Iceland Liechtenstein Norway grants

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

ANNUAL REPORTING
2024

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 Program (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 VI: Final data integration and joint analysis of the results

Phase results: scientific papers

Activities

6.1. Common analysis of data

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

6.3. Elaboration of scientific papers / communications; training activities (courses, training stage, bilateral visits)

PP participated in activities A6.1 and A6.3.

During this phase, we continued to update the clinical and paraclinical data of patients with ASD included in this study and of some new ASD patients who were also evaluated according to the protocol. We also continued to introduce patient data in the Registry for ASD. Clinical data was harmonized with the Norwegian registry for ASD (BUPGEN), this enables future scientific and clinical collaborations between the partners.

Phenotype-genotype correlations performed for cases with pathogenic or likely pathogenic variants, and the clinical data of our patients were compared with those of other cases with the same genetic anomaly from the literature. A follow-up plan was established for these patients according to their genetic syndrome.

P1 participated in activities A6.1, A6.2 and A6.3.

Whole Genome Sequencing (WGS) analysis of samples from ASD cohort (n=316) and controls (n=60) have been completed at The Norwegian Sequencing Centre Core Facility, Oslo University Hospital including : library preparation, 100 Gb 2 x 150 bp sequencing (6 x Full 25B FC Novaseq X, 6 x 25B Alignment) and clinical laboratory genomic alignment.

The alignment was done with the compute solution integrated on the sequencer - Illumina NovaSeq X DRAGEN. The following parameters were used in the sample sheet: SoftwareVersion 4.1.23 AppVersion 1.2.1 MapAlignOutFormat cram ReferenceGenomeDir hg38-noalt-with-decoy-cnv.rna-8-1667496006-2 VariantCallingMode AllVariantCalle rs and using the pipeline nf-core/sarek (https://nf-co.re/sarek/3.4.2).

Bam files from test samples have been transferred to TSD and forwarded to partners in Romania. Files on TSD are consisting of six folders, containing raw fastq files and aligned cram files. Complete dataset with bam files and variant calling (incl. short variants (SNP, Indel), CNVs and structural variants using DeepVariant, CNVkit and Manta respectively) has being added, as well and final total size is estimated to 30TB. Safe transfer optimalisation on

such amount of sensitive data is currently under discussion and will progress in very near future.

P2 participated in activities A6.1, A6.2 and A6.3.

CNVs validation and inheritance study continued in this phase, using the protocol described in the previous phases, on 7500 Fast PCR machine (Applied Biosystems, Foster City, CA, USA). Fifteen genes intersected by CNVs were assessed for copy number changes in 20 sample sets (proband plus both parents, if available). The majority of the CNVs tested were confirmed with only ten variants, covered by a small number of probes in microarrays studies, proved to be false positive and removed from analysis. The family segregation evaluation found both maternally and paternally inherited CNVs. In addition, the molecular characterization of potential clinically relevant genetic variants continued with direct sequencing of several genes. Primer design was done for *ALDHA1, PRKN* and *FMR1* genes. The first two genes, known to be involved in recessive conditions, encompassed deletions in our patient group, thus warranting the screening for sequence variants of the second allele. *FMR1* can cause fragile X syndrome when mutated, thus for the patients with highly suggestive phenotypes and normal methylation and repeat expansion pattern, screening for nucleotide defects may reveal the genetic etiology.

A6.1 Common analysis of data was performed with the contribution of all partners.

Data of all CNV calls from ASD subjects and controls, updated with the results of CNV validation, were centralized and filtered.

Burden analysis

Burden analysis was performed using a logistic regression model for control or ASD status against the number of rare CNVs, the total length of the genomic regions covered by rare CNVs (log10-transformed), and the number of genes overlapped by rare events, adjusting for sex. Rare CNV burden was significantly higher in the ASD group compared to the control group, overall ($p<0.001$), as well as when exonic CNVs, intronic CNVs, deletion and duplications were considered separately ($p<0.01$).

