p16.3 in a 4-year-old boy with speech delay, epileptic seizures, and dysmorphic facial features.
10. Duplication of 15q11.2q13.1 in a 5-year-old boy with speech delay, mild intellectual disability, dysmorphic facial features, and a family history of motor delay (his sister) and psychiatric disease
11. Deletion of 2q12.1q14.1 in a 4-year-old boy with mild global developmental delay, dysmorphic facial features, and brain malformation.
12. Deletion of 15q11.2 in a 14-year-old boy with speech delay, behavioral problems (hyperkinesia, aggressive behavior), dysmorphic features
13. Deletion of 3q29 in a 7-year-old girl with mild intellectual disability, speech delay, dysmorphic features, a small cyst of pineal gland on brain MRI, and a family history positive for psychiatric conditions.

The phenotype of these patients correlated with the clinical features of previously reported cases with the same variants.

Gene sets encompassed by genomic imbalances and pathway analysis

Overall, the pathogenic and likely pathogenic CNV covered 948 genes, while variants of uncertain significance (VOUS) covered 274 genes. Pathway analysis using online public resources (REACTOME) allowed the identification of 11 pathways enriched in the clinically relevant CNVs gene set. Similarly, 17 pathways were enriched in VOUS CNVs gene set.

Elaboration of scientific papers / communications; training activities

All partners contributed to the dissemination activities. The communications and published papers are detailed in the next section and Indicators table.

In addition, four researchers from PP and P2 Romanian institutions met P1 team in Norway in a visit focused on the consolidation team interaction within consortium and transfer of knowledge from the Norwegian team to Romanian team members. The visit took place in November 21-23 2022 at the site of Oslo University Hospital and involved the Norwegian project team members as well as other researchers from the hospital. The program included SNP-array data processing, GWAS data presentation, and genomic data management. The progress of genetic investigations of Romanian ASD patients, including a presentation of

variant calling using different programs staring from SNP array, was presented with subsequent joint discussions on genetic data. Discussion of clinical data files and models of registries for ASD took place with establishment of further directions. Sequencing wet lab protocols were presented during a visit to the sequencing core facility. The visit was concluded with discussion on the research strategies focused on deciphering the functional consequences of genome aberrations in patients with neurodevelopmental disabilities.

Overview of the progress of work towards the objectives of the project, including milestones and deliverables identified in the project contract. The report must include explanations justifying the differences between the work expected to be carried out in accordance with the project contract and that actually carried out. (max 10.000 characters, including spaces)

Our project **aims** to: improve early recognition and clinical diagnosis of ASD; develop and implement a comprehensive protocol for genetic testing and biomedical imaging; devise recommendations for patient-centered intervention plan; provide research-based knowledge for development of social/school/professional integration programs, and ultimately initiate the establishment the Romanian National Registry for ASDs.

The project **key targets** are:

- optimized protocol for clinical evaluation of ASDs patients;
- imagistic protocol for brain MRI of ASDs patients;
- genetic testing algorithm for ASD patients;
- project database integrating patient clinical and genetic data for the initiation of the Romanian National Registry for ASDs;
- establishment of communication channels and dissemination of results towards patient associations and other suitable stakeholders, general public, healthcare authorities.

Most of the delays caused by COVID pandemics in 2020 and 2021 were overcame. By the end of 2022, PP completed the enrollment of patient and control groups. However, after analysis of sex distribution of control individuals, the enrollment reopened for additional samples (~ 27).

P1 is currently working to ……

P2 completed the screening for genomic imbalances by array-CGH for all available control group samples and for the entire ASD study population. The screening for fragile X CGG repeats expansion continued with MLPA investigations and was completed by triplet primed PCR.

The data sets generated within the project were jointly analyzed, with extraction of genotype-phenotype correlations, syndromic / recurrent genomic imbalances and rare CNVs sets, as well as sets of genes with known contribution to ASD / neurodevelopmental disorders and genes not reported by date in ASD / neurodevelopmental disorders databases.

