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Influence of FUT2/FUT3 polymorphism on human breastmilk oligosaccharide composition in the EDEN mother-child cohort.

Eric Venot¹, Sylvain Dechaumet², Blanche Guillon¹, Mikail Berdi¹, Sophie Cholet², Ghita Tajeddine¹, Manon Mutaner³, Aline Meirhaeghe³, Barbara Heude⁴, Blandine de Lauzon-Guillain⁴, Etienne Thevenot², François Fenaille², and Karine Adel-Patient¹

Context

General goal : Search for biomarkers associated with the prevention of infant food allergies



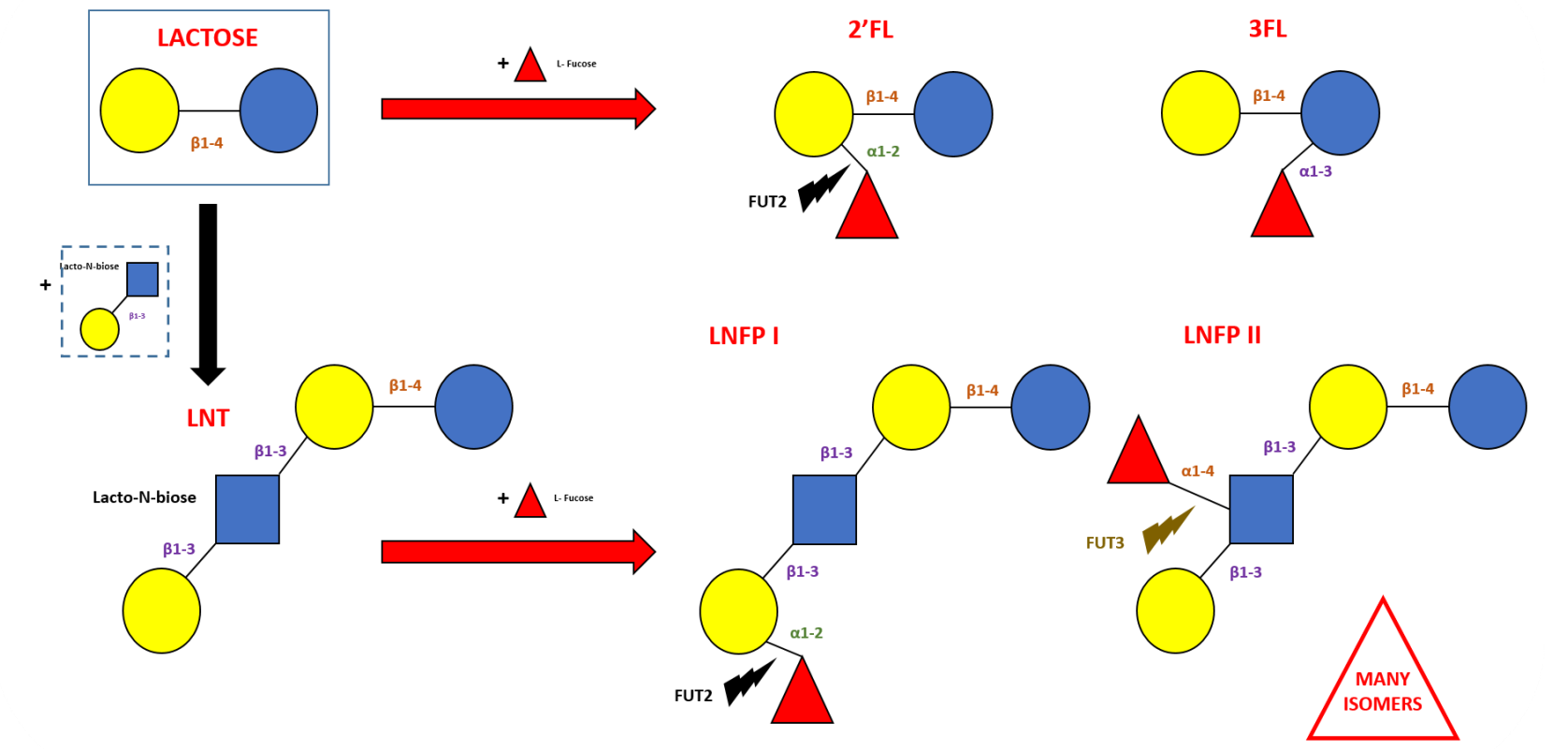
Focus of this study : Human Milk Oligosaccharide (HMO) composition of early breast milk

Material & Methods

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**.