Gene-set enrichment analysis

Classical gene sets associated with ASD and NDD were found to be significantly enriched in the ASD cohort, compared to the control group ($p<0.05$). These included:

- SFARI syndromic and SFARI 1 genes, as well as SFARI 2,3 genes.
- Gene lists reported in Satterstrom et al. (PMID: 31981491) and Rolland et al. (doi.org/10.1038/s41591-023-02408-2).
- DBD Tier 1 and AR genes, as well as DBD Tier 2,3,4.
- Synapse function genes (SynGO).
- Genes with probability of loss of function intolerance (pLI) >0.9.

We also found significant enrichment for gene sets known to be related to behaviour, sleep regulation, sensory perception, motor function and the immune system, extracted from HPO. Most of these results remained significant when adjusting for CNV burden.

Candidate genes

Because of the low sample size of our cohort, we could not perform meaningful formal statistical analysis to identify ASD candidate genes. Nonetheless, genes overlapped by VUS in the ASD group, which did not occur in the control group, included non-syndromic SFARI genes such as ASTN2 and TM9SF4, which are known to be intolerant to variation, as well as non-SFARI genes such as *FOXK1, GNB5, UBASH3B*, and *RABEPK*, which are highly expressed in the brain.

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.

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.

PP completed all proposed milestones and deliverables, including a protocol for early diagnosis and management for patients with ASD, a database with clinical, paraclinical, imaging and genetic data of over 300 patients with ASD, a registry for ASD patients. Following the protocol developed in this project, we will continue to evaluate children with ASD with an interdisciplinary approach, and to complete the database and the registry for ASD patients. The ASD patients with a genetic condition will be follow-up periodically with specific evaluations according to the specific recommendations for the respective genetic syndromes. For cases with variants of unknown significance which include gene with implications in the neurodevelopment, together with P2, we will continue to follow the international dedicated databases and scientific papers for eventually new data about the pathogenic role of these variants.

P2 already completed all its proposed milestones and deliverables. Additional approaches were also employed for further characterization of the variants detected by microarray, such as inheritance studies, studies of the second allele for genes with known recessive inheritance pattern or screening for different defects in relevant genes. The statistical studies evaluated the patient cohort in a case-control approach highlighting the involvement of genes with roles in neurodevelopment as well as higher genomic variation burden in individuals with ASDs.

The deliverables of this phase are:

-database with clinical, imaging and genetic data,

-scientific papers and communication of results to congresses and conferences

International publications in prep/submitted/in review:

In preparation:

1. Magdalena Budisteanu, Sorina Mihaela Papuc, Alina Erbescu, Adelina Glangher, Emanuela Andrei, Florina Rad, Mihail Eugen Hinescu, Aurora Arghir. A non-systematic review of genetic and neuroimaging findings in autism spectrum disorder – a clinical perspective
2. Adelina Glangher, Mihaela Oros, Sorina Mihaela Papuc, Alina Erbescu, Emanuela Andrei, Florina Rad, Florentina Linca, Doina Ioana, Mihail Eugen Hinescu, Aurora Arghir, Magdalena Budisteanu, Sleep disorders in children with autism spectrum disorders – clinical, genetic and therapeutic aspects.
3. Sorina Mihaela Papuc, Iuliana Ciocanea-Teodorescu, Alina Erbescu, Maria Dobre, Adelina Glangher, Gisela Gaina, Lucian Albulescu, Florina Rad, Mihail Eugen Hinescu, Magdalena Budisteanu, Aurora Arghir. Rare genomic copy number variants burden in a cohort of children with autism spectrum disorder from Romania
4. Maria Dobre, Gisela Gaina, Sorina Mihaela Papuc, Alina Erbescu, Mihail Eugen Hinescu, Magdalena Budisteanu, Aurora Arghir. FMR1 abnormal methylation and repeat expansion screening in a cohort of children (boys) with autism spectrum disorders: correlation of genetic findings with clinical presentations

