IMI1 Final Project Report
Public Summary

Project Acronym: EU-AIMS

Project Title: European Autism Interventions- A Multicentre Study for Developing New Medications

Grant Agreement: 115300
Project Duration: 04/2012 - 03/2018

1. Executive summary

1.1. Project rationale and overall objectives of the project

EU-AIMS is a world-wide unprecedented integrated, translational, effort of pre-clinical and clinical expertise to develop new effective therapies for the core and associated symptoms of Autism Spectrum Disorder (ASD). The project started in April 2012 and was completed in March 2018.

Rationale: The project was motivated by the recognition that there are no effective pharmacological treatments for the core symptoms of autism spectrum disorder (ASD). Several barriers to progress had been identified: This included a poor understanding of the pathophysiology of the disorder, a lack of valid and reliable cellular assays and animal models that are necessary to identify new treatment targets, an absence of tests that demonstrate efficacy in individuals with ASD from childhood to adulthood; and the reliance of clinical trials on biologically diverse groups of individuals as operationally-defined by DSM/ICD10 categories. Further, even if novel treatments were developed, there was no EU platform available to effectively run clinical trials. Despite these limitations, rapid advances in the identification of genetic risk factors for ASD provided unique opportunities to substantially improve this situation. Our guiding hypothesis was that although a variety of different genetic and environmental risk factors for ASD had been identified there was reason to assume that they may converge to perturbate a smaller common set of protein systems or downstream neurobiological pathways. This meant that new tractable treatment targets identified using the monogenic approach may potentially be applicable for broader patient groups.

We therefore aimed to harness these new discoveries and technological advances to develop treatments that are driven by the likely biological basis of ASD, and tests and tools that identify and stratify patients with distinct subtypes. To achieve this, we proposed an unprecedented integrated, translational, effort to deliver new research tools and standards for clinical development and pave the way for drug discovery and clinical trials. This brought together over 150 leading psychiatrists, psychologists, cognitive developmental neuroscientists, basic neuroscientists, neurobiologists, geneticists and others from over 16 academic institutions, 6 industry partners, and 3 SMEs

Objectives. Thus, the overall objectives of the EU-AIMS project were four-fold:

- to identify tractable treatment targets based on the underlying pathophysiological mechanisms,
- To develop and validate translational approaches for the advancement of novel therapies to treat ASD;
- To identify and validate risk and stratification biomarkers to parse this clinically and etiologically diverse condition into more homogeneous biological subgroups;
- to identify and develop expert clinical sites across Europe to run clinical studies and trials, and the creation of an interactive platform for ASD professionals and patients.

To address these objectives our project brought together 5 overlapping themes with associated deliverables that aimed to underpin new drug discovery for ASD.

WP 01 and 02 combined cellular assays and animal models to identify new tractable treatment targets. These work streams capitalised on rare monogenic forms of ASD that provided a breakthrough for the identification of treatment targets by enabling us to trace causal links from a gene to specific molecular alterations and biological pathways. We predominantly focused on risk genes that modulate pathways involved in synapse formation and function, as well as other cellular functions, such as chromatin remodeling and transcription, protein synthesis and degradation, and receptor signaling. Any of these mutations may alter essential developmental processes in utero or shortly after birth. For example, abnormalities in synapse development, function, and plasticity may broadly impact the balance between excitation (mainly modulated by glutamate) and inhibition (mainly modulated by gamma-aminobutyric acid, GABA) that are necessary for healthy network function and cognitive development.

Whereas animal models allowed us to link abnormalities between morphological, cellular, physiological, system level and behavioural levels, patient-derived pluripotent stem cells were a promising new approach that overcomes inter-species differences.

Translational Imaging: Workpackage 3 on translational imaging further aimed to bridge the gap between pre-clinical and clinical research by studying similar brain systems phenotypes (e.g., abnormalities in the E/I balance) in both animal models and individuals with ASD (using, e.g., PET scanning, MRS and functional imaging).

Clinical research development. To select individuals for a particular therapy, stratification biomarkers are needed. Thus, our clinical research WP04 aligned three longitudinal multi-disciplinary cohorts spanning infants at high-risk for autism, children adolescents and adults with autism, and individuals with particular monogenic forms of autism. Each cohort was comprehensively characterized in terms of clinical symptom profile, family psychiatric history, environmental risk factor, cognition, eye-tracking, EEG and MRI.

- The High-Risk Infant (HRI) cohort was designed to better understand early signs of autism in high-risk (HR) infants from families with already one child with ASD compared with low-risk (LR) infants born in families without previous cases of ASD. 300 high-risk and 100 low-risk infants were prospectively followed from 4 months to 3 years of age.
- The EU-AIMS Longitudinal European Autism Project (LEAP) is the largest multi-centre multidisciplinary observational study on ASD. LEAP includes 430 children and adults with ASD and 300 controls between the ages of 6 and 30 years with IQs varying between 50 and 148. It is the first multi-modal longitudinal autism project that has the power to identify stratification markers for autism.
- The synaptic (SynaG) cohort was designed to better understand the genotype-phenotype relationship in carriers of deleterious mutations in specifically synaptic genes associated with ASD. We focused mostly on patients carrying SHANK3 (Phelan McDermid Syndrome) or NRXN1 mutations.

Human Genomics. In 2014, we were able to obtain additional funding from the IMI scheme “Explore New Scientific Opportunities” (ENSO) to establish a comprehensive genomic profile of each individual. This allows us to complement and relate rich ‘phenotypic’ information of our cohorts with whole genome sequencing to understand how rare and common genetic variants impact brain development, function, cognitive processes and clinical symptoms.

Central data base. We created a central data base (WP05) to efficiently and safely share data from all study centres, and to perform quality control and pre-processing procedures, each led by experts in their fields. All EU-AIMS members could then access and download the data following a *pre-registration procedure* to increase transparency among colleagues and contribute to more robust and replicable science.

Together, EU-AIMS represents a world-wide unique integrated effort to pursue precision medicine approaches to ASD.

1.2. Overall deliverables of the project

Our overall strategy reflects our belief that neither ‘top-down’ clinical and translational studies, nor ‘bottom-up’ model system analysis alone are sufficient to develop new therapies for ASD. Rather, we need to integrate proven technologies and expertise including animal models and PET, together with new approaches (e.g. functional MRI (fMRI), induced pluripotent stem cells (iPSCs) and multi-omics). To achieve this, we defined 7 Work Packages linked to 7 major deliverables (Figure 1, below) which strike a pragmatic balance between technological advances (enhanced and enriched animal models,

new imaging techniques, advanced analyses) and lines of research that may carried more risk but were transformative (e.g., genetics for patient stratification and response prediction).

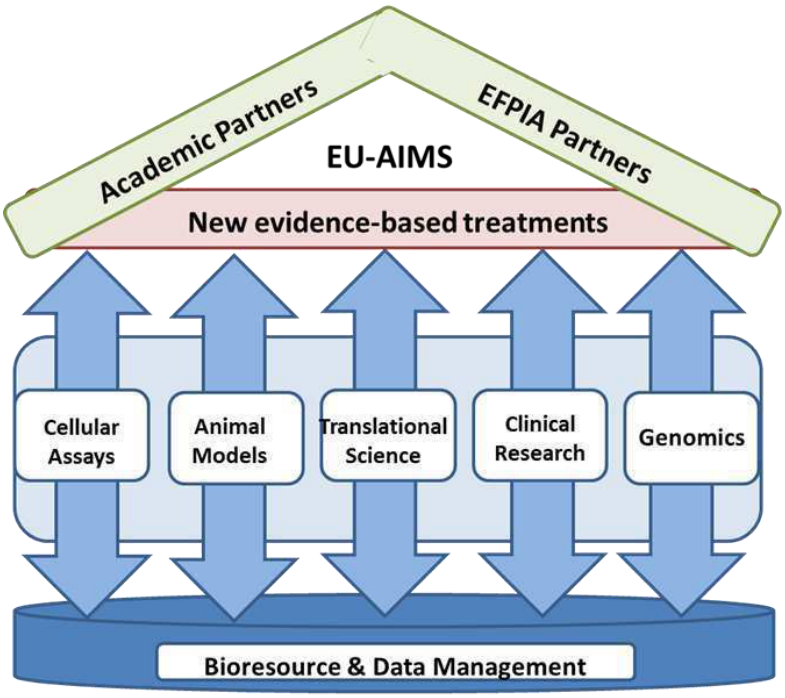

Figure 1: *The goal of EU-AIMS is to accelerate the development of Innovative Medicines through 7 main deliverables*

Overall Deliverables of the project

WP01, Deliverable A: An Integrated Approach Using Cellular Assays that Translate to Animal Models and Humans

A. ESTABLISHING CELLULAR ASSAYS
Tasks and their outputs: i. Bank keratinocytes/fibroblasts and iPSCs samples from specific patient cohorts and 'neurotypical' controls ii. Utilization of iPSCs and other cellular systems to define cellular phenotypes associated with ASD iii. Provide an aetiological link between the genetic and neurodevelopmental components of ASD

WP02, Deliverable B: Animal Models with a Close Link to the Neurobiology of ASD and that Support Translation from Animals to Patients

B. ANIMAL MODELS THAT SUPPORT TRANSLATION TO PATIENTS
Tasks and Outputs: i. Animal models that exhibit robust core ASD symptoms ii. Reproducible quantitative behavioural assays iii. Defined physiological deficits and biomarkers iv. Animal models for pharmacological intervention

WP03, Deliverable C: Validation of Biomarkers that Aid the Drug Discovery Process

C. VALIDATING BIOMARKERS
Tasks and Outputs: v. Establish animal translational endpoints in humans through cross-species anatomical and functional neuroimaging, and electrophysiology, that aid drug discovery vi. Apply existing, and develop new, molecular neuroimaging tools for drug discovery in ASD vii. Identify biomarkers for segmentation/stratification of patient groups.

WP04, Deliverable D: Clinical Research Development and Education

D: Clinical Research Development
Tasks and Outputs: i. Identify biomarkers which precede onset of clinical symptoms ii. Define the relationship between biomarkers and the autistic clinical phenotype in children and adults iii. Develop a clinical research infrastructure iv. Develop standardized clinical assessment methods, improved clinical trials methodology and regulatory guidelines v. Assess clinical standards, and outcome, of ASD individuals in the EU

WP05, Deliverable E: Bioresource and data management

E. BIORESOURCE AND DATA MANAGEMENT
Tasks and Associated Outputs: i. A repository of samples that facilitates identification of risk markers, stratification markers, and markers that predict therapeutic response. ii. Characterisation of genomic mechanisms underlying ASD iii. A data sharing platform with systems for data collection, integration, management, and access.

