Why do we need biomarkers for Autism Spectrum Disorders?

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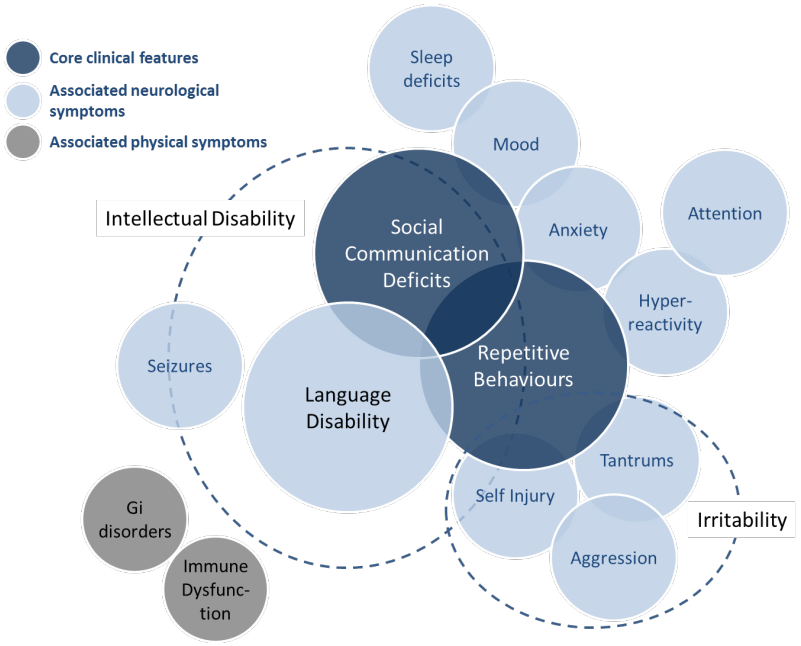
EU-AIMS– the work leading to these results has received funding from the Innovative Medicines Initiative Joint Undertaking (IMI)

ASD: Current situation

- 1 in 88 children and adults is affected
- Diagnosis and treatment solely rely on clinical observation, not cause or pathophysiology
- No medical treatments that significantly improve core symptoms;
- Which treatment works for which child/ person?



Challenges for treatment development

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- **Understanding of pathophysiological mechanisms is poor, due to:**
- **Phenotypic heterogeneity**
  - Symptom quality and severity varies between individuals
  - 2/3 of individuals with ASD have 1+ comorbidities (Simonoff et al., 2008)
- **Genetic heterogeneity**
  - Several hundred ASD risk genes identified;
  - Together account for 10-20% of cases, individually for <2% (Betancur, 2011)
- **Etiological heterogeneity**
  - Different “autisms” or one/ few final common pathway(s)?
- **Lack of biomarkers**
  - For patient selection in clinical trials
  - To estimate treatment response

Overview

1. **New approaches to identifying etiology-based treatment targets**
2. **Biomarker approaches for**
    1. (early) diagnosis
    2. Patient stratification
    3. Prognosis
3. **Qualification advice from an international regulator: the European Medicines Agency**

Five integrated programmes

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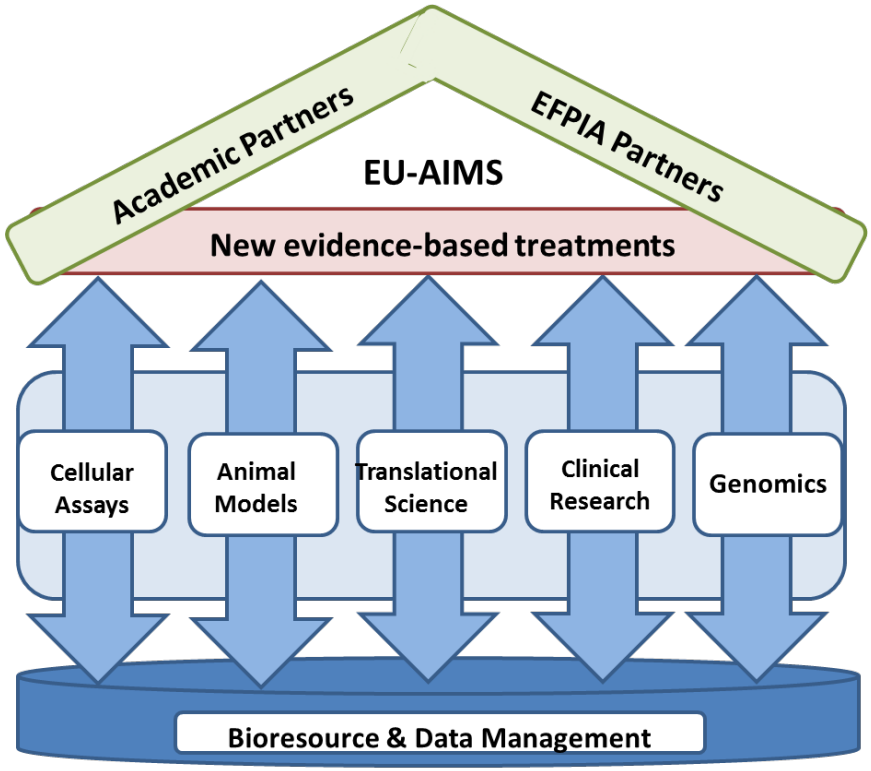

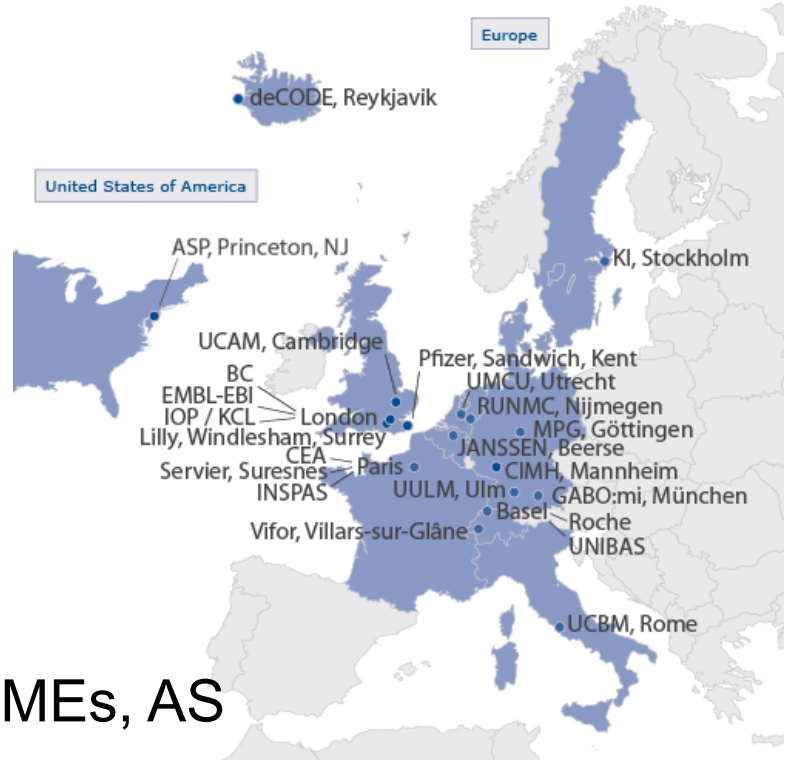

14 academic partners, 5 EFPIA, 3 SMEs, AS

In vitro and *in vivo* models

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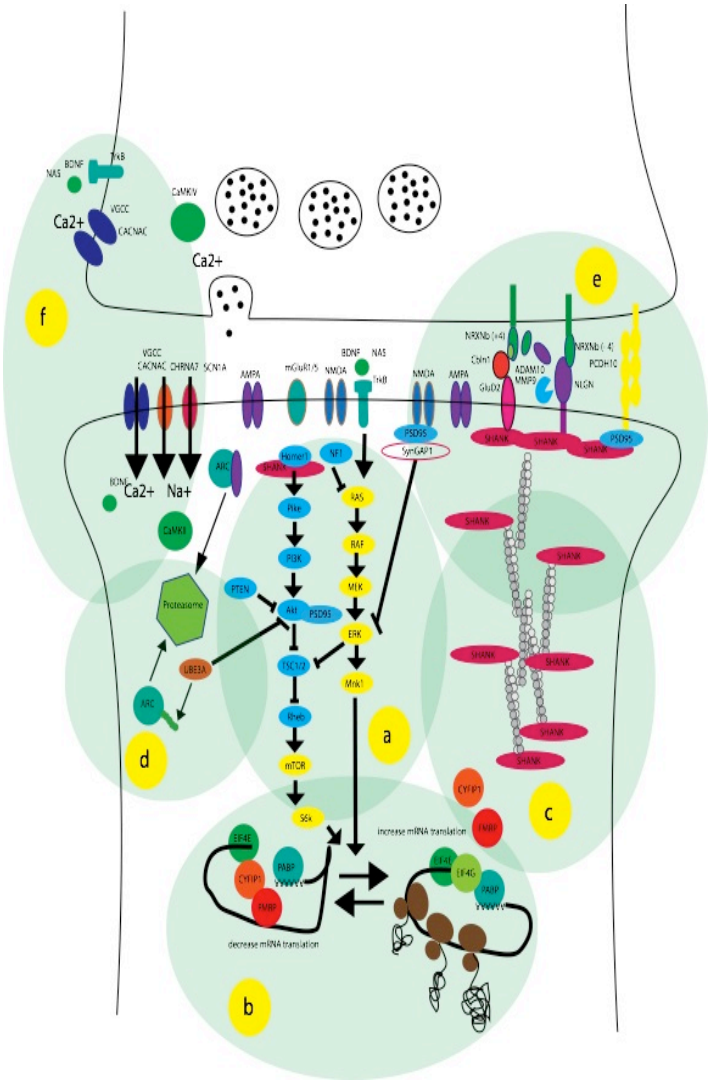
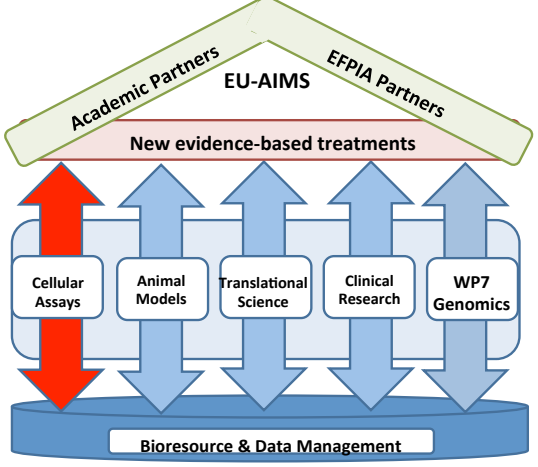
- Monogenic forms of ASD provide new window into a mechanistic understanding of ASD-symptoms
  - Genes involved in regulating synapse structure and function (SHANK3, CNTNAP2, NRXN1, NLGN3/4X)
  - Genes involved in transcriptional/ translational control (TSC1/2, MECP2, NF1, PTEN)
- Different risk genes converge on a limited number of molecular pathways (Voineagu et al., 2011)affecting synaptic homeostasis (Bourgeron, 2009)
- **Hypothesis:** Defects of Synaptogenesis affect excitatory-inhibitory balance.





Cellular Assays

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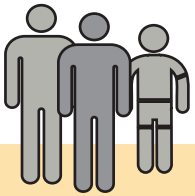
Lead

- Jack Price (KCL)
- Daniel Ursu (Lilly)



Synaptic gene deficits

Patient -derived induced pluripotent stem cells

Cellular and molecular phenotypes

Goal: Generate ASD patient-specific iPSCs

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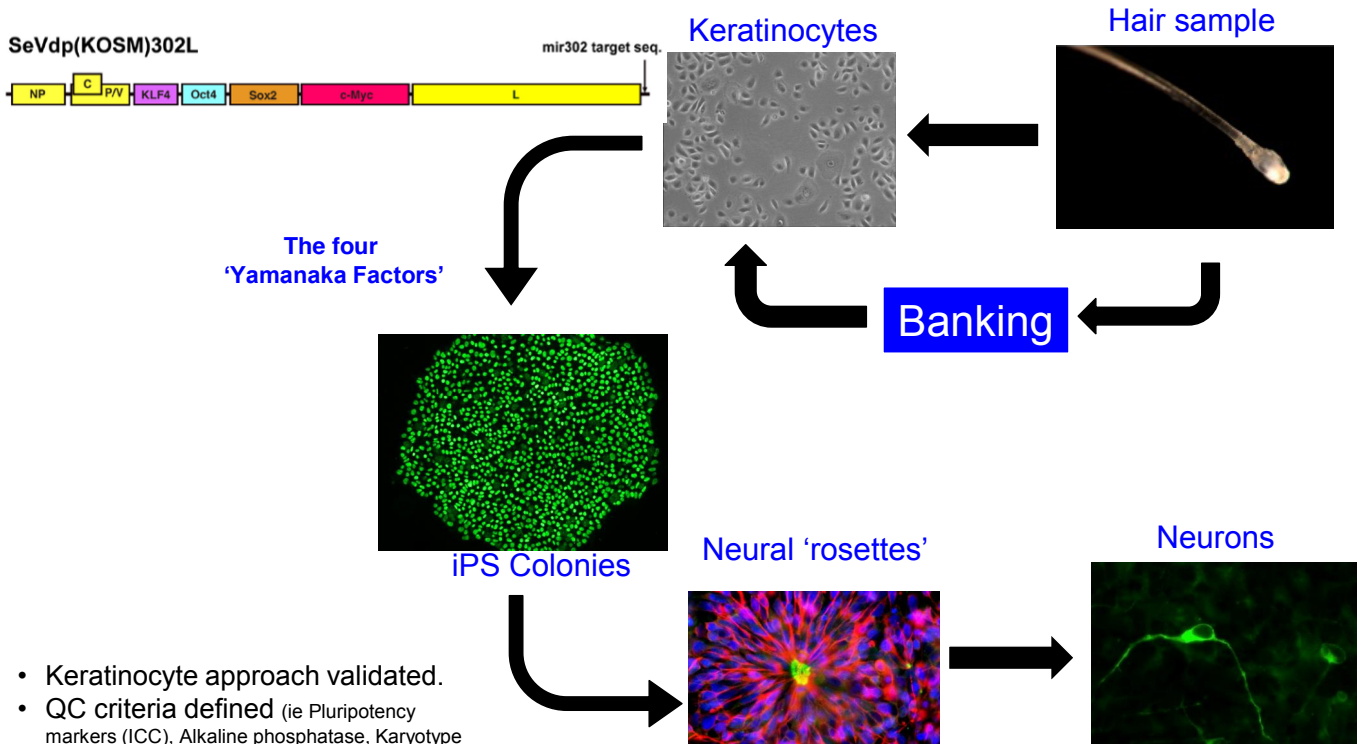

- Developed protocol to freeze hair biopsies (Price)
- Developed and validated robust differentiation protocol

Physiological properties of iPSC-derived neurons

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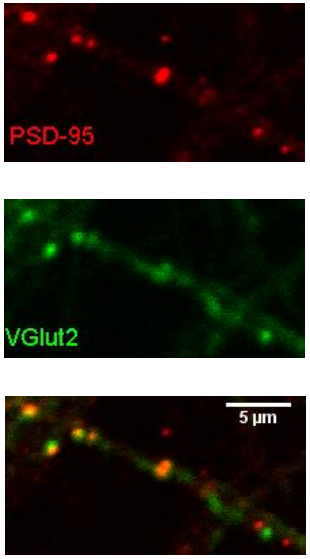
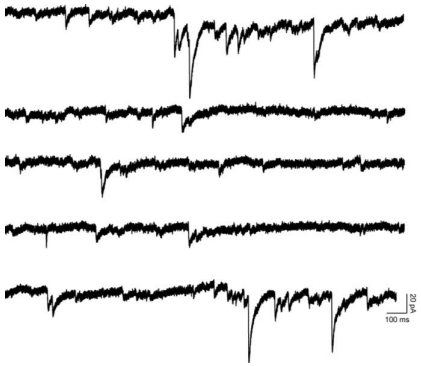
From Spines to Synaptic Proteins and Excitatory Synaptic Currents





