Evaluation of biochemical algorithms to screen dysbetalipoproteinemia and *APOE* mutations

Louise Michenaud[1,a], Nathanaël Marrié[1,a], Oriane Marmontel[1,2], Sybil Charrière[2, 3], Charles Gibert[1], Caroline Bouveyron[1], Jade Maami [1], Philippe Moulin[2,3], Mathilde Di Filippo[1,2*]

[a]Louise Michenaud and Nathanaël Marrié contributed equally.

[1]Hospices Civils de Lyon, UF Dyslipidémies, Service de Biochimie et de Biologie Moléculaire, Laboratoire de Biologie Médicale MultiSites, F-69677 Bron, France ;
[2]CarMen Laboratory, INSERM, INRA, INSA Lyon, Université Claude Bernard Lyon 1, Pierre-Bénite, France ;
[3]Hospices Civils de Lyon, Fédération d'endocrinologie, maladies métaboliques, diabète et nutrition, Hôpital Louis Pradel, Bron, France.

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INTRODUCTION

- **Dysbetalipoproteinemia** (DBL) is a mixed dyslipidemia, which increases the risk of **atherosclerosis** and cardiovascular diseases
- DBL occurs mostly in subjects with an ***APOE* ε2ε2 haplotype**, but also in subjects with ***APOE* rare variants**
- Diagnosis gold standard criteria require **molecular diagnosis** or **serum ultracentrifugation** to assess the presence of remnants, which are **not available** in most laboratories
- **Several algorithms** using different lipid parameters and ratios have been proposed in the literature
- We developed and tested a **performant new algorithm** based on routine lipid parameters and ratios: the "**HCL algorithm**"

OBJECTIVE

To compare the diagnostic performances for DBL of our algorithm *versus* 8 algorithms from the literature

MATERIALS AND METHODS

Retrospective study
HCL local biochemical database
(2001 to 2019)

Exclusion criteria : Triglycerides > 10 mmol/L; Incomplete data; Particular genotypes

Patients with *APOE* genotyping
n = 184

- ***ε2/ ε2***, n = 49
- ***APOE mut***, n = 20
  Rare APOE variants ≠ *ε2/ ε2*
- **Control**, n = 115
  Dyslipidemic patients,
  non carriers of *APOE* mutations

Complete lipid profile

1 Comparison of screening algorithms for DBL in ***ε2/ ε2* cohort**

Comparison of the algorithms' Se, Sp, PPV, NPV — **Gold standard** : APOE pathological genotype + TG > 1.7 mmol/L

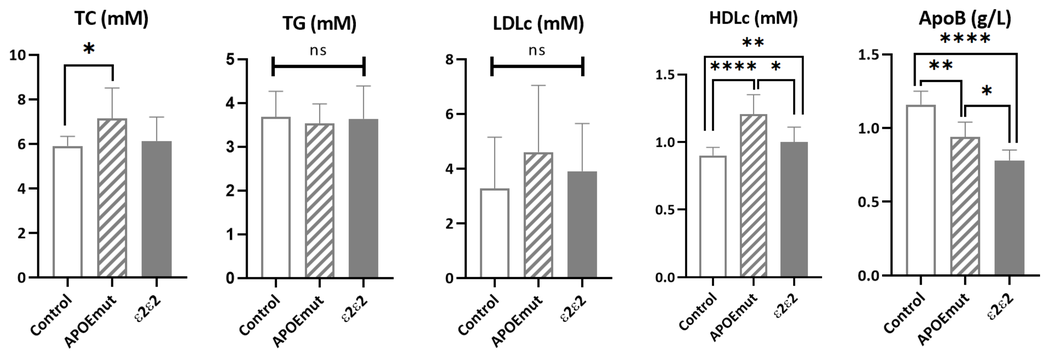
HCL	TG > 75th percentile	+ TC/ApoB > 6.58		
Fredrickson et al, 1975	1.7 < TG < 11.3	+ VLDLc/TG > 0.3		
Sniderman et al, 2007	TG > 75th percentile	+ TC/ApoB ≥ 6.2	+ TG/ApoB < 10	
De Graaf et al, 2008	TG > 1.50	+ TC/ApoB ≥ 6.2	+ TG/ApoB < 10	+ ApoB < 1.2
Adapted from Boot et al, 2019	TG > 1.50	+ TC > 5	+ NHDLc/ApoB ≥ 4.91	
Adapted from Paquette et al, 2020	TG > 2	+ TC ≥ 5.2	+ NHDLc/ApoB ≥ 3.69	
Sampson et al, 2022	1.7 < TG < 11.3	+ VLDLc/TG > 0.194 (obtained by Sampson formula)		
Enhanced Sampson, 2022	1.7 < TG < 11.3	+ VLDLc/TG > 0.209 (obtained by eSampson formula)		
Bea et al, 2023	TG > 1.7 or LDLc > 4.91 or NHDLc > 4.9	+ TG/ApoB ≥ 3.5	+ NHDLc/ApoB ≥ 4.4	

