Molecular and functional characterization of *NF1* locus recurrent deletions

Hortense Guillaume dit Taunière[1], Djihad Hadjadj, [1] Eric Pasmant[1]

[1] Institut Cochin, Inserm U1016 - CNRS UMR8104 - Université Paris Cité, CARPEM, Paris, France

BACKGROUND

- **Neurofibromatosis type 1** (NF1) is caused by **loss-of-function variants** in the *NF1* gene (17q11.2), among which 5-10% are **deletions** of the whole *NF1* locus. These deletions are associated with a **severe clinical presentation** in NF1 patients[1] (Figure 1).

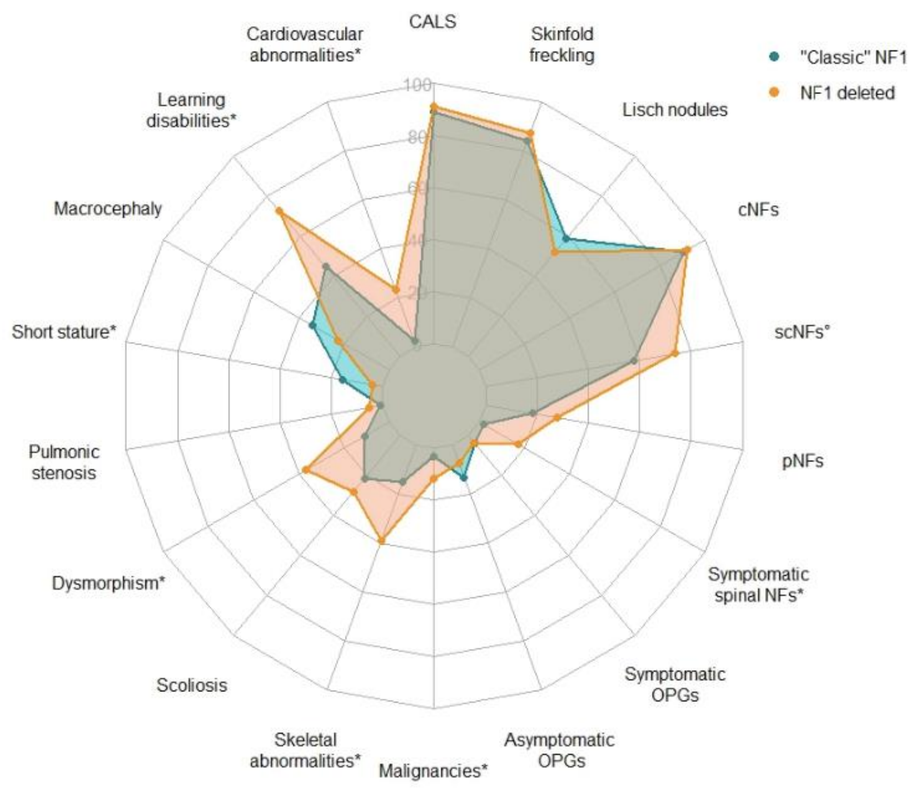

Figure 1. Radar chart of the frequency of the NF1 main symptoms in the French *NF1*-deleted cohort and in "classic" NF1 (adapted from Pacot et al., *Cancers* 2021).

- Three recurrent types of deletions have been described this far. They are caused by **non-allelic homologous recombination** (NAHR) mediated by low-copy repeats (LCR) at the *NF1* locus (namely, *NF1-REPa, NF1-REPb,* and *NF1-REPc*), or by NAHR events between *SUZ12* and its pseudogene, *SUZ12P*[2] (Figure 2).