Percentage of Patched Cells with Excitatory Synaptic Currents

Week *in vitro*	Control cells
7	57%
8	89%

- Demonstrate basic properties of excitatory and inhibitory synaptic currents in control lines (Andrae, KCL)
- Evaluate mechanisms of synaptic plasticity, in particular long-term depression and long-term potentiation (Bischofberger, Uni Basel)

Identify cellular phenotypes linked to ASD and specific CNVs

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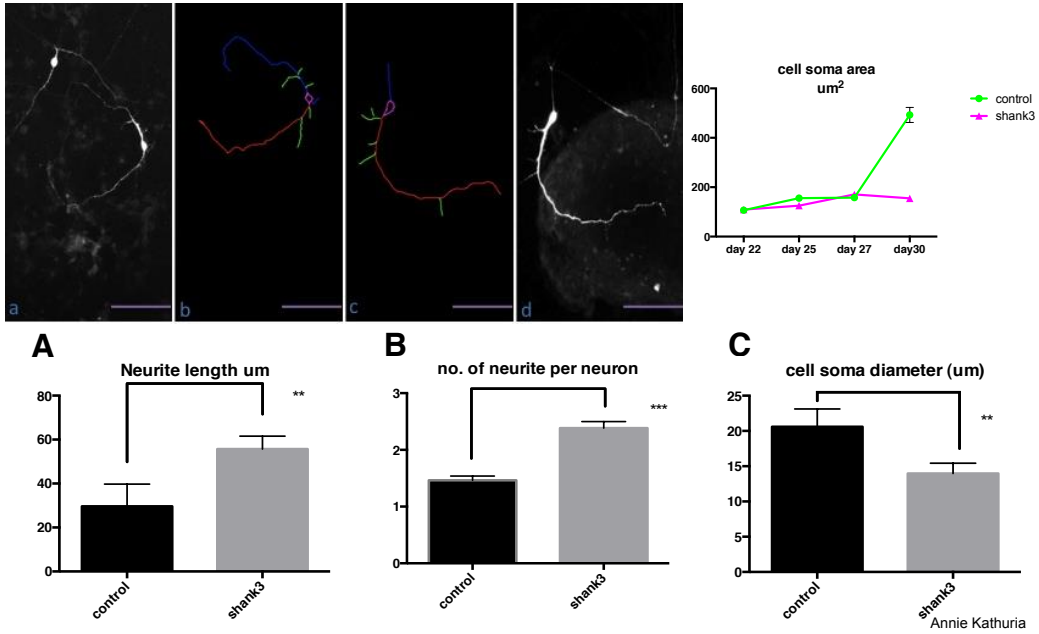
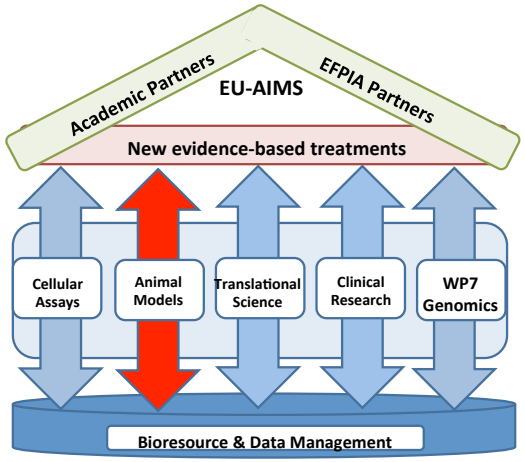
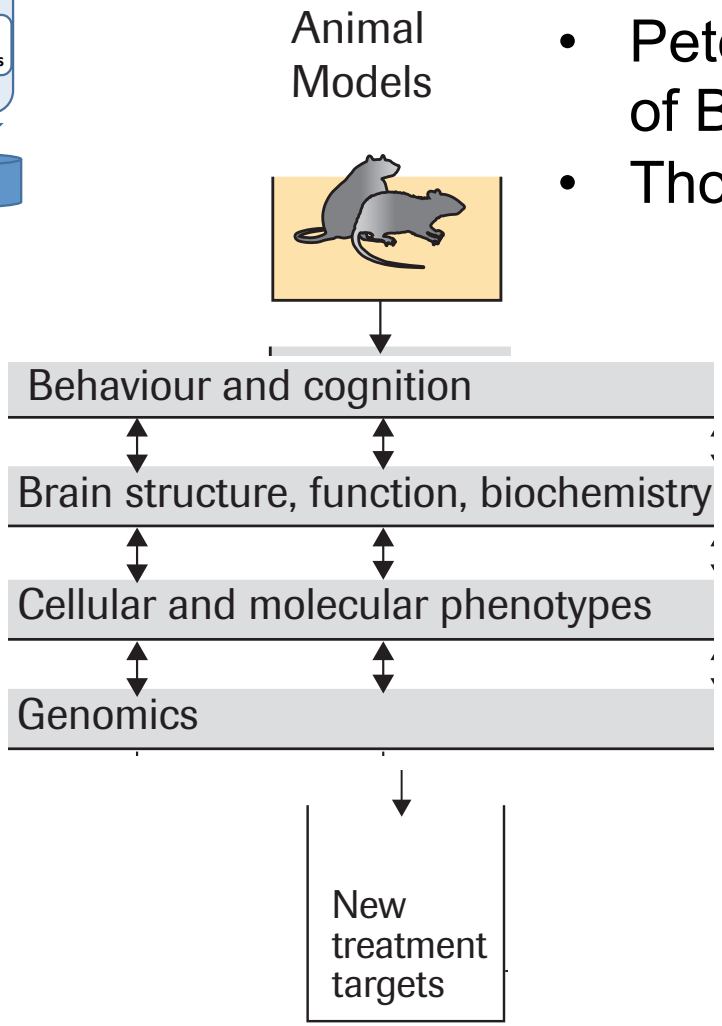


- **Preliminary findings:** Neurons from SHANK3 patients show abnormalities in terms of neuronal size and morphology (Jack Price, KCL)
- Characterisation of ion channels and glutamatergic/ GABA-ergic receptors: significant increase in AMPA response, indicating increased excitatory pathways (Ursu, Lilly)

Animal Models





Lead

- Peter Scheiffele (University of Basel)
- Thomas Steckler (J&J)

Nlgn3 mice as models for non-syndromic ASD

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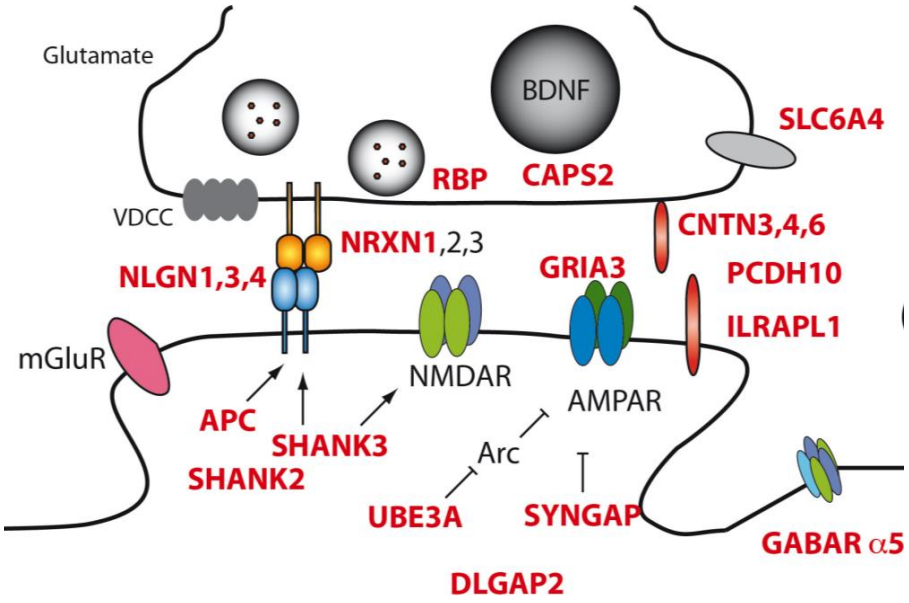

- **Neuroligins:** post-synaptic cell adhesion molecules (CAMs) at GABAergic and glutamatergic synapses
- Reversible Nlgn3 KO

Scheiffele group: Baudouin et al., 2012, *Science*

Nlgn3KO mice: cellular, morphological, electrophysiological phenotypes

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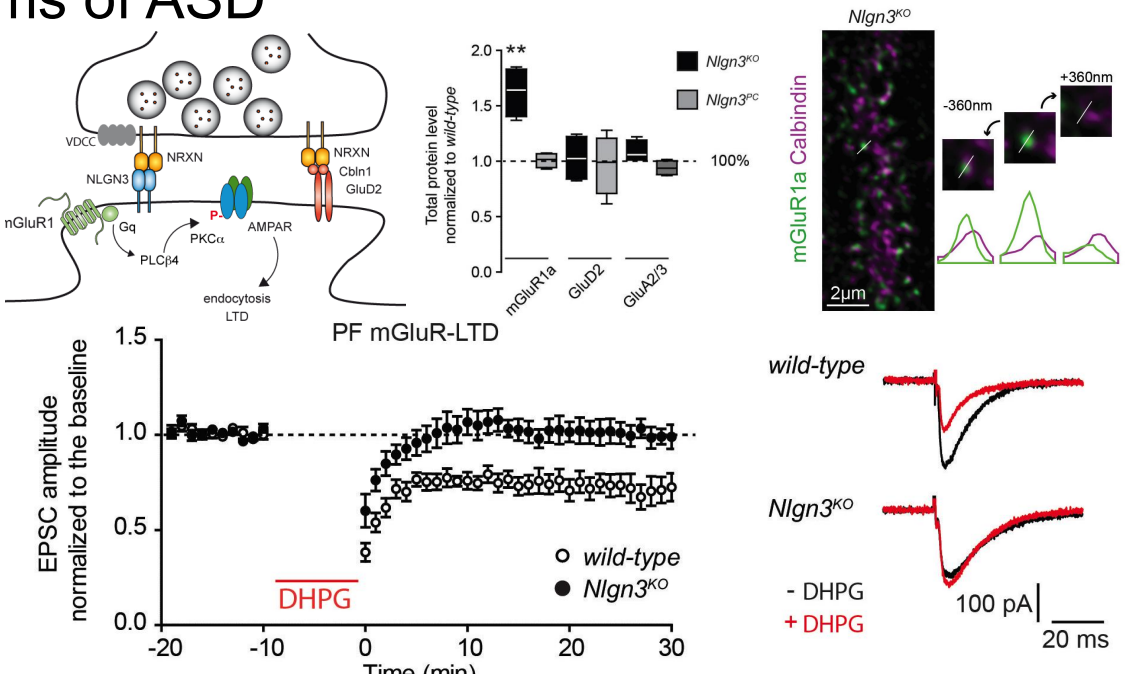
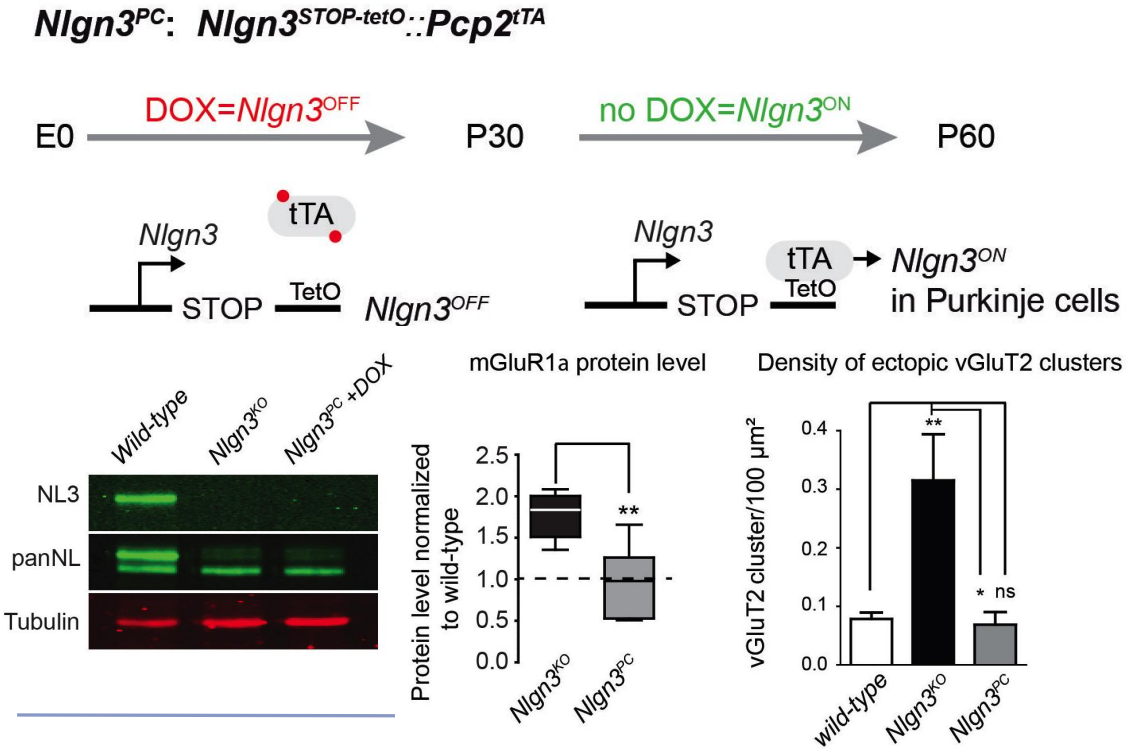
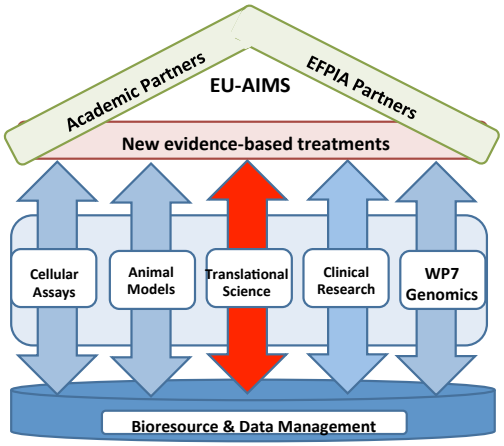
- Loss of NL3 from parallel fibre synapses results in increased mGluR1 expression,
- mGluR LTD at parallel fibre synapses is occluded
- Neurons in cerebellum form abnormal synaptic connections
- Reminiscent of pathophysiology in Fragile X (convergence of potential drug targets between syndromic/ non-syndromic forms of ASD



Genetic rescue in adulthood

- Gene was switched on after mice reached adulthood
  - mGluR1 expression and ectopic synapse formation could be reversed
  - Some behavioural deficits normalised
  - Potential of symptom amelioration after completion of development





