Book of abstracts

Quantitative microbiome profiling in health and disease.

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Alterations in the gut microbiota have been linked to various pathologies, ranging from inflammatory bowel disease and diabetes to cancer. Although large numbers of clinical studies aiming at microbiome-based disease markers are currently being performed, our basic knowledge about the normal variability of the human intestinal microbiota and its determining factors remains limited. Here, I will discuss our findings studying a large-scale study (Flemish Gut Flora Project; n=3400) of the gut microbiome variation in a geographically confined region (Flanders, Belgium), in which analysis of microbiome variability in health identified the primary parameters associated to microbiome composition. In this presentation, I will discuss our experiences in large-scale microbiome monitoring, show how the development of dedicated computational approaches can assist in microbiome analysis and interpretation, and which confounders are essential for inclusion in microbiome disease research. In addition I will show how Quantitative Microbiome Profiling (QMP; Vandeputte et al. Nature 2017), which combines microbiomics with flow cytometry-based cell counts, is profoundly changing our view on gut microbiota variation and allowed the identification of an inflammation-associated, cross-disease enterotype.

"The genetic architecture of autismS"

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The genetic architecture of autism is complex made of a combination of common and rare variants. Our previous studies pointed at one biological pathway associated with autism related to the synapse. Among the causative genes, synaptic cell adhesion molecules (neuroligins and neurexins) and scaffolding proteins (SHANK) are crucial for synapse formation/maintenance as well as correct balance between inhibitory and excitatory synaptic currents. These findings significantly advanced our knowledge on the possible causes of autism. However, they also (unintentionally) contributed to the emergence of a simplistic conception of autism as a binary trait: mutated vs. non-mutated or affected vs. non-affected. This simplification neglects the large phenotypic heterogeneity of autism, whose genetic architecture – like most complex diseases – cannot be reduced to a single gene. In this presentation, I will discuss our recent results coming from human studies in large populations and genetic isolates as well as mouse studies that shed new light on the inheritance of autism and some of the underlying mechanisms. Finally, I will illustrate how we are currently studying Resilience to understand why some carriers of deleterious mutations seem to be protected (The Resilients) while others are severely affected.

Implementing Global governance on human genome editing

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The recent application of tools, such as CRISPR-Cas9, to edit the human genome with the intention of treating or avoiding disease has highlighted the need for robust oversight in this area. The World Health Organization (WHO) has established a global, multi-disciplinary expert advisory committee to examine the scientific, ethical, social and legal challenges associated with human genome editing (both somatic and germline).[1] The Committee explores how best to promote transparent and trustworthy practices and how to ensure appropriate assessments are performed prior to any relevant work being undertaken. The Committee works in a consultative manner and build on existing initiatives to develop a responsible and responsive governance framework for the application of genome editing technologies going forward. It liaises with relevant UN and other international agencies, and communicate with Academies of Science and Medicine as well as with other national or professional bodies, patient groups and civil society organizations such as ARRIGE that have worked, or are working, in this area. The Committee adopted five guiding principles: (a) transparency; (b) the responsible stewardship of science; (c) inclusivity, (d) fairness and (e) social justice. Since march 2019 under the recommendations of the Committee, WHO established a registry of relevant research and development, including efforts to make use of WHO's International Clinical Trials Registry Platform; launched a global internet consultation and made hearings on responsible scientific stewardship, on whistleblowing and ethics dumping. The Committee believes those who prematurely attempt human genome germline editing for reproduction are engaged in unscrupulous behaviour. These individuals risk a backlash, making it impossible for science to progress to the point where applications might proceed one day. The Committee began to explore a range of scenarios to highlight key issues, explore challenges to governance, and foster greater engagement.

https://www.who.int/ethics/topics/human-genome-editing/en/

Dissecting the genetic and evolutionary factors driving immune response variation

