Hypertension, antihypertensives and mutations in the Von Hippel-Lindau gene in renal cell carcinoma: results from the Netherlands Cohort Study

Hypertension, antihypertensives and mutations in the *Von Hippel–Lindau* gene in renal cell carcinoma: results from the Netherlands Cohort Study

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Objectives **Hypertension and/or antihypertensive medication are reported to be risk factors of renal cell carcinoma (RCC). We investigated whether these risk factors are associated with *von Hippel-Lindau* gene (*VHL*) mutations in RCC.**

Methods **The Netherlands Cohort Study on Diet and Cancer (NLCS) started in 1986 ($n = 120\,852$ men and women) and uses the case–cohort methodology. After 11.3 years of follow-up, 337 RCC cases and 4774 subcohort members were available for analysis. DNA was isolated from paraffin-embedded tumour tissue for *VHL* analysis.**

Results **Cohort members who reported hypertension or use of antihypertensive medication had a slightly (non-significant) increased risk of RCC: rate ratios (RR) 1.22 [95% confidence interval (CI), 0.94–1.58] and 1.14 (95% CI, 0.85–1.52), respectively. RRs were adjusted for sex, age, body mass index (BMI) and cigarette smoking. Of the 235 patients for whom tumour tissue specimens were collected, 187 had a clear-cell RCC, of whom 114 had a *VHL* mutation. History of hypertension was associated with a non-significantly increased risk of clear-cell RCC with *VHL* mutations: RR = 1.34 (95% CI, 0.87–2.07), and was not associated with the risk of clear-cell RCC without *VHL* mutations; RR = 0.88 (95% CI, 0.51–1.53). Use of diuretics was associated with clear-cell RCC without *VHL* mutations; RR = 2.11 (95% CI, 1.16–3.83).**

Conclusions **In this study non-significantly increased risks for history of hypertension and use of antihypertensive medication with RCC were observed. The association with hypertension was stronger in RCC patients with *VHL* mutations, while there was a positive association of diuretics use and risk of RCC without *VHL* mutations.**
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Keywords: antihypertensive medication, cohort study, hypertension, renal cell carcinoma, The Netherlands, *VHL* mutations

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Introduction

Hypertension and use of diuretics or other antihypertensive medication have been found to be risk factors for renal cell carcinoma (RCC) in many epidemiological studies [1,2]. It is unclear, however, whether the increased risk is caused by hypertension itself, or by the use of antihypertensive medication. Some recent studies showed that diuretic medication was no longer a risk factor after controlling for the diagnosis of hypertension [3,4], suggesting that not medication but hypertension is a risk factor for RCC. Several prospective studies have studied the effect of hypertension on the risk of RCC [5–12], but few prospective cohort studies were also able to study the use of antihypertensive medication [8,11,12].

RCC is classified in different subtypes. The majority of RCC are of the clear-cell type (~80%); other subtypes are papillary RCC (10%), chromophobe RCC (5%), collecting-duct carcinoma (1%) and unclassified RCC (3–5%) [13]. Von Hippel–Lindau disease (VHL) is a rare inherited disorder associated with (amongst others) an increased risk for clear-cell RCC [14]. After the identification of the *VHL* gene on chromosome 3p25, it became evident that this gene is also involved in the development of sporadic clear-cell RCC. It is estimated that

approximately 75% of all sporadic clear-cell RCCs harbour bi-allelic *VHL* defects [15]. Two studies suggested that risk factors, such as occupational exposure to trichloroethylene and fruit consumption, are associated with mutations in the *VHL* gene in RCC [16,17].

Despite the fact that many studies have observed an association between the diagnosis of hypertension and/or use of antihypertensive medication and RCC risk, there is still much uncertainty with respect to the biological mechanism. Gago-Dominguez [18] suggested the 'lipid peroxidation' hypothesis as the underlying mechanism, but this remains to be proven. The different subtypes of RCC may have different aetiologies. The *VHL* gene is the main causative gene for sporadic clear-cell RCC [15]. Whether hypertension and/or use of antihypertensive medication are associated with clear-cell RCC, or more specifically with mutational status of the *VHL* gene, has not been investigated before. It is conceivable that these risk factors are associated with specific subtypes of RCC, or with mutational status of the *VHL* gene in clear-cell RCC.

We decided to study whether hypertension and use of antihypertensive medication were associated with risk of RCC, and more specifically with mutational status of the *VHL* gene in clear-cell RCC, within a large prospective cohort study.