Translational Imaging

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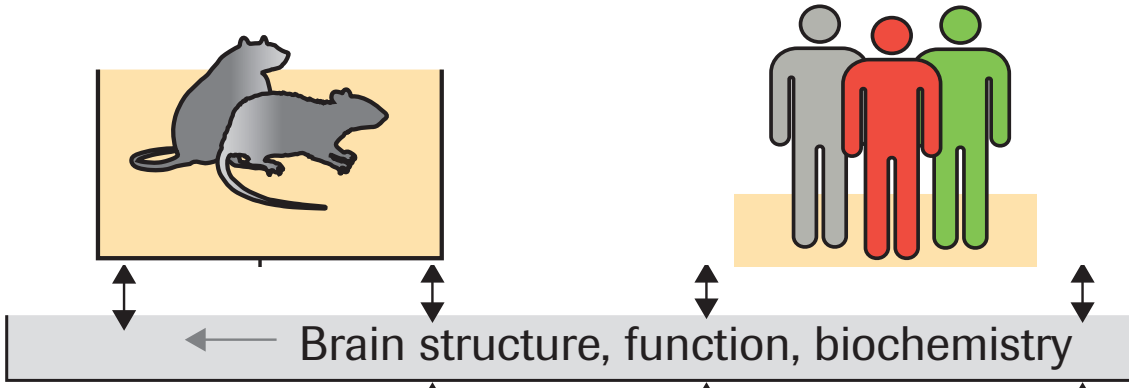
Lead

- Andreas Meyer-Lindenberg (CIMH, Germany)
- Gahan Pandina (J&J)



Translational method development

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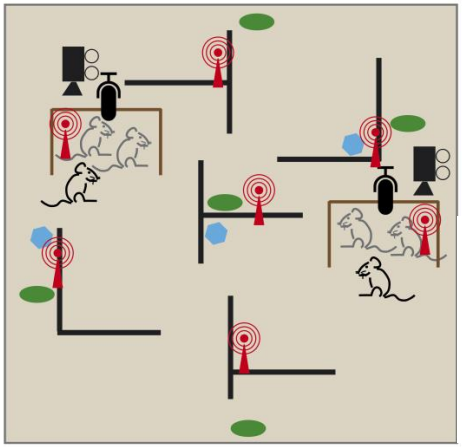
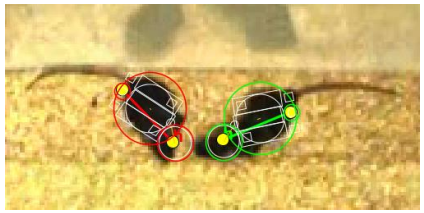
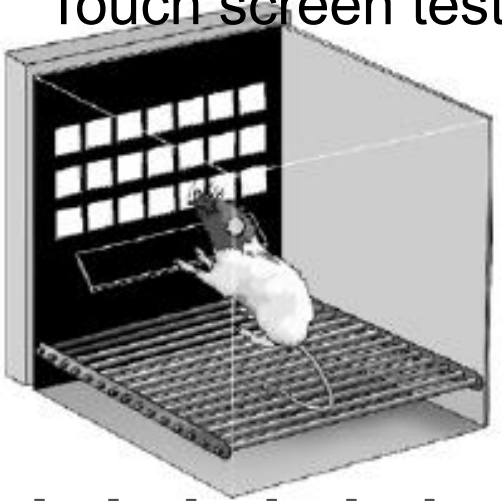
“Mouse city” (Pasteur)



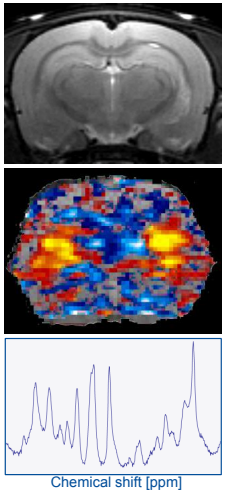


Touch screen tests (J&J)



Integrated behaviour-EEG analyses (UMCU)

Translational imaging



Anatomy — **Structural MRI**

Neural activity
Brain circuitry
Connectivity — **Functional MRI**

Neurotransmitters
Metabolites — **MR spectroscopy**

Proof-of-concept for E/I imbalance in animal models and patients

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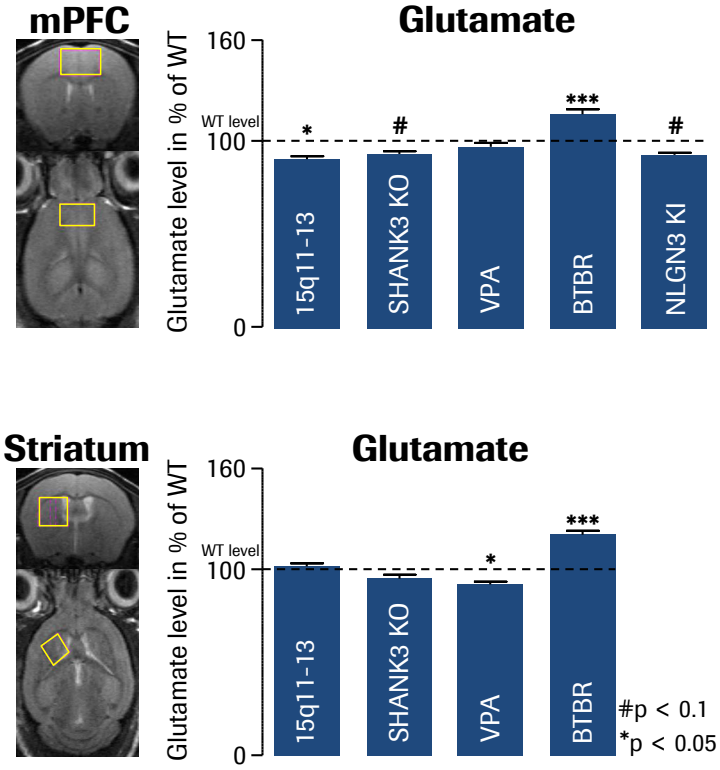
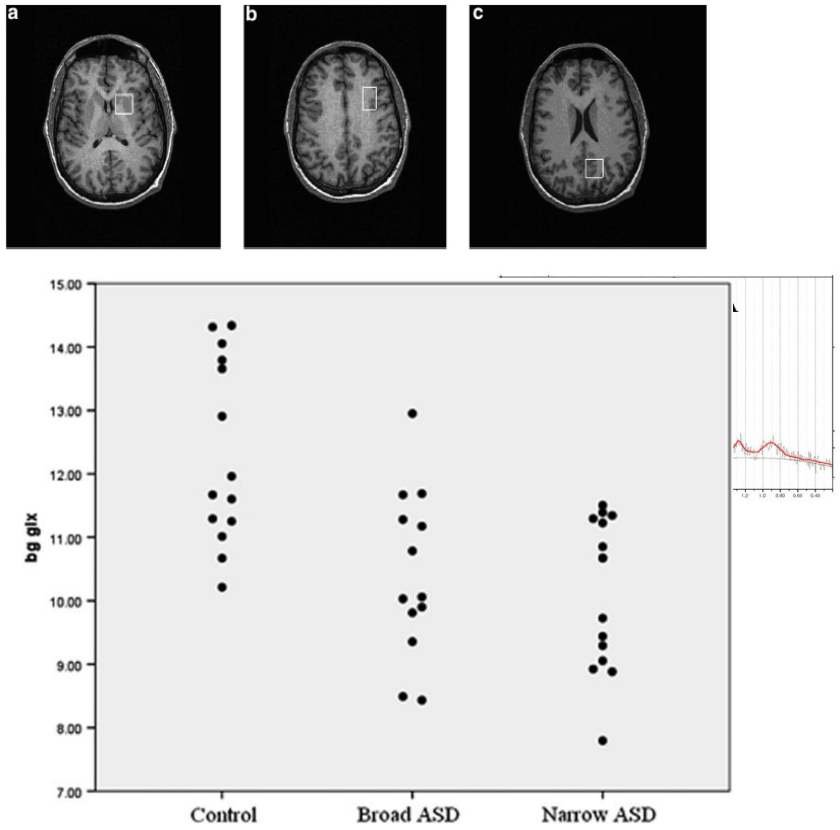


L Basal Ganglia



- ASD=28 ,Controls =14
- ASD: Reduced Glx in Basal Ganglia

Petrinovic, unpublished data

Horder et al., 2013, *Translational Psychiatry*

Challenges for clinical trials

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- Clinical and etiological heterogeneity:

If/ when new treatment targets are found…..Difficult to test treatment efficacy as some/ most treatments may only be effective in certain biological subgroups

Current situation

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- No validated universal or specific biomarkers for ASD
- Most (single-site) studies include **small** and **heterogeneous** samples -> often failure to replicate
- Limited **power** to stratify groups and identify more biologically homogeneous subgroups
- Use of different (often not standardized) experimental measures
- Need of outcome measures sensitive to change
- Many academics have limited experience with translational applications: Experiments are not planned to be used in clinical trials or to gain regulatory approval for new treatments

Why we need biomarkers

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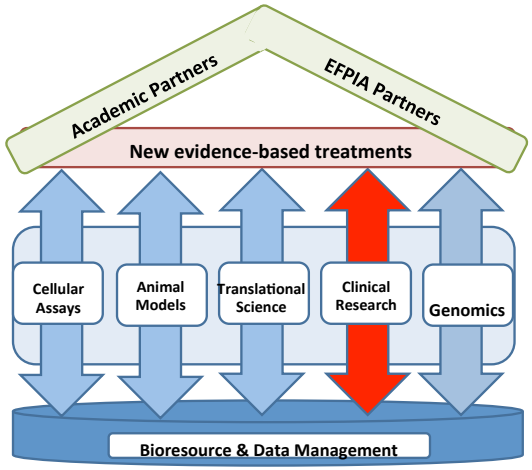
- **Objective measure of a quantifiable process**
- **Risk/ diagnostic biomarker:** predict which child will develop ASD, detect ASD as early as possible
- **Stratification biomarker:** group patients into biologically (more homogeneous) subtypes
- **Prognostic biomarker:** predict the progression of symptoms and 'outcome' in adulthood
- **Predictive biomarker:** Estimates/ predicts an individual's response to a treatment
- **Biomarker as surrogate outcome measure:** predicts clinical outcome and can therefore be used as a substitute for a clinical end-point



Clinical Research

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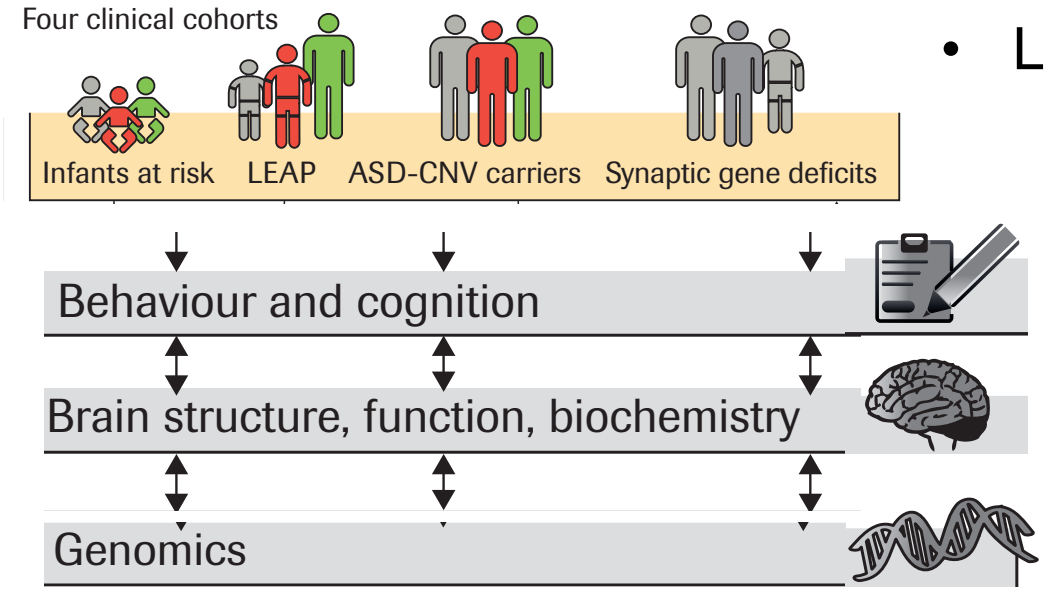
Lead

- Jan Buitelaar (Radboud University Medical Centre Nijmegen)
- Eva Loth (KCL)
- Lauren Boak (Roche)



Four unique patient cohorts

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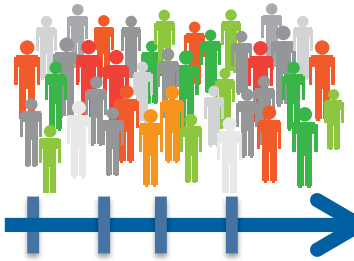
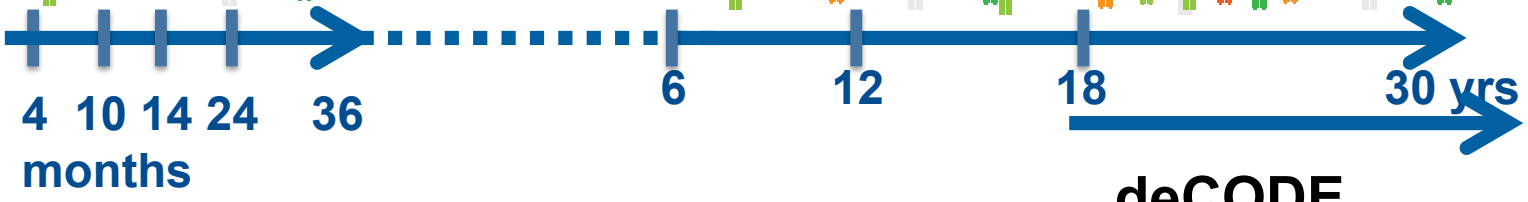
High-risk siblings
HR=300, LR=100