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Unravelling the contributions of host and environmental factors to inter-individual and inter-population variability in immune responses is crucial to understand immune pathology. There is growing biomedical interest in studies of the variation of the immune response and its determinants in the healthy population — in a strategy known as systems or population immunology. Here, I will present our recent studies aiming to dissect the genetic, non-genetic and evolutionary determinants of immune response variation. The role played by pathogens in shaping human immune diversity is clearly attested by population genetic studies, indicating that immunity and host defense functions are among those most frequently subject to natural selection, whether purifying, positive or balancing. Notably, we have shown that population adaptation to novel pathogen pressures can be facilitated by the acquisition, via admixture, of advantageous alleles from local "adapted" populations, such as Neanderthals or modern humans. I will also discuss our work on how genetic variation, whether adaptive or not, affects the diversity of molecular phenotypes (i.e., gene expression-eQTL, alternative splicing-sQTL, and DNA methylation-meQTL), and highlight the importance of inferring the causality behind the detected associations between genetic, epigenetic and transcriptional variation. Finally, I will present our recent data on the respective contributions of genetic and non-genetic factors, such as age and sex, to the diversity of both transcriptional responses to microbial challenges and epigenetic marks. This presentation will attempt to provide a glimpse into how population and functional genomic approaches can help to pinpoint evolutionarily important determinants of host immune responsiveness and, more generally, shed new light onto the foundations of precision medicine approaches.

Integrated analysis of the large-scale sequencing project « MYOCAPTURE » for diagnosis and gene identification in myopathies

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More than 200 different myopathies have been described but nearly half of patients are still devoid of a genetic diagnosis, suggesting the implication of yet unlinked genes and unforeseen clinical and genetic heterogeneity. Moreover, through comparative analyses of published mutations and DNA variant databases, we highlighted 256 published mutations that have similar characteristics as non disease-causing polymorphisms and several genes which implication in the disease needs to be confirmed. To tackle these issues, we launched a multi-disciplinary consortium ("Myocapture") and sequenced >1500 exomes/genomes from affected families. Overall statistical and integrated analyses will be presented. The data obtained highlighted several cases: 1) some patients were linked to mutations in known myopathy genes, mainly large genes that were not routinely tested through diagnosis; 2) some cases were due to mutations in genes previously implicated in other muscle diseases, thus enlarging the phenotypic spectrum; 3) several novel myopathy genes were identified. In several cases, the genetic diagnosis had a strong impact on patient care and treatment. Examples concerning the genetic diagnosis of myopathies and the identification of novel myopathy genes will be presented. The identified novel genes and pathways represent novel drug targets for further therapeutic development. While the Myocapture project has clearly helped to refine the classification of myopathies and identified novel causes, a significant proportion of patients were not resolved, calling for the ongoing development of novel strategies.

FREX: A general population panel of exomes with ancestries in different regions of France

Emmanuelle Génin

on behalf of the FREX consortium

The FREX project was initiated thanks to funds allocated by France Génomique (AAP 2013: "The French Exome Project"). The objective was to establish a reference panel of genetic variations based on genomic data from individuals from different regions of France.

Exomes were sequenced for a total 574 individuals sampled in 6 different regions of France. When comparing these exomes against the frequency data available in GnomAD, several variants were found to have significant allele frequency differences. We also found that, compared to the different public panels, the "French exomes" allow a more efficient filtering of variants leading to a substantial reduction in the number of candidate variants retained for validation.

Allele frequency differences are also detected within France between the different geographic regions, pointing out the need for a fine-scale geographic matching of cases and controls. Population-specific exome panels are clearly useful to avoid false positives findings due to population structure and to allow a better understanding of genetic diversity at fine geographic scales.

Observed allele frequencies in the six regions can be obtained on the FREX portal (http://lysine.univ-brest.fr/FrExAC/). This portal provides the French scientific community with a unique database allowing a more precise analysis of case-control association at the demographic level, and the identification of new genetic variants of low allelic frequency involved in monogenic and complex diseases.

Virus-induced STAT3 signaling is a risk factor for hepatic steatosis and hepatocellular carcinoma

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Chronic hepatitis C virus (HCV) is a major cause of chronic liver disease, steatosis, fibrosis and hepatocellular carcinoma (HCC). It is currently believed that chronic HCV infection contributes to the development of liver disease indirectly via chronic inflammation and directly by virus-induced alternations of the hepatocyte homeostasis. Since HCV does not develop latency nor integrate into the host genome it has developed refined strategies to evade antiviral responses and to optimize conditions for its replication and persistence. Thereto, the virus is twisting essential aspects of host cell biology, i.e, gene expression machinery, epigenetics, signaling pathways, and metabolism. These chronic alterations have important consequences for the development of liver disease and even may persist since the HCC risk cannot be fully eradicated after viral cure.