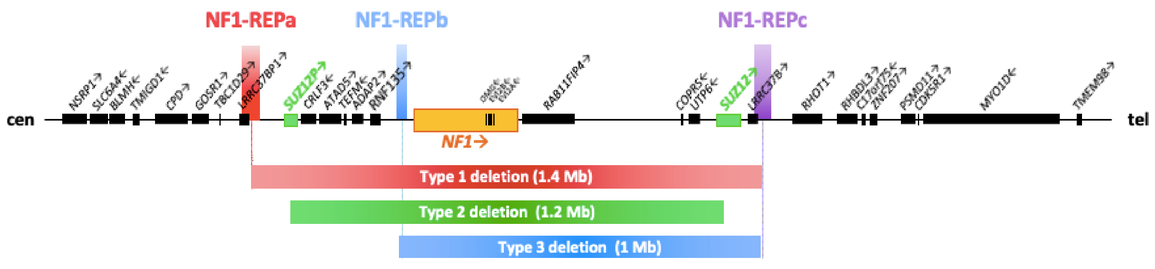

Figure 2. Illustration of the genomic region harboring the *NF1* and adjacent genes.

OBJECTIVES

1. To analyze precisely the **molecular mechanisms** of non-allelic homologous recombination (NAHR) between repeated sequences located at 17q11.2 using a **long-read sequencing approach** (*Oxford Nanopore Technologies, ONT*).

2. To study if the **blood methylome** of NF1 patients with large 17q11.2 locus deletions differs from that of NF1 patients with intragenic mutations. This **episignature** could include elements identified in (i) episignatures linked to syndromes caused by constitutional heterozygous loss-of-function mutations of PRC2 members (for SUZ12: Imagawa-Matsumoto syndrome, for EZH2: Weaver syndrome and for EED: Cohen-Gibson syndrome) and (ii) methylomes of tumours with somatic bi-allelic loss of-function mutations of PRC2 (Figure 3).

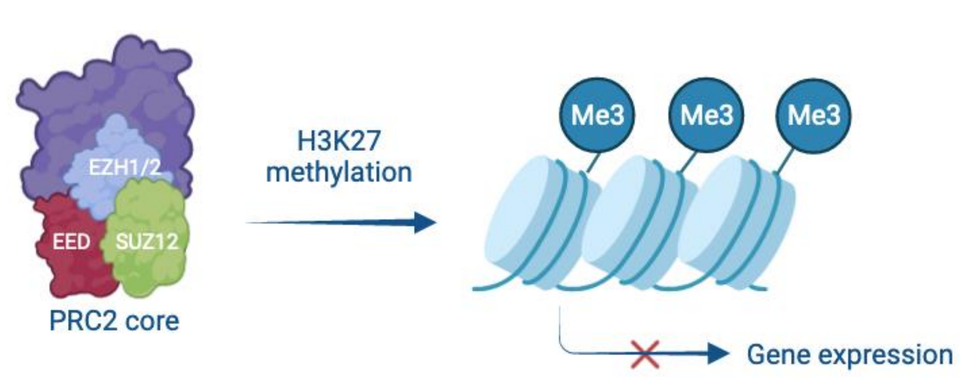

Figure 3. PRC2 complex catalyses H3K27 methylation.

MATERIALS & METHODS

- The team has recently implemented the innovative **nanopore approach** (ONT) for **genomic rearrangements** and **methylome** analysis, using an adaptive sequencing function to enrich on-target reads through real-time alignment.

- A total of **121 index cases** with an ***NF1* deletion** have been included. All patients were phenotypically described and had an MLPA (multiplex ligation-dependent probe amplification) genotyping of the three recurrent deletion types.

CONCLUSION

- A comprehensive description of the repeated sequences located at the 17q11.2 locus could significantly improve our understanding of the **specific NAHR mechanisms leading to *NF1* locus** recurrent deletions.
- The description of **episignatures** associated with these large deletions could reveal functional consequences and specifically implicate some of the deleted genes in the phenotype specifically associated with these deletions.
- Given the specific phenotype, it is important to finely characterize these rearrangements at the molecular level to allow more precise and **specific genetic counseling** in patients with large *NF1* deletions in the future.

References:

[1] Pacot *et al.,* Severe Phenotype in Patients with Large Deletions of *NF1*. *Cancers* 2021

[2] Kehrer-Sawatzki & Cooper. Classification of *NF1* microdeletions and its importance for establishing genotype/phenotype correlations in patients with *NF1* microdeletions. *Hum Genet* 2021

[3] Cyrus *et al.,* Rare *SUZ12* variants commonly cause an overg. *Am J Med Genet C Semin Med Genet* 2019