LEAP
ASD =500, Control =350

deCODE
CNV carriers
N=300,
Control=300

Synaptic Gene Deficit =~80,
Control =50

Longitudinal European Autism Project

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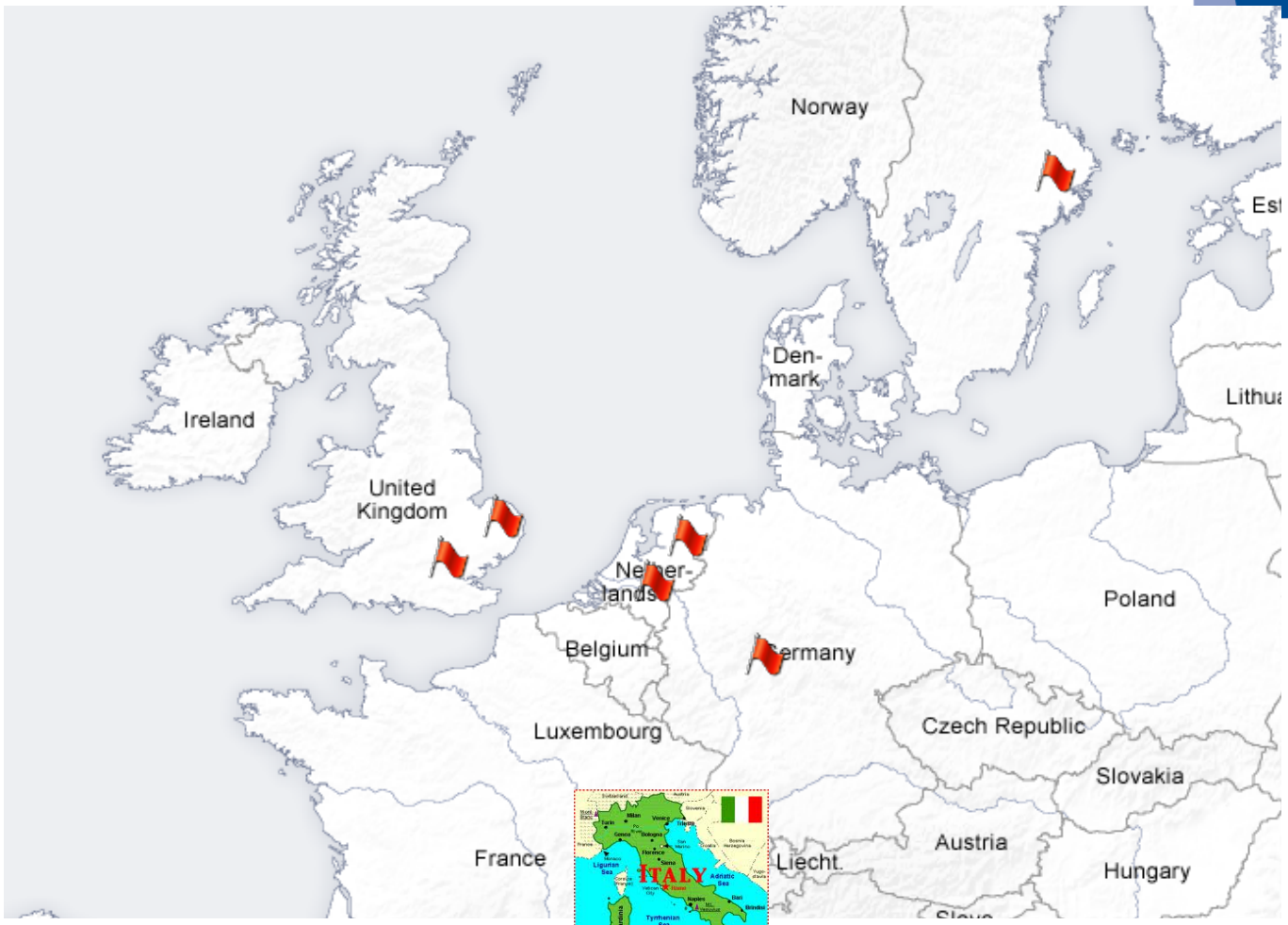

7 centres:
KCL
UCAM
RUNMC
UMCU
CIMH
UCBM
+KI twins

LEAP enrolls a large cohort of participants (400 ASD; 250 TD) diverse in age (6-30 years) and ability levels

Multi-modal profile of each volunteer

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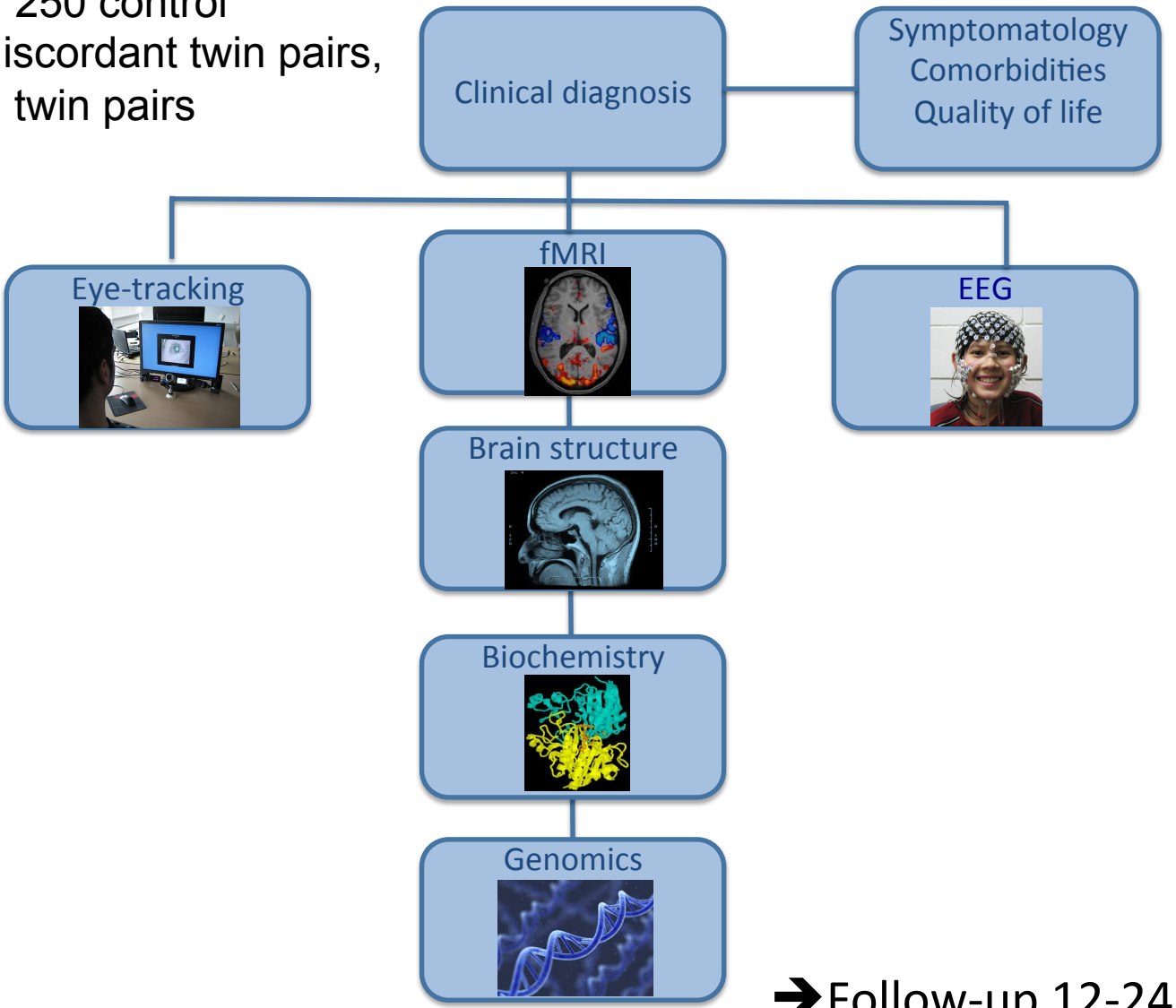
search in Europe

400 ASD, 250 control
50 ASD discordant twin pairs,
KI; 20 TD twin pairs



➔Follow-up 12-24 months later

Protocol Split in 4 schedules

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	A Adults 18-30 yrs	B Adolescents 12-17 yrs	C Children 6-11 yrs	D mild ID 12-30 yrs
Parent Interviews Parent Questionnaires Self-report Qs	✔ ✔ ✔	✔ ✔ ✔	✔ ✔ ✗	✔ ✔ ✗
MRI •Structural scan •Resting state •DTI •Task-related fMRI*	 ✔ ✔ ✔ ✔	 ✔ ✔ ✔ ✔	 ✔ ✔ ✔ ✔✗	 ✔ ✔ ✔ ✔✗
Eye-tracking	✔	✔	✔✗	✔✗
Cognition	✔	✔	✔ ✗	✔ ✗
EEG (KCL, RUNMC, CIMH, UMCU)	✔	✔	✔	✔
Blood, saliva, urine, hair sample	✔	✔	✔	✔

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Example of protocol in action

Parent and self-report on-line Questionnaire Platform

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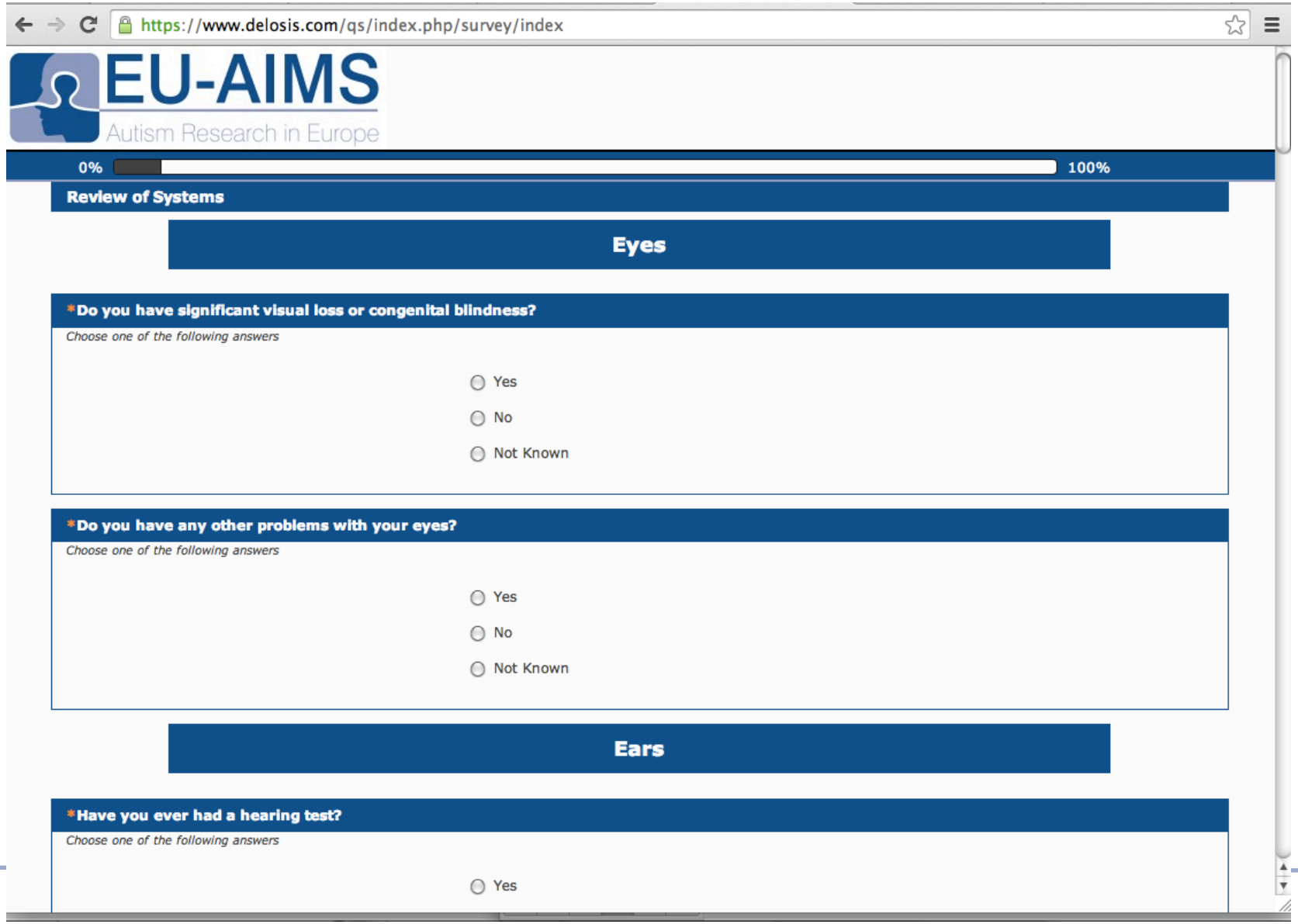

Example: First visit

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fMRI task Pre-training

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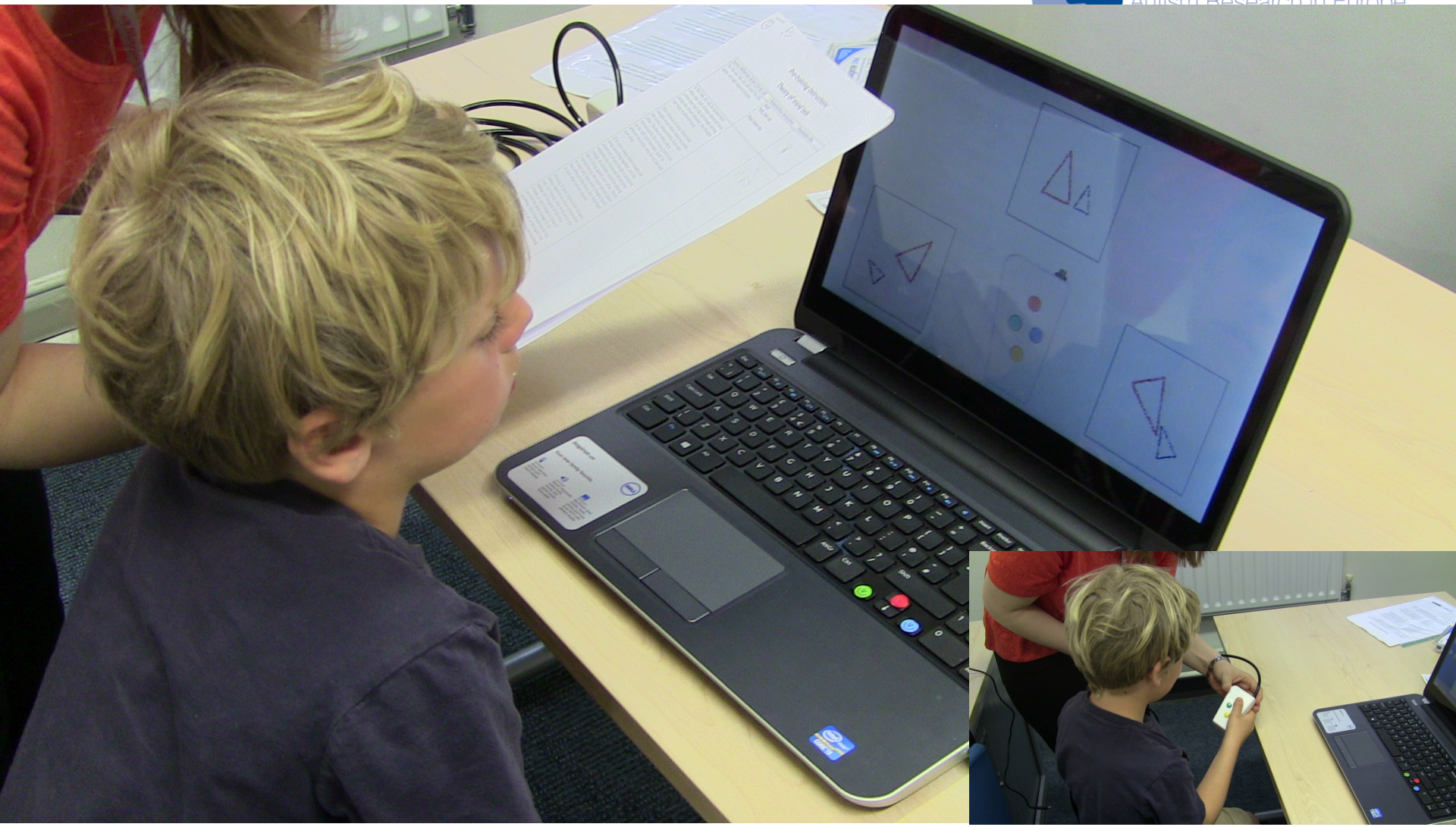