Using multiomics studies involving transcriptomics, proteomics and metabolomics combined with advanced bioinformatics our unit has developed a proteogenomic atlas recapitulating the molecular mechanisms of virus-host interactions and metabolic disease[1]. This atlas combines infection models using hepatocyte-like cells with liver tissue specimens from HCV-infected chimeric mice and HCV-infected patients. Key findings demonstrate that HCV infection induces persistent EGFR signaling[1-4], which is a main driver of fibrogenesis and HCC in animal models[5]. EGFR maintains STAT3 signaling and promotes viral evasion from IFN response[6]. Our studies revealed that HCV induces STAT3[7], a key player during liver regeneration which is suppressing peroxisomal function in hepatocytes[1]. This leads to an accumulation of very long-chain fatty acids in infected hepatocytes matching the clinical phenotype of HCV-induced steatosis in patients. Indeed, we demonstrated that impaired peroxisomal function associates with clinical outcomes and phenotypes in viral and metabolic liver disease[1]. Moreover, our unit identified a pro-oncogenic epigenetic footprint of HCV infection in the host genome that associates with transcriptional signatures of cancer risk genes and also involves regulators of STAT3 signaling[8]. Importantly, this epigenetic footprint persists after viral cure and thus may contribute to the residual HCC risk in HCV-cures patients[8].

Our unit is currently refining the role of the STAT3 regulatory network during liver disease development We aim to identify potential targets for a future therapeutic intervention to restore the metabolic phenotype and to reverse disease progression in risk patients with and without HCV infection and use the identified viral footprint to develop biomarkers to identified HCV-cured patients with elevated HCC risk.

References:

1. Lupberger J, et al. Gastroenterology 2019.
2. Lupberger J, et al. Nat Med 2011.
3. Mailly L, et al. Nat Biotechnol 2015.
4. Zona L, et al. Cell Host Microbe 2013.
5. Fuchs BC, et al. Hepatology 2014.
6. Lupberger J, et al. Hepatology 2013.
7. Van Renne N, et al. Gut 2018.
8. Hamdane N, et al. Gastroenterology 2019

Familial breast cancer and DNA repair genes

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Abstract

Women carrying a pathogenic variant (PV) in the *BRCA1* or *BRCA2* (*BRCA1/2*) gene are at high risk of developing breast and ovarian cancer, and these risks vary between 40 to 85% according to the families. Cancer risk for carriers increase with an increasing number of affected first- or second-degree relatives, suggesting that other genetic or familial factors modify cancer risks for PV carriers. Consistent with this observation, it was shown that common breast and ovarian cancer susceptibility single nucleotide polymorphisms (SNPs), identified through genome-wide association studies in the general population, also modify breast and ovarian cancer risks for *BRCA1/2* carriers. However, *BRCA1/2* PV explain only about 10% of hereditary breast and ovarian cancer (HBOC) families attending the French family cancer clinics. In such families, the absence of a known germline PV does not rule out any genetic predisposition and complicates recommendations for surveillance of at-risk individuals. Because of cost-effectiveness, multigene panel testing is now performed in HBOC families, but whether the identification of an alteration in some of the DNA repair genes screened in parallel of *BRCA1* and *BRCA2* is useful for clinical management of the patient and her/his relatives remains questionable. Moreover, the potential of recently developed Polygenic Risk Scores (PRS) reflecting the combined effect of susceptibility SNPs for risk stratification of high-risk population carrying or not a *BRCA1*/*2* PV is unclear. Here, I will present results from large-scale high-throughput sequencing and genotyping nation-wide projects whose aims are to assess the contribution of rare, damaging predicted variants in DNA repair genes, as well as the performance of PRS developed by the international consortia CIMBA (Consortium of Investigators of Modifiers of BRCA1/2) and BCAC (Breast Cancer Association Consortium) in the French high-risk population. The overall goal of these projects is to assess if rare predicted PV identified through multigene panel testing as well as population-based derived PRS can be used to improve management of at-risk women and should be incorporated into comprehensive cancer risk prediction tools.