WP07, Deliverable F: Human Genomics

F: HUMAN GENETICS
Tasks and Associated Outputs: i. Genetic profiling of the clinical cohorts (infants-at-risk for autism); Longitudinal European Autism Project (LEAP), patients with synaptic gene deficits; ii. Characterisation of genes and pathways associated with ASD; iii. Network-based stratification of patients with ASD; iv. Establishing associations between genetic subtypes and intermediate phenotypes of ASD

1.3. Summary of progress versus plan since last period

We report no major progress from WPs 1 and 2 since the periodic report year 5, because work conducted during the no-cost extension period only pertained to WPs 3, 4 and 7.

W01 – In vitro system development

WP01 has been mainly finalised in year 5. In Period 6 WP1 has identified morphogenetic and electrophysiological phenotypes associated with SHANK3, Tsc2, and sporadic ASD-derived neurons. SHANK3 is a structural protein found predominantly at the postsynaptic density. Mutations in the SHANK3 gene have been associated with risk for autism spectrum disorder (ASD). We generated induced pluripotent stem cells (iPSCs) from control individuals and from human donors with ASD carrying microdeletions of SHANK3. In addition, we used Zinc finger nucleases to generate isogenic SHANK3 knockout human embryonic stem (ES) cell lines. We differentiated pluripotent cells into either cortical or olfactory placodal neurons. We show that patient-derived placodal neurons make fewer synapses than control cells. Moreover, patient-derived cells display a developmental phenotype: young postmitotic neurons have smaller cell bodies, more extensively branched neurites, and reduced motility compared with controls. These phenotypes were mimicked by SHANK3-edited ES cells and rescued by transduction with a SHANK3 expression construct. This developmental phenotype is not observed in the same iPSC lines differentiated into cortical neurons. Therefore, we suggest that SHANK3 has a critical role in neuronal morphogenesis in placodal neurons and that early defects are associated with ASD-associated mutations. These findings were published in Molecular Psychiatry volume 23, pages 735–746 (2018) doi:10.1038/mp.2017.185.

W02 – Animal Model Development

During the additional year of EU-AIMS, WP2 has performed a cross-site study involving 3 sites. The objective was to replicate a previous experiment and provide reproducible results by standardizing the experimental protocol for the behavioral evaluation and drug testing in a genetic rat model.

To improve data robustness, several parameters, such as animal handling, standard operating procedures (SOPs), compound and animal provider, data analyses and reporting were carefully aligned between the participating partners.

SHANK2 knock-out (KO) rats were bred at Charles River (Wilmington, MA – USA) and sent to the 3 partners (Pfizer, Roche and University of Groningen) for phenotypic assessment. Previously, Pfizer have demonstrated hyperactivity and repetitive circling behavior in these animals.

Each test site was equipped with Phenotyper behavioral chambers, provided by SME partner Noldus technologies. Janssen provided the 3 sites with the metabotrobic glutamate receptor 1 (mGluR1) antagonist, JNJ 16259685. Raw and analyzed data were uploaded to a platform by Sylics (a collaborator linked to partner Noldus).

Using automated and manual scoring of the behavior, all three sites showed comparable results, both at the level of the genotype differences and the pharmacological effect. Some inconsistencies between manual and automated scoring were observed, however they were replicated across the sites, suggesting a beneficial effect of intense standardization. These findings reveal the importance of phenotype definition for the interpretation of the genotypic and pharmacological findings.

WP03 - Translational Neuroimaging

DeCODE/ Amgen: 190 genotyped individuals have been added to the large dataset on cognitive, structural and neurofunctional phenotypes (N~1500) in Iceland. Data are continuously analyzed and several manuscripts on genetic risk and associated phenotypes are in preparation or have already been published.

CIMH: We have advanced our analyses on the LEAP wave-1 dataset (N>600) and completed group-level standard analyses. The LEAP wave-2 dataset (N>500) has successfully been pre-processed and analyzed on the individual level. This data is currently subjected to quality control assessments and

will be released to the consortium as soon as the central database is finalized at Pasteur. Besides standard analyses, we have applied network segregation modelling approaches to the data. Results have been presented at international conferences and manuscripts are in preparation.

In a collaborative effort between Roche and KCL, we finalized a translational MRS study in persons with idiopathic ASD and in several rodent models of ASD. The study's aim was to ascertain potential common underlying substrates related to excitatory and inhibitory neurotransmission. In the current reporting period a manuscript on this study was submitted and accepted for publication in Translational Psychiatry (in press).

In joint projects at KI and KCL, we have successfully established a PET protocol for quantification of metabotropic glutamate mGluR5 receptors in ASD subjects and examined twin pairs discordant for ASD as well as control subjects. We have further, for the first time, developed a methodology for comparisons of GABA and glutamate measured with PET and MRS. One high impact publication has been finalized and several manuscripts on 5-HTT, GABA and glutamate imaging are in preparation.

In total, 74 individuals were recruited to the Synaptic Gene (SynaG) project. This includes 23 participants with Phelan McDermid Syndrome (SHANK3 deletion) (12 female, 11 male), 5 participants with a "developmental CNV" (2NRXN1 deletion, 3 16p11.2), 30 children with ASD (28 male, 2 female) and 16 typically developing children. Participants were comprehensively assessed in terms of clinical profile (ASD core symptoms, and comorbid psychiatric symptoms, level of adaptive function), family history, environmental risk factors, parent-child interaction, cognitive and social cognitive development (based on behavioural assessments and eye-tracking), EEG, and – in a subset of participants – MRI scans. Blood samples were collected and banked for genomic and fluid biomarker analyses; hair samples were collected and banked for selective iPSC generation. Findings on clinical symptom profile, cognition, eye-tracking and parent-child interactions are currently being prepared for publication.

WP04 - Clinical Research Development

Eurosibs (Infant-at-risk study). We have continued to collect follow-up data on our high-risk infant cohort (for which we exceeded our enrolment targets), with over 80% of our cohort followed to age 2. We have replicated our earlier observation of reduced temporal lobe specialisation in infant siblings and linked alterations in specialisation of the temporal lobe to later autism. Further, we have completed an initial case series study of infants with a known genetic disorder linked to autism (neurofibromatosis Type 1), essential to translational insights. We have also been developing and applying new techniques such as genetic-based machine-learning algorithms to both behavioural and neuroimaging data to move beyond group-based effects to individual-level prediction. Finally, we have continued to identify novel candidate biomarkers of later core and associated symptoms of autism, including alterations in neural microstates; diminished habituation of auditory gamma responses; and reduced dynamic modulation of EEG theta power. Additionally, the results were published in several publications.

Longitudinal European Autism Project (LEAP). During the last periodic report, we reported that the follow-up assessment wave of LEAP had been completed. Major progress in this final period includes:

- pre-processing and quality control of the follow-up data led by core analysis groups; this is complete for clinical, cognitive, fMRI, sMRI and DTI data; and ongoing for EEG and eye-tracking data;
- data analysis and manuscript preparation of 68 pre-registered projects based on LEAP data;
- integration of findings according to core questions;
- communication of findings to scientific audiences, including submission of the first four empirical papers to peer reviewed journals, and more than 25 oral or poster presentations at INSAR (International Society for Autism Research) and other international conferences.

WP05 - Database

In the last reporting period the EU-AIMS bioresource continued accepting and managing biological samples from the consortium. At the end of the project the number of proband EDTA blood samples for the LEAP study is 1130 (from 8 acquisition centres), additionally there are 804 parent samples (from both mothers and fathers). The bioresource also contains Tempus blood samples, plasma, urine, and saliva – please see the tables below for details.

In 2017, NeuroSpin continued the operation of the existing tools developed in the EU-AIMS project, and prepared the transfer of the data to Institut Pasteur Paris France.

New clinical raw datasets were collected during year 2017 for new or already included subjects. As of March 2018, data on 2333 independent subjects were collected. Anonymization and format harmonization were applied to the raw data. NeuroSpin continued the operation of the existing tools developed according to WP4 prescriptions during the project: (i) raw data were included in the data exposition server (eu-aims.cea.fr) and (ii) raw data entered the quality control and first-line consensus processing (QC&CP) stage established during the project. All the raw data collected were made available to the different core analysis groups. NeuroSpin was part of one core analysis group for structural image analysis and ran the Freesurfer analysis, as well as its quality control. The data that underwent QC&CP were sent back to NeuroSpin and included in the exposition server.

As decided with WP4 leaders of the clinical studies, the raw data collection was closed at the end of year 2017, and the status of the external processing carried out by external core analysis group was frozen. The exposition server of the clinical data – anonymized, harmonized, curated and QC&CP – was stopped on March 21th 2018.

Institut Pasteur was chosen by the EU-AIMS consortium to be the centre that will host EU-AIMS data in the future. NeuroSpin started the legal procedures to enable the transfer in May 2017 which is a pre-requisite to any data transfer. This legal procedure has been completed and data transferred to the Institut Pasteur.

In Q4'2016 work was started to expose some of the clinical data via a data exploration system tranSMART. EU-AIMS Data Sharing System provides means to find and download data for further analysis, while tranSMART allows users to perform some basic analysis on the fly, with an aim to aid hypothesis generation. Clinical, demographic and questionnaire data have been transformed to match the requirements of tranSMART, and were made available via this system.

WP07 – Human Genomics

There was no genetics study in the initial EU-AIMS project. It was only in 2014 that Work Package 7 (WP7) was added to obtain the genetic profiling of the patients and their parents collected in the EU-AIMS Longitudinal European Autism Project (LEAP), Eurosibs, the High-Risk Infant project (HRI), and the synaptic (SynaG) cohorts. We received funding from ENSO (Explore New Scientific Opportunities) to initiate this genetic project and to start the genetic profiling of a subset of individuals collected through EU-AIMS. The strategy described in the application was initially to perform a high-density SNP array (5 million SNPs) and a whole exome sequencing (WES) for a subset of the participants. Because of the drop in the cost of the whole genome sequencing (WGS) and our partnership with Autism Speaks, we decided to improve the genetic profiling by using the Illumina OmniExpress SNP array to genotype 700,000 SNPs and then to perform a WGS. The WGS of the HRI and the SynaG cohorts is currently performed by the National Human Genomic Research Centre (CNRGH formerly CNG). The WGS of the LEAP cohort will be performed through our collaboration with Autism Speaks through the MSSNG project (https://www.mss.ng/). The use of the SNP array before WGS was

justified for (i) having a very detailed QC of the DNA samples before the WGS and (ii) a validation of the relatively large copy-number variants (CNVs) (>50kb).

1.4. Significant achievements since last report

W01 – In vitro system development

WP1 has identified morphogenetic and electrophysiological phenotypes associated with SHANK3, Tsc2, and sporadic ASD-derived neurons: we generated induced pluripotent stem cells (iPSCs) from control individuals and from human donors with ASD carrying microdeletions of SHANK3. In addition, we used Zinc finger nucleases to generate isogenic SHANK3 knockout human embryonic stem (ES) cell lines. We differentiated pluripotent cells into either cortical or olfactory placodal neurons. We show that patient-derived placodal neurons make fewer synapses than control cells. Moreover, patient-derived cells display a developmental phenotype: young postmitotic neurons have smaller cell bodies, more extensively branched neurites, and reduced motility compared with controls. These phenotypes were mimicked by SHANK3-edited ES cells and rescued by transduction with a Shank3 expression construct. This developmental phenotype is not observed in the same iPSC lines differentiated into cortical neurons. Therefore, we suggest that SHANK3 has a critical role in neuronal morphogenesis in placodal neurons and that early defects are associated with ASD-associated mutations. These findings were published in Molecular Psychiatry volume 23, pages 735–746 (2018) doi:10.1038/mp.2017.185.