Materials and methods

Subjects

The Netherlands Cohort Study on diet and cancer is a prospective cohort study, which started in September 1986. The study design has been reported in detail elsewhere [19]. Briefly, the cohort included 120 852 men and women, aged 55–69 years, at the beginning of the study. The study was designed as a case–cohort study, using all cases and a random sample of 5000 persons from the cohort (subcohort), who have been followed to estimate the accumulated person-years in the entire cohort [20]. Follow-up for incident cancer has been established by computerized record linkage with the Netherlands Cancer Registry (NCR) and PALGA, a national database of pathology reports. The method of record linkage to obtain information on cancer incidence has been described previously [21]. The completeness of cancer follow-up was estimated to be more than 96% [22]. From 1986 to 1997 (11.3 years follow-up) 355 kidney cancer cases [*International Classification of Diseases for Oncology*, version 3 (ICD-O-3): C64.9] were identified. Urothelial cell carcinomas were excluded and only histologically confirmed epithelial cancers were included (ICD-O: M8010-8119, 8140-8570), leaving 337 cases. All subcohort members who reported prevalent cancer (excluding skin cancer) at baseline were excluded from analyses (leaving 4774 subcohort members).

VHL mutation analysis

Paraffin blocks of tumours were collected from 51 pathology laboratories, the procedures have been described in detail elsewhere [23]. We were able to collect material for 251 cases. One experienced pathologist (C.A.H.K.) revised all haematoxylin and eosin (HE)-stained slides. The RCCs were classified according to the World Health Organization (WHO) Classification of Tumours, 2002 [24]. The protocol for DNA isolation and mutation analyses have been described previously [23]. Briefly, paraffin was removed with xylene and tumour DNA was extracted by salt precipitation. The entire gene was amplified using six primer sets, as described before [23]. Samples were first subjected to polymerase chain reaction–single-strand conformational polymorphism (PCR-SSCP) analysis, which was followed by direct sequencing in the case of aberrant or unclear results. Mutations were identified by visual inspection of sequences provided by the ABI basecaller (Applied Biosystems, Nieuwerkerk a.d. IJssel, The Netherlands). After revision and *VHL* gene mutation analyses, data were available for 235 cases [23].

Questionnaire

At baseline, all cohort members completed a mailed, self-administered questionnaire on dietary habits and other risk factors for cancer [25]. Participants were asked to report whether a physician had ever diagnosed 'high blood pressure' and at what age the diagnosis was made (in 5-year age groups from 'younger than 30 years', 30–34 years' to '65–69 years'). Duration since diagnosis was calculated by subtracting the midpoint age in the age group of diagnosis from the age at baseline and was categorized into three broad categories: 0–9 years, 10–19 years and $\geq$20 years.

Participants were also asked to report on use of any drugs that they used longer than 6 months, for what condition and in what calendar period. All drugs were classified into therapeutic groups using the Anatomical Therapeutic Chemical (ATC) classification of the WHO Collaborative Centre for Drug Statistical Methodology [26,27].

Data analysis

Differences between cases with and without collected tumour material were assessed by calculating Student *t*-tests and chi-squared tests. RRs for RCC were calculated for history of hypertension and use of antihypertensive medication. Case groups were defined as follows: total RCC (all histologically confirmed cases of RCC detected by linkage to cancer and pathology registries; $n = 337$); clear-cell RCC (classified as clear-cell RCC after pathological revision; $n = 187$); mutated clear-cell RCC (clear-cell RCC with a mutation in the *VHL* gene; $n = 114$) and wild-type clear-cell RCC (clear-cell RCC without a mutation in the *VHL* gene; $n = 73$). Confounders considered were age at baseline (years), sex, current

cigarette smoking (yes/no), cigarettes smoked (number/day), years of cigarette smoking (years), alcohol consumption (g/day), body mass index (BMI; kg/m^2), a history of diabetes mellitus (yes/no), a history of RCC in first-grade family (yes/no), non-occupational physical activity (<30, 30–60, 60–90, >90 min/day), occupational physical activity for men only (<8, 8–12, >8 kJ/min) and socio-economic status (SES) based on education. Those variables that were associated with diagnosis of hypertension (and/or use of antihypertensive medication), that were an independent risk factor of RCC and that changed the risk estimates for the association of hypertension (and/or the use of antihypertensive medication) and RCC more than 10% were included as confounders in multivariable analyses. Using these criteria, confounders entered in the analyses were age, sex, BMI, current cigarette smoking, number of cigarettes smoked per day, and number of years of cigarette smoking.

RRs and corresponding 95% confidence intervals (CI) for RCC were estimated using Cox proportional hazard models processed with the STATA statistical software package (STATA statistical software, Release 7; STATA Corporation, College Station, Texas, USA; 2001), after testing the proportional hazards assumption using scaled Schoenfeld residuals [28]. Standard errors were estimated using the robust Huber–White sandwich estimator to account for additional variance introduced by sampling person-time from the cohort [29]. To obtain *P* values for dose–response trends, ordinal exposure variables were fitted as continuous terms.