Units : TG (mmol/L); TC (mmol/L); ApoB (g/L); VLDLc/TG (mM/g); NHDLc (mmol/L); LDLc (mmol/L)

2 Comparison of the algorithms' performances in ***APOEmut* versus in *ε2/ ε2* cohort**

RESULTS

Comparison of **descriptive parameters** for **control vs *ε2/ ε2* *vs* *APOEmut* cohorts**



* : ***p<0.05 ; ** : p<0.01 ; *** : p<0.001 ; **** : p<0.0001 ; ns : non significant***

Comparison of the **algorithms' performances** in ***APOEmut* *versus* in *ε2/ ε2* cohort**

Algorithm	Se (*ε2/ε2* cohort)	Se (*APOEmut* cohort)	One-tailed p-value summary	Sp (*ε2/ε2* cohort)	Sp (*APOEmut* cohort)	One-tailed p-value summary
Fredrickson	0.57	0.47	Ns	**0.98**	**0.97**	Ns
HCL	**0.86**	0.88	Ns	**0.98**	**0.97**	Ns
Sniderman	**0.86**	**0.94**	*	0.92	0.92	Ns
De Graaf	0.82	0.76	Ns	0.95	0.94	Ns
Adapted from Boot	0.69	0.76	Ns	0.86	0.86	Ns
Adapted from Paquette	0.63	0.65	Ns	0.47	0.46	Ns
Sampson	0.45	0.47	Ns	0.77	0.75	Ns
eSampson	0.78	0.88	*	0.87	0.85	Ns
Bea	0.82	0.65	*	0.70	0.71	Ns

* : ***p<0.05*** *APOEmut* vs *ε2/ ε2*

DISCUSSION

In *ε2/ ε2* cohort

Best ratios
TC/apoB
NHDLc/ApoB
(AUC = 0.96)

HCL, Sniderman: De Graaf, Bea 0.82 — Se 0.86 — eSampson 0.78

HCL, Fredrickson: De Graaf 0.95 — Sp 0.98 — Sniderman 0.92

Best Se+Sp-1
HCL
(0.84)

- **No single criterion alone** is suitable for DBL diagnosis : need for complex algorithms with ratios
- Algorithms with **personnalized TG thresholds** (Sniderman's, HCL's) according to age and sex perform better than algorithms with generic thresholds
- Paquette's and Boot's algorithms were **modified** (to exclude ultracentrifugation or genotyping, as these techniques are not available in most laboratories). This may lower their performances in our study.