WP02 - Animal models of ASD

During the additional year of EU-AIMS, WP2 has performed a cross-site study involving 3 sites (Pfizer, Roche and University of Groningen (collaboration partner)). The objective was to replicate a previous experiment and provide reproducible results by standardizing the experimental protocol for the behavioural evaluation and drug testing in a genetic rat model. Using automated and manual scoring of the behaviour, all three sites showed comparable results, both at the level of the genotype differences and the pharmacological effect. Some inconsistencies between manual and automated scoring were observed, however they were replicated across the sites, suggesting a beneficial effect of intense standardization. These findings reveal the importance of phenotype definition for the interpretation of the genotypic and pharmacological findings.

WP03 - Translational Neuroimaging

Our human imaging projects have advanced significantly with respect to data acquisition and data analysis. At deCODE/Amgen/Domus, 190 genotyped individuals have been added to the large dataset on cognitive, structural and neurofunctional phenotypes (N~1500). Data are continuously analysed and several manuscripts on genetic risk and associated phenotypes are in preparation or have already been published. The major achievement was reached in the analyses on the LEAP wave-1 dataset (N>600) and group-level standard analyses. The LEAP wave-2 dataset (N>500) has successfully been pre-processed and analysed on the individual level. Additionally, a translational MRS study in persons with idiopathic ASD and in several rodent models of ASD was finalised, a PET protocol for quantification of metabotropic glutamate mGluR5 receptors in ASD subjects and examined twin pairs discordant for ASD as well as control subjects was successfully established. Finally, for the first time, a methodology for comparisons of GABA and glutamate measured with PET and MRS was developed.

WP04 - Clinical Research Development

Eurosibs/High-risk infant sibling study. We have continued to collect follow-up data on our high-risk infant cohort (for which we exceeded our enrolment targets), with over 80% of our cohort followed to age 2. We have published a number of collaborative manuscripts on the first wave of siblings to reach outcome age, including a recent high-profile publication in Nature Communications on the predictive relation of alterations in the pupillary light reflex to later autism (Nystrom et al., 2018). We have also replicated our earlier observation of reduced temporal lobe specialisation in infant siblings (Braukmann et al., 2018), and linked alterations in specialisation of the temporal lobe to later autism (Lloyd-Fox et al., 2018). Further, we have completed an initial case series study of infants with a known genetic disorder linked to autism (neurofibromatosis Type 1), essential to translational insights (Kolesnik et al., 2018). Consistent with our emphasis on reproducibility, we have three other replication papers in preparation or review that replicate our findings on early connectivity and later autism (Haartsen et al. in review); and social attention engagement and later autism (Gui et al., in prep; Tye, Bussu et al., in prep). We have also submitted a paper demonstrating that we can collect eyetracking-based putative biomarkers with high degrees of precision across multiple European sites despite varied lab set-ups, critical to clinical utility (Jones et al., in review). We have also been developing and applying new techniques such as genetic-based machine-learning algorithms to both behavioural and neuroimaging data to move beyond group-based effects to individual-level prediction (Bussu et al., 2018; Tye, Bussu et al., in prep). Finally, we have continued to identify novel candidate biomarkers of later core and associated symptoms of autism, including alterations in neural microstates (Gui et al. in prep); diminished habituation of auditory gamma responses (Kolesnik et al., in review); and reduced dynamic modulation of EEG theta power (Jones et al., in review).

LEAP. We have completed the major top-down core analyses of our baseline cohort, including clinical characterisation, and analyses of cognition, resting-state EEG, task-related fMRI and resting-state fMRI. A summary of these findings is given in section 3.1. In addition, analyses of over 60 « bottom-up » projects are in progress. Together, our findings clearly demonstrate the heterogeneity of autism across the levels of behaviour, cognition, and brain structure/ function. This challenges the predominant practice in autism research (as well as in other areas of psychiatry) to infer from findings at the group level that a given deficit or abnormality applies to a particular individual. To move beyond such group-level comparisons, we used novel analysis approaches, such as normative modelling, which allow us to quantify the severity of deficits/ abnormalities of each indivdiual on a particular biological or cognitive measure, and the frequency of abnormalities/ deficits in a group context. Such scores can then be used as the basis to defining 'subgroups'. We applied normative modelling approach to cognitive data (Loth et al., in prep, Ahmad in prep, INSAR 2017,2018), EEG data (MMN) (Ahmad et al, INSAR 2018, in prep) and structural MRI data (Zabihi et al., submitted). These findings may ultimately have an important impact on clinical practice and healthcare as they provide the foundation for subtyping ASD into biologically more homogeneous « biotypes ». In turn, these can be the basis for developing and testing targeted treatments/ interventions tailored to the pathophysiology of an individual.

WP05 - Database

EU-AIMS bioresource continued to manage biological samples from the consortium. EDTA blood samples, Tempus blood samples, plasma, urine, and saliva are avilable, from study subjects, siblings, and parents.

Raw data (anonymized and harmonized), as well as QC reports and first-line processed data are indexed and made available via a custom-built infrastructure (the EU-AIMS DataSharingSystems, EU-AIMS DSS). Beyond indexing and offering web views of the consortium data, the infrastructure enables controlling the user access, and reviewing ongoing research projects.

A plan to sustain the clinical database beyond the end of the project has been developed and agreed: Institut Pasteur was chosen as the centre that will host existing (EU-AIMS period) and future (AIMS-2-TRIALS period) clinical data. During the one-year extension of the project CEA-NeuroSpin and Institut Pasteur worked on the legal agreement as a pre-requisite to data transfer.

Another component of the EU-AIMS data management, a system based on the tranSMART platform, was made available to the consortium partners. It provides means for quick, exploratory data analysis. Clinical, demographic and questionnaire data were made available via this system.

WP07 - Human Genomics

For the LEAP cohort, the total number of participant is 749 including 422 individuals with ASD, 34 with intellectual disability (ID) and 293 TD. In addition to these participants, we collected phenotypic information on 1,004 relatives (mostly parents from individuals with ASD). We collected blood for 77% of the participants (N=323 ASD, 23 ID and 230 TD) and 74% of the relatives (N=742). We then isolated DNA for 519 participants (N=291 ASD, 17 ID and 211 TD) and 589 relatives. The blood DNA was used for the genotyping of 700,000 SNPs (Illumina OmniExpress array) of 491 participants (N=266 ASD, 16 ID and 202 TD) and 562 relatives. This summer 2018, we will receive the last batch of DNA and genotyping (N= 361) and if all DNAs and SNP arrays pass the QC, we will have a complete cohort of 1,318 individuals including 323 with ASD (76% of the total EU-AIMS ASD participants), 23 individuals with ID (68% of the total ID participants), 230 TD (78% of the total TD participants) and 742 relatives (74% of the total relatives). Importantly, to detect de novo mutations, we will have complete families with both parents for 275 individuals with ASD, 13 ID and 82 TD. The DNA will be sent to our partner Autism Speaks (Laboratory of Steve Scherer) for WGS and for inclusion in the MSSNG project. To date, we have the SNP genotyping and CNV data results for 1,046 individuals.

For the **SynaG** cohort, the total number of individuals is 156 including 44 individuals with ASD, 9 TD and 103 relatives. We collected blood for 95 individuals including 27 individuals with ASD, 1 TD and 67 relatives. We will receive the DNA this summer and complete the SNP genotyping and the WGS. To date, we have the SNP genotyping and CNV data results for 8 individuals with ASD and 26 relatives.

For the **HRI** cohort, the total number of individuals is 485 including 56 individuals with ASD (the older sibling in the high-risk families), 146 HR-ASD babies, 29 HR-ADHD babies, and 82 LR-ASD/ADHD babies, and 172 relatives. We succeeded in collecting either blood (N=205) or saliva (N=280) for all the individuals. This sampling is now complete, and the DNA will be send to the National Human Genomic Research Centre (CNRGH) for both SNP genotyping and WGS.

Pipeline of analyses and results

In addition to the collection of the blood, DNA, SNP genotyping and WGS data, we (INSPAS) (i) designed the main pipelines of analysis of the cohort, (ii) analysed a cohort of 85 individuals with SHANK3 CNVs (Tabet et al. 2017), (iii) developed GRAVITY, a Cytoscape plug-in to visualize multiple hits in individuals https://research.pasteur.fr/en/software/gravity/, and STRATIPY, a new Python package for network-based stratification https://research.pasteur.fr/en/software/stratipy/.

1.5. Scientific and technical results/foregrounds of the project

WP01 - Cellular models of ASD

Examples of successful academic-industry partnerships include a collaboration between Basel University and Roche, which has identified molecular and cellular phenotypes in a human stem cell model of tuberous sclerosis: mTORC1 inhibition corrects neurodevelopmental and synaptic alterations in TSC2. Lead by Prof Price, researchers at KCL and Roche developed an assay to evaluate the interaction between genetic and environmental risk factors for ASD. They found that when neural progenitors from ipSCs derived from control individuals were treated with pro-inflammatory cytokines at stages equivalent to the first trimester, they phenocopy precisely the morphogenetic phenotypes seen in cells derived from SHANK3 ASD patients. However, this effect of pro-inflammatory cytokines is not seen in cells from ASD patients, where the phenotype is already in evidence, suggesting that the cytokines are genuinely reproducing an ASD phenotype. Neural progenitors 'remember' their exposure to cytokines when administered at this 1st trimester stage and augment their response to the same cytokine delivered at later neuronal stages. This cellular memory appears to be associated with 'PML nuclear bodies', a mechanism demonstrated in immune cells, but never before in neural derivatives. The achievement of D1.8 "Provide evidence for the 'two-hit' hypothesis in ASD" is a major success and brings to culmination the series of studies that comprise this work package. We had previously reported a morphogenetic and physiological phenotype for the SHNAK3-derived neurons. This models a genetic risk factor for autism and provides evidence for an early cellular/molecular phenotype associated with ASD. The question associated with the 'two-hit' hypothesis was whether a similar phenotype might be associated with an environmental risk of the disorder, and whether the two risks combined would show the same or an enhanced phenotype.