Results

Hypertension was reported somewhat more frequently among RCC cases than among subcohort members (29.4 versus 26.3%; Table 1). RCC cases also reported a slightly higher use of antihypertensive medication than

Table 1 Description of exposure variables and potential confounders in subcohort members (*N* = 4774), RCC cases (*N* = 337) and RCC cases with tissue blocks collected (*N* = 235), The Netherlands Cohort Study on Diet and Cancer (NLCS) 1986–1997

	Subcohort members (*N* = 4774) *n* (%)	RCC cases (*N* = 337) *n* (%)	RCC cases with collected tumour material (*N* = 235) *n* (%)
Hypertension and use of antihypertensive medication			
Diagnosis of hypertension			
No	3517 (73.7)	238 (70.6)	166 (70.6)
Yes	1257 (26.3)	99 (29.4)	69 (29.4)
Duration since diagnosis[a,b]			
0–9 years	664 (53.7)	56 (57.7)	35 (51.5)
10–19 years	367 (29.7)	27 (27.8)	24 (35.3)
20+ years	206 (16.7)	14 (14.4)	9 (13.2)
Ever use of hypertension medication			
No	3767 (78.9)	259 (76.9)	172 (73.2)
Yes	1007 (21.1)	78 (23.2)	63 (26.8)
Ever use of diuretics			
No	4238 (88.8)	298 (88.4)	206 (87.7)
Yes	536 (11.2)	39 (11.6)	29 (12.3)
Ever use of beta-blockers			
No	4203 (88.0)	287 (85.2)	195 (83.0)
Yes	571 (12.0)	50 (14.8)	40 (17.0)
Potentially confounding variables			
Age at baseline (years)[c]	61.4 (4.2)	61.9 (3.9)	62.0 (3.9)
BMI (kg/m^2)[c]	25.1 (3.1)	25.5 (3.0)	25.5 (2.9)
Current cigarette smoking[b]			
No	3397 (71.3)	213 (63.4)	155 (66.0)
Yes	1365 (28.7)	123 (36.6)	80 (34.0)
Number of cigarettes/day[c,d]	15.2 (10.2)	17.9 (12.3)	18.3 (12.4)
Years of smoking[c,d]	31.9 (12.3)	34.2 (12.2)	33.7 (12.1)
Alcohol consumption (g/day)[c]	10.4 (14.4)	11.5 (14.6)	10.6 (14.0)
Diagnosis of diabetes mellitus			
No	4588 (96.1)	326 (96.7)	226 (96.2)
Yes	186 (3.9)	11 (3.3)	9 (3.8)
Family history of RCC			
No	4716 (99.0)	332 (98.8)	232 (98.7)
Yes	47 (1.0)	4 (1.2)	3 (1.3)
Non-occupational physical activity[b]			
<30 min/day	1083 (23.2)	73 (22.1)	54 (23.4)
30–<60 min/day	1447 (30.9)	100 (30.2)	67 (29.0)
60–<90 min/day	939 (20.1)	63 (19.0)	47 (20.4)
≥90 min/day	1210 (25.9)	95 (28.7)	63 (27.3)
Social economic status[b]			
Primary school	1476 (31.0)	94 (28.0)	64 (27.2)
Lower vocational school	1036 (21.8)	78 (23.2)	57 (24.3)
Intermediate vocational school	1584 (33.3)	109 (32.4)	74 (31.5)
Higher vocational school or university	613 (12.9)	51 (15.2)	36 (15.3)

RCC, renal cell carcinoma; BMI, body mass index. [a]Only for persons who reported a diagnosis of hypertension. [b]Due to missing values totals do not add up to 4774, 337 and 235, respectively. [c]Mean (standard deviation). [d]Only for ever-smokers.

the subcohort members (23.2 versus 21.1%). RCC cases had a higher BMI, were more often current cigarette smokers and had been diagnosed less frequently with diabetes mellitus than subcohort members. When these variables were compared between patients with tumour material and patients without tumour material, no significant differences were observed. Only the percentage of antihypertensive medication use was significantly higher for cases with tumour tissue collected compared to cases for whom no tissue could be collected ($P = 0.02$).

Cohort members who reported a history of hypertension or use of antihypertensive medication at baseline, had a slightly increased risk of RCC: rate ratio (RR) 1.22 [95% confidence interval (95% CI), 0.94–1.58] and 1.14 (95% CI, 0.85–1.52), respectively (Table 2). These RRs (as all following RRs) were adjusted for sex, age, BMI, and cigarette smoking. There was no difference between men and women with respect to the association between history of hypertension and RCC risk (P for interaction = 0.99). A diagnosis of hypertension was associated with a RR of 1.21 (95% CI, 0.87–1.69) in men and a RR of 1.21 (95% CI, 0.80–1.84) in women. The RR for use of antihypertensive medication was also nearly the same in men and women (P for interaction = 0.92). With an increasing time interval between diagnosis of hypertension and baseline, hypertension was associated with slightly decreasing risks of RCC, with RRs of 1.27; 1.16 and 1.15, for time intervals of less than 10 years, 10–19 years, and 20 years or more, respectively.