Transcriptomics approaches to decipher immune cell diversity and cell communication in cancer

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Inter-cellular communication is critical to coordinate cellular function in tissue during steady state and inflammation. Few efforts have been done to reconstruct cell communication networks. In addition, the molecular events shaping communication and connectivity within inter-cellular networks are not known. This question is particularly relevant to immune cells during controlled and dysregulated inflammation, especially in cancer. We have used transcriptomics approaches to dissect immune cell heterogeneity in breast, and head and neck cancer. We have developed ICELLNET, an original systems biology framework to reconstruct cell connectivity networks based on transcriptomics data of purified cell types. ICELLNET is expert and manually curated, offering a detailed biological annotation, several visualisation options, and can be applied to bulk as well as single cell RNAseq datasets. We are now applying this and other strategies to the tumour microenvironment, in order to attempt deciphering the complex cellular networks engaged during tumour inflammation. These should be valuable tools to better understand the organisation of anti-tumour immune responses and guide therapeutic manipulations.

Elucidating the molecular basis of cardiac arrhythmias

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In partnership with the National Referral Center against inherited cardiac arrhythmia based in Nantes, we have been investigating for two decades the genetic bases of rare electrical cardiac diseases. Our aim is to better understand the molecular mechanisms leading to high risk of sudden cardiac death in the young adult.

By applying familial or trio-based approaches, we have recently identified new susceptibility genes for ventricular depolarization/repolarization and conduction defects. We are now applying whole-genome sequencing on still unresolved familial cases.

In parallel, by conducting a large association meta-analysis on 2,820 patients with Brugada syndrome (or BrS, a rare inherited arrhythmia disorder) compared to 10,000 ancestry-matched control individuals, we have recently identified 18 new loci associated with BrS, some of which have also been involved in more common cardiac disorders. In complement to this classical association scan, we are now exploring the full spectrum of variants putatively contributing to BrS phenotypes on the full genome sequences of 350 French and 425 British/Dutch index cases with BrS.

Identifying both rare and common genetic variants associated with rare electrical cardiac diseases will help in better stratifying the risk of sudden death among patients and adapt their management accordingly.

Microglia phenotype in human temporal lobe epilepsy

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Amongst other effects, an epileptic seizure induces an immune reaction. Microglia, the immune cells of the brain, have a controversial role in epilepsy. By adopting an anti-inflammatory phenotype, they may protect neurons and repair local damage but also may exacerbate pathological activities.

This work was based on tissue from patients with a defined, intractable epileptic syndrome: temporal lobe epilepsy with hippocampal sclerosis. We attempt to clarify the microglia immune response to a human seizure. In the temporal lobe, the degree of neuronal loss, gliosis and the excitability of remaining neurons differs between the CA1 and CA3 regions the dentate gyrus (DG) and the subiculum. However, differences between microglia in these regions have been little examined. We used anatomical, live imaging and transcriptomic data to define the morphological shape, activation and molecular markers of microglia in different regions.

We observe microglia in different regions of human epileptic hippocampus have different forms, express different markers and exhibit different functional responses. In sclerotic regions (CA1 and CA3) amoeboid microglia predominate (30-40% of microglia), are rapidly activate under purinergic activation (5-8 min), express inflammatory marker as MHCII and are associated with an IL-10 regulation of microglia's transcripts. However, in less sclerotic regions (Subiculum) most microglia are ramified (35-40%), respond slower to purine activation (10-15 min) and are associated with a microglia's transcript regulation by inflammatory cytokines as IL1b. Independently of the area, the rapid, local and transient innate immune response following a seizures is mediated in part by microglia and consisting of the release of inflammatory cytokines such as IL-1B and the human cytokine IL-8.

In this study we show for the first time the large variability of microglia shape and activation in the same brain region according to the neuronal loss. Moreover, an epileptic seizure induce a microglia local and transient activation. Chronic inflammatory process observed in epilepsy could be the consequence of a synergy between extracellular immune cells and microglia.