We have modelled the environmental risk by employing a cellular model of maternal immune activation (MIA). Activation of the maternal immune system during pregnancy by, for example, viral infection, is a known risk factor for ASD. In a Danish cohort study, the increased risk of a diagnosis of autism in a child exposed to MIA in utero as a consequence of severe influenza infection was almost three-fold (Atladóttir et al., 2010). We mimicked this in vitro by exposing neural progenitor cells at a stage equivalent to first trimester to pro-inflammatory cytokines, such as interferon gamma (INFg). We discovered that neurons subsequently differentiating from such exposed neural progenitor cells show a morphogenetic phenotype equivalent to that we previously observed in the SHANK3 cells (Kathuria et al., 2018)

The question then arose of whether similar phenotype could be observed in cells derived from sporadic ASD patients. The figure shows that these cells show the phenotype, even without the INFg exposure, just like the SHANK3 cells. Thus, we have demonstrated the same phenotype in three models of ASD: genetic, sporadic, and environmental. This gives us a degree of confidence that this phenotype is associated with the disorder, and not some by-product of (for instance) the genetic background.

WP02 - Animal models of ASD

WP02 delivered rodent models that exhibit several core ASD symptoms. The WP defined detailed protocols for existing quantitative behavioral assays of social behavior that are sufficiently robust and reproducible for drug discovery. The identified models and methodologies now provide the basis for competitive industry projects on ASD drug discovery and development of new therapeutics.

Increasing understanding of the neuropathology of ASD. Already during the early phase of EU-AIMS WP02 made important break-throughs. The genetic heterogeneity of autism poses a major challenge for identifying mechanism-based treatments. A number of rare mutations are associated with autism, and it is unclear whether these result in common neuronal alterations. We discovered in our WP02 study an unexpected convergence of synaptic pathophysiology in a nonsyndromic form of autism with those in fragile X syndrome. More specifically, neuroligin-3 knock-out mice exhibited

features characteristic of fragile X (which includes autism as one of their multifaceted symptoms); including disrupted heterosynaptic competition and perturbed metabotropic glutamate receptor-dependent synaptic plasticity. These phenotypes could be rescued by re-expression of neuroligin-3 in juvenile mice, highlighting the possibility of reverting neuronal circuit alterations in autism after the completion of development, as well as important implications for mechanisms underpinning the disorder. This highlighted the previously unknown role of mGlu1 in non-syndromic forms of ASD. The finding was published in Science (Baudouin et al., 2012) and further extended by also demonstrating pharmacological rescue.

We then translated this into clinical samples in less than a year by initiating the development of a PET Ligand to measure mGLU1 in ASD, and have linked this to ongoing PET-pilot studies of synaptic deficits in iPSCs from ASD individuals with other glutamatergic and GABergic receptor subtypes.

A second major step forward in the understanding of mechanism involved ASD was the testing of novel Mnk- inhibitors with better brain penetration and their action of specific rodent behaviour (WP2 – Scheiffele lab). The work on Mnk inhibition led to the filing of a patent (US 2015/0038506 A1) submitted by Peter Scheiffele (UNIBAS). The present invention relates to certain compounds (e. g., imidazopyrazine, imidazopyridine, imidazopyridaZine that act as inhibitors of the MAP kinase interacting kinases MNK2a, MNK2b, MNKla, and MNKlb. It further relates to pharmaceutical compositions comprising these compounds, and to the use of the compounds for the preparation of a medicament for the prophylaxis and treatment of diseases (e.g., prolifera tive diseases (e.g., cancer), neurodegenerative diseases (e.g., Alzheimer's disease), metabolic diseases (e.g. diabetes), neurodevelopmental disorders (e.g. autism), or psychiatric disorders (e.g. schizophrenia or anxiety)) as well as methods of treating them.

Transgenic models. WP2 has studied several transgenic models, as well as the inbred BTBR mice and environmental model using prenatal sodium valproate (VPA) exposure in mice.

Cross-site reproducibility was tested in the BTBR mice using a harmonized protocol for social interaction and automated scoring (EthoVision XT 9.0 – Noldus). The effect of oxytocin (50 mg/kg, ip) versus vehicle was tested on direct social interaction. Results showed a reproducible readout across the test sites. The testing of several cohorts of BTBR mice (Roche), revealed a robust and stable phenotype, whereas many transgenic models (Nlgn1, Nlgn3, Shank3, 16p11 KO, Cacna 1a KO, 15q duplication, Frm1 KO and Sapap3 KO) did not exhibit a robust and stable phenotype. Many of the factors contributing to the observed variability remain elusive. For drug discovery, however robustness is an essential aspect.

The cross-site study performed with SHANK2 KO rats also showed that through harmonization of protocols and using one cohort of animals a specific phenotype is reproducible in different laboratories. It seems however more of a challenge to produce animal models that exhibit a phenotype that is robust and stable over time.

The pharmacological effect of the shared mGluR1 antagonist (JNJ16259685) was equally reproducible across the sites. This study has also reproduced previously obtained data in the same strain (Pfizer).

Multi-centre study. The main objective of this WP2 multi-center study was to provide replicable and reproducible results by standardizing an experimental protocol for behavioral evaluation and drug testing using a genetic animal model for Autism Spectrum Disorder (ASD). To improve data reproducibility, several factors were aligned carefully between the three sites involved, such as lab protocol, compound and animal provider, data analysis and report of results across different sites. It was hypothesized that by sharing a series of experimental elements in detail, the variance across sites will be reduced and provide higher rates of reproducibility across sites.

Based on an earlier finding from one of the three sites involved in this study, a replication study focusing on this initial finding was initiated, namely the assessment and pharmacological reversal of a behavioral phenotype difference between a rodent genetic model for ASD and the wild type

littermate controls. As part of the replication study in SHANK2 mutant rats and their controls, a phenotypic assessment was carried out quasi-simultaneously in 3 different research facilities (Pfizer, Roche, University of Groningen (the latter through project partner UMCU)). In order to standardize the experiment as much as possible several rounds of discussions were held to reach consensus about the experimental design of the experiment. A working document was continuously shared between sites about the different aspects of the protocol to standardize. The elements that were standardized fell within one of the next categories: provider of animals, the design and timeline of the experiment, technical details about the equipment and software used (provided by Noldus I.T.), behavioral testing and room set up, animal related guidelines, drug provider and administration method (provided by JNJ R&D), and behavioral data analysis procedures.

After conducting the experiments, raw and analyzed data was uploaded to a platform powered by a consortium partner (Sylics), that allowed all the sites to share and access data from the partner sites. Using the automated scoring and manual scoring of the behavior, all three sites showed very comparable results both at the level of the genotype differences and the pharmacological effect. The alignment of the manual and automated scoring showed different frequencies for each of the behaviors analyzed; however, the tendency was the same for both methods of analysis for most of the behavioral categories (e.g. the group showing more grooming on the manual analysis also showed more grooming in the automated analysis, although the frequency was not the same for both analyses). These inconsistencies between the methods of analysis were replicated across sites, suggesting a beneficial impact of the intensive standardization followed in this project.

With regard to the discrepancies between the methods of analysis, it seems that both methods have different sensitivity to detect the behaviors, which may be the result of phenotype definition differences between the hand and automated scored data.

Following thorough standardization of the protocol for a multicenter study, it was shown that reproducibility of behavioral pharmacological data in rodents can be established. Furthermore, the consistent discrepancies at all three sites between automated and manually scored behavioral data reveals the importance of phenotype definition for interpretation of the genotypic and pharmacological findings. The main results from this study are currently being prepared for a collaborative publication from EFPIA, SME and academic partners.

Novel standardized and automated behavioral paradigms. In 2017 and 2018, the R&D efforts at Noldus Information Technology once more focused on further development of the EthoVision system for automated video tracking and behavior recognition. The software automatically recognizes 10 different mouse behaviors, without the need for training for different set-ups or strains. It works with an overhead video camera (e.g. the top unit of a PhenoTyper cage), which makes it compatible with cage enrichment, sensors and stimulus devices attached to the cage walls (with a horizontal camera these would block the view). It makes behavioral scoring repeatable, objective and consistent. Our R&D efforts in EU-AIMS were targeted at supporting the multi-site study of the Shank2 rat at Pfizer, Roche and University of Groningen. Two rat behaviors had our special attention: grooming and rotation behavior.

EthoVision analysis of rat behavior. The initial analysis (using EthoVision software) of the Shank2 rat video recordings, compared against human scoring, led to unexpected results: grooming overestimation and fluctuating rotations results. When doubts arise on the correctness of automated behavior recognition, the process of validation takes four steps. At first, the videos in question must be manually annotated to establish a ground truth. Next, comparison of the manual annotations versus the automated behavior recognition leads to insight in what might be wrong. Thirdly, when changes to the processing are expected to improve the detection of the behaviors, such changes are made and a re-evaluation of step 2 ensues. The final step is to discuss the new results with project partners and come to decisions. This process repeats until the best possible result is obtained.

Rotation behavior. In 2017, for rotations algorithm improvements, a lot of effort has been spent on steps 2 and 3 resulting in a documented improvement proposal for the rotations detection algorithm and the recommendation to change the original definition of what a rotation is. Detailed numeric analysis of the manually annotated rotations revealed that what to the eye looks like a rotation regularly does not conform to the stated definition of rotation. New criteria were examined, prototyped and their contribution to correct rotation detection evaluated. These include the use of:

- Turn angle,
- Distance moved by the body points Nose and Tail Base, respectively, during the rotation,
- Vector-length ratio of Nose to Body Center and Tail Base to Body Center,
- Elongation,
- Body Angle, and
- Degree of animal movement during the rotation.

Application of the new criteria resulted in much better automated detection, but further improvement must come from a better definition of what a rotation really is.

Grooming behavior. For the grooming overestimate, it was discovered that the videos in which this behavior was to be automatically detected were overexposed. Overexposure causes noise and movement everywhere in the video, and no movement on the animal. The result is confusion between Resting and Grooming. In many cases, grooming is scored while in fact the animal sits completely still. The confusion does not appear so much in the control group (vehicle) since here the animal is active most of the time. Attempts to filter the data to lower the noise did not improve the result. There are more options to deal with overexposed data, but time did not allow for this. A chance to create new videos did not offer itself.

Cognition. For cognition testing, EU-AIMS partner Sylics has developed the CognitionWall for the mouse, which has been successfully validated in various projects. In 2017 we jointly developed an enlarged version for the rat, which was tested at Pfizer. It turns out rats have no problem passing through the holes of the device. The test protocol, however, still has to be optimized. Rats need different settings with respect to the numbers of reward pellets and the criterion to be rewarded. The device is now available for parties within or outside the EU-AIMS consortium who are interested in further joint development of the CognitionWall protocol for the rat.