Studying the interaction of the diagnosis of hypertension and antihypertensive medication use did not reveal divergent results. The RRs for use of diuretics or beta-blockers in cohort members, who had not reported a diagnosis of hypertension, were relatively high, but the number of cases and person-years in the subcohort was small (Table 2).

Repeated analysis with exclusion of the first 2 years of follow-up did not alter the results considerably: RRs were slightly lower (data not shown).

Of the 235 patients for whom tissue specimens were available, 187 had a clear-cell RCC (80%). In 114 patients with clear-cell RCC a mutation in the *VHL* gene was

Table 2 Age-adjusted and multivariable-adjusted rate ratios (RR) for total RCC ($N = 337$) according to hypertension and antihypertensive medication use; The Netherlands Cohort Study on Diet and Cancer (NLCS) 1986–1997

	Age- and sex-adjusted analyses			Multivariable-adjusted analyses		
Variable	*n* cases/person-years subcohort	RR[a]	95% CI	*n* cases/person-years subcohort	RR[b]	95% CI
Diagnosis of hypertension						
No	238/36943	1	Ref	210/33554	1	Ref
Yes	99/13054	1.24	0.97–1.59	90/12097	1.22	0.94–1.58
Time interval between diagnosis of hypertension and baseline						
No diagnosis of hypertension	238/36943	1	Ref	210/33554	1	Ref
0–9 years	56/6918	1.32	0.97–1.79	51/6365	1.27	0.92–1.76
10–19 years	27/3753	1.16	0.77–1.76	24/3459	1.16	0.74–1.81
20+ years	14/2181	1.06	0.61–1.86	14/2075	1.15	0.66–2.03
P for trend			0.44			0.71
Antihypertensive medication use						
No	259/39787	1	Ref	231/36237	1	Ref
Yes	78/10210	1.20	0.92–1.56	69/9415	1.14	0.85–1.52
Diuretic use						
No	298/44553	1	Ref	265/40649	1	Ref
Yes	39/5444	1.16	0.82–1.64	35/5003	1.14	0.79–1.66
Beta-blocker use						
No	287/44157	1	Ref	255/40256	1	Ref
Yes	50/5839	1.30	0.94–1.78	45/5394	1.27	0.90–1.78
Diagnosis of hypertension (Hyp) and antihypertensive medication use (Med)						
No Hyp/No Med	219/34310	1	Ref	194/31148	1	Ref
No Hyp/Yes Med	19/2633	1.06	0.65–1.73	16/2408	0.95	0.56–1.64
Yes Hyp/No Med	40/5478	1.19	0.83–1.68	37/5090	1.16	0.80–1.68
Yes Hyp/Yes Med	59/7576	1.29	0.95–1.75	53/7008	1.25	0.91–1.74
Diagnosis of hypertension (Hyp)and diuretic use (Med)						
No Hyp/No Med	230/36155	1	Ref	203/32837	1	Ref
No Hyp/Yes Med	8/788	1.69	0.80–3.57	7/717	1.54	0.68–3.46
Yes Hyp/No Med	68/8398	1.32	0.99–1.75	62/7812	1.28	0.95–1.73
Yes Hyp/Yes Med	31/4657	1.15	0.77–1.69	28/4286	1.15	0.76–1.75
Diagnosis of hypertension (Hyp) and beta-blocker use (Med)						
No Hyp/No Med	222/35348	1	Ref	196/32098	1	Ref
No Hyp/Yes Med	16/1595	1.41	0.82–2.41	14/1456	1.36	0.76–2.44
Yes Hyp/No Med	65/8811	1.23	0.92–1.64	59/8159	1.21	0.89–1.64
Yes Hyp/Yes Med	34/4243	1.33	0.91–1.95	31/3939	1.31	0.87–1.96

RCC, renal cell carcinoma; RR, rate ratio; CI, confidence interval; Ref, reference. [a]Rate ratio adjusted for age (years) and sex. [b]Rate ratio adjusted for age (years), sex, body mass index (kg/m^2), current cigarette smoking at baseline (yes versus no), number of cigarettes smoked per day (continuous) and years of cigarette smoking (continuous).

detected (61%). The RR for diagnosis of hypertension was higher in cases with a *VHL* mutation than in cases with *VHL* wild type: RRs 1.34 (95% CI, 0.87–2.07) and 0.88 (95% CI, 0.51–1.53), respectively (Table 3). By contrast, the RR for use of antihypertensive medication was higher in cases with *VHL* wild type: RR = 1.53 (95% CI, 0.89–2.61). The RR for use of diuretics was statistically significantly increased in cases with *VHL* wild type, RR = 2.11 (95% CI, 1.16–3.83).

The RR was also increased in cohort members who used diuretics or beta-blockers and did not report a diagnosis of hypertension but, as in total RCC, numbers of cases and subcohort person-years were very small and the confidence intervals were wide.