MRI scan

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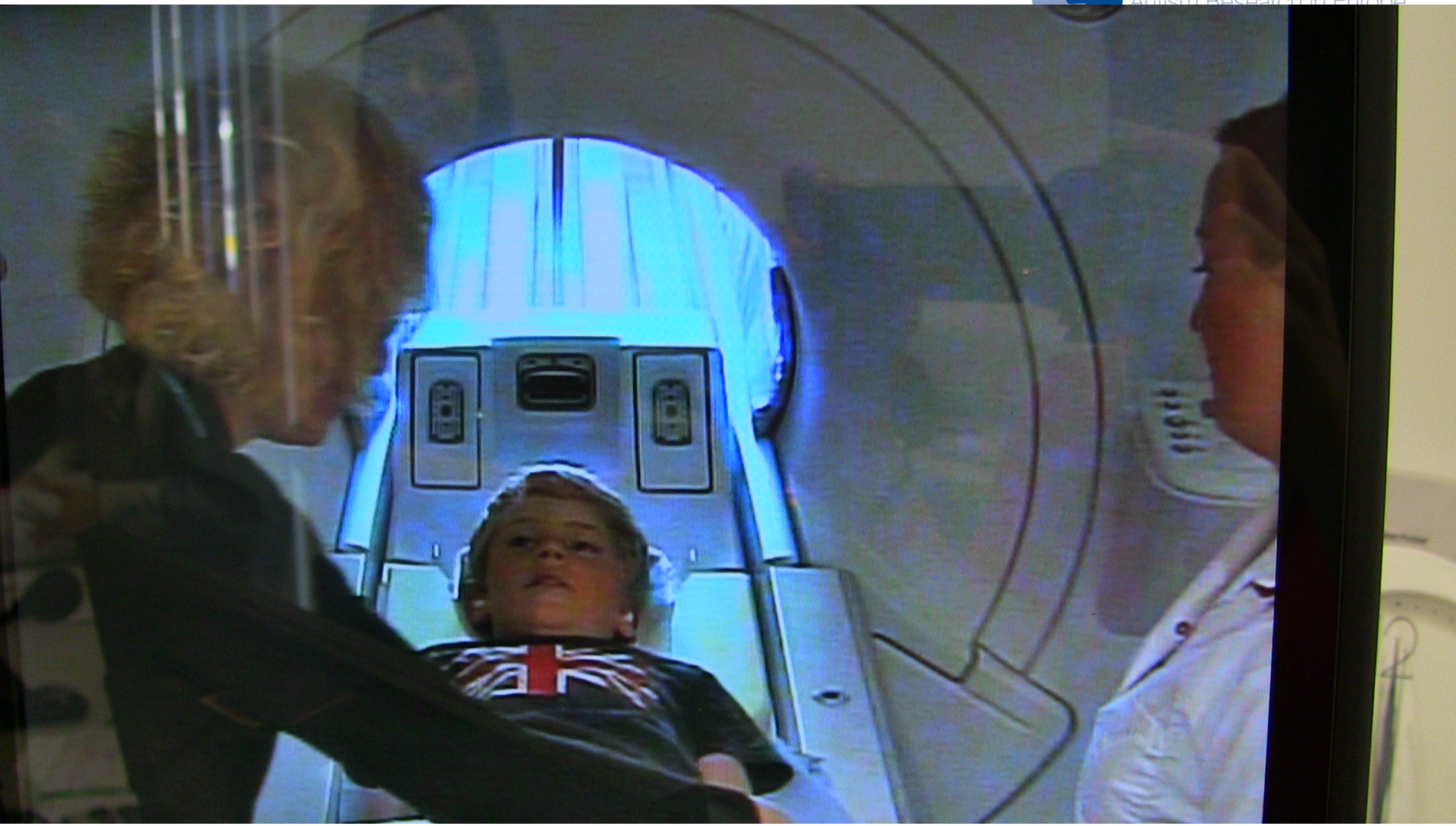

Breaks

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ADOS

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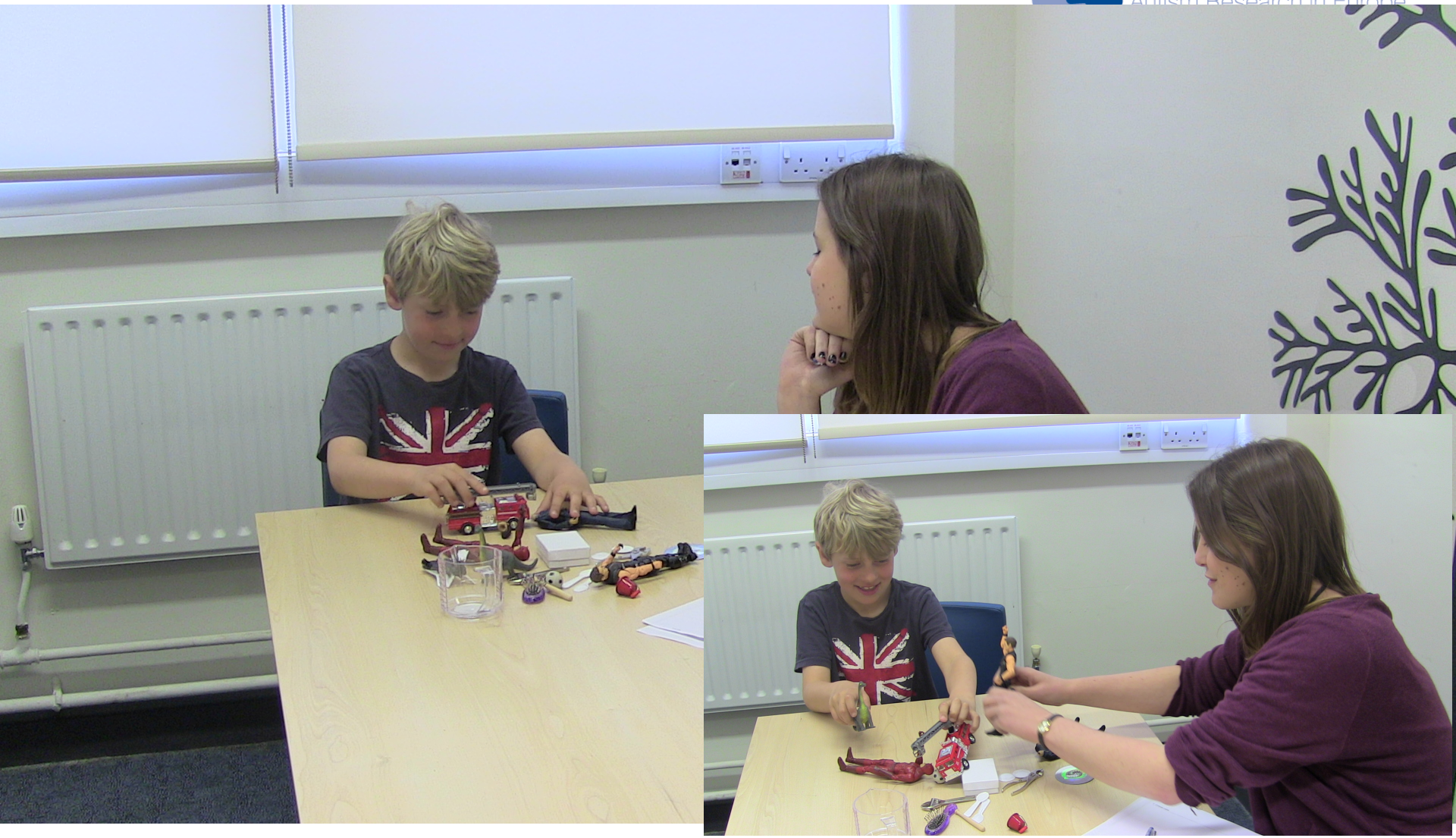

Parents: Vineland, ADI, DAWBA

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Family goes home or stays in hotel overnight

Second day: IQ testing

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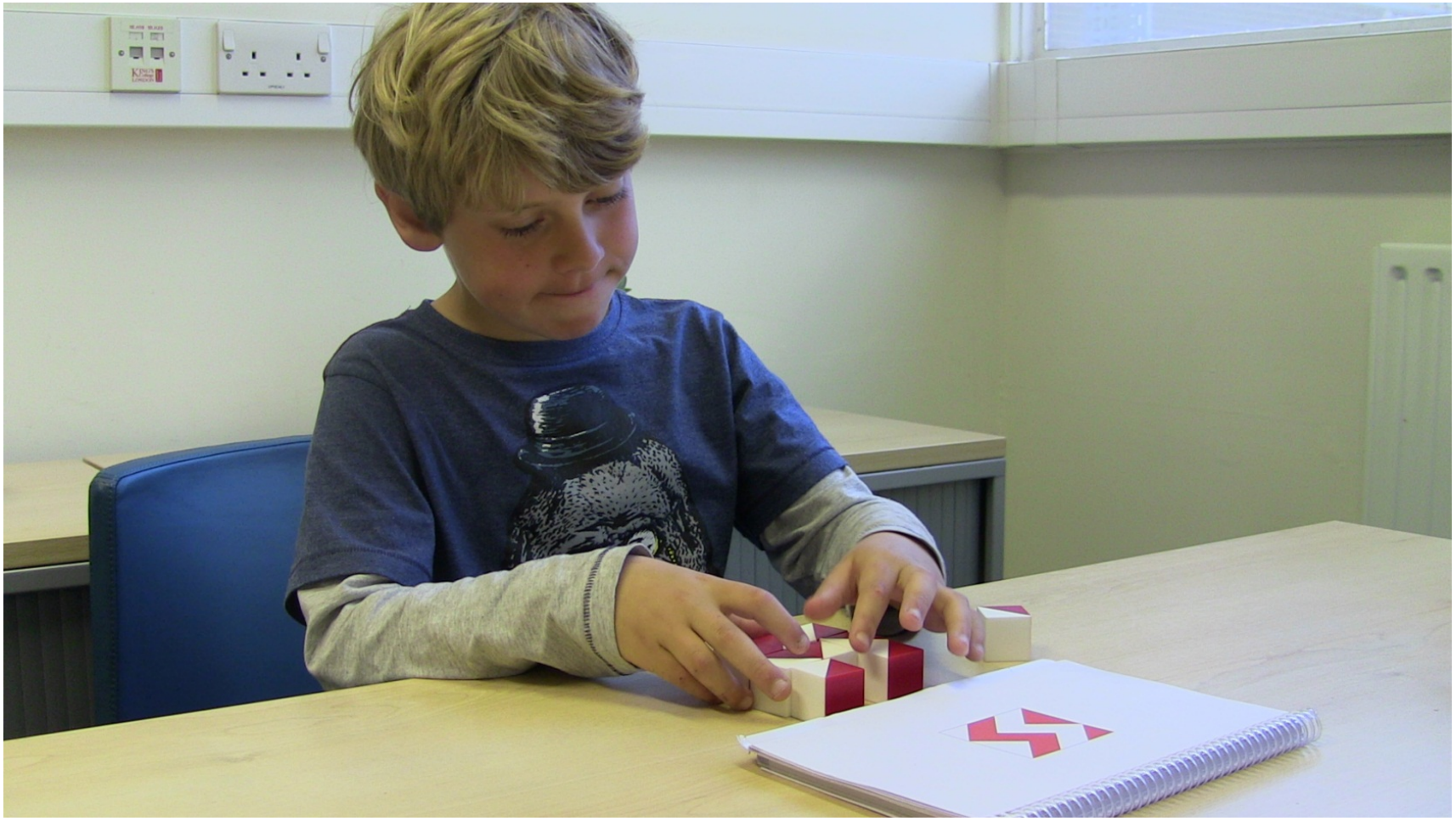
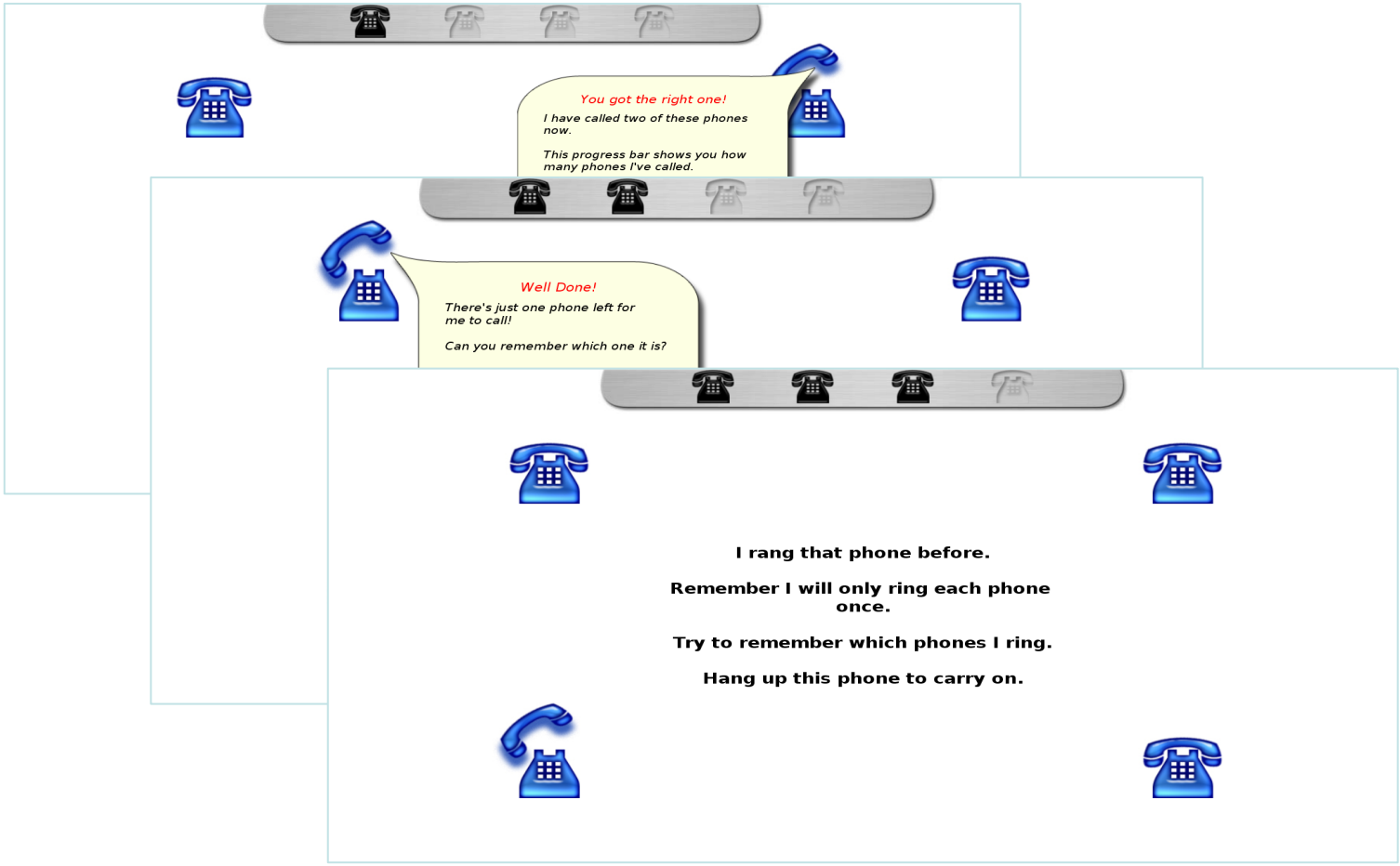