Submitted:

Alina Erbescu, Magdalena Budisteanu, Maria Dobre, Adelina Glangher, Sorina Mihaela Papuc, Aurora Arghir, Mihail Eugen Hinescu, Monica Neagu. Clinical variability of 15q11.2 BP1-BP2 genomic imbalances in individuals with autism spectrum disorders. Submitted to Frontiers in Pediatrics

Published:

Tissink, E.P., Shadrin, A.A., van der Meer, D. et al. Abundant pleiotropy across neuroimaging modalities identified through a multivariate genome-wide association study. Nat Commun 15, 2655 (2024). https://doi.org/10.1038/s41467-024-46817-4

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).

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 5	Scientific article and conference	Tissink, E.P., Shadrin, A.A., van der Meer, D. et al. Abundant pleiotropy across neuroimaging modalities identified

[1]Gold Open Acces, pending Open Acces, other

[2]Definition provided by Core indicators 2014-2021 Guideline (https://uefiscdi.gov.ro/eea-norway-grants)

		communication papers (oral presentations and posters)	through a multivariate genome-wide association study. Nat Commun 15, 2655 (2024). https://doi.org/10.1038/s41467-024-46817-4 **Conferences** 1. Rare copy number variations in a romanian pediatric cohort with autism spectrum disorders. Iuliana Ciocanea-Teodorescu, Sorina Mihaela Papuc, Alina Erbescu, Adelina Glangher, Crina Nedelcu, Florina Rad, Magdalena Budisteanu, Aurora Arghir. EuroNDD Workshop 2024, Lisbon, April 2024. 2. Rare copy number variants involving Parkinson associated genes in a cohort of individuals with autism spectrum disorders. Aurora Arghir, Sorina-Mihaela Papuc, Alina Erbescu, Maria Dobre, Magdalena Budișteanu. European Conference of Human Genetics, Berlin/virtual, June 2024 3. Magdalena Budișteanu, Sorina Mihaela Papuc, Florentina Linca, Adelina Glangher, Emanuela Andrei, Doina Ioana, Florin Rad[4], Aurora Arghir. Genetic aspects in autism spectrum disorders. Spring session of Romanian Scientists Academy. Bucharest May 2024. 4. M. Budisteanu , A. Glangher , E. Andrei, L. Mateescu, S.M. Papuc,, F. Linca, D. Ioana S. Riga, M. Ghinescu, C. Nedelcu, A. Arghir, F. Rad **N**euroimaging aspects in children with autism spectrum disorders. The mental health of child and adolescent conference. Sibiu, April 2024. 5. Magdalena Budisteanu, Sorina Mihaela Papuc, Alina Erbescu, Adelina Glangher, Emanuela Andrei, Florentina Linca, Doina Ioana, Laura Mateescu, Oana Tarta-Arsene, Cristina Motoescu, Dana Surlica, Florina Rad, Roxana

			Popescu, Cristina Rusu, Aurora Arghir. Phenotypic variability in microdeletion/microduplication 22q11.2 syndromes. The conference "Iasi Pediatrics Days". Iasi, June 2024. 6. Adelina Glangher, Emanuela Andrei, Laura Mateescu, Florentina, Linca, Doina Ioana, Magdalena Budisteanu. The importance of diagnosing and managing sleep disorders in children with ASD. The National Conference in Pediatrics, Guidelines and Protocols 2024. Bucharest, 3-6 April, 2024. 7. Magdalena Budisteanu, Florentina Linca, Adelina Glangher, Emanuela Andrei, Doina Ioana, Cristina Nedelcu, Florina Rad. Somatic Comorbidities In Children With Autism Spectrum Disorders. The 10th Congress of the European Academy Of Paediatric Societies. Vienna, 17-20 October 2024 (abstract accepted).
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