Discussion

In this study we observed that hypertension and use of anti-hypertensive medication were associated with a slightly increased, although statistically non-significant, risk of RCC. The association with hypertension was stronger in RCC patients with *VHL* mutations, while the association with use of antihypertensive medication was stronger in cases with *VHL* wild type. Diuretics were associated with an increased risk of clear-cell RCC with *VHL* wild type.

These results from the Netherlands Cohort Study on Diet and Cancer are most likely not affected by selection or information bias. Selection bias is unlikely given the high level of follow-up in terms of cases and subcohort person-years [22,30]. In theory, selection bias may have occurred in the collection of tissue samples [31]. For 235 of the 337 cases (70%), tumour material could be collected. There was no indication for bias in the selection of cases with tumour material according to the risk factors and potential confounders studied, except for the use of antihypertensive medication, which can be attributed most likely to chance. Information bias is unlikely in our study because the information with respect to the risk factors was collected before the diagnosis of RCC. Diagnosis of hypertension and use of antihypertensive medication were self-reported, however, and misclassification of exposure is a potential source of bias. In two studies conducted in the USA moderate agreement between self-report and actual measurement of blood pressure was observed; estimates of sensitivity for self-reported hypertension were between 62 and 82% [32] and 71% in the NHANES III study [33]. In an American validation study, sensitivity of recall for use of antihypertensive medication among controls was 86% after 2 years and 79% after 8 years [34]. In a small validation study (207 subjects) within our cohort study, use of medication for the cardiovascular system was recalled correctly by 66% of the users [26]. The subgroup of cases and subcohort members that reported use of antihypertensive medication, without reporting a diagnosis of hypertension, may indicate misclassification. Examining the indications for medication reported by the participants in the questionnaire at baseline revealed that only 11% of the persons in this group reported that the medication was used because of hypertension, suggesting that misclassification is limited. The misclassification of self-reported hypertension and use of antihypertensive medication is expected to be non-differential, attenuating the rate ratios towards one.

In a meta-analysis [2] based on 13 case–control studies, a pooled adjusted odds ratio of 1.75 (95% CI, 1.61–1.90) was calculated for the association between hypertension and RCC. This pooled odds ratio did not include results from prospective cohort studies. Four prospective cohort studies measured blood pressure at baseline and followed the cohort members for the occurrence of RCC [5,6,9,10]. All studies found increased risks of RCC with increasing blood pressure. The outcomes of these studies are difficult to compare with our study, because of the different exposure measurement. Three prospective cohort studies used self-reported diagnosis of hypertension to define the exposure [8,11,12] and show therefore the highest resemblance with our study design. Two of these studies reported increased RRs for diagnosis of hypertension and RCC risk [11,12]. These studies were relatively small, however, with 14 and 62 RCC cases, respectively. The Cancer Prevention Study II [8] included 1.2 million subjects and 335 RCC deaths after 7 years of follow-up. Self-reported diagnosis of hypertension was associated with RCC deaths in females (RR, 2.2; 95% CI, 1.5–3.2), but not in males (RR, 1.1; 95% CI, 0.9–1.5) [8]. The RRs for hypertension observed in the current study are lower than the pooled odds ratio for case–control studies [2], but our estimates point in the same direction. Also, several other studies have published RRs comparable to ours, especially for males [4,8, 35–37].

In another meta-analysis, a pooled odds ratio was calculated for use of diuretics and risk of RCC [1]. Based on nine case–control studies, an average odds ratio was calculated of 1.55 (95% CI, 1.42–1.71). Some of the included studies used self-report, while others used medical files or data from a pharmacy database [4,36,38,39]. Three prospective cohort studies also showed an increased risk for use of diuretics, with an exception for males in the Cancer Prevention Study II [7,8,11].

Hypertension may be a possible cause, but it may also be an early symptom of RCC [40]. The increased risk of hypertension may therefore reflect detection bias. Whether this bias is present may be evaluated by excluding cases from the analysis that were detected shortly after baseline measurement, or by investigating the relative risks according to time interval between diagnosis of hypertension and baseline. In our study, relative

Table 3 Multivariable adjusted rate ratios (RR) for RCC with *VHL* mutation status according to hypertension and antihypertensive medication use, The Netherlands Cohort Study on Diet and Cancer (NLCS) 1986–1997