Mechanims of Repression by Polycomb Proteins

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The role of the Polycomb machinery is to maintain gene silencing throughout development. It is composed of several multiprotein complexes that orchestrate this epigenetic memory through the regulation of chromatin structure. Interfering with the activity of the Polycomb machinery impairs development and can be driver to tumor progression.

While therapies based on the systemic inhibition of Polycomb complex PRC2 are currently being developed, the role of this machinery in germ cells remains poorly understood. Moreover, the long-term transcriptional consequences of disrupting this epigenetic memory are still not well appreciated. I will discuss our recent efforts to address those two questions.

Decoding microRNA mode of binding and its regulation

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There is a growing body of evidence about the presence and the activity of the miRISC in the nucleus of mammalian cells. Here, we showed by quantitative proteomic analysis that Ago2 interacts with nucleoplasmic Sfpq in a RNA-dependent fashion. By HITS-CLIP and transcriptomic analyses in P19 stem cells, we demonstrated that Sfpq directly controls the miRNA targeting of a subset of miRNA-target mRNAs when it binds locally (for both ectopic let-7a and endogenously expressed miRNAs). These data were validated in both mouse P19 and human NTERA-2 stem cells. Although Sfpq interacts with Ago2/miRNAs only in the nucleoplasm, it modulates miRNA targeting in both nucleoplasm and cytoplasm, indicating a nucleoplasmic imprinting of Sfpq-target mRNAs. Mechanistically, Sfpq binds to a set of long 3'UTR recognizing two specific binding motifs with a core composed by UGU sequence, as we determined by de novo motif analysis. Sfpq uses these two binding motifs to form long aggregates to optimize miRNA position/recruitment to selected binding sites, as we showed by HITS-CLIP analysis and on Lin28A 3'UTR using gene reporter assay and atomic force microscope. Finally, Sfpq regulates the let-7-dependent gene expression program to ultimately elicit the exit from stem cell status and to promote differentiation towards a neuron-like phenotype, while in prostate cancer the upregulation of Sfpq correlates to Lin28A expression levels and therefore to the aggressiveness of the tumor.

In conclusion, these data extend the miRNA-mediated post-transcriptional gene silencing into the nucleoplasm and indicate that a unique Sfpq-dependent post-transcriptional strategy for controlling miRNA-dependent program takes place in cells.

The different ways through which gene expression and cell identity are impacted by NF-Y's role in establishing a correct chromatin landscape

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NF-Y, also known as the CCAAT-binding factor CBF, is a ubiquitously expressed heterotrimeric TF composed of NF-YA, NF-YB, and NF-YC subunits, all of which are conserved from yeast to human. In recent years, we and others have proposed that the NF-Y complex behaves like a pioneer factor through its capacity to remodel the chromatin landscape.

Our studies were able to show that NF-Y binding is not restricted to gene promoters as was previously believed, but that in close to 50% of the cases, it binds to distal regulatory elements (enhancers). When binding to enhancers, NF-Y does so in a cell-type specific manner, which in mouse ES cells is necessary to maintain pluripotency through the facilitated recruitment of other members of the core pluripotency network.

We further revealed a novel regulatory mechanism for NF-Y at gene promoters. NF-Y binds promoters in a ubiquitous manner, mostly regulating cell-cycle and housekeeping genes. We were able to establish that NF-Y binding at these promoters is essential to (1) direct PIC recruitment to the promoter region through its interactions with TBP and the TAFs, (2) prevent ectopic nucleosome binding within the nucleosome-depleted region, and (3) occlude alternative transcription initiation sites to ensure correct TSS usage.

Our research highlights how NF-Y's nucleosome-like structural properties and DNA-binding mode have a broad impact on both gene expression and cell identity.

