

Human Genetics and Cognitive Functions

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Our group gathers psychiatrists, neuroscientists and geneticists to understand the causes of autism spectrum disorders (ASD). We previously identified one synaptic pathway associated with ASD – the *NLGN-NRXN-SHANK* pathway. This pathway is known for playing a role in synapse formation and in the balance of excitation and inhibition within the brain. In parallel, we identified the first mutations within the melatonin pathway, which could contribute to the sleep problems observed in patients with ASD. Our results highlight the genetic heterogeneity of ASD, but also point at common pathways that could constitute relevant targets for new treatments.

We are currently performing a thorough genomic and clinical profiling of >500 patients using high-throughput genotyping/sequencing and brain imaging. In parallel, we are focusing on a set of mutations that we identified within the *NLGN* and *SHANK* families by studying in depth their functional impact at the clinical and neuronal levels, in patients and animal models. We will make a special effort to test whether the observed deficits could be reversible, by using human induced pluripotent stem cells (iPSC) and animal models. Our group is developing new methods for analyzing whole genome and brain imaging data as well as new paradigms for characterizing mouse social and vocal behavior.

Autism spectrum disorders

Autism spectrum disorders (ASD) are a heterogeneous group of pervasive neurodevelopmental disorders affecting 1% of the population. The diagnosis of ASD is based on impairments in reciprocal social communication and stereotyped behaviors. Behind the apparently unifying definition of ASD lies an extreme degree of clinical and genetic heterogeneity. Autism is not anymore considered as a single entity, but rather as a complex phenotype, ranging from patients with severe forms of autism (presenting an IQ<70), to patients with high-functioning autism and Asperger syndrome (IQ>70).

ASD are more frequent in males than females with a ratio of 4.1:1. This ratio is of 5.5:1 among high-functioning ASD patients (HF-ASD), 2:1 in populations with intellectual disability, and becomes more balanced if dysmorphic features are present. The diagnosis of ASD is also associated with the presence of seizures, immune system dysregulation, sleep disorders, aggression, hyperactivity, self-injury, anxiety and depression. In addition to these clinical and behavioral phenotypes, a number of endophenotypes shared by a significant proportion of the patients and among their non-affected relatives have been reported. For instance, macrocephaly is observed in up to 20% of patients and their 1st degree relatives, and abnormal serotonin/melatonin blood levels are recurrently observed in patients and their unaffected relatives. Although these traits are not specific to ASD, they most likely act as risk factors for developing the disorder.

Twin and family studies have conclusively described ASD as the most “genetic” of neuropsychiatric disorders, with concordance rates of 82-92% in monozygotic twins versus 1-10% in dizygotic twins; sibling recurrence risk is 6%. It is now understood that autism symptoms can be caused either by gene mutations or by chromosomal aberrations. In approximately 10-25% of the affected individuals, autism is “syndromic”, i.e., occurring in a child with a known genetic or environmental disorder, such as fragile X syndrome, tuberous sclerosis, neurofibromatosis, valproate syndrome, or caused by brain herpes simplex

infection. In the last years, various independent studies and large-scale international efforts have identified rare variants, copy number variants (CNVs) and single nucleotide polymorphisms (SNPs) associated with ASD and suggested a set of mechanisms that could underlie the ASD phenotype.

Synaptic genes and ASD

In 2003 we were the first to report mutations of two X-linked neuroligins, *NLGN3* and *NLGN4X*, in patients with autism and Asperger syndrome. The third gene that we identified within this pathway was *SHANK3* located on chromosome 22q13, a region deleted in several individuals with ASD. Finally, the fourth gene within this pathway was *NRXN1* on chromosome 2p. Neuroligins are cell adhesion molecules with a crucial role in the formation of functional synapses. They are located at the postsynaptic side of the synapse and bind to neuroligins located on the pre-synaptic side of the synapse (Figure 1). *SHANK3* is a scaffolding protein of the postsynaptic density, which binds to NLGN, and is known to regulate the structural organization of dendritic spines.

Since then, we have identified new synaptic genes associated with ASD such as *SHANK2* and *DLGAP1* that are part of the same synaptic pathway. In addition, following the discovery of two *de novo SHANK2* deletions by the Autism Genome Project, we screened *SHANK2* for CNVs and coding mutations in patients with ASD (Leblond *et al. unpublished results*). These new results strengthen the role of synaptic gene dysfunction in ASD, but also highlight the presence of putative modifier genes, which agrees with a “multiple hit” model for ASD.