	Clear-cell RCC			Clear-cell RCC with a mutation in the *VHL* gene			Clear-cell RCC, *VHL* wild type		
Variable	*n* cases/person-years subcohort	Multivariate adjusted RR[a]	95% CI	*n* cases/person-years subcohort	Multivariate adjusted RR	95% CI	*n* cases/person-years subcohort	Multivariate adjusted RR	95% CI
Diagnosis of hypertension									
No	117/33554	1	Ref	66/33554	1	Ref	51/33554	1	Ref
Yes	50/12097	1.14	0.81–1.61	33/12097	1.34	0.87–2.07	17/12097	0.88	0.51–1.53
Time interval between diagnosis of hypertension and baseline									
No hypertension	117/33554	1	Ref	66/33554	1	Ref	51/33554	1	Ref
0–9 years	24/6365	1.01	0.64–1.59	16/6365	1.20	0.68–2.12	8/6365	0.76	0.36–1.62
10–19 years	21/3459	1.72	1.05–2.80	14/3459	2.03	1.11–3.73	7/3459	1.30	0.58–2.94
20+ years	5/2075	0.69	0.28–1.72	3/2075	0.74	0.23–2.36	2/2075	0.64	0.16–2.64
P for trend			0.72			0.90			0.84
Antihypertensive medication use									
No	123/36237	1	Ref	75/36237	1	Ref	48/36237	1	Ref
Yes	44/9415	1.38	0.90–1.88	24/9415	1.15	0.70–1.90	20/9415	1.53	0.89–2.61
Diuretic use									
No	143/40649	1	Ref	89/40649	1	Ref	54/40649	1	Ref
Yes	24/5003	1.36	0.86–2.14	10/5003	0.91	0.45–1.81	14/5003	2.11	1.16–3.83
Beta-blocker use									
No	139/40257	1	Ref	82/40257	1	Ref	57/40257	1	Ref
Yes	28/5394	1.39	0.91–2.13	17/5394	1.43	0.82–2.47	11/5394	1.34	0.69–2.59
Diagnosis of hypertension (Hyp) and antihypertensive medication use (Med)									
No Hyp/No Med	103/31147	1	Ref	59/31147	1	Ref	44/31147	1	Ref
No Hyp/Yes Med	14/2407	1.53	0.85–2.78	7/2407	1.32	0.59–2.98	7/2407	1.82	0.78–4.22
Yes Hyp/No Med	20/5090	1.12	0.69–1.84	16/5090	1.57	0.90–2.75	4/5090	0.53	0.19–1.48
Yes Hyp/Yes Med	30/7008	1.25	0.81–1.92	17/7008	1.24	0.69–2.21	13/7008	1.26	0.68–2.34
Diagnosis of hypertension (Hyp) and diuretic use (Med)									
No Hyp/No Med	110/32837	1	Ref	63/32837	1	Ref	47/32837	1	Ref
No Hyp/Yes Med	7/717	2.60	1.13–5.99	3/717	1.98	0.60–6.59	4/717	3.37	1.11–10.26
Yes Hyp/No Med	33/7812	1.19	0.80–1.78	26/7812	1.64	1.03–2.61	7/7812	0.59	0.27–1.33
Yes Hyp/Yes Med	17/4286	1.19	0.70–2.04	7/4286	0.86	0.38–1.97	10/4286	1.64	0.83–3.25
Diagnosis of hypertension (Hyp) and beta-blocker use (Med)									
No Hyp/No Med	60/32098	1	Ref	60/32098	1	Ref	45/32098	1	Ref
No Hyp/Yes Med	6/1456	2.23	1.18–4.22	6/1456	1.91	0.80–4.56	6/1456	2.67	1.09–6.57
Yes Hyp/No Med	22/8159	1.23	0.83–1.83	22/8159	1.40	0.85–2.30	12/8159	1.00	0.53–1.90
Yes Hyp/Yes Med	11/3939	1.17	0.67–2.02	11/3939	1.41	0.71–2.77	5/3939	0.85	0.34–2.15

RCC, renal cell carcinoma; RR, rate ratio; CI, confidence interval; ref, reference. [a]All rate ratios are adjusted for age (years), sex, body mass index (kg/m^2), current cigarette smoking at baseline (yes versus no), number of cigarettes smoked per day (continuous) and years of cigarette smoking (continuous).

risks were slightly lower after exclusion of cases in the first 2 years of follow-up, and duration between diagnosis of hypertension and baseline was associated with slightly decreasing risks estimates. In a Danish record-linkage study [7] a U-shaped pattern was observed, with highest risks for the shortest and the longest time intervals. Other studies did not observe a modification of the risk estimates according to time interval [4,10,38,41,42].

Despite the large number of epidemiological studies, there is little convincing evidence with respect to the biological mechanism between hypertension or use of antihypertensive medication and the development of RCC. Gago-Dominguez *et al.* [18] suggested that lipid peroxidation, which is increased in obese and hypertensive individuals, might be responsible – at least in part – for the increased risk of RCC. By-products of lipid peroxidation have been shown to react with renal DNA to form adducts [18]. Diuretic therapy might be carcinogenic through conversion in the stomach to carcinogenic nitroso derivates or through a low-grade carcinogenic effect on the renal tubular cell, its principal target [1].

