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### ► **To cite this version:**

Virginie Rondeau, Albert Iron, Luc Letenneur, Daniel Commenges, François Duchêne, et al.. Analysis of the effect of aluminum in drinking water and transferrin C2 allele on Alzheimer's disease.. Eur J Neurol, 2006, 13 (9), pp.1022-5. 10.1111/j.1468-1331.2006.01339.x . inserm-00138508

**HAL Id: inserm-00138508**

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Submitted on 27 Mar 2007

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## **Analysis of the effect of aluminum in drinking water and transferrin C2 allele on Alzheimer's disease.**

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*Key words:* aluminum, drinking water, transferrin, apolipoprotein E, Alzheimer's disease

*Word count* : 1797

## **Abstract**

Although highly controversial, the hypothesis of a link between aluminum in drinking water and Alzheimer's disease (AD) has been supported by several epidemiological studies. Transferrin (Tf) is a major transport protein for both iron and aluminum. Moreover, it has been demonstrated that defective binding of iron and aluminum to the transferrin variant C2 could be present in Alzheimer's disease. Individuals carrying the Tf C2 allele might therefore be at greater risk of developing AD. We investigated whether the transferrin C2 allele (Tf C2) might be responsible for susceptibility to Alzheimer's disease in a sample of 292 subjects (with 55 AD) aged 75 years and over from south west France, some exposed to high levels of aluminum in tap water (n=181 subjects) and others to low levels of aluminum (n=111 subjects). We also examined the combined genetic effects of Tf C2 and  $\epsilon$ 4 allele of apolipoprotein E gene (ApoE). Logistic regression analysis showed that neither Tf C2 itself nor its interaction with aluminum or with the  $\epsilon$ 4 allele of the ApoE were significantly associated with the risk of AD.

## **Introduction**

The etiology of Alzheimer's disease (AD) is complex and both environmental and genetic causes are involved. The  $\epsilon 4$  allele of apolipoprotein E gene (ApoE) is a major susceptibility factor for AD. The presence of one or two  $\epsilon 4$  alleles is associated with both familial and sporadic late-onset AD across all ages, and in both men and women (Saunders et al., 1993). Moreover, although highly controversial, it has been suggested that aluminum (Al) could play a role in AD (Exley et al., 2001; Rondeau et al., 2002). The hypothesis of a link between Al from drinking water and AD has been supported by several epidemiological studies (Martyn et al., 1989; Rondeau et al., 2000).

Disorders of iron or Al metabolism in the brain of patients with AD could explain the therapeutic effects of iron and Al chelator desferrioxamine for these patients (Savory et al., 1998; McLachlan et al., 1991). Transferrin (Tf) is a major transport protein for both iron and aluminum. The Tf protein has many variants, but C1 and C2 variants account for the majority of the population of all races (Kamboh & Ferrell, 1987). Farrar et al. (1990) have suggested that deficiency of aluminum binding to transferrin may increase the amount of unbound aluminum which could cross the blood brain barrier, thereby increasing the risk of neurotoxic effects. Individuals carrying the C2 allele could therefore be at greater risk of developing AD. In a case-control study, Namekata et al. (1997a) obtained a significantly higher C2 allele frequency in AD patients than in age-matched controls. More recently however Hussain et al. (2002) did not find a significant association between the C2 allele and AD. They obtained a significant excess of the C2 allele only in AD cases without  $\epsilon 4$

allele. On the contrary, Namateka et al. (1997a) suggested that the C2 allele increases the risk of late-onset AD and is highly associated with  $\epsilon$ 4-homozygous AD patients.

Following these contradictory results, we wanted to investigate whether carrying the common C1 and C2 alleles of the Tf gene and exposure to aluminum in tap water predisposes individuals to a greater risk of developing Alzheimer's disease. We also studied the combined genetic effects of Tf and  $\epsilon$ 4 allele of apolipoprotein E.

### **Subjects and methods**

The data come from the Paquid cohort (Dartigues et al., 1992), complemented by the ALMA+ cohort (*for aluminium – maladie d'Alzheimer*).