In *APOEmut* cohort

Sniderman: HCL, eSampson 0.88 — Se 0.94 — Boot, De Graaf 0.76

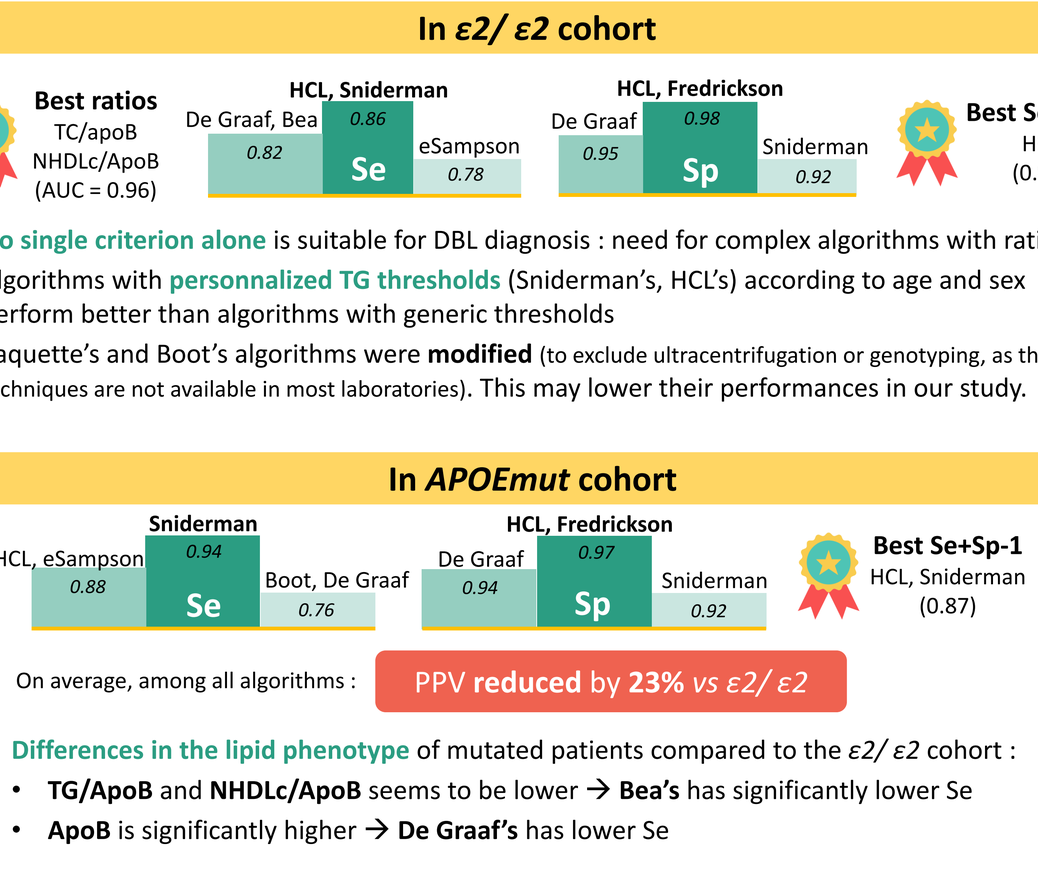
HCL, Fredrickson: De Graaf 0.94 — Sp 0.97 — Sniderman 0.92

Best Se+Sp-1
HCL, Sniderman
(0.87)

On average, among all algorithms : PPV **reduced** by **23%** *vs ε2/ ε2*

- **Differences in the lipid phenotype** of mutated patients compared to the *ε2/ ε2* cohort :
  - **TG/ApoB** and **NHDLc/ApoB** seems to be lower → **Bea's** has significantly lower Se
  - **ApoB** is significantly higher → **De Graaf's** has lower Se

Recommended workflow to effectively diagnose DBL



CONCLUSION

***APOE* genotyping, associated with dyslipidemia, should be the gold standard to definitively diagnose DBL and to identify carriers of *APOE* variants**

- **ApoB measurement** : **critical** in cases of mixed dyslipidemia
- **TC/ApoB ratio** : best for DBL detection, even if non-*ε2/ε2,* using the 6.58 mmol/g as **optimal threshold**
- It is essential to detect **both** *ε2/ε2* and **other mutations** forms of *APOE,* to consider genetic counseling

→ We propose a new workflow that offers a good and cost-effective DBL diagnosis as well as easy implementation in practice