The deliverables of this phase are:

- the personal file for each patient – this file includes data about pregnancy, birth, psychomotor development, association of other medical conditions, familial history positive for a neuropsychiatric condition (ASD, developmental delay, intellectual disability, speech delay, hyperkinesia etc.), vaccinations, general clinical examination, anthropometric parameters, dysmorphological features, neurological examination, psychiatric and psychological evaluation, the results of specific investigations (neuroimaging studies – computed tomography, magnetic resonance imaging, electroencephalographic studies, blood tests, ultrasounds etc.);
-a database of the patients – with recorded patient data;
-biological samples collection with blood samples for genetic tests taken from the entire ASD

and control groups;
-genomic profiles obtained by chromosomal microarray technique; methylation profiles of FMR1 and AFF2 by MLPA technique; high resolution melting curves for FMR1 triplet repeat expansion evaluation by TP-PCR;
-lists of known genes and new genes potentially contributing to the autistic phenotype;
-lists of pathways/functional gene modules that include the above mentioned genes;
-database for initiation of Romanian National Registry for ASDs patients
-scientific papers

International publications in prep/submitted/in review:

Details on the exploitation and dissemination of the results and of the activities (max 3.000 characters, including spaces)

Dissemination activities included online and in person scientific conferences, where scientific communications related to the project were presented (see list of indicators). Two scientific articles were published in 2022; one additional paper submitted in 2022 was accepted for publication in January 2023; other two papers were submitted in 2022 and are currently under review.

Indicators:

Indicator	***Unit of measurement***	***Type of scientific publication***[1]	***Description*** *Definition provided by Core indicators 2014-2021 Guideline (https://uefiscdi.gov.ro/eea-norway-grants)*[2]
Number of peer-reviewed scientific publications submitted	Number	Scientific article and conference communication papers (oral presentations and posters)	1. Erbescu A, Papuc SM, Budisteanu M, Arghir A, Neagu M. Re-emerging concepts of immune dysregulation in autism spectrum disorders. Front Psychiatry. 2022 Oct 19;13:1006612. doi: 10.3389/fpsyt.2022.1006612. 2. Papuc SM, Erbescu A, Glangher A, Streata I, Riza A-L, Budisteanu M, Arghir A. Autistic Behavior as Novel Clinical Finding in OFD1 Syndrome. *Genes*. 2023; 14(2):327. https://doi.org/10.3390/genes14020327

[1]Gold Open Acces, pending Open Acces, other
[2]Definition provided by Core indicators 2014-2021 Guideline (https://uefiscdi.gov.ro/eea-norway-grants)

			In 2022 – under review. 3. Alina Erbescu, Sorina Mihaela Papuc, Laura Mateescu, Emanuela Andrei, Florentina, Linca, Doina Ioana, Cristina Nedelcu, Florina Rad, Magdalena Budisteanu, Monica Neagu, Aurora Arghir. Microduplication syndromes in autism spectrum disorders: report of 5 cases (oral presentation), 12th National Conference in Medical Genetics (SRGM), ONLINE. 26-27.02.2022. 4. Magdalena Budisteanu, Sorina Mihaela Papuc, Alina Erbescu, Florentina Linca, Doina Ioana, Cristina Nedelcu, Aurora Arghir. Potocki-Lupsky syndrome in a patient with autism and intellectual disability (oral presentation) 12th National Conference in Medical Genetics (SRGM), ONLINE. 26- 27.02.2022 - EEA Grant 2014-2021, No 6/2019. 5. Magdalena Budisteanu, Sorina Mihaela Papuc, Alina Erbescu, Maria Dobre, Gisela Gaina, Lucian Albulescu, Adelina Glangher, Emanuela Andrei, Florentina Linca, Doina Ioana, Cristina Nedelcu, Florina Rad, Aurora Arghir. Investigation of neurodevelopmental disorders within the Medical Genetics Laboratory of "Victor Babes" National Institute of Pathology, Bucharest (oral presentation), VI International Congress of Medical Genetics, Craiova, 22-25.09.2022; 6. S. M. Papuc, A. Erbescu, L. Albulescu, F. Rad, G. Gaina, L. Mateescu, R. Grozavescu, M. Dobre, E. Andrei, B. Budisteanu, A. Glangher, F. Linca, D. Ioana, I. Dobrescu, M. Budisteanu, A. Arghir. Detection of rare genetic variants in a group of patients with autism spectrum disorders (Digital Poster). 7. European Human Genetics Conference

