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Development of an automated bioinformatic workflow to assess oligosaccharide composition of early breast milk samples from LC-HRMS data



Eric Venot¹, Sylvain Dechaumet², Blanche Guillon¹, Mikail Berdi¹, Ghita Tajeddine¹, Sophie Cholet², Blandine de Lauzon-Guillain³, Etienne Thevenot², François Fenaille², and Karine Adel-Patient¹



Context

General goal

Search for biomarkers associated with the prevention of infant food allergies



Focus of this study

Human Milk Oligosaccharide (HMO) composition of early breast milk

Material & Methods

EDEN Early Breast Milk samples



317 samples of early breast milk collected between 1 and 6 days after delivery in the EDEN mother-child cohort between 2003 and 2006.

LC-HRMS analysis

The samples were analyzed by Liquid Chromatography coupled to High Resolution Mass Spectrometry (Q-TOF™, resolution: 40000, PGC Hypercarb column)

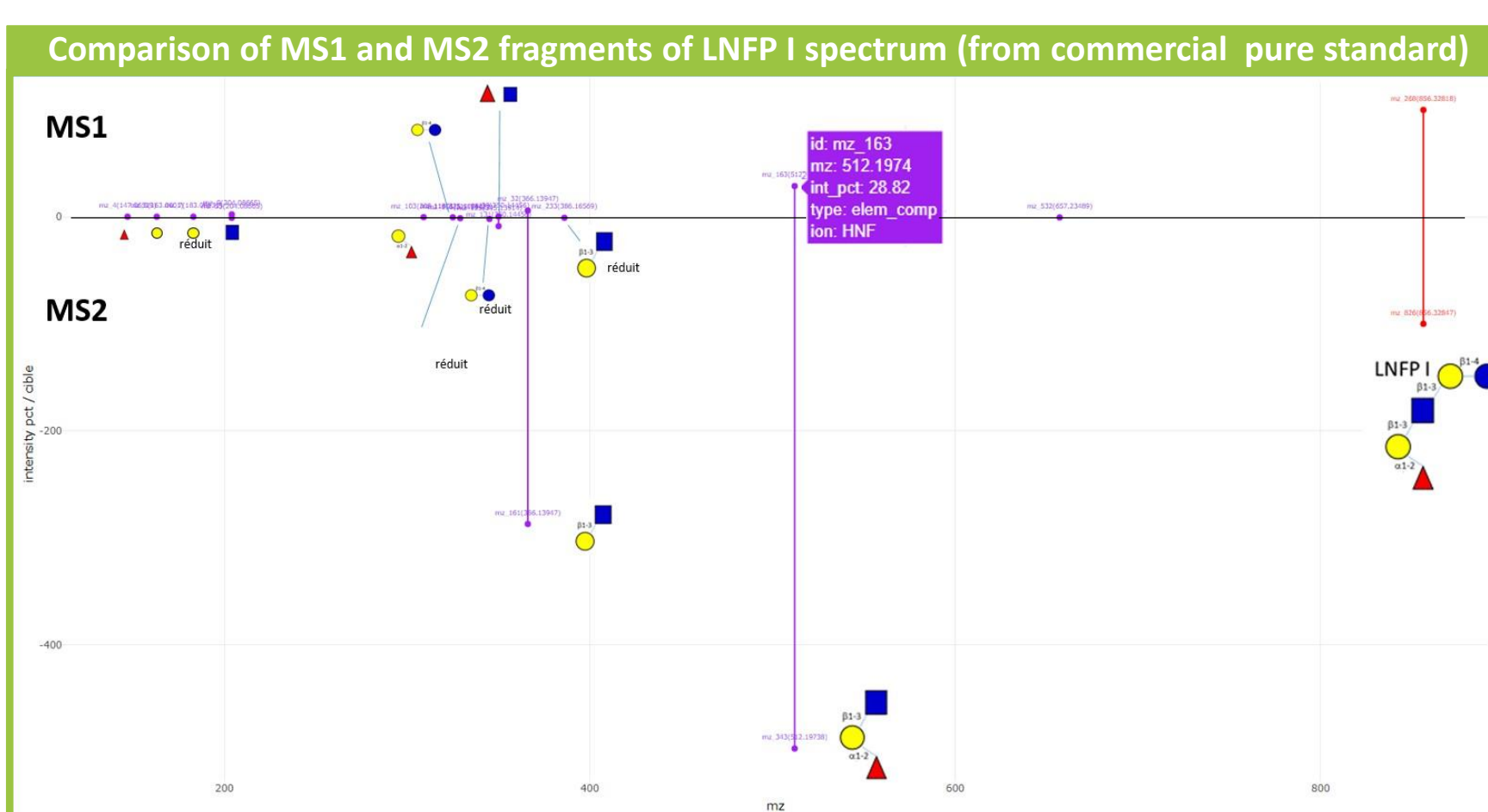


HMO references

- 19 commercial standards analyzed following the same analytical LC-HRMS method
- m/z of 8 953 HMO computed in silico based on all possible monosaccharide combinations