At baseline, in 1988-1989, the Paquid cohort included 3,777 people aged 65 years or over, living at home in one of the 75 randomized parishes of the administrative areas of Gironde or Dordogne in southwestern France. Subjects were randomly selected from electoral rolls and were followed up for 10 years. In brief, prevalent and incident cases of dementia were detected by a two-step procedure. First, all participants underwent a 1-hour home interview and a psychometric evaluation with a trained psychologist who systematically completed a standardized questionnaire designed to obtain criteria for dementia according to the DSM-III-R (McKhann et al., 1984). Second, subjects positive for these criteria were examined by a senior neurologist who confirmed the diagnosis and applied the NINCDS-ADRDA (Hachinski et al., 1975) criteria for AD. Subjects were then reevaluated with the same procedure as the baseline screening 1, 3, 5, 8 and 10 years after the initial visit.

At the same time as the 10 year follow-up of the Paquid cohort, another cohort of 400 subjects was randomly selected from electoral rolls (ALMA+). These subjects aged 75 years and over at entry lived at home in one of the 14 parishes of the administrative area of Dordogne in southwestern France with mean levels of aluminum in tap water greater than 50 microgram per liter, 5 parishes with levels of Al: [50-100[ and 9 parishes with levels of Al  $\geq 100\mu\text{g/l}$ . These subjects, seen in 1999, were expected to be comparable to the subjects seen at the 10 years of follow-up of the Paquid cohort.

For this study we analyzed a sub-sample of 292 subjects from the Paquid cohort (seen at the 10 years of follow-up) or from the new cohort, who volunteered to give a blood sample for ApoE and Tf genotyping, and for whom the data on aluminum in tap water were available. The subjects from the Paquid cohort and from the new cohort were similar in age (mean age=84.8 versus 82.6), sex (65.2% of women versus 61.6 % of women in the new cohort). There were slightly more educated persons in the Paquid cohort than in the new cohort (68.7% versus 57.1% of persons with at least a primary school diploma). The sample included 181 subjects exposed to high levels of aluminum (greater than 100 microgram/liter), living in 13 parishes of the administrative area of Dordogne and 111 subjects exposed to low levels of Al (lower than 100 microgram/liter) living in 25 parishes of the two administrative areas of Gironde and Dordogne, with rural and urban areas. This threshold of 100 microgram/liter has been used previously in the literature (Rondeau et al., 2000).

For each parish, we computed a weighted mean of all measurements of Al by using the results of chemical analyses of drinking water carried out by the sanitary administration between 1991 and 1994 for the Paquid cohort (Rondeau et al., 2000)

and between 1991 and 1997 for the new cohort. The measurement values were stable during the lag period 1994-1997.

Venous blood samples were collected on EDTA anticoagulant and frozen until leukocyte DNA extraction and subsequent genotyping of ApoE and Tf. As regards the determination of ApoE status, the 3 common alleles ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) were characterized by a PCR amplification of a specific 292 bp sequence of exon 4 of apo E gene followed by *Hha* I endonuclease restriction digestion of PCR products (Richard et al., 1994). The differentiation of C1 and C2 Tf alleles was obtained by *Bst*E II endonuclease restriction digestion of PCR amplification product of a specific sequence of 110 bp located in exon 15 of Tf gene (Namateka et al., 1997b). Subjects homozygous and heterozygous for ApoE  $\epsilon 4$  were combined and called  $\epsilon 4$  carriers; the other genotypes were called  $\epsilon 4$  non-carriers. The same was done for Tf C2 carriers.

To study the variables associated with Alzheimer's disease, we used a logistic regression model, with adjustment for some potential confounders : age at inclusion, gender, and educational level in two categories 1) no education or primary school (ages 6 to 12 years) without a diploma and 2) at least primary school with a diploma. Parameters were estimated with the SAS software (SAS/STAT computer program; SAS Institute, Inc., Cary, North Carolina).

## **Results**

A total of 292 subjects including 55 cases of AD were studied. The mean age of the participants was 83.5 (4.9) years, range 75.5 to 99.1.

The Tf C2 allele frequency among the controls (Table 1) was similar to that obtained by Namateka et al. (1997a) in Japan (22%) and slightly higher than that obtained in the North of England (12%) by Hussain et al. (2002). The frequency of Tf C2 polymorphism (Table 1) was not significantly different between AD and control patients although it was slightly higher in AD patients than in controls (25.9% vs. 20.0%,  $p=0.68$ ). Moreover, as regards the frequencies of the different Tf genotypes, there was no difference between the control group and the AD population.