In our study, we also investigated whether hypertension and/or use of antihypertensive medication were associated with mutational status of the *VHL* gene. The *VHL* gene is a tumour suppressor gene. Loss of function is an early event in most cases of clear-cell RCC, by mutation or methylation of the promoter region. When hypertension and/or use of antihypertensive drugs are related to RCC risk, it is conceivable that these risk factors are associated with specific subtypes of RCC, e.g. clear-cell RCC with mutations in the *VHL* gene. In our analysis somewhat higher risks were found for hypertension in relation to the risk of clear-cell RCC with a mutation in the *VHL* gene, and slightly decreased risks in cases with *VHL* wild type. For use of antihypertensive medication and especially diuretic treatment, we observed the opposite; RRs were increased in cases with *VHL* wild type and only slightly increased in cases with mutations in the *VHL* gene. It is possible that diagnosis of hypertension and use of diuretics and risk of clear-cell RCC work through different pathways (through a mutation in the *VHL* gene or not). However, false-positive findings because of multiple testing and small numbers cannot be excluded.

This is the first study to evaluate the association of hypertension and/or use of antihypertensive medication with mutations in the *VHL* gene. Without a specific *a priori* hypothesis it is difficult to exclude chance findings, especially with the small numbers in the subanalyses. The findings with respect to the possible association with the *VHL* gene need to be confirmed in future studies.

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References

1 Grossman E, Messerli FH, Goldbourt U. Does diuretic therapy increase the risk of renal cell carcinoma? [editorial; see comments]. *Am J Cardiol* 1999; **83**:1090–1093.
2 Grossman E, Messerli FH, Boyko V, Goldbourt U. Is there an association between hypertension and cancer mortality? *Am J Med* 2002; **112**:479–486.
3 McLaughlin JK, Chow WH, Mandel JS, Mellemgaard A, McCredie M, Lindblad P, *et al.* International renal-cell cancer study. VIII. Role of diuretics, other anti-hypertensive medications and hypertension. *Int J Cancer* 1995; **63**:216–221.
4 Shapiro JA, Williams MA, Weiss NS, Stergachis A, LaCroix AZ, Barlow WE. Hypertension, antihypertensive medication use, and risk of renal cell carcinoma. *Am J Epidemiol* 1999; **149**:521–530.
5 Chow WH, Gridley G, Fraumeni JF Jr, Jarvholm B. Obesity, hypertension, and the risk of kidney cancer in men. *N Engl J Med* 2000; **343**:1305–1311.
6 Rosengren A, Himmelmann A, Wilhelmsen L, Branehog I, Wedel H. Hypertension and long-term cancer incidence and mortality among Swedish men. *J Hypertens* 1998; **16**:933–940.
7 Mellemgaard A, Moller H, Olsen JH. Diuretics may increase risk of renal cell carcinoma. *Cancer Causes Control* 1992; **3**:309–312.
8 Heath CW Jr, Lally CA, Calle EE, McLaughlin JK, Thun MJ. Hypertension, diuretics, and antihypertensive medications as possible risk factors for renal cell cancer. *Am J Epidemiol* 1997; **145**:607–613.
9 Grove JS, Nomura A, Severson RK, Stemmermann GN. The association of blood pressure with cancer incidence in a prospective study. *Am J Epidemiol* 1991; **134**:942–947.
10 Coughlin SS, Neaton JD, Randall B, Sengupta A. Predictors of mortality from kidney cancer in 332,547 men screened for the Multiple Risk Factor Intervention Trial. *Cancer* 1997; **79**:2171–2177.
11 Prineas RJ, Folsom AR, Zhang ZM, Sellers TA, Potter J. Nutrition and other risk factors for renal cell carcinoma in postmenopausal women. *Epidemiology* 1997; **8**:31–36.
12 Fraser GE, Phillips RL, Beeson WL. Hypertension, antihypertensive medication and risk of renal carcinoma in California Seventh-Day Adventists. *Int J Epidemiol* 1990; **19**:832–838.
13 Kovacs G, Akhtar M, Beckwith BJ, Bugert P, Cooper CS, Delahunt B, *et al.* The Heidelberg classification of renal cell tumours [editorial]. *J Pathol* 1997; **183**:131–133.
14 Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, *et al.* Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet* 1994; **7**:85–90.
15 Cohen HT. Advances in the molecular basis of renal neoplasia. *Curr Opin Nephrol Hypertens* 1999; **8**:325–331.
16 Brauch H, Weirich G, Hornauer MA, Storkel S, Wohl T, Bruning T. Trichloroethylene exposure and specific somatic mutations in patients with renal cell carcinoma. *J Natl Cancer Inst* 1999; **91**:854–861.
17 Hemminki K, Jiang Y, Ma X, Yang K, Egevad L, Lindblad P. Molecular epidemiology of VHL gene mutations in renal cell carcinoma patients: relation to dietary and other factors. *Carcinogenesis* 2002; **23**:809–815.
18 Gago-Dominguez M, Castelao JE, Yuan JM, Ross RK, Yu MC. Lipid peroxidation: a novel and unifying concept of the etiology of renal cell carcinoma (United States). *Cancer Causes Control* 2002; **13**:287–293.
19 Van den Brandt PA, Goldbohm RA, Van't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in The Netherlands. *J Clin Epidemiol* 1990; **43**:285–295.

