INFLUENCE OF DELTA-AMINOLEVULINIC ACID DEHYDRATASE (ALAD) POLYMORPHISM ON RENAL TOXICITY OF LEAD IN WORKERS WITH PREVIOUS LEAD OCCUPATIONAL EXPOSURE

Ariane LEROYER, Catherine NISSE, Bruno LELEU, André KLEIN, Betty DEHON, Franck BROLY
Lille 2 University / Regional University Hospital Center, Lille, **FRANCE**

Cancun, ICOH 2012

Local context

- A foundry in the north of France, specialized in non-ferrous metal metallurgy
  - 1 pyrometallurgic smelter for lead
  - 1 pyrometallurgic smelter for lead and zinc
- The company ceased its activities in early 2003

Scientific context

- Major lead toxicity: neurological system, kidney, blood cells, cardiovascular system (blood pressure), probably carcinogene (2A IARC for inorganic lead compounds), …
- Renal toxicity
  - impairment of proximal tubular function (reversible)
  - chronic interstitial nephritis (irreversible)

Scientific context

- Lead has the specificy to decreases heme biosynthesis by inhibiting δ-aminolevulinic acid dehydratase (ALAD)
- ALAD polymorphism
  - Gene on chromosome 9q34 (Kelada et al. 2001)
  - G to C transversion at position 177 of the coding region of ALAD creates a variant allele ALAD 2 / wild-type allele = ALAD 1 (Wetmur et al. 1991)
  - Frequency of *ALAD 2* ≈ 10% in Caucasian populations (Kelada et al. 2001)
  - The ALAD enzyme is the principal lead-binding site in erythrocytes, and the ALAD 2 protein binds lead more tightly than does the ALAD 1 protein (Bergdahl et al. 1997)
  - This change alters the toxicokinetics of lead and may modify risk associated with lead exposure (Kelada et al. 2001)

Scientific context

- ALAD polymorphism and renal effects: 2 reviews at the early 2000
  - Onalaja et al. (2000): remain cautious…
    - publications suggesting ALAD-2 allele to be linked to an increase of renal effects of lead
    - publications suggesting ALAD-2 allele to be linked to a decrease of renal effects of lead
  - Kelada et al. (2001): ALAD-2 is associated with elevated BLL and an increase of toxic effects of lead on kidney, but only at very high levels of BLL

Aims of the study

- To investigate the impact of G177C δ-aminolevulinic acid dehydratase (ALAD) polymorphism (rs1800435) on the renal toxicity of lead
- To explore potential gene - environment interactions

Population

- Cross-sectional survey conducted 2008-2009
- Population of interest: cohort of ex-workers of the closed foundry
- Inclusion criteria
  - men
  - to sign the informed consent form

    ⇨ 615 potential participants
- Invitation to participate by post

Data gathering

- Questionnaire to collect
  - Socio-demographic characteristics
  - Occupational history
  - Other sources of lead exposure (domestic/leisure activities, smoking habits, food consumption, …)
- Blood samples for determination of
  - Lead (BLL)
  - urea, creatinine
  - ALAD G177C polymorphism
- Urinary samples for determination of
  - cadmium,
  - retinol-binding-protein (RBP),
  - N-acetyl-glucosaminidase (NAG) and its isoenzymes A and B

Statistical analysis

- Exploration of renal function:
  - 4 markers of renal function
    - urea
    - serum creatinine
    - creatinine clearance (formula of Cockcroft et Gault)

      clearance = [(140 – age) x weigth x S] / (7.2 x serum creatinine)

      (S=1.04 for men / S=0.85 for women)
    - estimated glomerular filtration rate (based on CKD-EPI equation of the « Chronic Kidney Disease Epidemiology Collaboration) », Levey et al. 2009 – using age, sex and serum creatinine)
  - 4 markers of proximal tubular dysfunction
    - RBP
    - NAG-A
    - NAG-B

Statistical analysis

- Lead exposure
  - Duration of occupational lead exposure (years)
  - BLL at the time of the study participation (2008-2009)
  - CBLI = Cumulated Blood Lead level Index* (in 2008-2009)
    - calculated with all the blood lead determinations realized between the 1rst occupational lead exposure and the study participation
    - formula:

$$CBLI = \int BLL \, . \, dt = \Sigma \, 0{,}5 \, (BLL_i + BLL_{i+1}) \, . \, \Delta t$$

* Fleming et al. (1997) – BLL_i and BLL_{i+1} = BLL respectively at the time i and (i+1)