The logistic regression analyses did not demonstrate an increased risk of AD associated with Tf C2 carriers ( $OR=0.95$ ,  $p=0.88$ ). We did not obtain a significant interaction between Tf C2 allelic polymorphism and aluminum exposure. The risks of different combinations are shown in Table 2.

In our sample, almost one out of five subjects was a  $\epsilon 4$  carrier (22.5% for the non-exposed subjects and 15.5% for the exposed subjects). The presence of ApoE  $\epsilon 4$  displayed a higher risk of AD ( $OR=3.97$ ,  $95\%CI=1.94-8.12$ ,  $p<0.001$ ). This association between ApoE  $\epsilon 4$  and AD was more pronounced for non-exposed subjects ( $OR=5.98$ ,  $95\%CI=2.13-16.84$ ,  $p<0.001$ ) than for those exposed to aluminum ( $OR=1.96/0.72=2.72$ ,  $95\%CI=0.99-7.43$ ,  $p<0.05$ ), (see Table 2). However the overall interaction between ApoE  $\epsilon 4$  and aluminum exposure was not significant ( $p=0.27$ ). We also studied the interaction between ApoE  $\epsilon 4$  and Tf C2 (results not shown); the overall interaction was not significant ( $p=0.84$ ). The effect of Tf C2 was neither significant for ApoE  $\epsilon 4$  carriers ( $OR=0.86$ ,  $95\%CI=0.26-2.81$ ,  $p=0.80$ ) nor for ApoE  $\epsilon 4$  non-carriers ( $OR=0.99$ ,  $95\% CI=0.45-2.23$ ,  $p=0.99$ ).

## **Discussion**



Our findings suggest that neither the Tf C2 allele, nor the combination Tf C2 / Al contribute to the development of Alzheimer's disease. These results could be attributed to a lack of power in the statistical analyses due to small sample size. To our knowledge, this is the only epidemiological study which addresses the issue of the interaction between aluminum in tap water and the influence of Tf C2 allele in AD.

A possible limitation of our study is whether the subcohort was a truly representative sample, as it was composed of people who accepted a blood sample procedure. However, the frequency of the individual genotypes observed in our sample were comparable with other published reports. The exposed subjects were included ten years after the non-exposed subjects; we chose to analyze the two samples jointly but the diagnosis of AD could be slightly different. The association of aluminum with AD could have been biased, but it is unlikely that a differential of AD diagnosis would have been associated with a differential in the Tf C2 carrier distribution in the two samples.

Namateka et al. (1997a) have found an excess of Tf C2 alleles in different AD populations. It has been suggested by Van Rensburg et al. (1993) that if the C2 allele promotes free radical production, then it may accelerate pathological damage caused as a result of the ApoE  $\epsilon$ 4 allele. The C2 allele may predispose ApoE  $\epsilon$ 4 homozygotes to AD. This has been confirmed by Namateka et al. (1997a) in Japan, who found that in AD patients homozygous for  $\epsilon$ 4, the C2 allele frequency was twice as high as that in the remaining AD groups, although Tf and ApoE genes are located on different chromosomes, viz., chromosomes 3 and 19, respectively. The results of Hussain et al. (2002) contrasted with these findings, they did not find a significant association between the frequency of the C2 allele and AD, but this association

became significant for subjects without  $\epsilon 4$  allele. We did not find any significant interaction between ApoE  $\epsilon 4$  allele and Tf C2. These contrasting results could be due to a lack of statistical power, but we should not exclude the fact that the previous results may have been obtained at random. It is also possible that the influence of the C2 allele varies in different populations. These aspects will be of particular interest for follow up studies.

A recent article of Exley (2004) has proposed a mechanism to explain the pro-oxidant activity of aluminum. If the effect of Tf C2 works also through its putative ability to generate free radicals, the diet of the subjects will have an effect on whether the presence of Tf C2 is a risk factor or not. We analyzed the interaction between the consumption of wine, which has an anti-oxidant activity, and the Tf C2 on our data. The results did not show a significant different effect of the Tf C2 for regular wine consumers compared to non-consumers.

The exact effect of transferrin and exposure to aluminum remains unclear and more studies need to be undertaken to elucidate this further and to lead to future preventive and therapeutic possibilities.