Example of the synthesis of 5 HMOs (2'FL, 3FL, LNT, LNFP I & LNFP II) from Lactose core



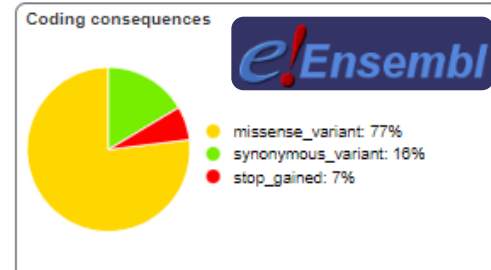
EDEN Early Breast Milk samples



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

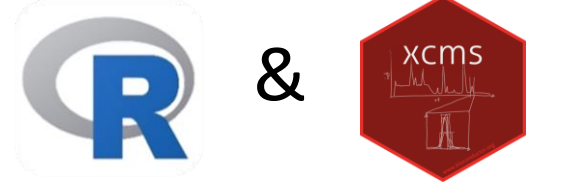
Secretor/Lewis genotypes

- Maternal genetic predispositions** induce HMO synthesis differences (Lefebvre et al., 2020): **2 fucosyltransferase enzymes** production depends on mother **FUT2 (Secretor)** and **FUT3 (Lewis)** genes : - active **FUT2 (Secretor +)** ⇔ **2'FL & LNFP I** synthesis - active **FUT3 (Lewis +)** ⇔ **LNFP II** synthesis
- 220 out of the 317 EDEN cohort mothers were genotyped** : **62 SNPs** associated to **FUT2** and **FUT3** genes were extracted. After SNP quality filters, selection of non homozygous SNPs for all EDEN mothers and selection of representative SNPs in complete Linkage Disequilibrium with others, **17 SNPs** were kept.
- Ensembl Variant Effect Predictor** tool was also applied on the 62 SNPs.



LC-HRMS analysis & HMO workflow

- The samples were analyzed by **Liquid Chromatography coupled to High Resolution Mass Spectrometry (Q-TOF™, res: 40000, PGC Hypercarb column)**
- HMO references:**
 - **19 commercial standards** analyzed
 - **m/z ratios of 8 953 HMO** computed **in silico** based on all possible monosaccharide combinations.
- specific in house HMO workflow** from mzML files developed with R & XCMS to detect m/z ratios corresponding to potential HMOs
 - ➔ **130 HMOs detected** after selection criteria including **18 HMOs annotated** based on commercial standard (including **2'FL, LNFP I & LNFP II**)



Statistical analysis

- The **significance of the difference in intensity between genotype groups** was assessed by **univariate hypothesis testing with multiple testing correction FDR** (Benjamini et al., 1995), (phenomis R package (Thévenot et al., 2023)), with : - **Student t test** when minor allele-minor allele genotype was compared to the 2 others gathered. - **Analysis of variance** when the 3 different genotypes were considered.
- Hierarchical cluster analysis (HCA)** (ComplexHeatmap R package (Gu et al., 2016)) was performed on all samples and all (annotated and potential) HMOs.

Results

FUT2/FUT3 effects on HMO intensities

- HMO composition highly depending of FUT2 and FUT3 polymorphisms :**
 - FUT2** has an **impact on 78%** of the 130 detected HMOs (p-values limit : 0.001)
 - FUT3** effect is also important but weaker than FUT2 ones (**27%** of the HMOs impacted with 0.05 p-value limit)