20 Volovics A, van den Brandt PA. Methods for the analyses of case-cohort studies. *Biom J* 1997; **39**:159–214.
21 Van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. *Int J Epidemiol* 1990; **19**:553–558.
22 Goldbohm RA, Van den Brandt PA, Dorant E. Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. *Tijdschr Soc Gezondheidsz* 1994; **72**:80–84.
23 Van Houwelingen KP, van Dijk BA, Hulsbergen-van de Kaa CA, Schouten LJ, Gorissen HJ, Schalken JA, *et al.* Prevalence of von Hippel–Lindau gene mutations in sporadic renal cell carcinoma: results from The Netherlands cohort study. *BMC Cancer* 2005; **5**:57.
24 Eble J, Sauter G, Epstein J, Sesterhenn I. *World Health Organization classification of tumours. Pathology and genetics. Tumours of the urinary system and male genital organs*. Lyon: IARC Press; 2004.
25 Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F, Hermus RJ. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 1994; **48**:253–265.
26 Van den Brandt PA, Petri H, Dorant E, Goldbohm RA, Van de Crommert S. Comparison of questionnaire information and pharmacy data on drug use. *Pharm Weekbl (Sci)* 1991; **13**:91–96.
27 Anonymous. *About the ATC/DDD System*. Oslo, Norway: WHO Collaborating Centre for Drug Statistics Methodology; 2004.
28 Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika* 1982; **69**:239–241.
29 Lin DY, Wei LJ. The robust inference for the Cox Proportional Hazards Model. *J Am Stat Assoc* 1989; **84**:1074–1078.
30 Van den Brandt PA, van't Veer P, Goldbohm RA, Dorant E, Volovics A, Hermus RJ, Sturmans F. A prospective cohort study on dietary fat and the risk of postmenopausal breast cancer. *Cancer Res* 1993; **53**:75–82.
31 Hoppin JA, Tolbert PE, Taylor JA, Schroeder JC, Holly EA. Potential for selection bias with tumor tissue retrieval in molecular epidemiology studies. *Ann Epidemiol* 2002; **12**:1–6.
32 Giles WH, Croft JB, Keenan NL, Lane MJ, Wheeler FC. The validity of self-reported hypertension and correlates of hypertension awareness among blacks and whites within the stroke belt. *Am J Prev Med* 1995; **11**:163–169.
33 Vargas CM, Burt VL, Gillum RF, Pamuk ER. Validity of self-reported hypertension in the National Health and Nutrition Examination Survey III, 1988–1991. *Prev Med* 1997; **26**:678–685.
34 Boudreau DM, Daling JR, Malone KE, Gardner JS, Blough DK, Heckbert SR. A validation study of patient interview data and pharmacy records for antihypertensive, statin, and antidepressant medication use among older women. *Am J Epidemiol* 2004; **159**:308–317.
35 Kreiger N, Marrett LD, Dodds L, Hilditch S, Darlington GA. Risk factors for renal cell carcinoma: results of a population-based case-control study. *Cancer Causes Control* 1993; **4**:101–110.
36 Weinmann S, Glass AG, Weiss NS, Psaty BM, Siscovick DS, White E. Use of diuretics and other antihypertensive medications in relation to the risk of renal cell cancer. *Am J Epidemiol* 1994; **140**:792–804.
37 Chow WH, McLaughlin JK, Mandel JS, Wacholder S, Niwa S, Fraumeni JF. Jr. Risk of renal cell cancer in relation to diuretics, antihypertensive drugs, and hypertension. *Cancer Epidemiol Biomarkers Prev* 1995; **4**:327–331.
38 Finkle WD, McLaughlin JK, Rasgon SA, Yeoh HH, Low JE. Increased risk of renal cell cancer among women using diuretics in the United States. *Cancer Causes Control* 1993; **4**:555–558.
39 Hiatt RA, Tolan K, Quesenberry CP. Jr. Renal cell carcinoma and thiazide use: a historical, case-control study (California, USA). *Cancer Causes Control* 1994; **5**:319–325.
40 Novick AC, Campbell SC. 75. Renal tumors. In: Walsh PC, Retik AB, Darracott Vaughan E Jr, Wein AJ, Kavoussi LR, Novick AC, *et al.*, editors. *Campbell's urology*. 8th ed. Philadelphia: Saunders; 2002. pp.2672–2719.
41 Yuan JM, Castelao JE, Gago Dominguez M, Ross RK, Yu MC. Hypertension, obesity and their medications in relation to renal cell carcinoma. *Br J Cancer* 1998; **77**:1508–1513.
42 Rosenberg L, Rao RS, Palmer JR, Strom BL, Stolley PD, Zauber AG, *et al.* Calcium channel blockers and the risk of cancer. *JAMA* 1998; **279**:1000–1004.