## **Acknowledgements**

This work was supported by the Agence de Bassin Seine-Normandie, Aluminum P echiney, ARMA (Institut du Cerveau), Association Recherche et Partage, AXA-AGIPI, Groupe Danone, Caisse Nationale d'Assurance Maladie des Travailleurs Salari es, Caisse Primaire d'Assurance Maladie de la Dordogne, CAPIMMEC, Conseil G en eral de la Dordogne, Conseil G en eral de la Gironde, Conseil R egional

d'Aquitaine, Caisse de Retraite Interentreprises, Direction Régionale des Affaires Sanitaires et Sociales d'Aquitaine, 2010 Média, Fondation de France, INSERM, M.G.E.N., Ministère de la Recherche et de la Technologie, M.S.A. de la Dordogne, M.S.A. de la Gironde, Novartis Pharma. We thank the Paquid staff for their collaboration in the study, and the DDASS Dordogne and DDASS Gironde who took part in the study by providing data on drinking water components. We gratefully acknowledge Guénaëlle Lancelot for technical assistance.

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## **TABLE LEGENDS**

**Table1 : Allele frequency of Tf C1 and C2 polymorphism in AD and non AD patients and exposure to aluminum in tap water.**

**Table2 : Odds ratios for Alzheimer's disease risk according to the interaction between Tf C2 and aluminum or according to the interaction between ApoE  $\epsilon$ 4 and aluminum exposure in tap water.**



**Table1 : Allele frequency of Tf C1 and C2 polymorphism in AD and non AD patients and exposure to aluminum in tap water.**

	Controls	AD patients
	(n=237)	(n=55)
	% (n)	% (n)
<b>Tf genotype</b>		
C1/C1	64.6 (153)	63.6 (35)
C1/C2	30.8 (73)	29.1 (16)
C2/C2	4.6 (11)	7.3 (4)
<b>Tf allele Frequencies</b>		
C1	80.0 %	74.1 %
C2	20.0 %	25.9 %

**Table2 : Odds ratios for Alzheimer’s disease risk according to the interaction between Tf C2 and aluminum or according to the interaction between ApoE ε4 and aluminum exposure in tap water.**

	OR <sup>a</sup>	95% CI <sup>a</sup>	P value
Tf C2+ / Al+ (11/56) <sup>b</sup>	0.55	0.22-1.38	0.20
Tf C2+ / Al- (9/28)	0.91	0.34-2.45	0.85
Tf C2- / Al+ (17/97)	0.55	0.24-1.25	0.16
Tf C2- / Al- (18/56)	Reference		
ApoE ε4+ <sup>c</sup>	3.97	1.94-8.12	<b>&lt;0.001</b>
Gender (women vs Men)	1.04	0.50-2.14	0.92
Age at inclusion	1.04	0.98-1.11	0.21
Educational level <sup>d</sup>	0.28	0.14-0.55	<b>&lt;0.001</b>
ApoE ε4+ / Al+ (8/20) <sup>b</sup>	1.96	0.66-5.78	0.22
ApoE ε4+ / Al- (13/12)	5.98	2.13-16.84	<b>&lt;0.001</b>
ApoE ε4- / Al+ (20/133)	0.72	0.33-1.59	0.42
ApoE ε4- / Al- (14/72)	Reference		
Tf C2+ <sup>c</sup>	0.97	0.50-1.90	0.93
Gender (women vs Men)	1.03	0.50-2.13	0.94
Age at inclusion	1.05	0.98-1.12	0.18
Educational level <sup>d</sup>	0.28	0.14-0.55	<b>&lt;0.001</b>

<sup>a</sup> OR, odds Ratio; CI, Confidence Interval

<sup>b</sup> Tf C2 +, Tf C2 carriers; Tf C2 -, Tf C2 non-carriers; Al+, Al ≥ 100 microgram per liter; Al-, Al < 100 microgram per liter; number of AD and control patients shown in brackets

<sup>c</sup> ApoE ε4+, ApoE ε4 carriers; ApoE ε4-, ApoE ε4 non-carriers; Al+, Al ≥ 100 microgram per liter; Al-, Al < 100 microgram per liter; number of AD and Control patients shown in parentheses

<sup>d</sup> primary school with diploma versus no education or primary school (ages 6 through 12 years) without diploma.