Cognitive testing



Example: Spatial working memory – Psytools Platform

EEG + Eye-tracking

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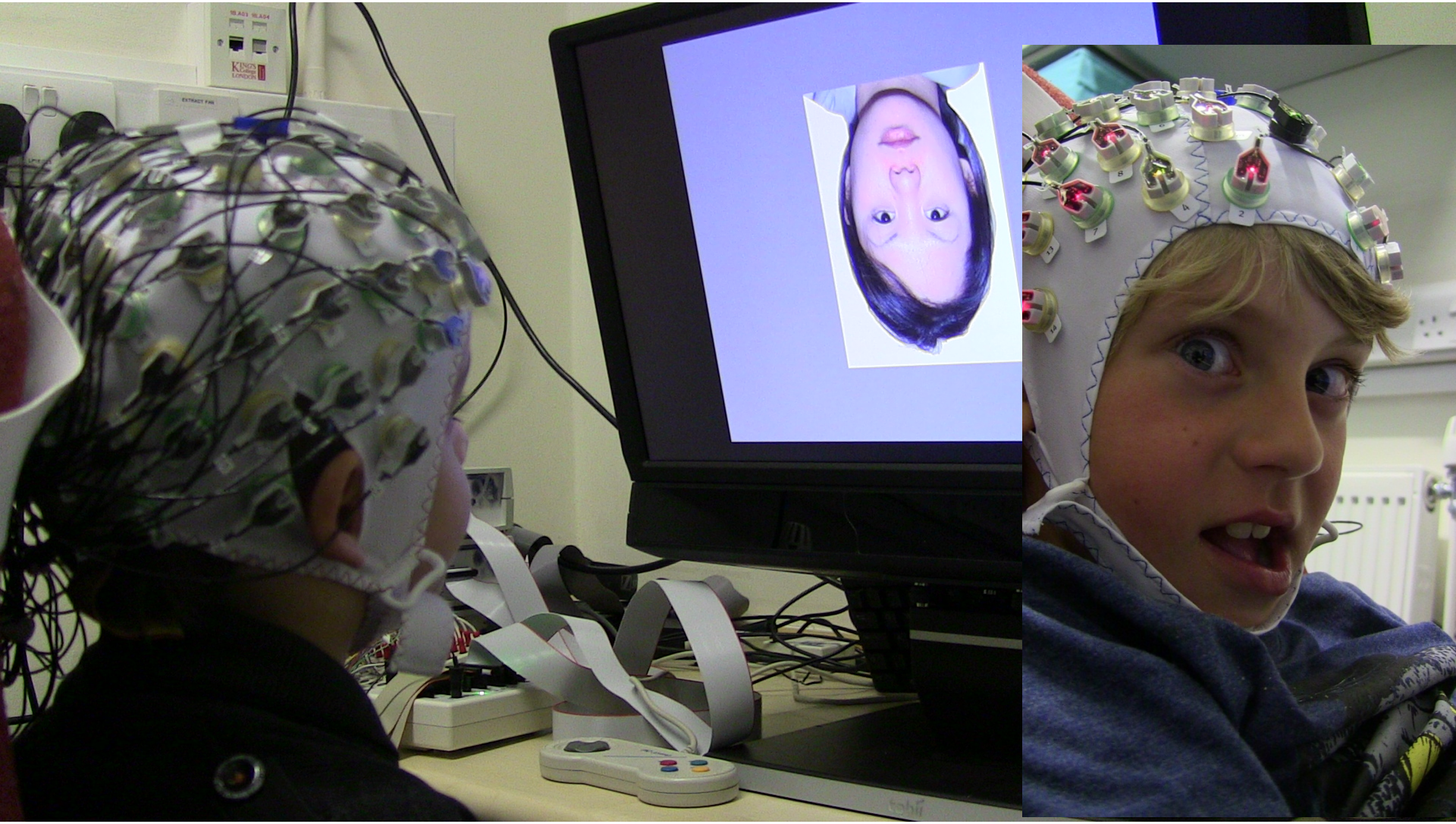

Biological samples

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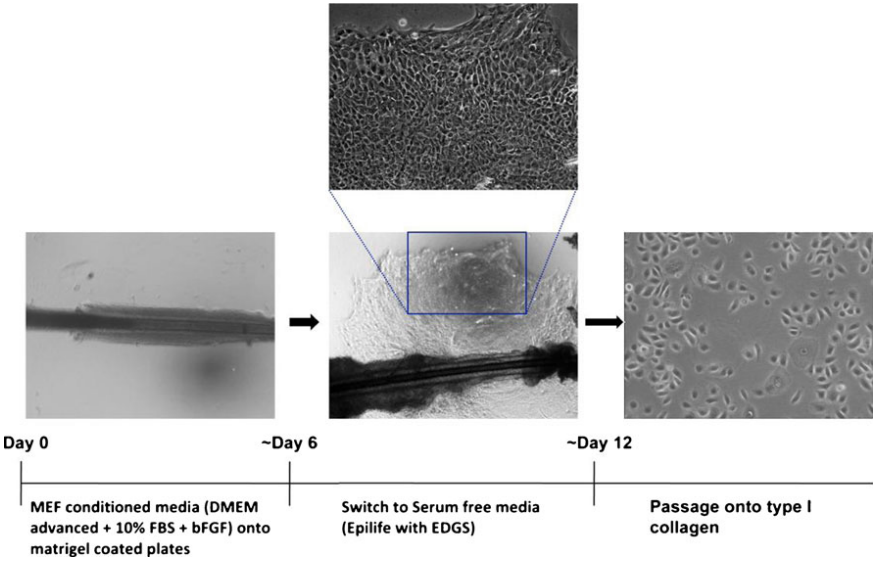
Blood or saliva sample

Hair biopsies (frozen for iPSC generation)



Urine sample (at home)

Challenges for a multi-site study

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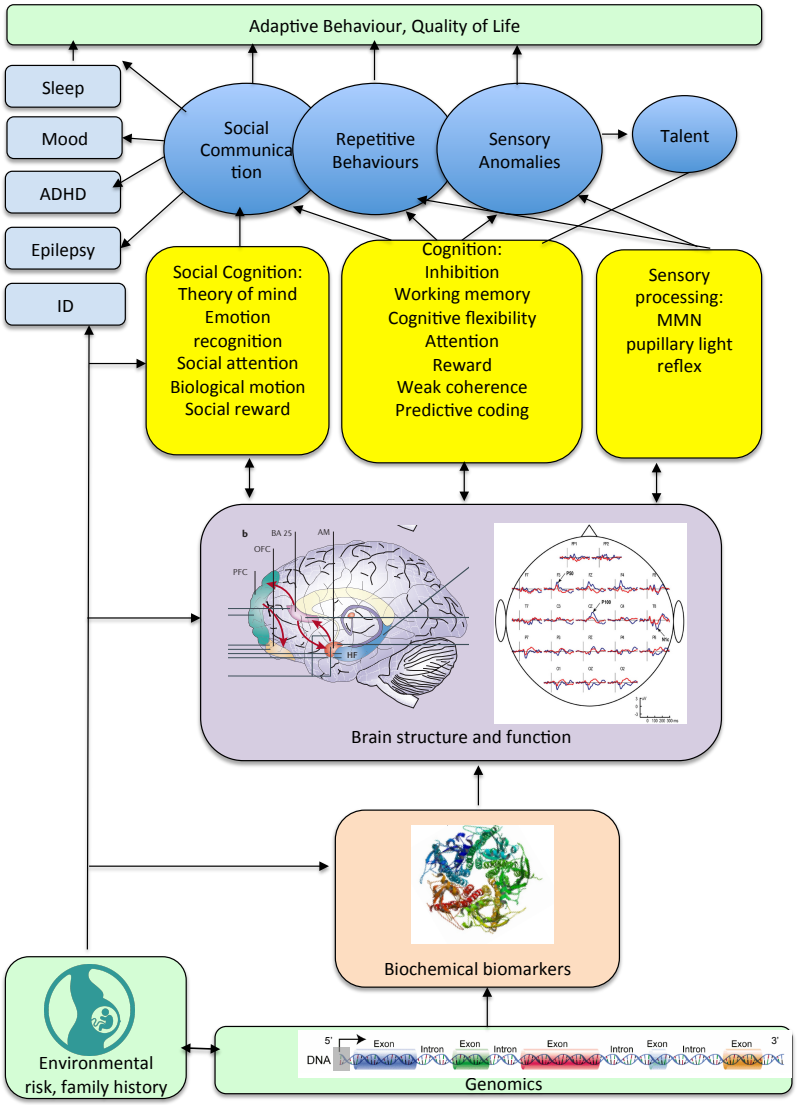
- **Standardization:**
  - SOPs for every assessment module
  - Training of RAs in all assessment modules
  - Translation
  - Reliability meetings
- **Quality control procedures, MRI: standardized pre-processing**
- **New generation of young researchers trained in multi-modal assessments (and analyses)**
- **Study carried out at ICH GCP level**

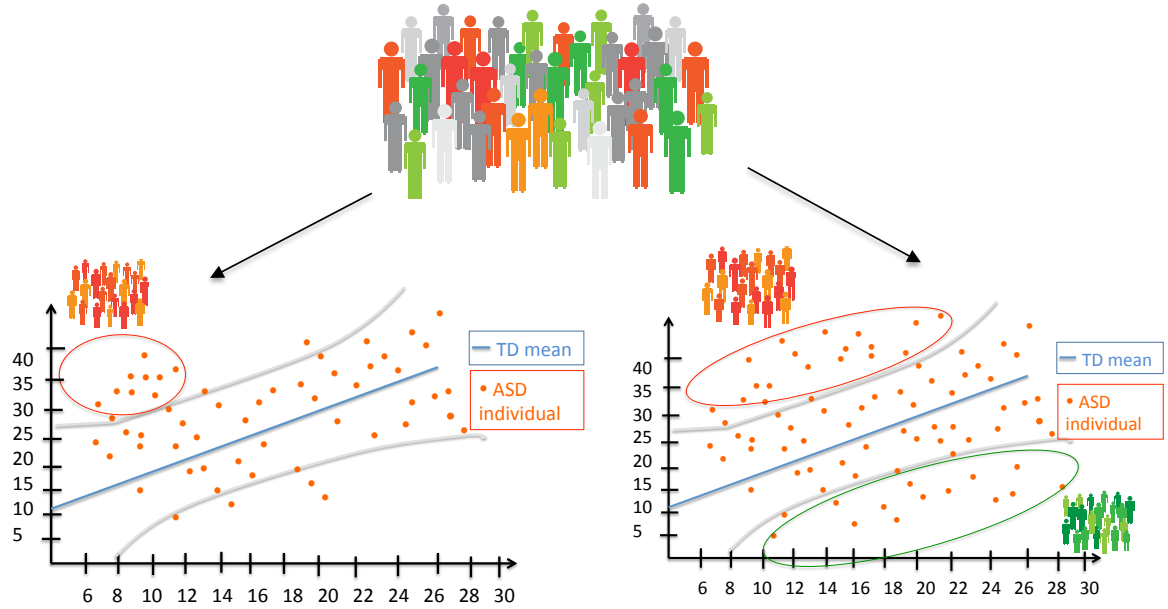
Biomarker approaches



Stratify by sex

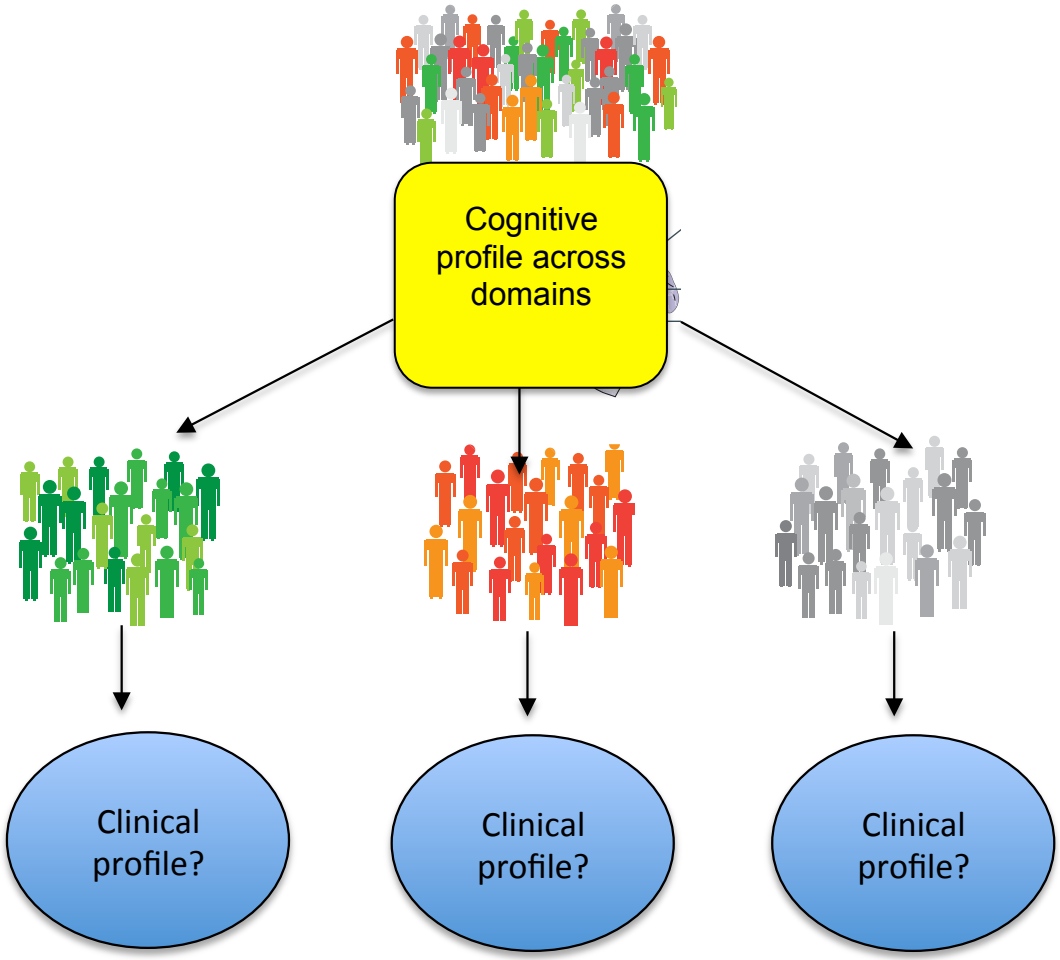
- **Sex differences in normal development**
  - Many aspects of (social) cognition
  - Brain development and function
  - Gene expression
- **Sex differences in ASD**
  - Cognitive profile (Lai et al., 2011, *PLOS1*
  - Brain function (Lai et al., 2013, *Biol Psychiatry)*
- **Over-recruitment of females:**
  - male: female ratio: 3:1

Developmental trajectories



- Derive trajectory for typical development from cross-sectional data
- Situate each person with ASD on the TD trajectory
- Construct a trajectory for the whole disorder group and compare to TD group
- Some biomarkers may only be detectable at certain developmental stages

Cognitive profile



Create a profile of cognitive strengths and weaknesses across domains to predict clinical outcome e.g., EF (deficits) as aggravating vs. compensatory factors

Mapping Brain to behaviour: mediating factors

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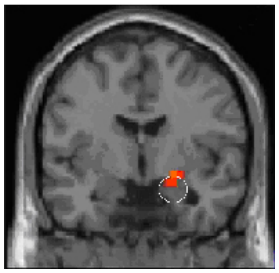
Emotion recognition

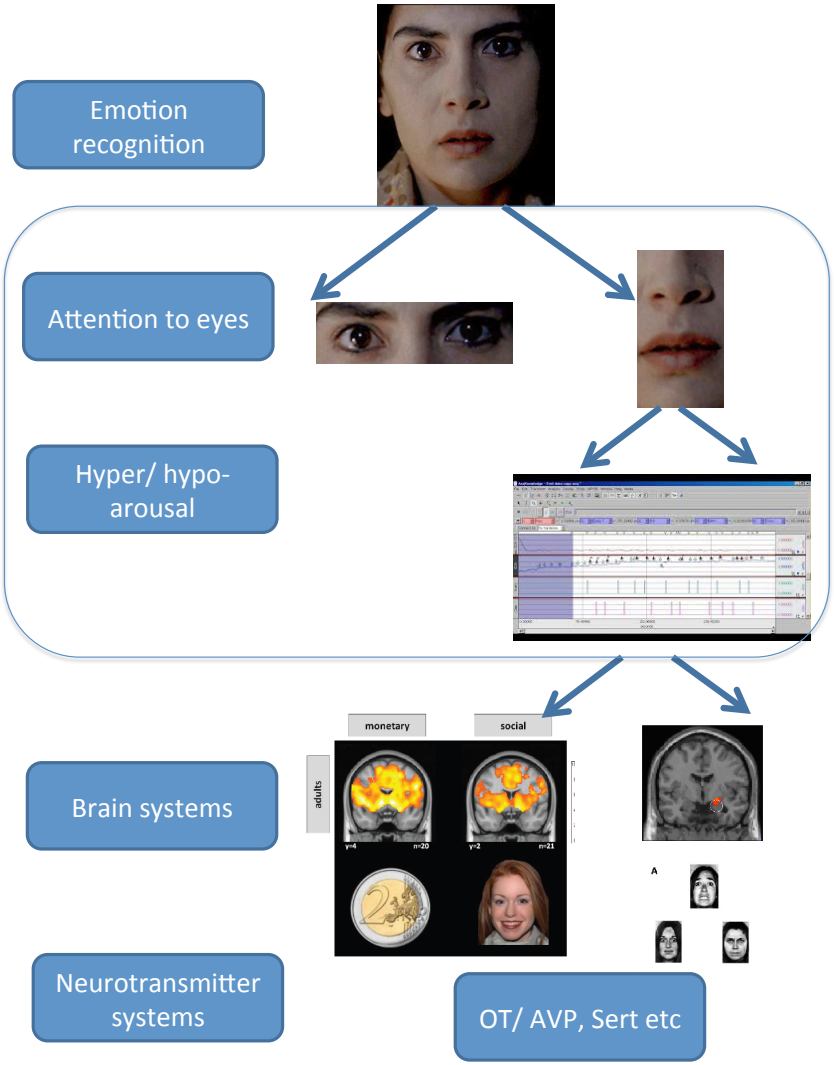
Multi-modal assessment

Brain systems



Neurotransmitter systems

OT/ AVP, Sert etc



Shared behavioural deficit may result from different causes that may warrant different treatments.