EthoVision XT 13. During all of 2017, development of EthoVision XT 13 resulted in the release of the product in June 2017. This latest version offers a number of significant improvements and innovations relative to previous versions:

- Logging manually scored events during acquisition to trigger actions in the PhenoTyper as the trial unfolds. For instance, “Drop Pellet” when the user presses the “F” key.
- Post-acquisition manual scoring of events including the ability to edit already manually scored behaviors. Apart from being a much asked for general purpose feature, in the light of the project activities this year, this allowed to make a much more accurate ground truth baseline, especially for the rotations which in previous years required The Observer XT to be used as separate product and involving additional data import and export work.
- Manual scoring of point events during or after acquisition: that is, events without a duration such as twitching, flinching, blinking, yawning.
- Free Interval Selection: a very flexible and powerful method to define time windows in behavioral analysis based on a user-defined start criterion and a user-defined end criterion. It is now possible to add user-defined leading and lagging time intervals to the events limiting the desired interval.
- A new Multi-condition Analysis parameter, allowing users to simplify complex analyses.
- A JavaScript custom parameter intended to allow end users to request specific custom parameters. An important aspect of this new feature is that such customers no longer need to

wait for Noldus to release the next version of EthoVision XT but can use their custom parameter directly after its development has completed.

WP03 - Translational Research Development

Translational Neuroimaging

Human Neuroimaging – MRI

- deCODE/Amgen, in collaboration with Domus: In 2017, approximately 190 genetically profiled (deCODE/Amgen) individuals were enrolled for imaging at Domus. While enrollment for the study has been concluded, a number of projects are leveraging this dataset. As noted in the previous report, analyses were focused on the effect of the 15q11.2(BP1-BP2) copy number variations (CNV) on cognitive, structural and functional imaging data which were published in Translational Psychiatry (Ulfarsson MO, et al, 2017). This is being followed up with analyses using diffusion tensor imaging (DTI) to investigate the effect of 15q11.2(BP1-BP2) dosage on WM integrity and will be submitted to Biological Psychiatry in the near future (Silva A, et al). Furthermore, structural imaging data from carriers of the 16p11.2 distal CNV was used to replicate findings from the ENIGMA consortium and is in preparation for submission to Molecular Psychiatry (Sønderby IE, et al). We discovered three rare protein truncating variants in the MAP1B gene that segregate with intellectual disability (with some carriers' also on the autism spectrum) and the carriers have significantly reduced white matter most profound in the corpus callosum (Walters GB, et al. 2018, in review). Since Attention deficit/Hyperactivity disorder (ADHD) is a common comorbid disorder in ASD, we have evaluated the risk on ADHD, conferred by the CNVs associated with ASD and schizophrenia. A manuscript reporting these results will be submitted presently. Finally, we followed up on our initial report (Stefansson H, et al, 2013) that demonstrated effects of genetic risk variants not only on cognition and brain structural traits, but also fecundity. We expanded the analysis of carrying risk variants for neurodevelopmental disorders to modelling the polygenic risk of autism on fecundity and found that both risk factors associate with having fewer children (Mullins N, et al, 2017).
- CIMH performed standard group-level analyses of functional MRI data of the LEAP wave-1 data set. Case-control differences were evaluated for several fMRI measures of activation and connectivity. We currently run in-depth analyses to characterize the impact of demographic and clinical variables on functional brain responses to a subset of tasks (Theory of Mind, reward anticipation). Manuscripts on case-control differences and influencing variables are in preparation (Moessnang et al., in preparation; Baumeister et al., in preparation). In addition, we have completed first-level analyses of LEAP wave-2 data which is prepared for release to the central database. We also collaborate closely with our partners of WP04 for defining joint research questions and performing multimodal analyses of the LEAP data (e.g. comparison of functional activation between subgroups of ASD subjects resulting from the application of stratification approaches on behavioral data using hierarchical clustering).

Animal Neuroimaging – MRI

- Roche/KCL: Interpretation of data obtained in a previously conducted translational MRS study in persons with idiopathic ASD and in several etiologically different rodent models of ASD was refined based on reviewers' input. Commonalities among animal lines and between man and animal models in terms of excitatory and inhibitory neurotransmission were investigated and reported in a manuscript recently accepted for publication in Translational Psychiatry. The key findings are that high functioning adults with idiopathic ASD have on average reduced glutamate in the striatum whereas no change in GABA was observed. The reduction in striatal glutamate is related to core ASD symptom severity. Moreover, reduction in striatal glutamate was

recapitulated in rodents carrying mutations in Nlgn3 or being prenatally exposed to VPA. These translational findings support the notion of glutamatergic dysfunction and are opposing the original hypothesis of neural excitability in ASD.

Molecular neuroimaging

- KCL/KI combined human PET and mouse autoradiography study of GABAA receptors has been accepted for publication in Science Translational Medicine, authored by Jamie Horder (KCL) and Max Andersson (Karolinska). This is the first paper to combine human and animal molecular imaging in the study of ASD. We show no alterations in the levels of GABAA receptors in human adults with ASD nor in three mouse models (SHANK3, CNTNAP2 and 16p11.2).
- KI: A manuscript for the case-control study of 5-HTT in 15 ASD/15 control subjects has been submitted to JAMA Psychiatry and is awaiting review. New methods for PET-MRS GABA quantification comparisons have been developed and a manuscript is in preparation and will be submitted by the end of Q2 2018. For mGluR5, the test-retest study of 18F-FPEB radioligand in 8 control subjects is finalized and data are currently analyzed. New methodology for PET-MRS glutamate comparison has been developed, and a manuscript is in preparation. Six ASD discordant twin pairs have been examined with PET and data are being analyzed during Q2.
- KCL has published the first Proof of Concept that GABA response to pharmacological challenge (with a single dose of Riluzole) is different in ASD and controls (Ajram et al., 2017). In addition, an increase in GABA fraction in the prefrontal lobe was accompanied by a 'normalization' of an ASD connectivity deficit between the prefrontal lobe and posterior brain cortices. Thus, the biology of ASD can be shifted, even in adults.

Biomarker integration

- Based on the successful application of network modeling approaches on human and animal imaging data outlined in the previous report (reviewed in Braun et al., 2018), CIMH continued this line of analysis and adopted a network segregation modeling approach on the LEAP wave-1 data.

The results suggest a higher sensitivity of this approach for age effects as compared to standard measures of functional brain activation and connectivity. In addition, we detected a specific loss of functional compartmentalization involving the so-called social brain (i.e. the functional network involved in social cognition) in ASD (Moessnang et al., in preparation). Based on these promising results, network segregation modeling approaches will be incorporated into the biomarker integration strategy of AIMS-2-TRIALS (lead: Nijmegen).

WP04 - Clinical Research Development

- Identification of risk biomarkers in infants at high familial risk for autism: We have published a number of papers identifying and in some cases replicating findings concerning candidate biomarkers of later autism or autism-relevant traits. These include enhanced visual search (Cheung et al., 2016), altered pupillary light reflex (Nystrom et al., 2018), altered neural responses to gaze (Tye, Bussu et al., in prep); diminished social specialisation of the temporal lobe (Braukmann et al., 2018, Lloyd-Fox et al., 2018); reduced habituation of auditory gamma (Kolesnik et al., in review); overconnectivity in functional brain activity during spontaneous attention (Haartsen et al., in review); altered Vineland and Mullen profiles (Bussu et al., 2018); and altered attention salience within dynamic naturalistic scenes (Mason et al., in review).
- **Relationship defined between biomarkers and the autistic clinical phenotype in children and adults:** We show that performance on several cognitive measures (theory of mind, executive function) as well as cross-domain cognitive profiles, functional connectivity patterns within and between networks as revealed by resting-state fMRI, as well as wide-spread, individual, diffuse

structural abnormalities dimensionally relate to ASD core and (in terms of cognition) associated symptoms. These findings are currently being prepared for or have been submitted for publication.

- **Development of a clinical research infrastructure:** Throughout 2017 and 2018, we have added additional sites to the clinical network, which now includes 106 sites from 37 countries. We have intensified the collaboration across the network by moving ahead with two proposals to utilise the clinical pooled data (N = 7,000 individuals with ASD from 18 sites in 9 countries) and generate new research. We have published a first manuscript from this effort to investigate sex- and age-related differences in ADI-R and ADOS scores in 2018 in Journal of Autism and Developmental Disorders (Tillmann et al. 2018. The findings were also disseminated at the International Meeting for Autism Research (IMFAR) in May 2017 in San Francisco, USA. We are currently moving ahead with a second proposal on behavioural and emotional difficulties in children with ASD with/without intellectual disabilities. This effort will analyse the pooled dataset of the EU-AIMS clinical network in combination with the USA-based Simons Simplex Collection (SSC). In terms of clinical trial network development, from the wider network we identified 24 'trial ready' sites following rigorous and systematic information gathering and QC and they are partners in the funded IMI2 AIMS-2-TRIALS consortium. They will participate in the first phase of academic-, charity- and industry-sponsored autism treatment trials to be run. Further expansion, training and consolidation of the clinical network will take place as part of the new programme of work.
- **Development of standardized clinical assessment methods, improved clinical trials methodology and regulatory guidelines**:
  - Based on an expert meeting at the start of EU-AIMS (April 2013) with European academic group, EFPIA and USA experts on outcome measures in clinical trials and observation studies in autism we included a broad range of clinical outcome measures in the LEAP data collection: for instance:
  - We also submitted to EMA a proposal for potential biomarkers in autism and had a meeting with EMA (April 2014) for qualification advice. Their recommendations were incorporated in the protocols for LEAP (Task 2) and the sibling study (Task 1).
  - We extensively commented on the draft "Guideline on the clinical development of medicinal products for the treatment of Autism Spectrum Disorder (ASD)" and contributed to its final version (9 November 2017, EMA/CHMP/598082/2013).
  - We currently collaborate with partner Roche on a clinical study that compares various RRBI outcome measures to one another and to objective outcome measure

WP05 - Database

A **repository of samples** (**a bioresource**) acquired as part of WP 4 was established at IOP/KCL; this facilitates identification of markers of risk, severity, and therapeutic response. The repository holds blood samples - EDTA-stabilised blood (2495 samples), Tempus™ Blood RNA Tubes (1208), and plasma (2279), urine (3675), and saliva (2042). Samples were recruited from the High Risk Infants (HRI) study, the Longitudinal European Autism Project (LEAP), as well as the Synaptic Gene study (SynaG). Samples come from 8 different sources: King's College London (KCL), participants recruited from the Special Needs and Autism Project (SNAP) to take part in LEAP at KCL, University of Cambridge (UCAM), Mannheim Central Institute of Mental Health (CIMH), Karolinska Institutet (KI/KIND), Radboud University Nijmegen (RUNMC), Biomedical Campus University of Rome (UCBM), and University Medical Center Utrecht (UMCU)..

The other result of the work package 5 is a **data sharing platform**, with systems for data collection, integration, management, and access. This work can be further split into 3 components:

- The clinical **data collection system,** operational since December 2014. All centres, for both WP4 studies (LEAP and HRI) were uploading data. Data acquisition was multi-channel:

- via the data collection platform as built by WP5, for MRI, EEG, eye-tracking, MRS, questionnaires;
- via the Delosis system for questionnaires.

