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)

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

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

Lead has the specificity 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)
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 weight x S] / (7.2 x serum creatinine)
    - 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 1st occupational lead exposure and the study participation
    - formula:
      \[ \text{CBLI} = \int \text{BLL} \cdot dt = \sum 0.5 \left( \text{BLL}_i + \text{BLL}_{i+1} \right) \cdot \Delta t \]
      Fleming et al. (1997) and \( \text{BLL}_{i+1} = \text{BLL} \) respectively at the time \( i \) and \( i+1 \)

Results: the studied population

- 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

615 postal invitations
- 378 no responses
- 33 refusals to participate
- 204 participants
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)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Min</th>
<th>P25</th>
<th>P50</th>
<th>P75</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (g/L)</td>
<td>204</td>
<td>0.1</td>
<td>0.31</td>
<td>0.35</td>
<td>0.41</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum creatinine (mg/L)</td>
<td>204</td>
<td>4.9</td>
<td>7</td>
<td>8</td>
<td>9.8</td>
<td>17.1</td>
</tr>
<tr>
<td>Clearance (mL/min)</td>
<td>204</td>
<td>47.3</td>
<td>102</td>
<td>128.3</td>
<td>157.5</td>
<td>327.1</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>204</td>
<td>42.6</td>
<td>86.8</td>
<td>101.6</td>
<td>110.1</td>
<td>133.7</td>
</tr>
<tr>
<td>NAG-A (UI/L)</td>
<td>187</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>NAG-B (UI/L)</td>
<td>187</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>16</td>
<td>61</td>
</tr>
<tr>
<td>Total-NAG (UI/L)</td>
<td>187</td>
<td>3</td>
<td>9</td>
<td>13</td>
<td>19.1</td>
<td>66</td>
</tr>
<tr>
<td>RBP (mg/L)</td>
<td>186</td>
<td>0.25</td>
<td>52</td>
<td>82</td>
<td>116</td>
<td>1780</td>
</tr>
</tbody>
</table>

Renal toxicity

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

- 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