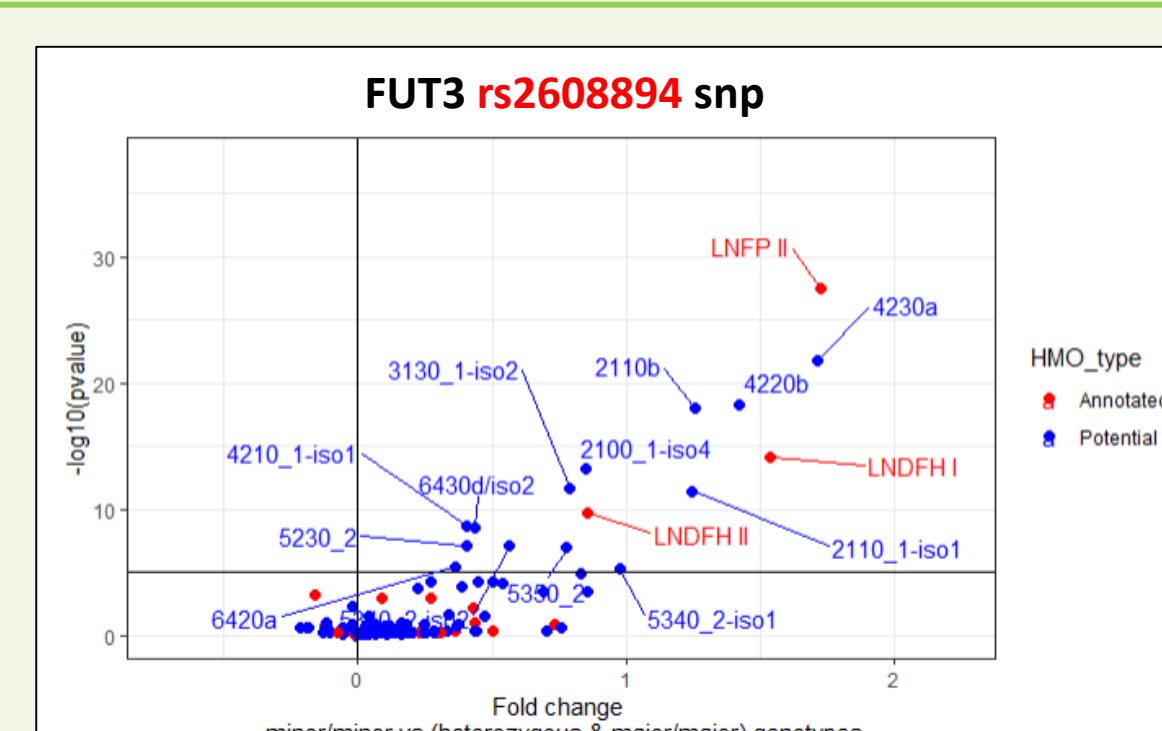
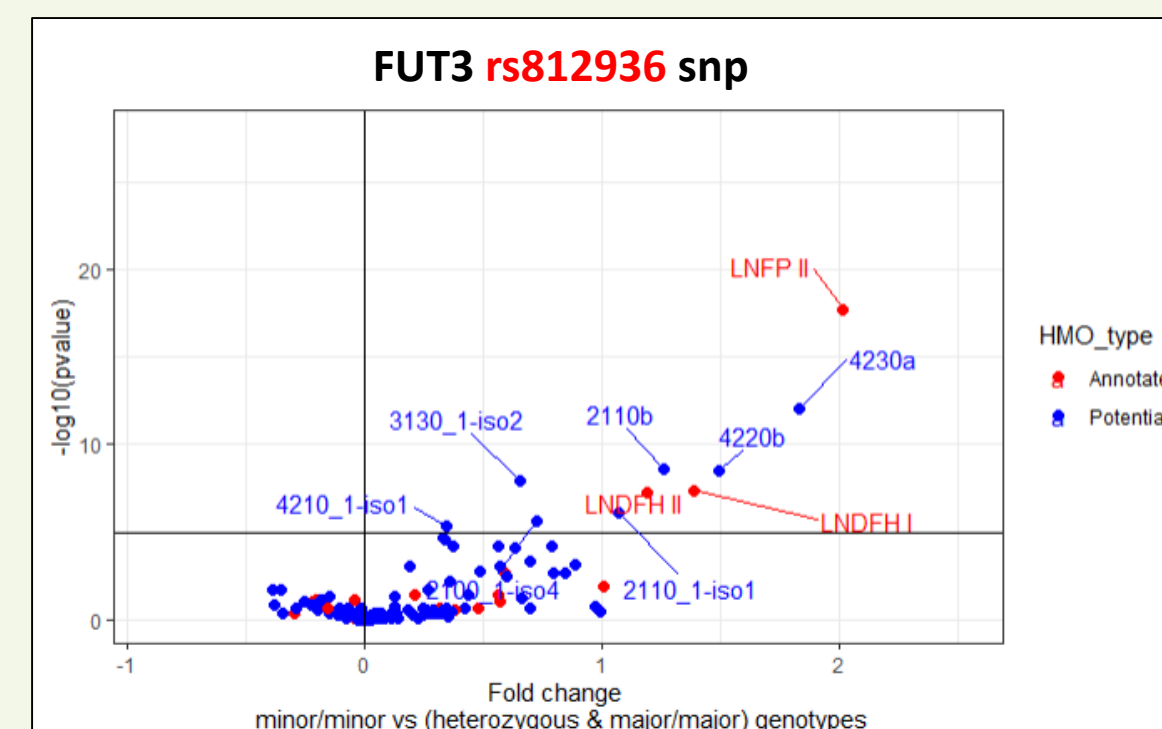
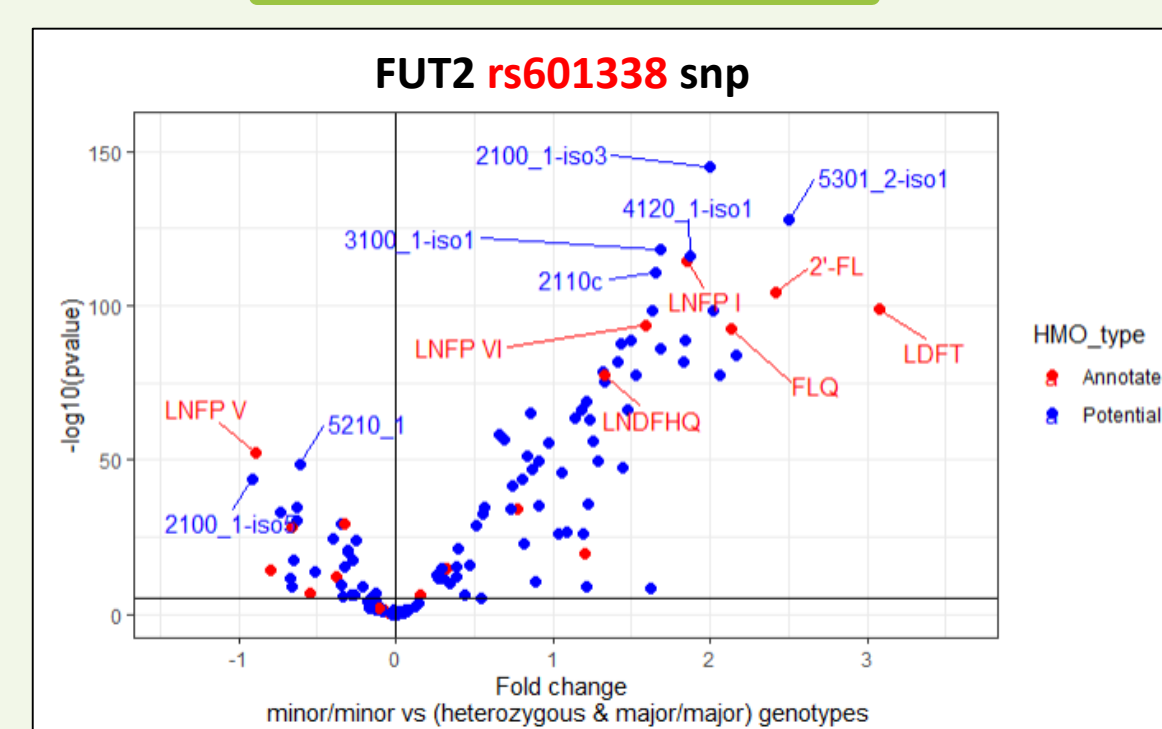
gene	snp	Ensembl Variant Effect Predictor type	Genotype distribution in 220 EDEN mothers			Considering the 3 genotypes separated			Considering the minor/minor genotype versus 2 other genotypes							
			minor/minor	minor/major	major/major	number of HMOs	median	pvalues	max	number of HMOs	median	pvalues	max	log10 difference	min	max
FUT2	rs601338*	stop_gained	46	114	60	101	1.23e-34	1.91e-145	7.06e-05	98	2.27e-26	1.76E-54	7.04e-05	0.78	-1	3.06
FUT3	rs812936*	missense variant	130	82	8	35	7.86e-04	1.95e-18	4.81e-02	24	6.60e-03	3.20e-10	4.39e-02	0.59	-0.44	2.2
FUT3	rs778986	missense variant	134	79	7	35	5.75e-04	2.07e-18	4.20e-02	24	6.60e-03	3.20e-10	4.39e-02	0.62	-0.44	2.2
FUT3	rs2608894	regulatory region variant - promoter	108	96	16	33	1.46e-05	2.71e-28	3.22e-02	29	1.51e-04	4.54e-12	4.82e-02	0.51	-0.2	1.88