Stratify by ‘intermediate phenotypes’

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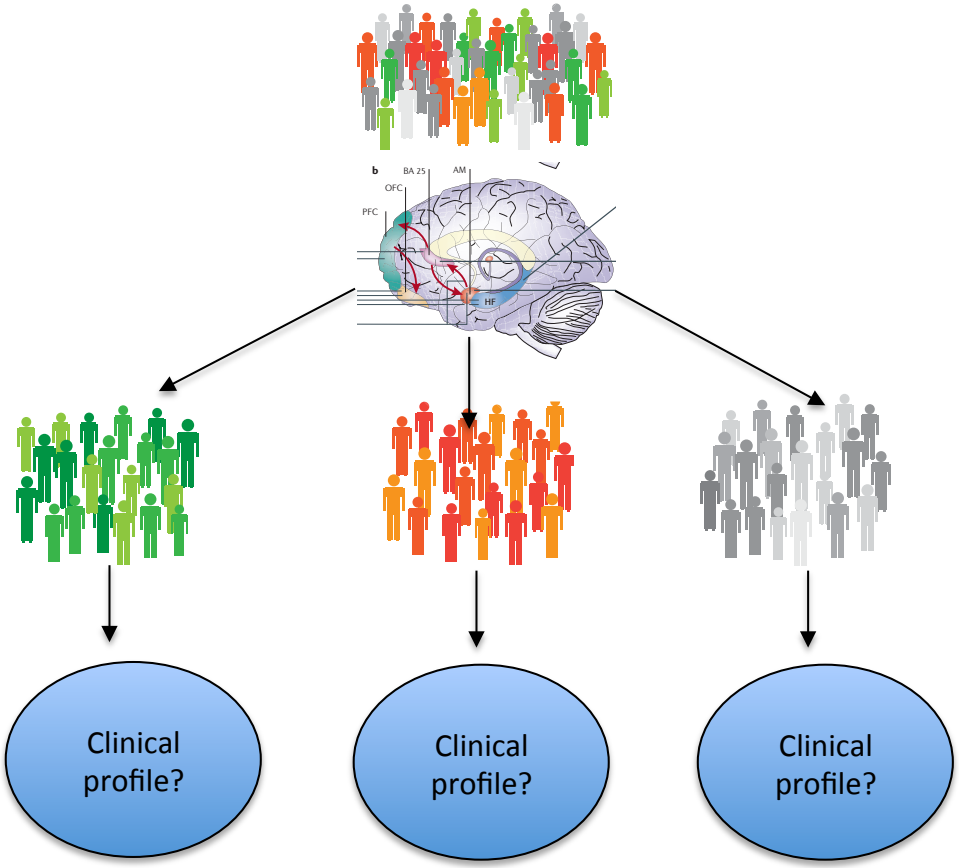

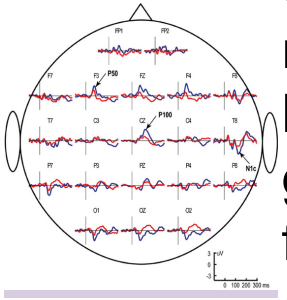

e.g., indirect markers of E/I balance (EEG gamma, functional connectivity)

Eye-tracking/ EEG/ fMRI also as potential surrogate outcome measure indicative of the mechanism underlying treatment effect

Novel ‘Translatable’ Imaging Measures

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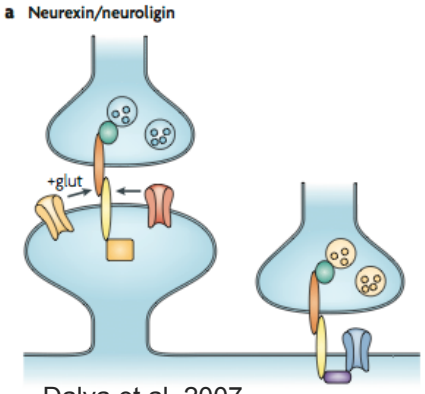
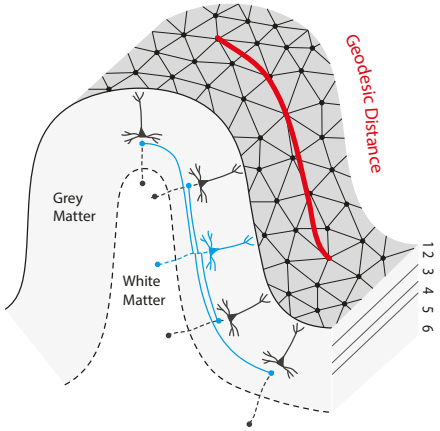
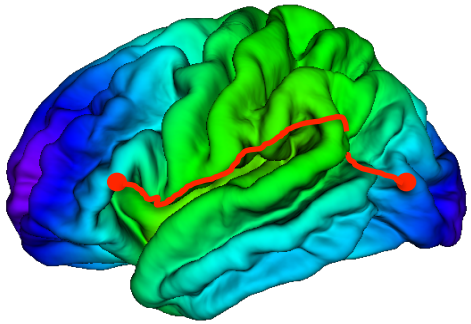


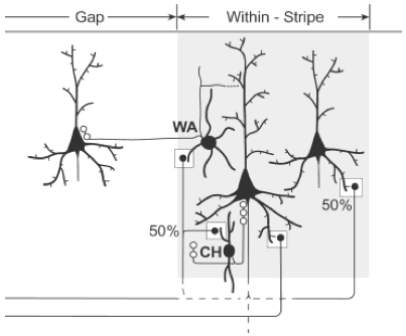
Dalva et al. 2007



Intrinsic ‘Wiring Costs’

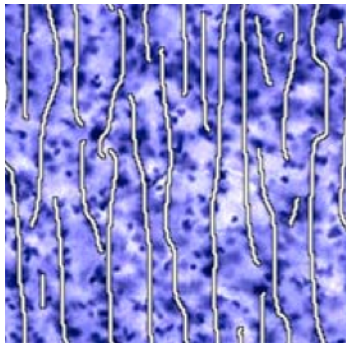


I/E (Im)balance in ASD



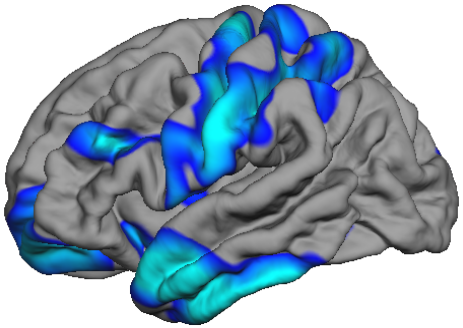
Melchitzky et al. 2001

Minicolumn-Pathology in ASD



Casanova et al. 2006

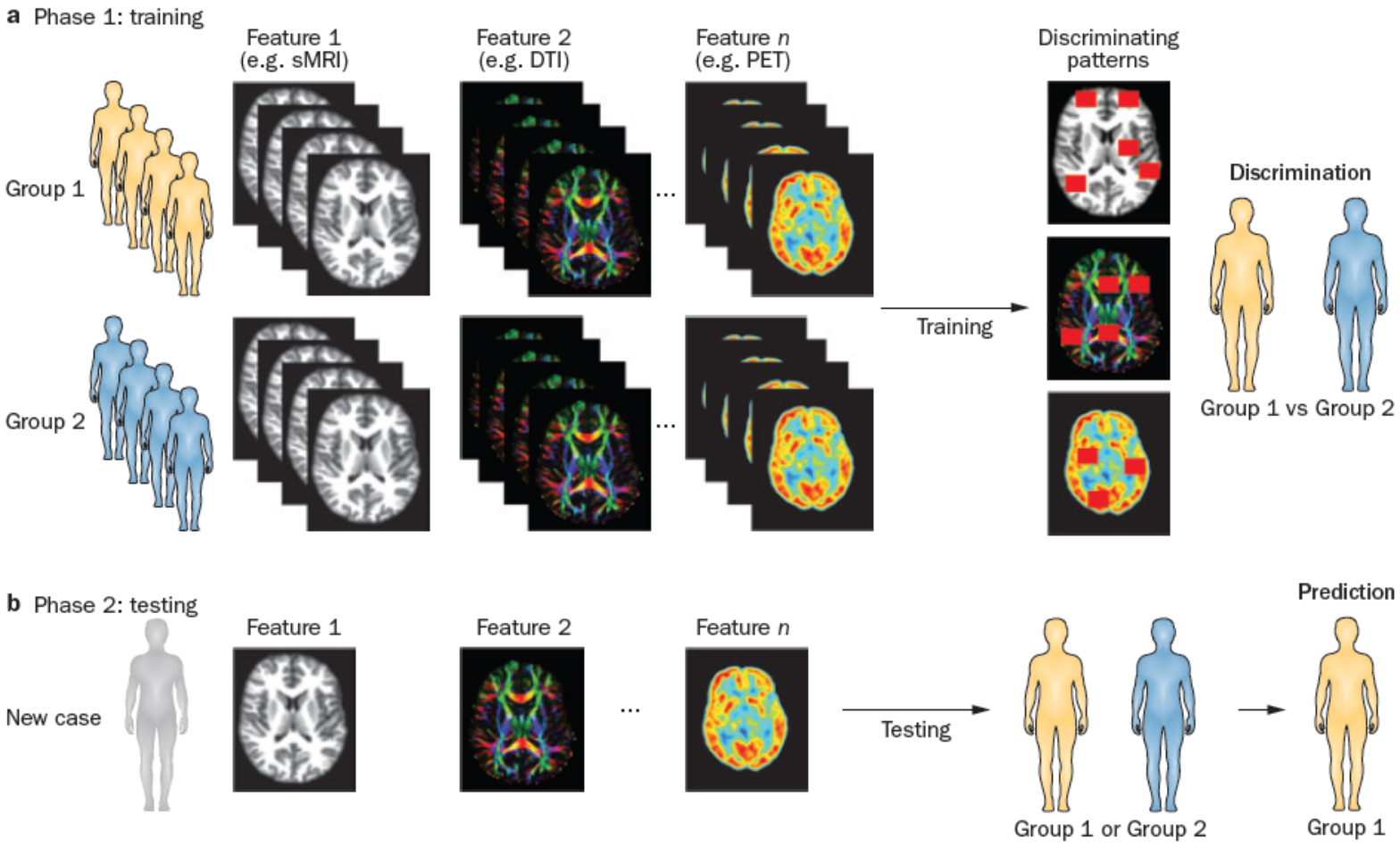
ASD < Controls



Ecker et al. (2013) PNAS

Slide Christine Ecker

Multivariate pattern classification



Ecker & Murphy, 2014, *Nat Rev Neurol*

Genetically-driven molecular subgroup

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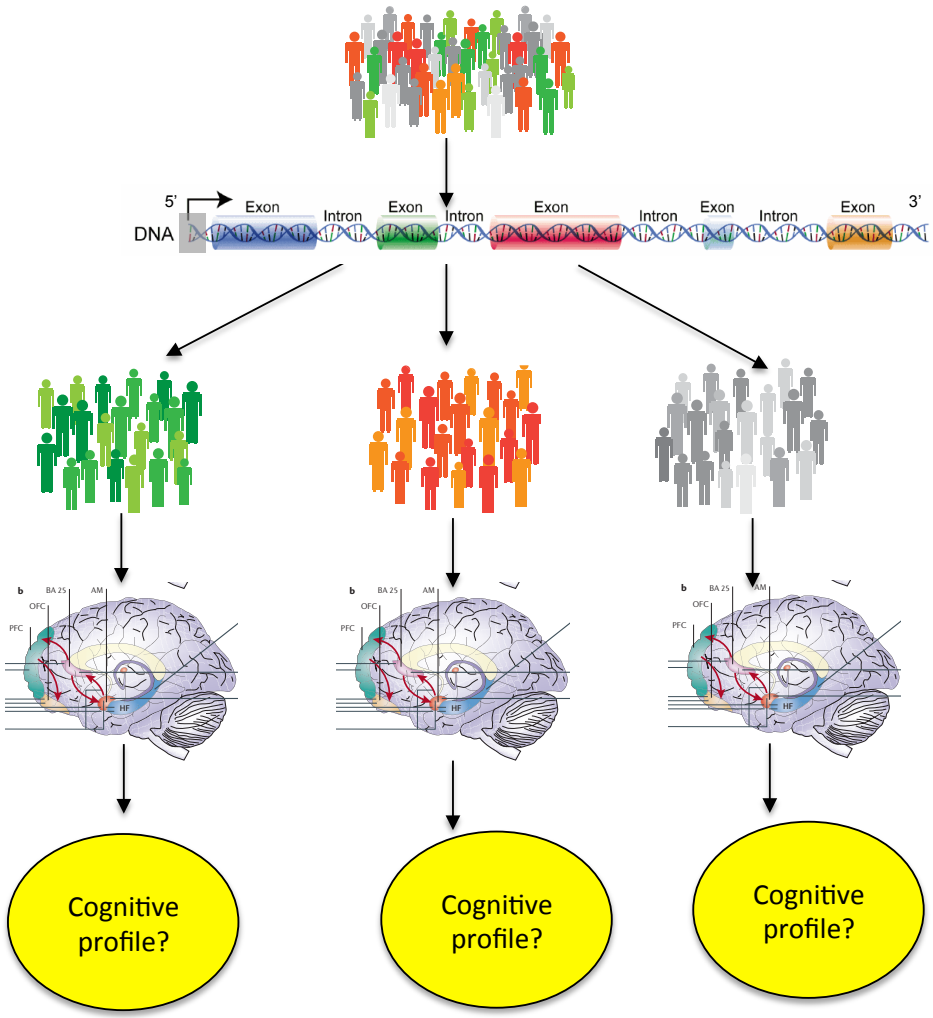

