Author's response to reviews

Title: On the association of metals and metalloids with kidney size and function in adult offspring of patients with Balkan endemic nephropathy. A two-year follow-up study.

Authors:

Wilfried Karmaus (karmaus@sc.edu)
Plamen Dimitrov (p.dimitrov@ncphp.government.bg)
Valeri Simeonov (p.dimitrov@ncphp.government.bg)
Svetla Tsolova (sv.tzolova@ncphp.government.bg)
Angel Bonev (p.dimitrov@ncphp.government.bg)
Rossitza Georgieva (r.georgieva@ncphp.government.bg)

Version: 2 Date: 18 November 2007

Author's response to reviews: see over
Point-by-point response to the Reviewer’s critique

We thank the reviewers for their insightful comments and suggestions. Their critiques have helped us to revise and improve the manuscript. Both reviewers acknowledged that the manuscript provides an important contribution to the field.

1. Reviewer's report
Title: Selenium may be adversely associated with kidney function and kidney length in adult offspring of parents with Balkan Endemic Nephropathy and controls. A two-year follow-up study.
Version: 1 Date: 7 August 2007
Reviewer: vesselin nenov

Reviewer's report:
Balkan nephropathy is a generalized proximal tubular disorder with associated Fanconi syndrome (see Nenov VD, Am J Nephrol, 2002), for which hyperuricosuria and hypouricemia are characteristic. Patients with lead nephropathy, on the contrary, typically have hyperuricemia and this is sufficient to rule out lead as a cause of Balkan nephropathy.

This study is important, because it demonstrates two important findings: first, that the offspring of patients with BEN who currently live in endemic areas are not exposed to toxic levels of lead, selenium, arsenic or cadmium. Second, from the age distribution of the studied BEN offspring (67.6% above 41 years of age) and not finding a single patient with renal failure among 102 subjects, who are offspring of BEN patients, this study serves to prove unequivocally that BEN is not a familial/genetic disease.

This issue is not addressed and not discussed in this paper. It is in part tackled in other publications of this ongoing project in Bulgaria (see below). However, an evaluation of familial and genetic influences cannot be conducted within the focus of this paper on metals and metalloid:

- Dimitrov PS, Simeonov VA, Tsolova SD, Bonev AG, Georgieva RB, Karmaus WJ. Increased blood pressure in adult offspring of families with Balkan endemic nephropathy: a prospective study. BMC Nephrol 2006;7:12.

The authors should place the emphasis on these two findings, both of which are very important, instead of emphasizing on a very weak relationship between selenium levels and some kidney parameters. If a parameter, such as selenium level, is weakly associated with kidney size and function to change them with only 1-2%, this cannot be called an "adverse effect on kidney function and size".

We revised the manuscript and substituted ‘adverse effects’ by more cautious expressions:
Page 2, Abstract, Result section: "weak but significant negative association with two of the four kidney parameters, namely creatinine clearance and $\beta_2$-microglobulin. It was positively related to kidney length."

Page 3, Abstract, Conclusion section: “may be a risk factor for renal effects”

Page 16, last paragraph: “Based on other findings, we expected that selenium would have a protective effect (hypothesis 1). However, we found three unexpected findings: Serum selenium was associated with increased $\beta_2$-microglobulin, with increased kidney length, and decreased CCR.”

Page 17, Conclusions: We deleted the part of the discussion of the selenium effects.

Furthermore, calculated statistical significance using complex statistical methods may have occurred by chance, especially with selenium, for which the average level was different between the two years of investigation (56.9 in 2003 and 72.1 in 2004).

As traditionally used in science, we has set chance occurrences of associations to 5% by setting the p-value to 0.05.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
2. Reviewer’s report
Title: Selenium may be adversely associated with kidney function and kidney length in adult offspring of parents with Balkan Endemic Nephropathy and controls. A two-year follow-up study.
Version: 1 Date: 26 August 2007
Reviewer: vecihi batuman
Reviewer’s report:
General
This is an important study evaluating the possible role of cadmium, lead, arsenic and selenium in Balkan Endemic Nephropathy (BEN). The study is well designed and examines in a longitudinal manner the role of these metals and metalloids in a well-defined population, 102 adult offspring of patients with an established diagnosis of BEN, a population clearly at risk of developing BEN. A control group of 99 patients, the adult offspring of hospitalized patients without BEN served as controls. The investigators evaluated blood levels of Pb and Se and urinary Cd and As along with creatinine clearance, kidney dimensions, blood pressure, urine total protein, and #2-microglobulin at two different time periods, in 2003-4, and 2004-5. Although the results of these investigations essentially are negative, i.e., without significant association between these metals/metalloids with possible exception of selenium and kidney parameters, the manuscript is significant because it is helpful in putting to rest the question whether environmental contaminants play a role in the pathogenesis of BEN.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The authors tested five hypotheses on the potential role of these metals/metalloids, and concluded that Cd, Pb, or As does not pose a risk for the development of BEN, nor that Se is protective. This is one of the best studies and the most recent one that have reached this conclusion, and is helpful in resting the metal/metalloid hypothesis and guiding investigators into different avenues in their search of the etiology of BEN. I am concerned however on the authors’ emphasis on Se, and their conclusion that Se may adversely affect kidney function. Although their statistical analysis supports this statement, a sweeping conclusion is premature, and does not deserve to be featured in the title. I therefore propose the authors temper their discussion of the Se findings using more cautious language on this apparently contradictory finding, and focus more on the important finding that Se is not protective. It would also be appropriate to delete reference to Se in the title and change it to something like," On the association of metals and metalloids with kidney size and function in the adult offspring of patients with Balkan endemic nephropathy. A two-year follow-up study."

We agree and accepted the proposed title. We also expressed our findings for Se with more caution. See Reviewer 1, point 2.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. In table 1, moderate hypertension is defined as systolic blood pressure \# 140 and/or diastolic \# 90 mm Hg. This obviously needs an upper limit and should probably be systolic 140-159 and/or diastolic 90-99, the definition of stage 1 hypertension by JNC-7.

   Thank you for this clarification. We added on page 11, 1st paragraph:
   “We defined hypertension (Table 1) according to the The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[42]”

2. Correlations with individual dimensions of kidney may not be meaningful. A better measure for kidney size is kidney volume calculated from kidney length, width, and depth using the ellipsoid volume formula. The investigators have kept these measurements; could the correlations with kidney size be recalculated using kidney volume?

   We agree that it would be preferable to estimate the kidney volume. However, we started this investigation in the Vratza hospital with two kidney ultra measurements: kidney length and minimal kidney cortex width. These were the measures, which were highly or sufficiently reproducible (see Table 3). We have electronically stored all kidney ultrasound pictures, but we cannot determine width and depth of the kidneys.

3. The glomerular filtration rates estimated from the 4-h urine collections are nearly 50% lower than the rates calculated using the Cockroft-Gault formula. Is there an explanation for this discrepancy? Also, by either measure, GFRs are significantly lower during the 2004/05 period compared to the 2003/04 period suggesting a trend. Such a trend, if real, would be of interest especially in the BEN offspring. Was the decline in GFR more marked in the BEN group compared with the controls?

   In most studies, CCR is adjusted for body surface area (for instance, mL/min/1.73m\(^2\)). We did not follow this rule, but attempted to adjusted body surface area in the model as predictor. This led to largely different values of CCR and the 4-hour GFR: CCR was nearly twice as large as the 4-hour glomerular filtration rate (4-hour GFR). We corrected this and directly adjusted for individual body surface area. Now the 4-hour GFR and the CCR are in a comparable range (Table 3).

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions
Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.