Building an atlas of the human airways at the single-cell level: From cell droplets to oceans of data

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To understand the preservation of homeostasis in the human airway epithelium, it is crucial to identify drivers of differentiation dynamics and cell fate. In chronic airway diseases, the injured epithelium frequently displays defective regeneration leading to tissue remodeling, characterized by a loss of multiciliated cells and mucus hyper-secretion. We have used single cell transcriptomics (1) to characterize the sequence of cellular and molecular processes taking place during human airway epithelium regeneration in vitro and (2) to explore cellular heterogeneity of human airways by building an atlas from 12 healthy volunteers. From our single-cell data obtained from air-liquid interface primary cell cultures and lineage inference algorithms, we have unraveled cell trajectories from basal to multiciliated cells, providing markers for specific cell populations, such as deuterosomal cells, i.e. precursors of multiciliated cells. We report that goblet cells, like club/secretory cells, can act as precursors of multiciliated cells. We have extensively characterized epithelial cell populations at the molecular level and have confirmed our findings by collecting about 80 000 cells of healthy volunteers, by bronchoscopy at 35 distinct locations, from the nose to the 12th division of the airway tree, either by forceps, or brush biopsies. This atlas has revealed a strong gradient of gene expression in suprabasal, secretory, or multiciliated cells between nose and bronchial airways. By contrast, a much smaller gradient was detected between proximal, intermediate, and distal bronchial airway samples. Our atlas has also improved the description of 244 rare cells (0.3% of total), detecting ionocytes, pulmonary neuro-endocrine and brush cells, which likely derive from a unique population of precursor cells.

Altogether, the combination of in vitro data with the robust annotation of an unprecedented large single-cell cohort of 12 well-characterized human healthy volunteers establishes an important resource for future single cell investigations.

Development of brain immune cells: intrinsic factors and microbiota impact

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Microglia, the resident macrophages of the central nervous system, continuously sense their environment to control brain homeostasis in physiological conditions and are able to quickly adapt to inflammation and injury. These cells colonize the brain during early embryonic development and persist throughout life. While much is known about microglia physiology in the adult, we still have limited information on the developmental phases and factors that regulate their differentiation during brain development. Here, we used transcriptomic analyses to delineate specific stages of microglia differentiation during embryogenesis and early life. We identified and validated embryonic microglia markers that are up-regulated during this developmental time period and potentially mediate specific functions in brain wiring. In spite of showing specific profiles, embryonic microglia expressed the main components of its "sensome" very early on. Consistently, embryonic microglia was affected by the absence of microbiota in germ free (GF) animals. Indeed, while brain patterning and morphogenesis were not affected in embryonic GF animals, microglia density in the neocortex, the preoptic area and the striatum was increased with altered morphology. By comparing control and GF embryonic microglia, we highlighted pathways dysregulated by the absence of microbiota as compared to those that are not changed in GF animals. Our study reveals that from embryonic stage to later on, microglia follows discrete developmental steps coordinated by intrinsic developmental programs and microbiota-derived environmental signals.

Integrated clinical and Omics approach to Rare Diseases: Unexplored variants, novel genes and oligogenic inheritance in Holoprosencephaly

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Holoprosencephaly (HPE) is a pathology of forebrain development characterized by high phenotypic and locus heterogeneity. The disease presents with various clinical manifestations at the cerebral or facial levels. Several genes have been implicated in HPE but its genetic basis remains unclear. Conventional molecular testing approaches result in a very low diagnostic yield and most cases remain unsolved. We address the possibility that genetically unsolved cases of HPE present an oligogenic origin and result from combined inherited mutations in several genes including the possible effect of unexplored synonymous variants in the Sonic Hedgehog (SHH) gene. All patients initially underwent a routine diagnostic procedure (CGH-array and gene panel or whole exome sequencing). (1) 26 unrelated families with exome sequencing and no established molecular diagnosis (following the ACMG guidelines) were tested for the hypothesis of oligogenic inheritance and (2) 931 patients were analysed for synonymous variants in the SHH gene.

(1) To identify variants of clinical interest, we used an integrative approach combining variant- and gene-based prioritization involving clinical ontologies and analysis of gene co-expression networks. Clinical phenotyping and exploration of cross-species similarities with known mouse mutants were further performed on a family-by-family basis. Variants of clinical interest were identified in 180 genes significantly associated with key pathways of forebrain development including Sonic Hedgehog and Primary Cilia. Oligogenic events were observed in 10 families and involved both known and novel HPE genes including recurrently mutated FAT1, NDST1, COL2A1 and SCUBE2. We also show that depending on the affected genes and pathways, patients present with particular secondary clinical phenotypes. Analysis of control populations (FReX and GoNL) show that the incidence of oligogenic combinations was significantly higher in HPE patients ($p<10-14$ and $p<10-11$, respectively).