- The **data exposition database**, with the first demonstrator released in April 2015, and the beta version online since September 2015. Raw data (anonymized and harmonized), as well as QC reports and first-line processed data were indexed and made available via a custom-built infrastructure (the EU-AIMS DataSharingSystems) based on an open source web semantic framework (CubicWeb, Logilab, France). Beyond indexing and offering web views of the consortium data, the EU-AIMS DataSharingSystems enable controlling the user access (sign-up, sign-in, lost-password), granting the access (moderator roles), and reviewing and following up the ongoing research projects performed by defined research groups.
- The **data exploration system**, based on the tranSMART platform, allowing users to perform basic data analysis on the fly, with an aim to aid hypothesis generation. After extensive cleaning and transformations that match the EU-AIMS data to the requirements of this platform, clinical, demographic and questionnaire data have been made available via this system.

The sustainability of the main component of the data sharing platform, i.e., the exposition database, was addressed by transferring the responsibility for data hosting to Pasteur Institute (see D05.04). This is in alignment with the on-going internal effort of Pasteur Institute to build a global platform for data sharing and open access for clinical/biological data (National Project INCEPTION), which will provide medium-term resources, and will ensure co-localization of genomics information with the information currently acquired by WP5 on EU-AIMS WP4 subjects, which will provide a better legal and ethical management. Institut Pasteur also participates in the AIMS-2-TRIALS project.

Another strand of work in WP5 was related to using the existing samples and data in order to investigate for markers of autism related traits. This work was carried out during the first years of the project, and the IMAGEN cohort was used. It was found that a common OXTR-variant affects brain responsiveness to negative social cues, and that in “risk-carriers” reduced sensitivity is simultaneously associated with more social-affective problems in “favourable environments” and greater resilience against stressful experiences.

WP07 – Human Genomics

Genotyping/Sequencing: The complete genetic profile of the EU-AIMS cohorts is still in progress, but we already have results for the subgroup of individuals with available genotypes.

Genetic analyses: We first ascertained the ethnic background and the inbreeding status of the EU-AIMS cohort by using the identical-by-state (IBS) distance . As expected, the vast majority of the individuals were clustered in the European populations and the inbreeding status was similar to the one observed for the European population. We found no significant difference between ASD and TD. The inbreeding status will be used in order to investigate the contribution of recessive mutations to ASD in the EU-AIMS cohort when the WGS data is available.

The ASD polygenic risk score (ASD-PRS) was calculated from a previous genome wide association study (GWAS) using over 16,000 individuals with ASD who do not overlap with the EU-AIMS sample. Interestingly, even before SNP imputation (that we will complete when all the samples are available), we found a significantly higher ASD-PRS in individuals with ASD compared to controls (P=0.0019). This higher ASD-PRS in patients compared to controls was a trend in almost all sites and reached significance in Cambridge (P=0.014) and Nijmegen (P =0.0081).

Using the SNP array data, we ran two CNV detection algorithms “QuantiSNP” and “PennCNV” in order to detect large CNVs (>50 kb). This analysis will be completed for the full cohort in 2018. As in previous cohorts of individuals with ASD, we found de novo or inherited CNVs affecting exons of genes previously involved in ASD (SFARI genes) or intolerant to loss of function mutations (pLI > 0.9)

or expressed in the brain (+1SD for both specificity and level of expression). This first analysis revealed an excess of deletions of such genes in patients diagnosed with ASD and ID. An example of four families with patients carrying de novo deletions affecting compelling ASD-risk genes is provided in D7.3.

Analysis of patients with SHANK3 CNVs: We investigated 85 patients with different 22q13 rearrangements (78 deletions and 7 duplications) that include the gene coding the synaptic scaffolding protein SHANK3. We first explored their clinical features and provided evidence for frequent corpus callosum abnormalities. We then mapped candidate genomic regions at the 22q13 locus associated with risk of clinical features, and suggested that a second locus is associated with absence of speech. Finally, in some cases, we identified additional rearrangements at loci associated with ASD, potentially modulating the severity of the syndrome. We also reported the first SHANK3 deletion transmitted to five affected daughters by a mother without intellectual disability nor ASD - suggesting that some individuals could compensate for such mutations. This study was published in Npj Genomic Medicine in 2017. We are currently including more patients in order to better characterize the contributing genes at 22q13 and to detect modifier genes.

A tool for multiple hits visualization: We developed GRAVITY, a tool to visualize genotyping, whole exome and whole genome sequencing data, in the context of protein-protein interactions. GRAVITY allows to dynamically filter on sequencing quality or allelic frequency, or to focus on specific pathways of interest. It also includes analyses of mutation pattern across cohorts of patients and their relatives, allowing the identification of multiple hits in individuals and providing a precise functional annotation of the variants. This tool helps in the interpretation of the combined effect of multiple variants in the patients, setting the stage for a better understanding of disease mechanisms, and potentially more precise and personalized therapeutic strategies. GRAVITY is an open source tool that is freely and openly available at http://gravity.pasteur.fr. An article reporting GRAVITY is under review.

A tool for Network-Based Stratification: We developed STRATIPY, a full Network Based Stratification (NBS) python pipeline based on the original method introduced in cancer research. The NBS approach is similar to one recently used for tumor stratification in cancer (Hofree et al. 2013). This method integrates the genomic information of each patient with functional gene networks. In cancer research, this method was used to successfully cluster together patients with mutations in similar network regions. For example, NBS could identify subtypes of cancer that are predictive of clinical outcomes such as patient survival, response to therapy or tumor histology. The underlying functional network will be constructed using different sources of data including the genotyping data of each patient and any relevant information available to annotate the variants and the genes.

1.6. Potential impact and main dissemination activities and exploitation of results

EU-AIMS had contributed towards the Innovative Medicines Initiative (IMI) goals to improve health by speeding up the development of, and patient access to, innovative medicines. The EU-AIMS consortium facilitated collaborations between the key players involved in healthcare research, including universities, research centres, the pharmaceutical and other industries, small and medium-sized enterprises (Noldus, patient organisations (Autism Europe, Autism Speaks), and regulators (e.g. EMA). EU-AIMS is one of the most successful examples for public-private partnerships (PPP) in the life sciences.

Indicators of the impact of EU-AIMS include

- a report by Thomson Reuters showing that EU-AIMS was 'by far the most prolific Call 3 project with app 300 publications by the end of 2018. The citation impact of this research was more than three times the world average' and 'the highest percentage of highly cited papers (42%) in the top decile';
- establishment of the first European Clinical Network in ASD that can provide a platform for developing both a clinical trials network, and cohorts to help identify risk, stratification, and predictive biomarkers,
- obtaining, for the first time in ASD research, qualification advice from the EMA to an industry-academic consortium on biomarker methodologies;
- demonstration that brain abnormalities can be modulated in both rodent models and people with ASD.
- as the first neuroscience consortium receipt of the Roche Pharma Research and Early Development (pRED) award.

EU-AIMS had two major goals to enable a precision medicine approach to ASD:

1) to identify tractable treatment targets based on the underlying pathophysiological mechanisms, and 2) to identify and validate (stratification) biomarkers for ASD.

Identification of pathophysiological mechanisms underlying ASD: One of our major achievements is the two-hit hypothesis study (WP1 – Price lab). For instance, the latest results in WP01 presents the novel idea that SHANK3 haploinsufficiency causes early structural deficits in neurons, which impact synaptic development. Moreover, we show a phenotype arising in olfactory placodal neurons, destined for the hypothalamus, consistent with suggestions of a functional association between ASD and this brain structure. This result has not only defined a critical period during development when cellular deficits occur in ASD but has also determined a period for successful rescue of these deficits (Kathuria et al, 2018). The importance of the work is that for the first time, it draws together a cellular/molecular phenotype associated not only with the genetic risk of autism, but also sporadic, and environmentally provoked autism. This gives us some degree of confidence that our cellular studies reflect the pathophysiology underlying the condition not just the genetics of the condition.

Increasing understanding of the neuropathology of ASD. A second major step forward in the understanding of mechanism involved ASD was reached in WP2. We discovered in our WP02 study an unexpected convergence of synaptic pathophysiology in a nonsyndromic form of autism with those in fragile X syndrome. More specifically, neuroligin-3 knock-out mice exhibited features characteristic of fragile X (which includes autism as one of their multifaceted symptoms); including disrupted heterosynaptic competition and perturbed metabotropic glutamate receptor-dependent synaptic plasticity. These phenotypes could be rescued by re-expression of neuroligin-3 in juvenile mice, highlighting the possibility of reverting neuronal circuit alterations in autism after the completion of development, as well as important implications for mechanisms underpinning the disorder. This highlighted the previously unknown role of mGlu1 in non-syndromic forms of ASD. The finding was published in Science (Baudouin et al., 2012) and further extended by also demonstrating pharmacological rescue.

We then translated this into clinical samples in less than a year by initiating the development of a PET Ligand to measure mGLU1 in ASD and have linked this to ongoing PET-pilot studies of synaptic deficits in iPSCs from ASD individuals with other glutamatergic and GABergic receptor subtypes.

A further step forward in the understanding of mechanism involved ASD was the testing of novel Mnk- inhibitors with better brain penetration and their action of specific rodent behaviour (WP2 – Scheiffele lab). The work on Mnk inhibition led to the filing of a patent (US 2015/0038506 A1) submitted by Peter Scheiffele (UNIBAS). The present invention relates to certain compounds (e. g., imidazopyrazine, imidazopyridine, imidazopyridaZine and that act as inhibitors of the MAP kinase interacting kinases MNK2a, MNK2b, MNKla, and MNKlb. It further relates to pharmaceutical

compositions comprising these compounds, and to the use of the compounds for the preparation of a medicament for the prophylaxis and treatment of diseases (e.g., prolifera tive diseases (e.g., cancer), neurodegenerative diseases (e.g., Alzheimer's disease), metabolic diseases (e.g. diabetes), neurodevelopmental disorders (e.g. autism), or psychiatric disorders (e.g. schizophrenia or anxiety)) as well as methods of treating them.

Tool development. WP02 has developed and validated tools for better assessment of social behavior in rodents. The robustness of read-outs has been improved, extensive phenotyping of various rodent mutant strains has added to a better understanding of their use, in particular in drug discovery. These insights have been fundamental for Pharma partners in selecting the most suitable models, procedures, and read-outs for drug testing.

Another important output has been the advancements in quantitative automated analysis and tracking of rodent behaviors, combined with extensive cross-site comparison. These advances have directly impacted the development of tracking tools developed by a participating SME. For example, the software EthoVison XT 12, was made available to the scientific community in November 2016, which features significant improvement and innovations relative to the previous versions.