- 2 SNPs (* in the table) mentioned in Lefebvre et al. paper (2020) confirmed in this study.**
- 2 new SNPs with high impact detected in this study (rs778986 & rs2608894)**

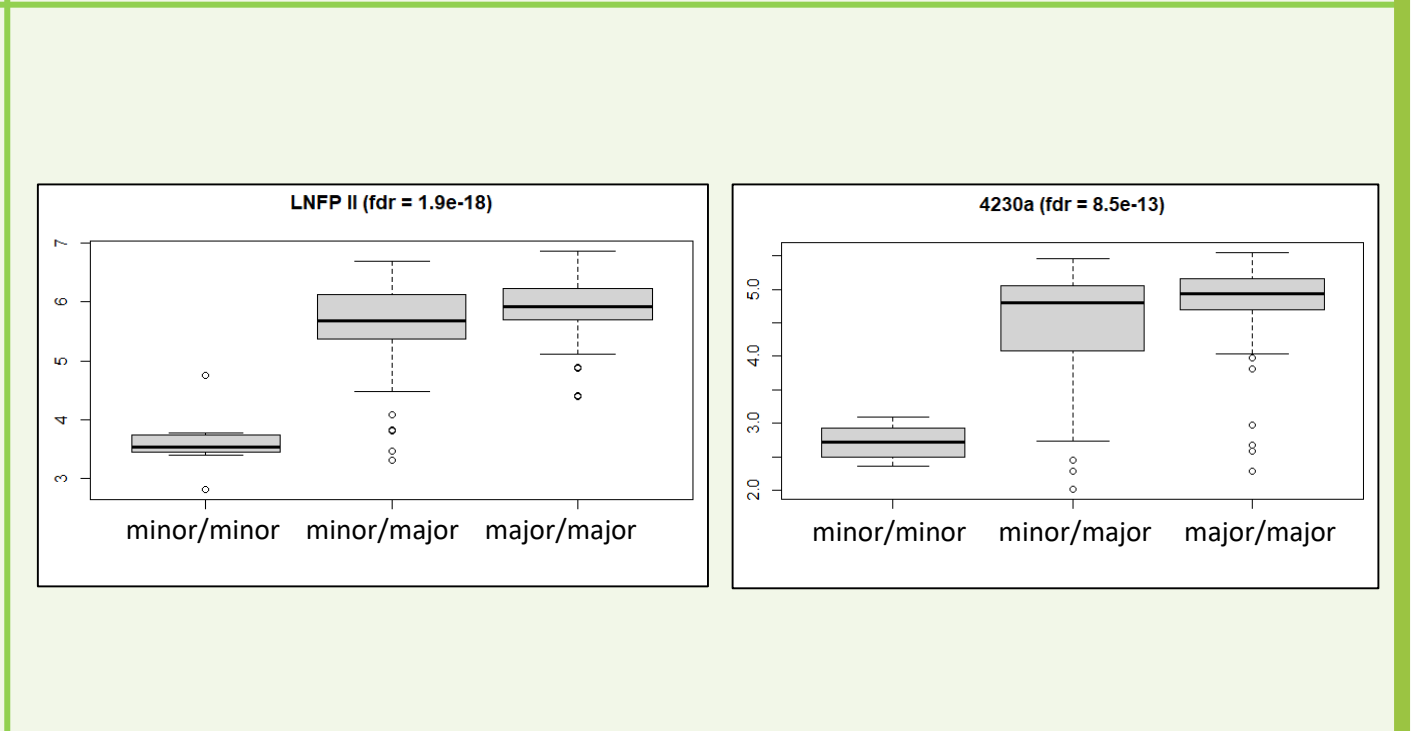