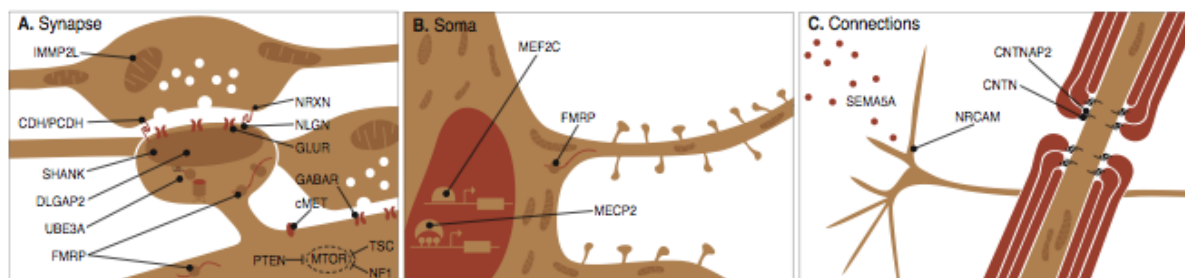


Figure 1. Examples of proteins contributing to ASD (reviewed in Belmonte and Bourgeron, *Nature Neuroscience*, 2006; Persico and Bourgeron, *Trends in Neuroscience*, 2006; Bourgeron, *Curr. Opin. Neurobiol.* 2009 and Toro *et al. Trends in Genetics* 2010).

Beside the NRXN-NLGN-SHANK pathway, our group has also explored the genetic variability of several candidate genes in ASD such as *GRIK2*, *SLC6A4*, *PCDH11Y*, *TPH2* and more recently *RPL10*, *NOS1AP* and *SEZ6L2*. Finally, we could detect families with high risk of carrying X-linked mutations by ascertaining the X chromosomal inactivation status of the mothers. Based on these results, our project is to focus on the identification of new susceptibility genes to ASD using high-throughput genotyping and whole-exome sequencing methods as well as functional characterization of the mutations.

To better understand the mechanisms leading to ASD, we cloned *Nlgn4* – the mouse orthologue of the human *NLGN4X* gene. We then characterized a gene-trap ES cell line and generated the mutant mice for *Nlgn4* in collaboration with Nils Brose (*Max Planck Institute of Experimental Medicine, Germany*). We showed that adult male mice lacking *Nlgn4* display larger latency to start vocalizing and a lower call rate in presence of an estrus female compared with wild-type males (Figure 2). *Nlgn4* KO mice also present a small but significant reduction in total brain, cerebellum and brainstem volumes, and significant deficits

in social behaviors, but no gross sensory or locomotion deficits and no repetitive behaviors. We recently reviewed the behavioral profile of the most relevant mouse models of ASD. Additional knockout mice are under investigation in our laboratory in order to understand the consequences of these synaptic mutations on brain anatomy as well as behavior.

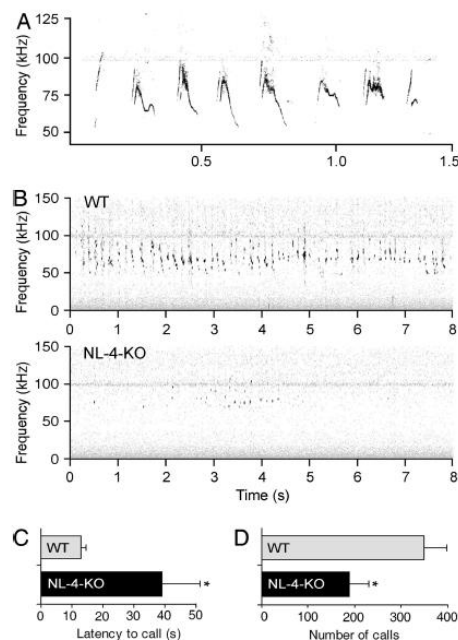


Figure 2. Reduced ultrasound vocalization in NL-4-KO mice. Ultrasonic vocalizations of individual male WT and NL-4-KO mice were measured upon contact of the male test mouse with an unfamiliar female mouse in estrous. (A) Frequency spectrogram of typical ultrasonic vocalizations of a male WT mouse. (B) Frequency spectrograms of ultrasonic vocalizations of a male WT (*Upper*) and a male NL-4-KO (*Lower*) mouse, indicating the reduced number of vocalizations in NL-4-KOs. (C) Quantitative analysis of the latency between the time the female mouse was put into the arena of the male test mouse and the first ultrasonic call of the male in WT ($n=20$) and NL-4-KO ($n=16$) mice. Data are presented as mean \pm SEM. The asterisk indicates a significant increase in latency in NL-4-KO mice ($P = 0.03$). (D) Quantitative analysis of the number of ultrasonic calls made by male WT ($n=20$) and NL-4-KO ($n=16$) mice during a 3-min session. Data are presented as mean \pm SEM. The asterisk indicates a significant reduction in the number of calls made by NL-4-KO mice ($P = 0.02$). Adapted from Jamain et al. *Proc Natl Acad Sci U S A*. 2008