Validation of Biomarkers that Aid the Drug Discovery Process. Insights gained from analyzing the effects of neurodevelopmental disorder risk variants on multiple cognitive and brain imaging traits (deCODE/Amgen) has the potential to improve diagnostic accuracy, clarify disorder nosology and etiology, as well as expand our knowledge of neurodevelopmental processes and networks that when impaired increase susceptibility for autism spectrum disorder development. Endophenotyping of rodent models of ASD by neuroimaging modalities (Roche) has had an impact on the selection of relevant models for compound testing in drug discovery projects. Translational molecular imaging methodology developed in this project, can be used as a highly precise strategy in drug development for ASD and other disorders, directly measuring relationships between targets and behavioural effects. The negative findings in our translational GABA-A receptor studies, can serve as an example of methodology to be used for decision-making in where to focus resources in brain disorder research. By performing direct studies of putative targets in patients, indications of what targets should be pursued and not can be obtained before conducting expensive clinical trials. Further, the knowledge gained from our studies combining molecular PET imaging and deep phenotyping contribute to understanding of the characteristics of different ASD subgroups and can aid stratification of patients for future treatment development. The methods developed for combining PET and MRS measurements of GABA and glutamate, aids interpretation of prior and future findings and can disentangle unspecific findings made with MRS, into specific molecular targets measured with PET.

Identifying biomarkers for ASD. To our best knowledge, EU-AIMS created the most extensively characterized longitudinal ASD cohorts in the world (including clinical profile, cognition/ eye-tracking, MRI, EEG, and genomics in >1200 cases and controls) that span 'high risk' infants followed from 4 months to 3 years (Eurosibs, individuals with idiopathic ASD aged 6-30 years (LEAP, and individuals with specific monogenic forms of ASD – Phelan McDermid Syndrome (SynaG). We have collected data to GCP standards; and we have developed new data analysis methods (e.g. normative modelling and network based stratification). We have also very recently identified potential candidate biomarkers that could be further developed. For example we found that; 1) brain 'over-connectivity' at 14 months predicts more repetitive behaviours whereas slower engagement with faces at 6 months predicts more social-communication symptoms (BU); 2) biological sex is associated with significant variation in the brain phenotype of ASD (KCL/ GU) 3) differences in cortical connectivity and 'shiftability' in brain function following serotonergic and glutamatergic challenge are associated with variation in clinical phenotype (KCL); and 4) differential responses to behavioural interventions depend on genotype (KI).

Endorsement of biomarker approaches from the European Medicines Agency. The EMA has published the letters of support on the LEAP biomarker qualification advice on their website (December 2015).

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000319.jsp&mid=WC0b01ac0580022bb0

A correspondence paper reporting on the outcome of the QA procedure between EU-AIMS LEAP and the EMA has been published in Nature Reviews Drug Discovery ("Identification and validation of biomarkers for autism spectrum disorders).

Establishing a Europe-wide clinical network for ASD: Apart from adding new members to the network (now includes 100 sites), the network has been actively collaborating with 18 of those sites from 8 European countries to pool a total of 28 clinical datasets for secondary analysis. We have identified two research questions that we would like to address (1) Sex- and age-related differences in ADI-R and ADOS scores and (2) Behavioural and emotional difficulties in children with ASD and with/without intellectual disabilities. We presented the overall aims of the initiative at IMFAR in Baltimore in 2016 and followed this up with a poster describing preliminary findings for the first proposal at IMFAR 2017 in San Francisco. We expect at least one publication by the end of this year based on this effort. *The collaboration between EU-AIMS and ASD was also topic of a presentation at the Autism Europe conference in Edinburgh.* In addition, we have assessed trial-readiness in 43 sites through an online survey and followed this up with in-person interviews to better understand trial-specific requirements for clinical trials in ASD. Part of this work has also helped to match trial sites in the network (23 in total) with Roche to participate in a potential Phase III trial.

Collaboration with other groups: To facilitate global data pooling and replication, EU-AIMS has set up several data sharing agreements with a number of international ASD consortia. This includes the Australian Cooperative Research Centres (CRC) Fondation Fondamental, the Chinese Key 973 program, the FNIH Autism Biomarker Consortium – Clinical Trials (ABC-CT), the Canadian PONDS consortium and Monash University, Australia. An exchange programmes between EU-AIMS researchers at KCL and collaborators of the Chinese Key 973 program were initiated in November 2014. These programmes were expanded to facilitate further student/ staff exchanges with students and researchers from the above consortia. Further agreements were set up with: Ghent University and Hansen Research Services LLC.

Further details, split by task, are given below.

Infant sibling program. Within the infant sibling program, we have continued to lead the field both empirically and conceptually. We have proposed new theoretical frameworks for understanding autism, particularly the view that autism may represent an adaptive alternate trajectory of brain development. The emerging interest in this view was reflected in the invitation of our project lead Mark Johnson to present a keynote at the International Society for Autism Research Annual Meeting (2018). Further, the work of early stage researchers within our Eurosibs and our related BRAINVIEW Marie-Curie training network was showcased in a symposium at this conference. Empirically, we have continued to demonstrate that early markers of autism extend beyond the social brain to a variety of lower-level processes, critical to developing new models of autism emergence and building new proxy markers of treatment outcome. Indeed, we have also shown that we have promising biomarkers that may be sensitive to early parent-mediated intervention (Jones et al., 2017), an intervention type that can produce long term amelioration of symptoms (Green et al., 2017). Finally, we have developed tools to allow us to implement biomarkers across a variety of equipment and training levels, essential to clinical deployment. Specifically, we have developed the TaskEngine framework for this purpose (Mason). Since 2013 Task Engine has been used by us and by our collaborators in 11 studies in nine countries, with over 10,000 experimental sessions performed over several thousand hours of stimulus presentation. We have used Task Engine throughout Eurosibs and have submitted a paper demonstrating that its use can allow the robust cross-site collection of

neurocognitive biomarkers that are more consistent across locations than behavioural measures (Jones et al., in review). This is a critical step towards the implementation of neurocognitive biomarkers in clinical settings. We have also continued to engage with the broader public, including recent talks at Google (Jones, 2018) and Accenture (Jones, Charman 2017). Our work is also to be featured in an upcoming BBC television documentary called the "Wonderful World of Babies', allowing us to reach a broad global audience.

The EU-AIMS LEAP study is probably the only autism study world-wide that has the necessary power to identify **multi-modal stratification biomarkers** for ASD. Major achievements of the past period include completion of the baseline assessments and follow-up with very high acquisition rates across data modalities and schedules. For example, on the baseline cohort, for cognitive tests, acquisition rates ranged between 92-86% in the ASD group and 96-87% in the TD group. For EEG measures, acquisition rates in the ASD group ranged between 83-75% and 80-79% in the TD group. These lower acquisition rates for EEG measures reflect the fact that one site (UCAM) did not acquire EEG data. For eye-tracking, acquisition rates of the four main task sets ranged between 91-86% vs 87% in the ASD and TD groups, respectively. Careful QC procedures of each data modality were carried out by expert analysis groups. We have published a protocol paper on our biomarker methodologies and analyses approaches (Loth et al., 2017) and a companion paper reporting on the clinical characterisation of the cohort (Charman et al., 2017). As indicated before, initial data analyses have across biomarker modalities led to a number of conference presentations (ECNP and IMFAR, 2017 where we also present 12+ individual talks/ posters from LEAP) and publications that are currently in preparation. A summary of participant characteristics is given in Table 1. This dataset offers a unique characterization of functional brain responses in four major cognitive domains from childhood to adulthood and allows for cross-sectional and longitudinal analysis of case-control differences. Importantly, our top-down analyses suggest that effect sizes of previously reported case-control difference are moderate to low and that the heterogeneity of ASD phenotypes is higher than previously expected. In addition to replication efforts, this dataset also allows for testing novel analysis routines, such as network modelling approaches and patient stratification approaches, which might open up new routes to biopharmaceutical research and development.

The project helped to pave the way for precision medicine approaches in ASD. Findings from LEAP dispel the current 'myth' that « autism is characterised by particular deficits, such as impaired theory of mind, or brain over/ underconnectivity». Instead, we show that the pattern of deficits/ abnormalities is extremely fragmented across individuals with autism and that virtually no single abnormality is universally present in all individuals with autism. A better understanding of individual neurocognitive/ biological 'fingerprints' of people with ASD will be vital to identify what treatment/ intervention a given person with a specific pathophysiological / neurobiological profile needs, and which treatment or intervention will likely be effective for that person. Adequate treatment/ intervention will in turn help a person with ASD to increase their QoL or reach their potential, and so have important repercussions on well-being both of people with ASD as well as their families. This will likely have socio-economic benefits for European citizens, as on average, many families affected by autism have a lower income relative to families of typically developing children, due to loss of income of at least one parent (usually the mother, who acts as full-time carer), loss of potential and financial income of many individuals with ASD, and considerable costs incurred by intensive behavioural interventions.

Table 1: LEAP participation characteristics. Case-control cohort by sex and schedule.

		Total		Adults		Adolescents		Children		Mild ID	
		ASD	TD/ID	ASD	TD	ASD	TD	ASD	TD	ASD	ID
Sex	N	437	300	142	109	126	94	101	68	68	29
	Males (%)	72.3	65	72.5	67	77	69.1	71.3	61.8	64.7	51.7
	Females (%)	27.7	35	27.5	33	23	30.9	28.7	38.2	35.3	48.3
Age (in years)	M	16.68	17.22	22.79	23.10	14.86	15.33	9.40	9.52	18.09	19.30
	SD	5.80	5.94	3.37	3.27	1.73	1.73	1.58	1.54	4.27	4.97
	Range	6.08 - 30.60	6.24 - 30.78	18.02 - 30.60	18.07 - 30.78	12.07 - 17.90	12.04 - 17.99	6.08 - 11.97	6.24 - 11.98	11.50 - 30.19	12.92 – 30.24
Full-scale IQ	M	97.61	104.57	103.99	109.15	101.59	106.58	105.29	111.46	65.84	63.39
	SD	19.74	18.26	14.82	12.60	15.68	13.18	14.76	12.69	7.70	8.00
	Range	40[a]-148	50-142	76-148	76-142	75-143	77-140	74-148	76-142	40[a]-74	50-74

Note: ASD (autism spectrum disorder), TD (typically developing), Mild ID (intellectual disability)
[a] There are 3 individuals with a Full-scale IQ <50

Developing a Clinical infrastructure. The EU-AIMS clinical network includes a total of 106 sites across 37 European countries. This includes over 40 partners from EU-AIMS/COST/ECNP, as well as additional sites across Europe (see Figure 6). As part of our efforts to develop a clinical infrastructure we are:

- collecting information about ASD patient cohorts and diagnostic characterisation undertaken across centres. The results of this survey were published in 2015 in ECAP (see "Press and Publications" section on the left)
- pooling existing clinical datasets across the network to provide a resource for researchers to address informative questions on a larger scale
- developing a European 'Trial-ready' network (see figure below)

For more information please see the newsletter EU-AIMS Clinical Network Newsletter_Summer_2017 published on our webpage: http://www.eu-aims.eu/clinical-network/

Finally, the scientific network assembled under EU-AIMS across academic centres, SMEs, and industry partners will continue to accelerate information exchange, critical discourse, and collaboration between leaders of the European ASD community. This will have a long-lasting impact on the competitiveness and quality of ASD research in Europe that extends far beyond the duration of the project.