HMO signature

HMOs are rather labile molecules: LCHRMS ionization step leads to partial HMO fragmentation. HMO structures can be confirmed by the presence of characteristic in-source fragment ions



HMO workflow

mzML files



Peak picking

Peak refining

1st peak grouping

RT Adjustement

2nd peak grouping

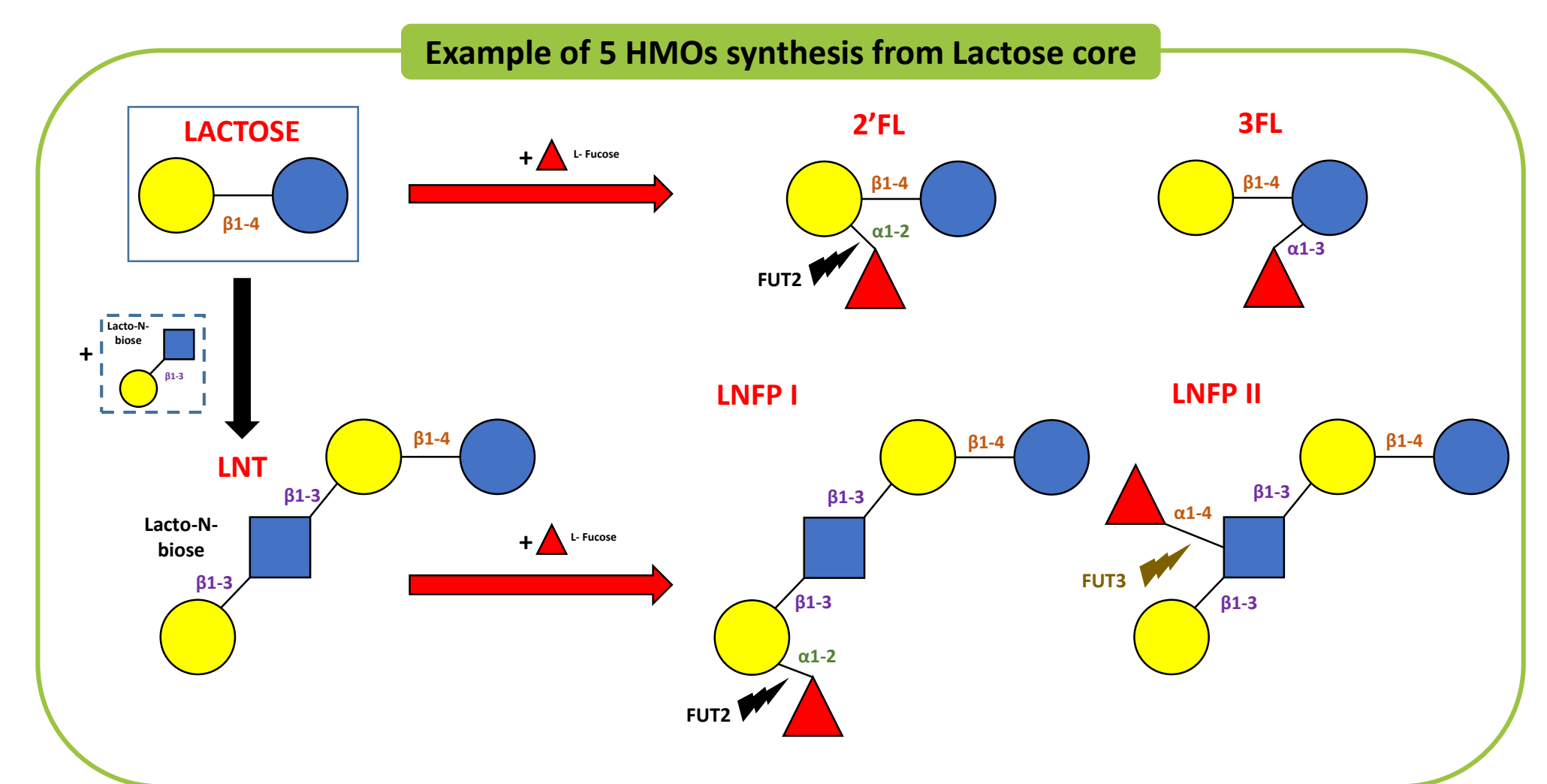
Missing peak filling

Potential HMO detection

Quality filters (CV QC, QC dilution)

HMO structures

HMOs are complex glycans composed of 5 different monosaccharides (D-galactose ●, D-glucose ●, L-Fucose ▲, N-Acetylglucosamine ■, Sialic Acid ◆) assembled from lactose core in highly diverse structures of 3 to more than 20 sugar units (many isomers).