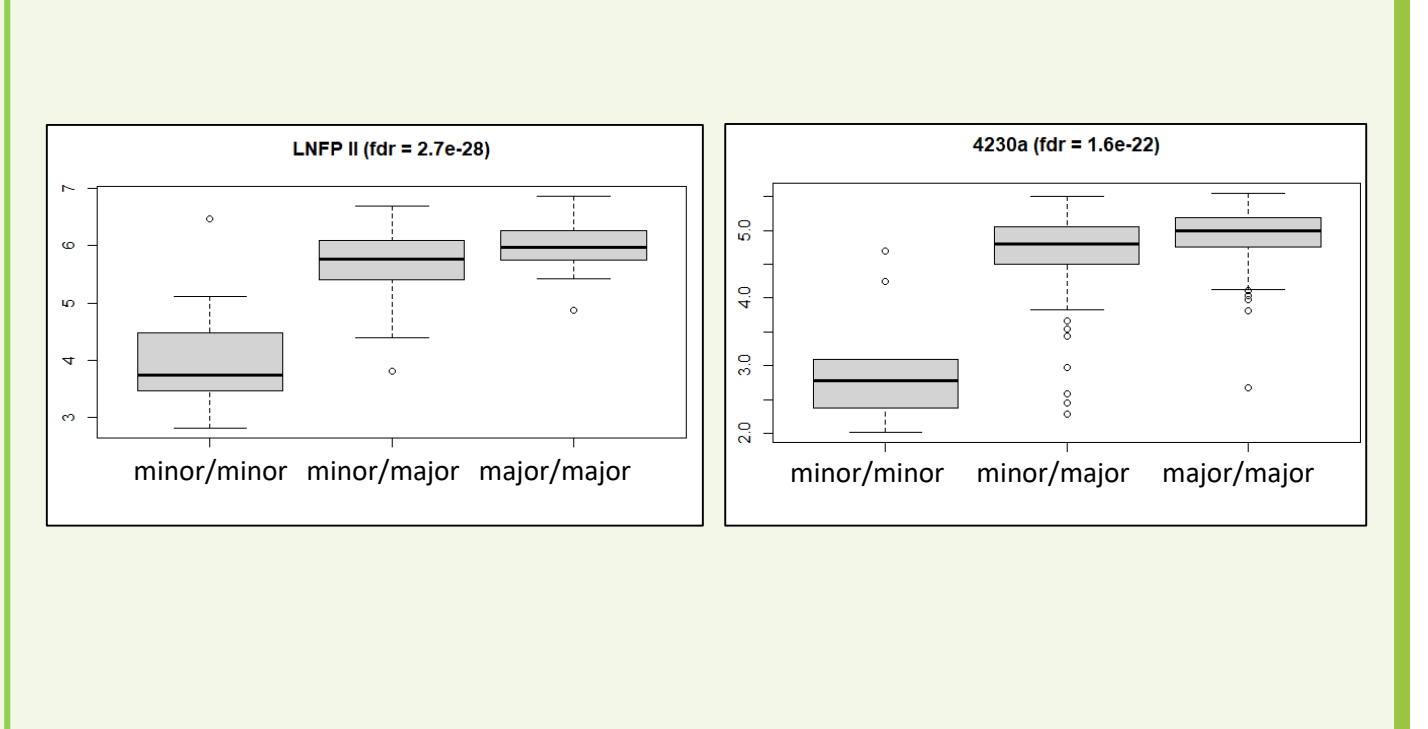
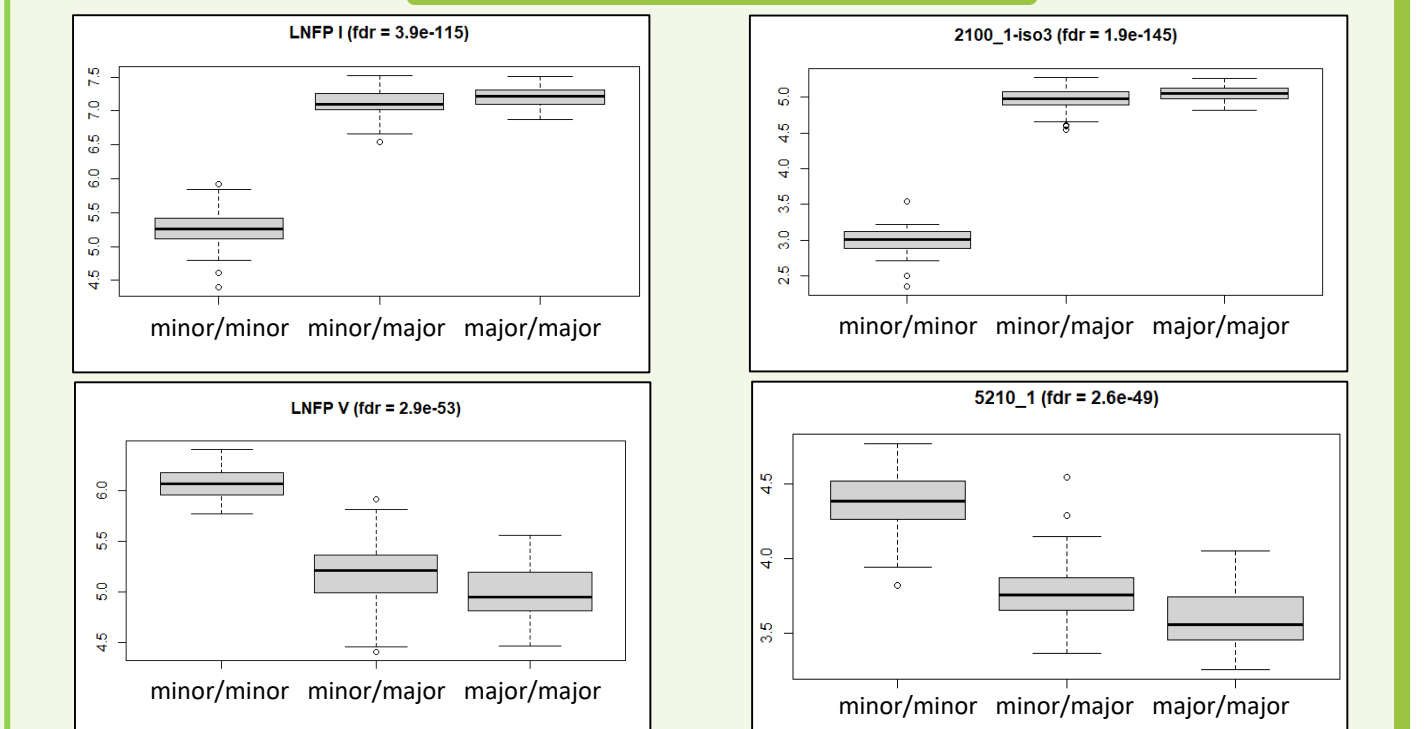
New impacted HMOs identified