The serotonin-melatonin pathway and susceptibility to ASD

Sleep difficulties are a major concern for families of ASD patients, but often considered as an epiphenomenon. In 2008, we reported genetic and biochemical alterations of the melatonin pathway in patients with ASD. We identified deletions, partial duplications and loss of function mutations of *ASMT*, coding for the last enzyme of the melatonin synthesis pathway, in a subset of patients with ASD. In addition, two polymorphisms (rs4446909 and rs5989681) located in the *ASMT* promoter were more frequent in ASD compared with controls ($P=0.0006$) and were associated with a decrease in *ASMT* transcripts ($P=2 \times 10^{-10}$). Biochemical analyses performed on blood platelets revealed a highly significant decrease in *ASMT* activity ($P=2 \times 10^{-12}$) and melatonin levels ($P=3 \times 10^{-11}$) in ASD patients compared with controls.

Following these results, we developed new tools to study the *ASMT* protein by purifying the recombinant human protein and producing antibodies with high affinity. We also screened for mutations in the melatonin receptor *MTNR1A*, *MTNR1B* and *GPR50* and identified pathway-biased and deleterious mutants in ASD and in the general population. We then extended our screening to patients with Attention Deficit and Hyperactivity Disorders (ADHD) and patients with intellectual disability. In all populations, we could detect deleterious mutations associated with functional alterations of enzyme/receptor activities. However, there was no significant enrichment of deleterious mutations in the clinical

populations compared with the general population and the impact at the clinical level is still not fully understood. Remarkably, four recent clinical studies report a great efficacy of melatonin treatment for treating sleep problems in ASD. In this line, we are part of the Mends project (<http://www.controlled-trials.com/ISRCTN05534585>), the largest randomized control trial for testing the efficacy of melatonin on sleep and behavior in children with neurodevelopmental disorders.

Brain Imaging and genetics

The high incidence of macrocephaly among ASD patients has been suggested to result from an abnormally fast brain growth during infancy, that levels out with age. Magnetic resonance imaging studies have shown that this augmentation is principally due to an increase in frontal white matter, but also a larger frontal grey matter volume and degree of folding. These results have been interpreted in the framework of the influential central coherence theory of Uta Frith (1989), which suggests that the lack of "high-level" and "low-level" coherence among autistics could be due to an abnormal profusion of local frontal lobe connections in detriment of the rest of the brain, which would become virtually "disconnected".

In 2008, we started to explore the link between the genetics and neuroimaging of ASD patients (ANR AGIR). In the laboratory, Roberto Toro has a strong expertise in the analysis of brain variability in large populations. He has developed various methods for the analysis of cortical folding, the multivariate allometry of brain anatomy, and brain functional connectivity (<http://brainfolding.sourceforge.net>, <http://coactivationmap.sourceforge.net>).