Secretor/Lewis genotypes

Maternal genetic predispositions induce HMO synthesis differences: 2 fucosyltransferase enzymes production depends on mother Secretor (Se) and Lewis (Le) genes (enzymes FUT2 and FUT3 respectively)

- active FUT2 (Se +) ⇔ 2'FL & LNFP I synthesis
- active FUT3 (Le +) ⇔ LNFP II synthesis

link between Se - Le & Human Milk Groups

Human Milk Groups	Secretor	
	+	-
Lewis +	Group I	Group II
Lewis -	Group III	Group IV

Specific XCMS parameters

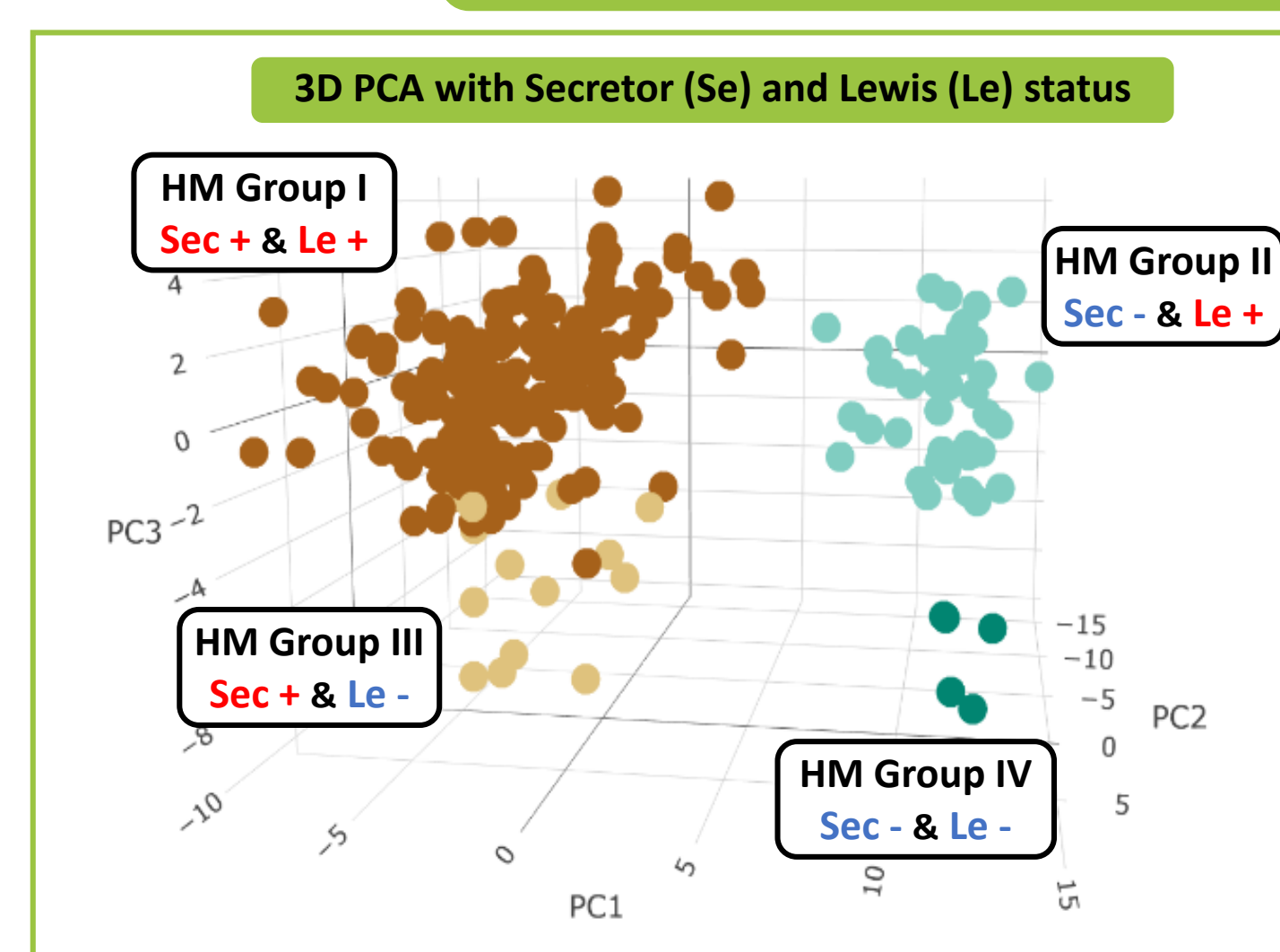
Preliminary analysis were run to define the best XCMS parameters adapted to HMO detection.

Results

Detection of 634 potential HMOs

- 20 610 features detected
- 1348 m/z ratios corresponding to potential HMOs
- 350 potential HMOs (⇔ 634 potential isomers) after selection criteria
 - 18 HMOs annotated based on pure standard (including 2'FL, LNFP I & LNFP II)
 - use of oligosaccharide fragmentation patterns needed in the next future for the others

Human Milk Groups in the EDEN cohort



Human Milk Groups	Prevalence	
	EDEN mothers	Oriol et al., 1986
Group I	72 %	69 %
Group II	19 %	20 %
Group III	7 %	9 %
Group IV	2 %	1 %

Conclusion

- Development of an automated R workflow dedicated to HMO detection from LC-HRMS mzML files
- Secretor & Lewis mother genotypes traced back from specific HMO composition (2'FL & LNFP II)

What's next?

- Finalization of HMO global milk composition in EDEN samples
- Assessment of influence of environmental factors on milk HMO composition & association between HMO composition and health outcomes in childhood
- Application of this HMO workflow on ELFE nationwide birth cohort