Statistical analysis

- Exclusion of too diluted or too concentrated urines (urinary creatinine <0.3 or >3.0 g/L)
- Multiple linear regression models with interaction term were used, using the logarithm of each renal markers
  - Covariates : age, urinary Cd, duration since end of occupational lead exposure, smoking habits and medications which interfere with renal function + urinary creatinine for urinary markers
  - 3 models for each of the 8 renal biomarkers, function of the choice of lead exposure evaluation

Results: the studied population

615 postal invitations

→ 378 no responses

→ 33 refusals to participate

204 participants

- 32 to 66 years old at the time of inclusion (med=53)
- 68% 50 years old or more
- 57% manual workers
- 82% worked 20 years or more in the foundry

Lead exposure

- Cumulated duration of lead exposure:
  from 4 to 44 years (median 26 years)
- BLL at the time of the study:
  from 9 to 397 µg/L (med=137)
- CBLI: from 516 to 29736 µg/L x years
  (median 11809) (equiv. to 500 µg/L during 20 years)

ALAD polymorphism

- The frequency of ALAD-2 allele was 9.3%.
  - 34 subjects were heterozygotes (ALAD1-2) and 2 homozygotes (ALAD 2-2)
  - Comparisons: ALAD 1-1 vs ALAD 1-2 or ALAD2-2.

Renal functioning

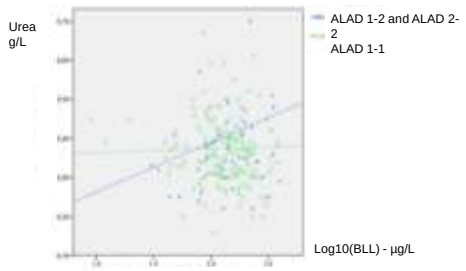
Most of the ex-workers have normal or slightly abnormal renal parameters:

- 11 (5%) have an elevated urea (>0.50 g/L)
- 3 (1,4%) an elevated serum creatinine (>14,0 mg/L)

	N	Min	P25	P50	P75	Max
Urea (g/L)	204	0.1	0.31	0.35	0.41	0.7
Serum creatinine (mg/L)	204	4.9	7	8	9.8	17.1
Clearance (mL/min)	204	47.3	102	128.3	157.5	327.1
eGRF (mL/min/1,73 m²)	204	42.6	86.8	101.6	110.1	133.7
NAG-A (UI/L)	187	1	2	2	3	11
NAG-B (UI/L)	187	2	7	11	16	61
Total-NAG (UI/L)	187	3	9	13	19,1	66

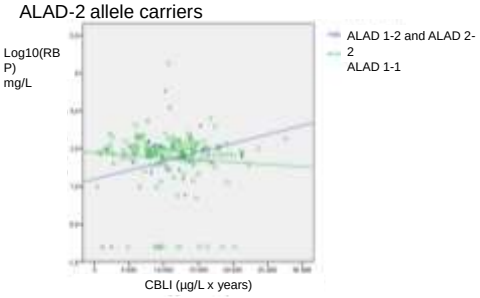
Renal toxicity

- Higher increase of blood urea with BLL at the date of the study (p = 0.06) for ALAD-2 allele carriers



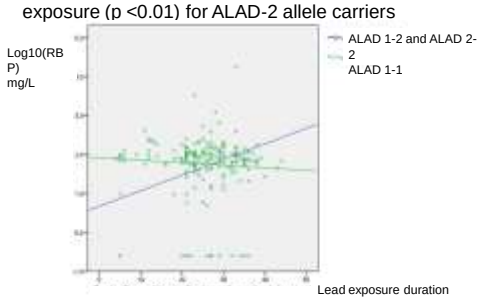
Renal toxicity

- Higher increase of urinary RBP with CBLI (p = 0.06) for ALAD-2 allele carriers



Renal toxicity

- Higher increase of urinary RBP with duration of lead exposure (p <0.01) for ALAD-2 allele carriers



Strengths and limits of the study

- Strengths
  - Long time of lead exposure (20 y. or more for 82%)
  - High levels of lead exposure
  - Large variability of lead exposure (to study dose-effect responses)
  - Past lead exposure: permit to distinguish reversible and irreversible renal effects (only constituted damages here)
  - Use of 4 markers of renal function / 4 of proximal tubular dysfunction
- Limits
  - Despite age and exposure, few observed renal damages (HWE)
  - Lack of statistical power (only 204 participants)

Conclusion

- These results are broadly consistent with those of the literature and reinforce the idea that the nephrotoxicity of lead may be influenced by ALAD polymorphisms
- Mechanism of ALAD polymorphism on renal effects remains unclear
- Other polymorphisms seem to modulate renal effects (VDR, eNOS, …) and explorations have to continue

Thank you for your attention!

- Thanks to
  - All participants
  - Study supported by:
    - ANSES
    - North – Pas-de-Calais Region