Genetically driven molecular subgroups

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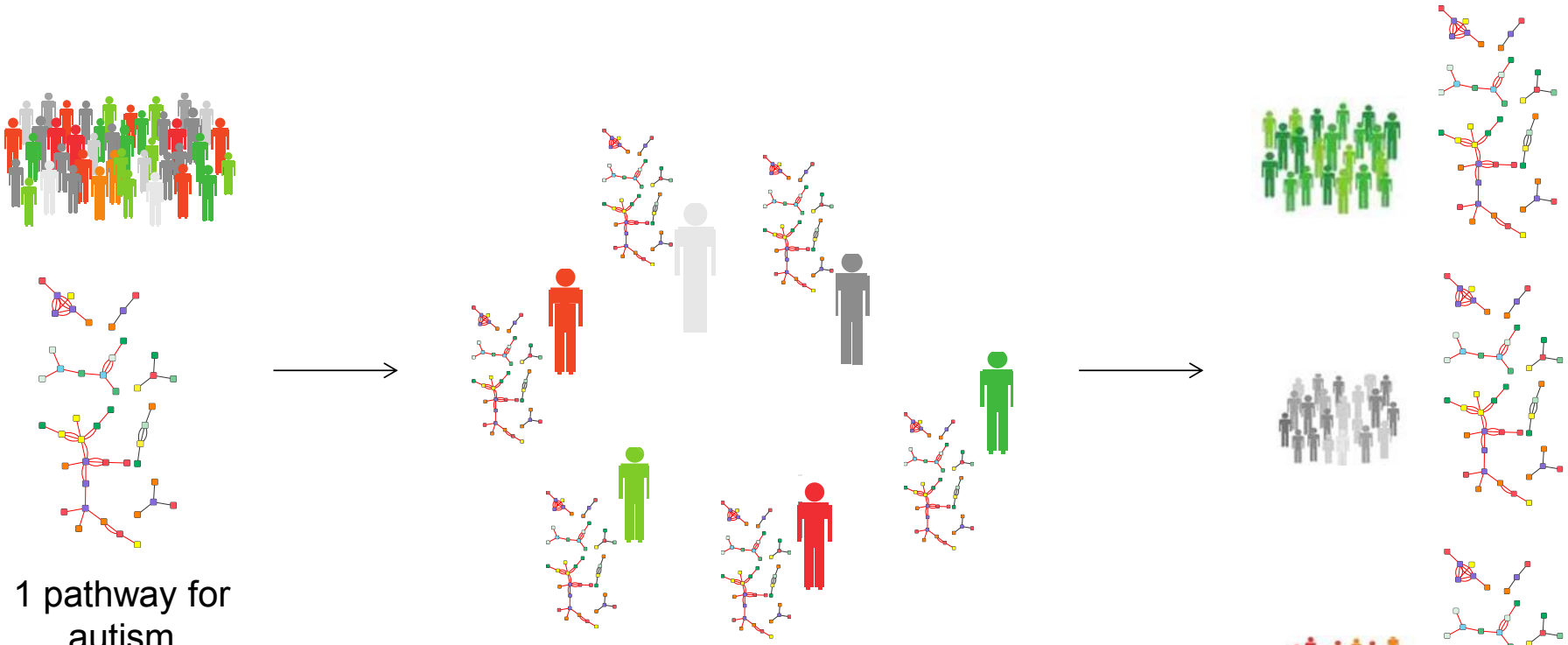


1 pathway for autism

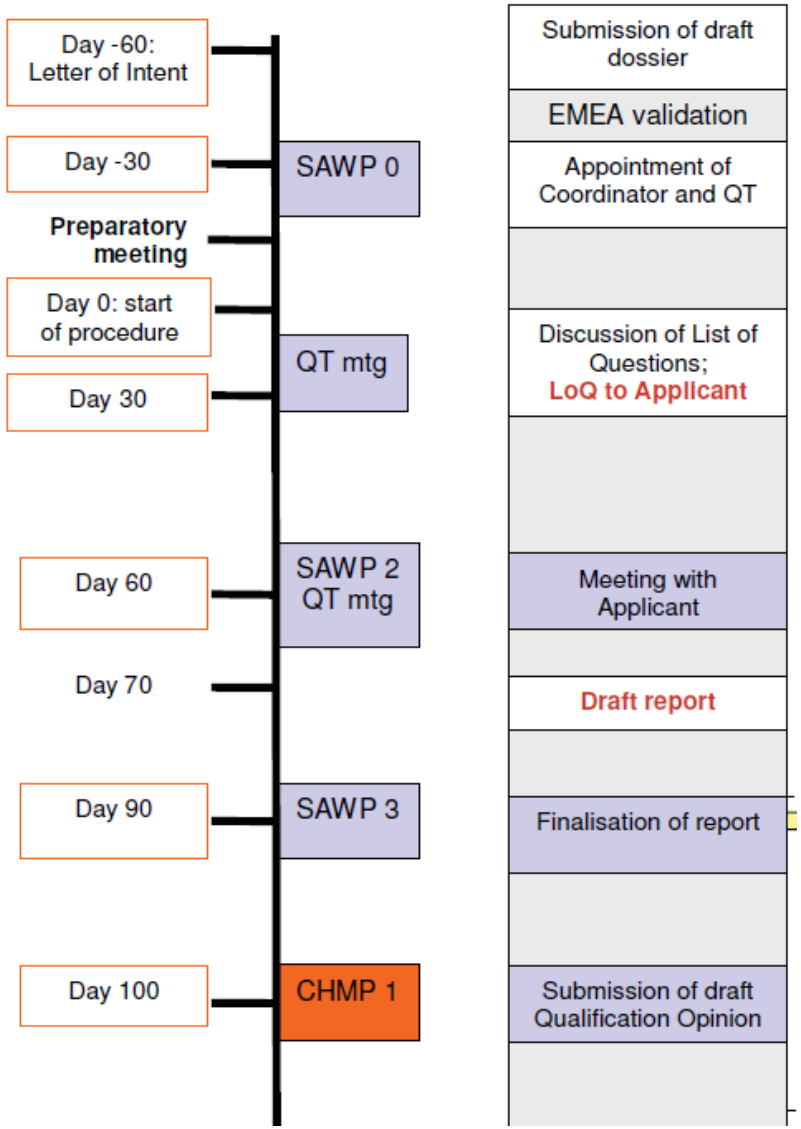
1 pathway for each individual with autism

N pathways for N subgroups of patients with autismS

Longitudinal reassessment after ~18 months (12- 24 months) to ascertain how cognitive or biological biomarkers change over time

Qualification Advice from EMA

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



Qualification Advice from EMA

EU-AIMS
Autism Research in Europe

- EMA broadly endorsed population selection criteria, biomarker approaches and methodologies
- Key recommendations:
- Need to establish sensitivity and specificity across all biomarker modalities
- Need to define cut-offs for stratification biomarkers
- Large number of endpoints/ analyses recognized: Replication will be required, particularly to validate biomarkers as surrogate end points.

EU-AIMS international collaborations

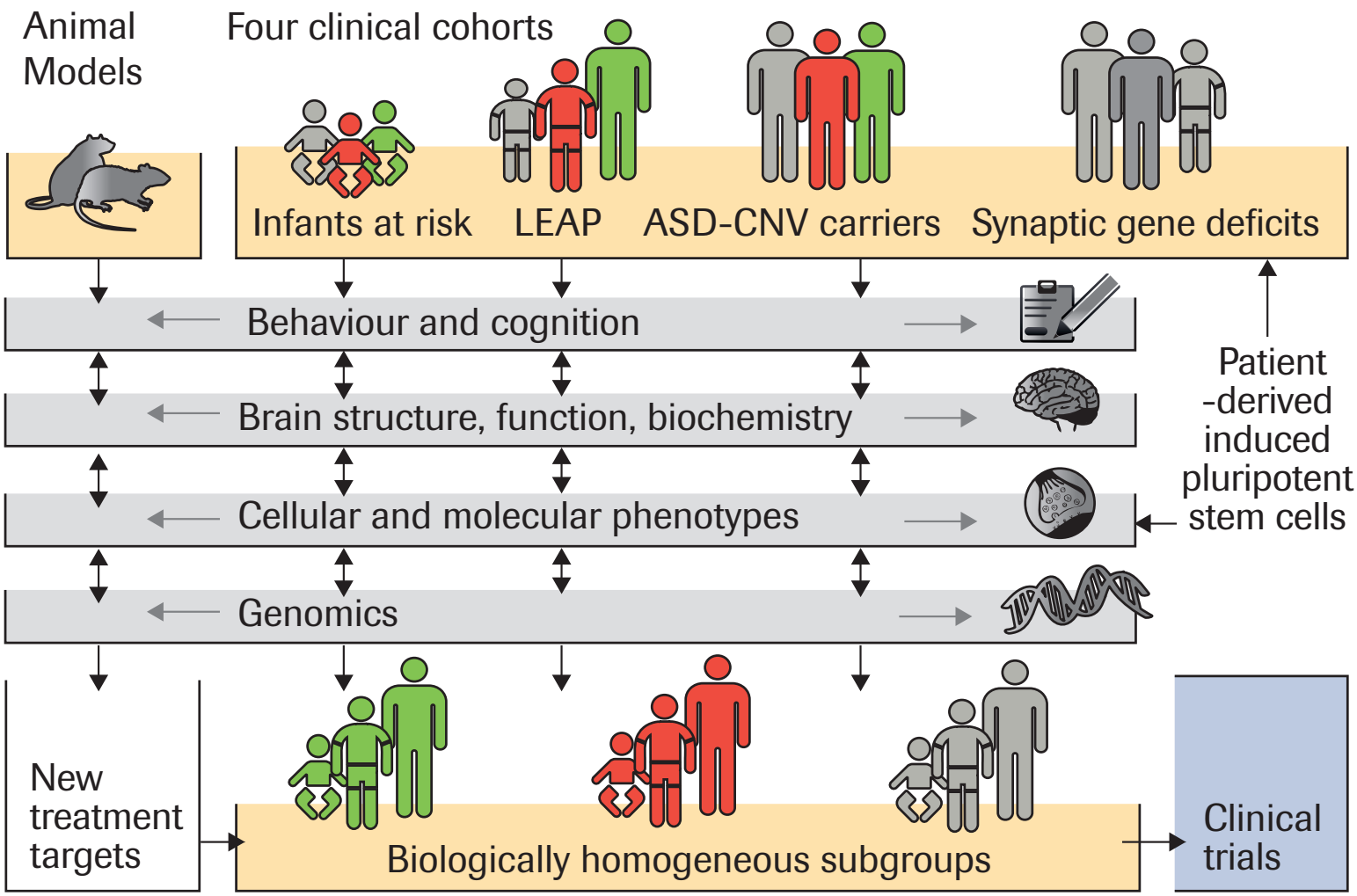
Sharing protocols, SOPs, data-sharing agreements for pooling and replication

So, we need biomarkers

EU-AIMS
Autism Research in Europe

- To guide clinical diagnosis
- Stratify people according to ‘biology’
- Evaluate prognosis
- When validated/ qualified, aid in population selection for clinical trials
- Assess effect of treatment/ intervention on symptom progression
- EU-AIMS – EMA collaboration: Important step towards a shared understanding of biomarker criteria between academia, industry, regulators.

Summary



Loth, Spooren, Murphy, *Lancet Psychiatry,* 2014

Thank you!

KCL: Declan Murphy, Tony Charman, Emily Simonoff, Steve Williams, Hannah Hayward, Daisy Crawley, Antonia San Jose Caceres, Jess Faulkner
BBK: Mark Johnson, Luke Mason, Emily Jones
UCAM: Simon Baron-Cohen, Rosie Holt, Jack Waldman, Meng-Chuan Lai
RUNMC: Jan Buitelaar, Larry O'Dwyer
UMCU: Sarah Durston, Sara Ambrosino, Bob Oranje
CIMH: Tobias Banaschewski, Luise Poustka, Niko Mueller, Sarah Baumeister
KI: Sven Boelte, Ela, Elodie Cauvet
UCMB: Tony Persico, Roberto Sacco

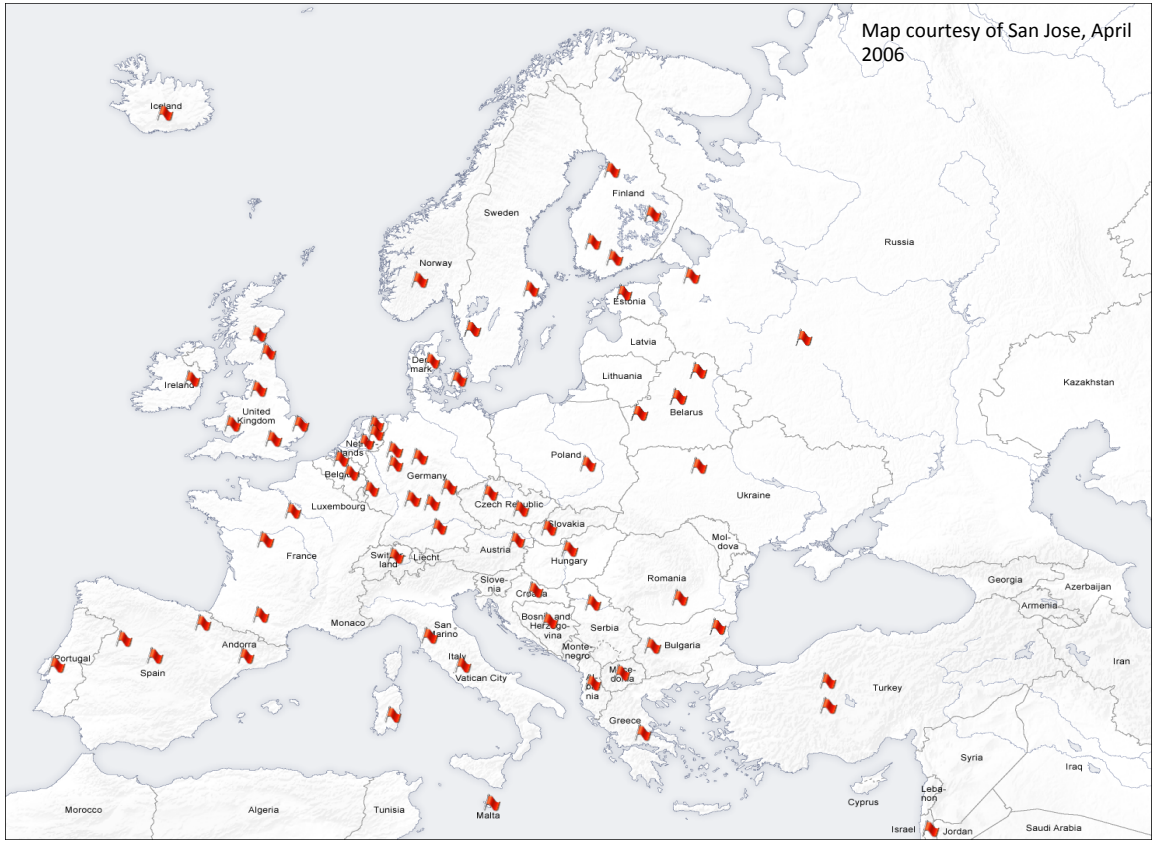
Thank you!

EU-AIMS Clinical Network

EU-AIMS
Autism Research in Europe

Aim: to facilitate future (transnational) clinical trials

78 sites from 37 countries



May indicate multiple sites in the same city/ area

- Currently 78 partners from EU-AIMS/ COST/ ECNP and new centres
- Goal: to collect information about ASD patient cohorts and assessment methods
- On-line survey, 50 partners responded
- Next: collaborative platform for data-sharing

Lead: Tony Charman, KCL