Figure 2: Clinical trial network of EU-AIMS

Dissemination, training and public engagement

The broad dissemination of data resulting from and concepts for the design and execution of preclinical studies of ASD will significantly accelerate studies on disease mechanism and drug discovery.

Training. We organised two translational Neuroscience Trainings workshop: the first EU-AIMS Translational Neuroscience Workshop was held in in Lisbon (15-17 October 2014). Building on this success, EU-AIMS partnered with the prestigious Neuroscience School of Advanced Studies **http://www.nsas.it/** and co-organised a one-week workshop on Autism in Bressanone, Italy (3-10 October 2015). The course format enabled 1-2 faculty to discuss one cutting-edge topic in the field for one entire day. The aim is to stimulate and foster cross-disciplinary discussions and ideas. The workshop was intended for PhD students, postdocs and senior scientists to learn basic concepts as well as hear about advanced approaches from across a range of disciplines focused on ASD, including cellular models, animal models, translational imaging, biomarker approaches, infant scanning, clinical diagnosis, and industry perspectives. Twelve experts taught during this 2-day course and over 40 PhD students, postdocs and senior scientists participated. We provided 5 stipends for EU-AIMS Internal PhD students and postdocs and 6 stipends for PhD students/ postdocs from among the wider clinical network.

Webinar series. In 2017 we have started a webinar series to disseminate key findings from the EU-AIMS project primarily to scientists and professionals, but also other stakeholders such as participants in our projects and families affected by autism. This has included a kick-off webinar by Prof Murphy and Dr Spooren that gave an overview of EU-AIMS, a dedicated webinar on the biomarker approaches adopted in the Longitudinal European Autism Project by Dr Loth, and a dedicated webinar on sex/ gender differences in autism by Dr Lai This was followed by a webinar on

genetic approaches to autism, by Prof Bourgeron, Prof Meyer-Lindenberg, and a webinar on preclinical advances by Prof Scheiffele.

Videos: KCL has produced a video that explains to families what study participation involves, created a facebook page for families SynaG: www.facebook.com/euaimssynag | Facebook LEAP: www.facebook.com/euaims , and created a puppet movie (Pip and the Brain Explorers) which shows families and young children what an MRI scan involves (see figure 4).

Figure 3. Storyboard (first page) of Pip and the Brain Explorers

Videos, podcasts and other events. The EU-AIMS group recorded a video at the third GA meeting of EU-AIMS in Paris. This video was financed by Servier. The goal of this video is to give an overview about what EU-AIMS is doing for Autism Research. This video aroused a great interested in the wider community with almost 3000 visitors.

The EU-AIMS Ethics Advisory Board organised a public dialogue at the Institute of Psychiatry in London on 22 October 2014. Over 250 people attended this lively and provocative event. Audience members represented a range of publics, including people with autism and their families, autism support groups, clinicians and researchers. Speakers included Professor Richard Ashcroft (QMUL), Virginia Bovell (Ambitious About Autism), Professor Declan Murphy (IoP) and Russell Stronach (Autistic UK). Sandy Starr from Progress Educational Trust chaired the evening. The pod casts are available on the EU-AIMS website. As a result of this event a very active interaction between the patient groups, families and the research community of EU-AIMS could be initiated.

Additionally, the Ethics board prepared a video in which EU-AIMS scientists answer questions from the autism community about EU-AIMS research. This video is available on you tube (https://youtu.be/kBisyFGx3tk).

Social Media. Wit project start we setup a linked in and a twitter account to create awareness about ASD in the wider public and (take advantage of social media to) foster the dialogue with the wider public. EU-AIMS has a linkedin account with 1700 followers and the number of the members is still growing.

Overview about EU-AIMS publication

EU-AIMS is a very active project up to now we have almost 300 publications in high-ranking journals. The publications are published on the EU-AIMS webpage.

1.7. Lessons learned and further opportunities for research

The Innovative Medicines Initiative (IMI) is a large-scale public–private partnership between the European Commission and the European Federation of Pharmaceutical Industries and Associations (EFPIA). The IMI aims to boost the development of new medicines across Europe by implementing new collaborative endeavours between large pharmaceutical companies and other key actors in the health-care ecosystem, i.e., academic institutions, small and medium enterprises, patients, and regulatory authorities.

Public-private partnerships represent attractive means to leverage resources dispersed across industry, academia, and voluntary health organisations in order to address its multiple challenges, in an era of constrained resources. The fact that IMI generated such opportunities for academia, SMEs and industry to collaborate and create a broad platform to make technologies and patient material accessible, bringing academia and SMEs in the clinic is a major achievement. This new type of collaborations created trust and new partnerships with other areas of expertise, such as regulatory bodies, and patient representative groups, to bring new and better products or treatments faster to the patients. The new collaboration models may have lasting effects beyond the existence of the IMI funded projects, as the added value of working cross disciplinary, becomes more obvious.

EU-AIMS demonstrates that this approach can lead to significant advances for the development of innovative drugs (as well as non-medical interventions). EU-AIMS has been instrumental for creating major synergies between preclinical and clinical researchers as well as academic scientists, scientists in the SME sector, and industry. The concepts and findings generated in preclinical academic research studies have led to innovations in the experimental approaches and concepts applied to target identification and prioritization of rodent models and functional read-outs in industry. These preclinical findings have also advanced the neurobiological insights into ASD, which are fundamental for rational, phenotype and circuit-based stratification of patient populations. In turn, the vast experience at the industry partners in drug discovery as well as the insights into the most salient features of the phenotype of the disorder have been instrumental for the design and focus of the preclinical work. These unique strengths arising from the organization of work under the IMI mechanism should be transferable to other challenging psychiatric as well as neurodegenerative disorders.

Our academic-industry collaborations exemplify how a public private partnership has helped to increase the standard of clinical autism research. For example, industry partners emphasised the need to conduct our clinical research studies to GCP standards, to have a study operation monitor continuously monitor assessment progress/ standardisation across all study sites, and to use Standard Operating Procedures (SOPs) across all assessments (LEAP, EUROSIBS). Industry and academic partners converged in their recognition of the need for robustness and replication for results to be useful both in clinical practice and for drug testing. LEAP has therefore been one of the first studies in autism research that uses a common pre-registration procedure whereby for each project, hypotheses, predictions, time-lines, methodological approaches (including identification of replication samples, multiple-comparison correction procedures etc) are specified before project start.

At the beginning of our project we recognised the importance of working with regulators – and our interactions with them (in collaboration with EFPIA) were even better than we expected. We obtained qualification advice from a regulator (the European Medicines Agency (EMA)). The EMA published their letters of support about our LEAP biomarker qualification advice on their website

(December 2015). Also, we jointly published a position paper with the EMA in a high impact peer reviewed journal (Nature Reviews Drug Discovery). Moreover, the EMA quoted our work when they developed new EU policies on drug testing in autism. Hence, we improved the regulatory tools needed to achieve greater effectiveness and efficiency in the drug development process. The early success of LEAP also helped to initiative an fNIH funded research collaboration, the Autism Biomarker Consortium-Clinical Trials. This study, which focused on biomarkers for social impairments of autism, includes several of our EEG and eye-tracking measures for data-sharing and independent replication.

Lessons learnt from LEAP include the need to conduct a feasibility study before the start of the multi-site assessments, to include more participants/ parents in the study design, to conduct focus groups to understand which measures are tolerated/ well understood by participants, conduct interim analyses, and avoid bottlenecks for data analyses. We are keen to transport both the experiences gained in EU-AIMS including our successes and lessons learnt for the development of our new and continued clinical research studies in AIMS-2-TRIALS.

Sustainability is an essential aspect in EU-AIMS. Thus, the main component of the data sharing platform, i.e., the exposition database, was addressed by transferring the responsibility for data hosting to the Institute Pasteur (see D05.04). This is in alignment with the on-going internal effort of the Pasteur Institute to build a global platform for data sharing and open access for clinical/biological data, which will ensure co-localization of genomics with other phenotyping measures. This data sharing platform is a part of the newly launched IMI2 project AIMS-2-TRIALS. Although sustainability is necessary; it is difficult to get funding to maintain and update a curated data base that holds the scientific standards of the pre-registration model. Thus, we would appreciate if the IMI would initiate a separate funding scheme to underpin these efforts.

In 2016, the 'FAIR Guiding Principles for scientific data management and stewardship' were published in Scientific Data. "FAIR" stands for "Findability, Accessibility, Interoperability, and Reuse", which, among other things, means that data are described with rich metadata, a clear data usage licence is used, etc. The European Commission stipulates the use of FAIR principles for data management in the projects supported by EC and requires a well-developed Data Management Plan. Other funding organizations also are starting to require that research projects consider data management and sharing more seriously. In this respect EU-AIMS already set a good example, with a substantial part of a work package devoted to data management. In the future PPPs more, involvement of the industry partners in those problems would help ensure optimal knowledge and data flow in the project, with external connections as appropriate.

In our original application we recognized the scientific value of genomic profiling, and this was to be contributed by an EFPIA partner. Unfortunately, they withdrew just before submission, and so funding for genotyping/sequencing could not be accommodated in the original budget. This was a significant limitation and missed the unique opportunity to create a truly integrated genotype-phenotype platform to identify new treatment targets. Therefore, we applied for additional funding (ENSO –Exploration of new scientific opportunities in on-going IMI JU collaborative projects). This additional funding gave us the opportunity to identify genetic and molecular ASD subgroups, which dramatically improves our ability to find novel treatment targets through back-translation. Thus, we strongly recommend that the IMI reactivates the option to request additional funding.

Technically, the software for detection of structural variants using WGS data have now greatly improved and a confirmation of the CNVs (>50kb) using SNP array data might not be necessary. However, the SNP arrays were found to be very helpful for a reliable QC before performing WGS.

The deep genotyping and phenotyping of the EU-AIMS cohorts represents a gold standard and a practical framework for such fine-grained genotype-phenotype studies. We are now using this knowledge to perform such analyses for other European or National projects.

EU-AIMS supports the incorporation of SMEs in IMI projects. The participation of SMEs is of major importance as they are a key component in driving the competitiveness of the European health industry. The SMEs have a potential to become future mid-size enterprises and potentially new big European pharmaceutical companies in the future. For instance, deCODE (SME in EU-AIMS) merged with AMGEN which joined EU-AIMS in year3. Thus, the IMI should make additional efforts to facilitate the participation of SMEs.

EU-AIMS encouraged the participation of patient organisations (e.g. Autism Europe, Autism Speaks). The involvement with patient groups is essential to better understand their problems and needs. So they act as a guides or controllers. For instance, Autism Europe trained us in using language that is acceptable to the community in all our communications. For instance, we no longer talk about 'patients', they are people with autism. As "Lessons learned" we recommend closer integration with patient groups and charities in the preparation of proposals - as we did for AIMS-2-TRIALS.

One challenge for maximizing the output of consortia can be the shifting priorities at the participant sites. In particular, restructuring processes in industry can lead to substantial changes in the engagement and focus of partners. This can impact on the completion of work streams and limit dissemination of information.

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