- Association between FUT2/FUT3 polymorphisms and several HMOs (2'FL, LNFP I, LNFP II...)** already described in the literature (Lefebvre et al., 2020) **found back in this study.**
- But new potential HMOs with higher or similar association levels with FUT2/FUT3 polymorphisms were also identified.**

VolcanoPlots



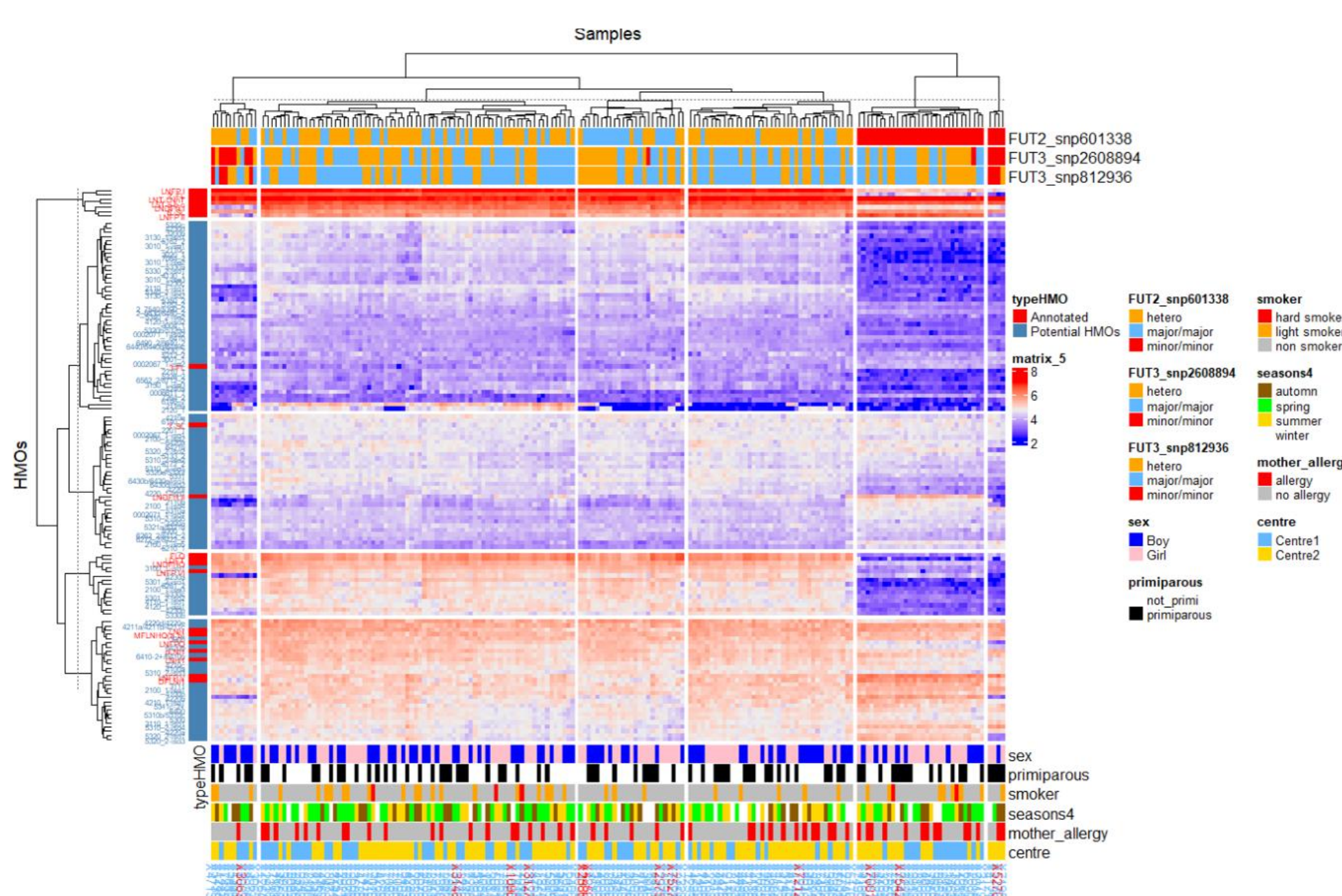
ANOVA results



HCA results & different HMOs patterns identified

- specific HMO composition associated to FUT2/FUT3 polymorphisms**

- other patterns identified to be deeper characterized**



Conclusion

- High impact of FUT2 & FUT3 polymorphisms on HMOs composition
- Results for 2 Lefebvre et al. SNPs confirmed and larger list of impacted HMOs identified
- Different clusters of HMOs identified

What's next?

- Deeper insight into different HMOs clusters (HMOs mother profiling?)
- Assessment of **influence of environmental factors** on milk HMO composition & association between **HMO composition and health outcomes in childhood**

References

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