(2) Analysis of control populations (FReX and GoNL) also revealed the existence of a significant enrichment of synonymous SHH variants in the cohort of HPE patients, suggesting their role in the etiology of this disease. We thus systematically evaluated the impact of these variants on the transcription and translation of the SHH gene using computational approaches (miRNA binding sites, mRNA structure, codon and bicodon usage bias) and in vitro validations. These analyses demonstrated that five identified SHH synonymous variants did not lead to mRNA splicing or microRNA binding defects, or even to changes in the predicted mRNA structure. However, they did indicate a highly probable effect of these variants on the translation dynamics of the SHH gene. Functional testing demonstrated that these five SHH synonymous variants result in significantly reduced expression of the SHH protein (ranging from 5% to 23%, $p < 10-9$) and ruled out any possible splicing-related defects. By inhibiting the proteasome, we were able to restore the protein quantities for four of these five synonymous variants, confirming their impact on the stability and folding of the SHH protein. In addition, we were able to show a significant correlation between the experimentally evaluated protein reduction values and computational measures of codon usage ($R2=0.83$; $P=0.0016$), underscoring the relevance of certain models for predicting the impact of synonymous variants on translation.

This study reports novel HPE and HPE-related phenotype genes and supports oligogenicity as clinically relevant model in this disease. Considering the critical role of SHH in brain development, our results indicate the importance of synonymous variants in holoprosencephaly and highlight that synonymous variants may have a major role in genetic pathologies independently of a possible alteration in mRNA splicing. It also underlines that integrating clinical phenotyping in genetic studies will improve the identification of causal variants in rare disorders and points out the importance of having access to genomic data from control cohorts.

HPV genomics and cancers

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To comprehensively characterized the human papilloma virus DNA associated with carcinomas, we developed a Capture-NGS method to identify at once the complete HPV genotype, the HPV status (absence, episomal or integrated), the viral load, the viral-host chromosomal DNA junctions, as well as the associated genome rearrangements at the insertion locus (deletion or amplification). This technology was first validated on 72 cervical carcinomas using frozen biopsies and circulating DNA. Today, at the Institut Curie and other medical research centers and NGS platforms, it has been applied to a larger cohort of cervical tumors (>700) as well as for anal, vulval and head and neck samples. Highly significant differences of HPV genomic parameters are observed among tumor localizations.

Then, to characterize the multiplicity of HPV-chromosomal junctions in certain tumor samples (>2 chromosomally clustered or scattered hybrid junctions), we developed a long-read Capture-HPV PacBio method and in parallel performed Illumina and PacBio Whole Genome Sequencing. So far, it begun to partially resolve the structural complexity of certain chromosomal insertion events in cervical tumor samples but not all. Our HPV genomic technologies are adaptable to other virus-associated diseases. The development of such HPV genomics technologies should be useful in medical research, diagnosis and follow-up of patient treatment.

References:

1. Holmes et al, (2016) Npj Genomic Medicine 1, 16004
2. Jackson et al., (2016) BMC Genomics 17, 851
3. Jeannot et al. (2018) Genes Chromosomes Cancer, 57, 638
4. Morel et al., (2019) Cancers.11,1846
5. Thomas et al. (2019) J. of Lower Genital Tract Disease, 24, 53

Single-cell RNAseq reveals the immungenic properties of genomically stable rhabdoid tumors

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Rhabdoid tumors (RTs) are genomically simple pediatric cancers driven by the biallelic inactivation of SMARCB1, leading to SWI/SNF chromatin remodeler complex deficiency. Bulk RNA sequencing and DNA methylation profiling studies reaveled an unsuspected variety within these tumors, likely to be related to various cells of origin. Deconvolution analyses also revealed un unexpected high immune signature in some molecular subtypes of rhabdoid tumors. Single-cell RNAseq and single-cell TCR sequencing highlighted the presence of therapeutically targetable exhausted effector and clonally expanded tissue resident memory CD8+ T subpopulations. Finally, we show that one mechanism mediating RTs immunogenicity involves SMARCB1-dependent re-expression of endogenous retroviruses and interferon-signaling activation.