			Hybrid Conference Vienna, Austria JUNE 11–14, 2022. 8. Adelina Glangher, Magdalena Budisteanu, Florentina Linca. Correlation between mean age of diagnosis of ASD, specific symptoms and residence area in a cohort of patients from Romania (Poster) 14th Congress of the European Paediatric Neurology Society (EPNS) 22.04.- 02.05.2022, Glasgow, UK. 9. Sorina Mihaela Papuc, Alina Erbescu, Florina Rad, Gisela Gaina, Laura Mateescu, Raluca Grozavescu, Maria Dobre, Lucian Albulescu, Emanuela Andrei, Bogdan Budisteanu, Catrinel Iliescu, Carmen Burloiu, Diana Barca, Cristina Motoescu, Cristina Anghelescu, Dana Craiu, Adelina Glangher, Florentina Linca, Doina Ioana, Iuliana Dobrescu, Magdalena Budisteanu, Aurora Arghir. Genetic and clinical characteristics in a group of Romanian patients with autism spectrum disorders. (Poster) 14th Congress of the European Paediatric Neurology Society (EPNS) 22.04.- 02.05.2022, (EPNS) 22.04.- 02.05.2022, Glasgow, UK. 10. M. Budisteanu, S. Papuc, A. Erbescu, L. Albulescu, A. Arghir. Genomic imbalances of chromosome 15 in patients with autistic features and global developmental delay. (e-Poster Presentation) 30th European Congress of Psychiatry – 04.- 07.06.2022, Virtual. 11. Magdalena Budisteanu, Sorina Mihaela Papuc, Alina Erbescu, Cristina Nedelcu, Aurora Arghir Oral-facial-digital type 1 syndrome in a child with autism spectrum disorder. The 14th Excellence in Pediatrics Conference. Amsterdam/ hybrid. December 1-3, 2022. 12. M. Budisteanu, S.M. Papuc, A. Erbescu, L. Albulescu, L. Mateescu, F.

			Linca, D. Ioana, C. Nedelcu, I. Dobrescu, F. Rad, A. Arghir. Recurrent deletions in autism spectrum disorders. 9th Congress of the European Academy of Paediatric Societies. Barcelona/ hybrid, October, 7-11, 2022. 13. Magdalena Budișteanu, Sorina Mihaela Papuc, Florentina Linca, Adelina Glangher, Emanuela Andrei, Doina Ioana, Florin Rad, Aurora Arghir. Genetic mechanisms in autism spectrum disorder. National Conference of Psychiatry, Cluj-Napoca/hybrid, 12-15.07.2022. 14. Florentina Lincă, Lucia-Emanuela Andrei, Adelina Glangher, Ioana Doina, Laura Mateescu, Raluca Grozăvescu, Bogdan Budișteanu, Cristina Nedelcu, Florina Rad, Magdalena Budișteanu. Hyperkinesia in children with autism spectrum disorder. National Conference of Psychiatry, Cluj-Napoca/hybrid, 12-15.07.2022. 15. Magdalena Budisteanu, Florentina Linca, Emanuela Andrei, Laura Mateescu, Adelina Glangher, Doina Ioana, Cristina Nedelcu, Sorin Riga, Rad Florina. Early signs and symptoms in autism spectrum disorder. National Conference of Family Medicine. Bucharest/hybrid; 26-29.10.2022. 16. Magdalena Budisteanu. Genetic aspects in neuropsychiatric disorders. International Autism Neurodiversity Conference 2022. Bucharest; 10-12.11.2022.
Number of joint, peer-reviewed, scientific publications submitted	Number		Submission confirmation from editorial board
Number of jointly registered applications for Intellectual Property Protection	Number	N/A	Proof of submission and register number from IP office

Number of joint applications for further funding	Number	N/A	Proof of applications submission from the funding body
Number of jobs created	Number	N/A	Project records
Number of (pro) Roma organisations involved in projects	Number	N/A	Semi-annual

Signature
Principal Investigator