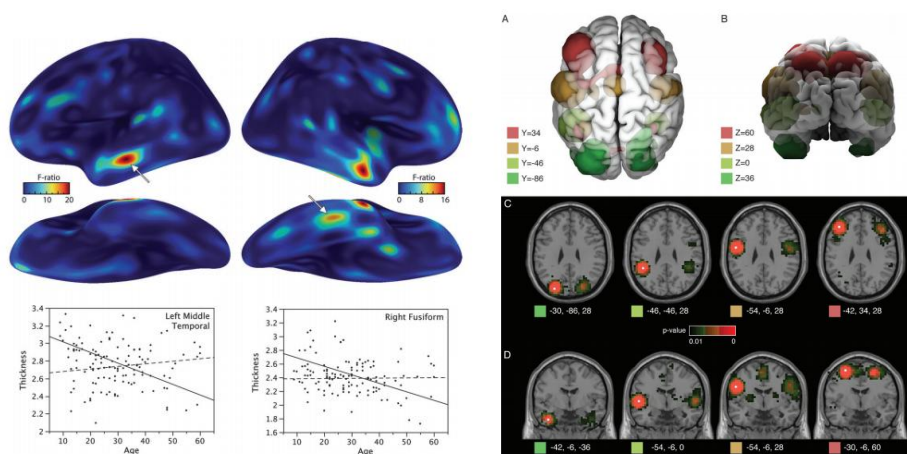


Figure 3 Brain imaging analyses of cortical anatomy in ASD and functional connectivity map of the human brain. Left. Results of surface-based analysis of age-by-group interactions across both cerebral hemispheres. Color maps show F ratio statistic with “warmer” colors indicating a larger magnitude of age-by-group interaction. See Raznahan, Toro et al. 2009 for details of those regions in which the F statistic exceeded the threshold imposed by FDR correction for multiple comparisons. Scatter plots of mean cortical thickness against age for controls (solid line) and cases (dashed line) are shown for 2 of these regions. Right. Symmetric interhemispheric coactivations. Coactivations of regions in the left hemisphere included most of the time the symmetric region in the right hemisphere, and vice versa. The figure shows 3-dimensional reconstructions (A, B) and stereotaxic slices of 4 networks corresponding to 4 seed-voxels in an axial plane (C), and 4 networks in a coronal plane (D). The network clusters are isosurfaces for $P = 0.01$, and the location of the seed-voxels is indicated by white squares in the stereotaxic slices. Adapted from Toro et al. 2008.

In collaboration with Armin Raznahan (Institut of Psychiatry, King’s College, London, UK) we recently analyzed the difference in cortical volume between patients and controls in more detail (Figure 3). Using cortical surface reconstruction methods we were able to decompose cortical volume into its 2 main components: cortical surface and cortical thickness. These 2 processes likely reflect different genetic factors. We observed that most of the difference in cortical volume was due to variations in cortical thickness: whether there is a marked decrease in cortical thickness with age in typically developing subjects, we did not

observed such a decrease in our patient group. Age-related decreases in cortical thickness were first thought to be due to synaptic pruning, however, given the small fraction of the cortical volume that synapses occupy, we think today that the apparent thinning may in fact reflect an increase in intra-cortical myelination. Intra-cortical myelin would make the T1W signal look whiter, producing a systematic bias in the automatic segmentation of the cortical grey that would appear thinner. The lack of decrease in cortical thickness that we observed would then reflect an abnormal intra-cortical myelination, probably due to defects in brain connectivity. Indeed, various DTI studies have reported recently differences in the volume, length and microstructure of white matter fiber tracts of ASD patients compared with controls.

The functional impact of these connectivity abnormalities remains still poorly understood. In the last decade, a new approach has emerged to study the global functional connectivity of the brain using Resting State fMRI (rsfMRI). rsfMRI is especially interesting as a method for studying functional connectivity in ASD, because subjects are not required to perform any particular task, and because rsfMRI data can be acquired even under anesthesia (rsfMRI may be the only way of obtaining functional MRI data from low-functioning ASD patients). One of the main functional networks observed in rsfMRI, the "default network" has been recently found to be weaker in ASD subjects than in controls. We have recently shown the existence of a remarkable correspondence between the functional networks observed at rest, and those observed in a large automatic meta-analysis of cognitive tasks (Figure 3). This will allow us to relate the eventual differences in functional connectivity observed at rest back to the cognitive domains that could be affected.

Perspectives

In the last decade, many candidate susceptibility genes for ASD have been reported. But in complex disorders, such as ASD, the identified variants may not fully segregate with the trait and are usually present in a small subset of patients. Therefore, one focus of the laboratory is now to understand the interplay between the different biological pathways (such as the synaptogenetic, clock and mTOR pathways) in the susceptibility to ASD. To achieve this goal, we are performing a detailed analysis of the genome of patients with ASD, their relatives, and controls using high-throughput genotyping/sequencing. In addition, for a subset of genes, we will characterize the functional impact of the mutations using induced pluripotent stem cells (iPSC) and animal models. This knowledge should also shed light on the origin of our ability to communicate, a complex process influenced by genetic/epigenetic factors and the environment.

Autism and society

Because of their high incidence, ASD are a major issue for our public health and educational systems. Besides the autistic symptomatology, psychiatric comorbidities are frequently observed in ASD patients, leading to further, sometimes severe, consequences in personal and public health terms. It is thus of great importance to systematically perform a thorough assessment of all comorbid disorders and conditions in order to specifically adapt treatment approaches to the needs of this group of patients.

However, even in individuals without medical problems, difficulties in social interaction and adaptive behavior very often lead to difficult schooling, inability to live independently from parents, and lack of employment as adults. The long-term outcome of individuals with High-Functioning ASD (HF-ASD), i.e. ASD with $IQ > 70$, was shown to be considerably better than that of individuals with ASD and intellectual disability, but nevertheless worse than what could be expected given their good cognitive abilities.

Especially in France, ASD are a major societal challenge: the lack of early diagnosis and educational interventions has led to a condemnation by the Council of Europe (Resolution ResChS(2004)). Our research aims to offer an evidence-based approach to ASD that should help improve their diagnosis, care and integration.

Consortia

Our group leads the European EU-HFAUTISM project focused on high functioning ASD and is part of Autism Genome Project, the largest international consortium for the study of the genetics of ASD.

Main publications

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