The Rare Cancers Genomics initiative unveils a new entity of pulmonary carcinoids and identifies clinically relevant molecular profiles of malignant pleural mesothelioma

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The Rare Cancers Genomics initiative (www.rarecancersgenomics.com) aims at the molecular characterization of "large" series of rare cancers through the integration of muti-omic data and detailed epidemiological and clinical information to generate a better understanding of these understudied diseases. Within this initiative we have already: (i) unveiled the existence of new molecular subtypes of pulmonary carcinoids, and particularly of interest a group named supra-carcinoids (Alcala *et al. Nat Commun* 2019); (ii) redefined malignant pleural mesothelioma types as a continuum uncovering immune-vascular interactions with clinical implications (Alcala *et al. EBioMedicine* 2019); (iii) contributed to provide recommendations for the classification of both malignant mesothelioma (Nicholson *et al. JTO* 2019) and neuroendocrine neoplasms (Fernandez-Cuesta and Foll *Transl Lung Cancer Res* 2019); and (iv) created the first molecular maps (http://tumormap.ucsc.edu; Gabriel *et al.* Submitted) for malignant mesothelioma and lung neuroendocrine neoplasms, which would help the research and translational impact of molecular studies in these rare cancers. We will be discussing all these results and describe the computational workflows that we have implemented for the analyses.

Epigenomic analyses in purified cell populations for personalized treatment decisions in complex human diseases

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Epigenetics and epigenomics have come to center stage for many questions in biomedical research in the last decade. Epigenetics has been attracted a lot of interest as a possible solution for resolving the missing heritability, coming with the disappointing realization that large-scale genetic studies particularly genome-wide association studies did not fulfill the initial promise of fully explaining complex diseases.

However, epigenetic research comes with a number of challenges, in particular epigenetic changes are established in a cell-type specific manner and are highly dynamic as responsive to a variety of environmental factors. However, these dynamics might make them interesting as a potential biomarker for stratification of patients and potentially predicting the response of an individual to a certain treatment, especially in diseases that are not characterized by strong genetic markers. In the current presentation we will present how we think that epigenetics can contribute to the elucidation of disease etiology and improved patient management using data from the laboratory in complex human diseases.

High Resolution Functional and Structural Mapping of the HIV-1 Genome Using Mutational Interference Mapping Experiments (MIME)

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Viruses concentrate numerous functional elements in short genomes. This is especially true for the 5' region of the HIV-1 genome, which regulates RNA transcription, polyadenylation, splicing, packaging, and reverse transcription. Selective packaging of the genomic RNA (gRNA) is thought to be dictated by interactions between Pr55Gag, the precursor of the structural proteins of the virus, and poorly defined packaging signals

We developed mutational interference mapping experiments (MIME) to comprehensively analyze packaging of the HIV-1 gRNA. MIME relies on (i) the introduction of random substitutions in the RNA, (ii) separation of functional from non-functional mutants, and (iii) the analysis of the mutation frequencies in each pool.

Applying MIME to study the interactions between gRNA and Pr55Gag in vitro, we identified a stretch of 110 nucleotides constituting the Pr55Gag core binding site. Within this region, stem-loop 1 (SL1) is the primary binding site, while SL3 and a long-range interaction supposed to play key roles in RNA packaging have relatively minor effects on Pr55Gag binding (1). We also showed that MIME can identify RNA helices important for function (1).

We next adapted MIME to cellular processes and showed that sequences required for Pr55Gag binding constitutes packaging signals. Unexpectedly, the repressed 5' polyadenylation signal also plays a key role in gRNA packaging even though it does not directly bind Pr55Gag (2). Repression of this polyadenylation signal in order to prevent gRNA premature cleavage requires a stable stem-loop and binding of snRNA U1 to the downstream major 5' splice site (2). However, repression is not complete and we uncover an unexpected balance between premature cleavage of the HIV-1 gRNA and its packaging, as mutations that prevent premature cleavage decrease gRNA packaging and vice-versa (2).

Altogether, our data reveal an unsuspected role of the cleavage and polyadenylation machinery in the packaging of the HIV-1 gRNA.

(1) Smyth et al. (2015) Nat. Methods 12, 866–872.
(2) Smyth et al. (2018) Nucleic Acids Res 46, e57

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