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# Glutathione peroxidase-1 gene (*GPX1*) variants, oxidative stress and risk of kidney complications in people with type 1 diabetes

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ABSTRACT

*Background and Aim.* Glutathione peroxidase (GPX) is a class of antioxidant enzymes that catalyze the reduction of hydrogen peroxide to water. GPX1 is the most abundant isoform and is expressed in all kidney cells. Isoprostane and advanced oxidation protein products (AOPP) were identified as markers of oxidative stress in patients with kidney disease. We investigated associations of GPX1 genotypes with kidney complications, and with plasma concentrations of isoprostane and AOPP in type 1 diabetic patients.

*Methods.* Four SNPs in the *GPX1* gene region were genotyped in SURGENE (n = 340; 10-year follow-up); GENEDIAB (n = 461) and GENESIS (n = 584) cohorts of type 1 diabetic patients. Subsets of GENEDIAB (n = 237) and GENESIS (n = 466) participants were followed

*Abbreviations:* ACE, angiotensin converting enzyme; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AOPP, advanced oxidation protein products; CI, confidence intervals; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GENEDIAB, "Génétique de la Néphropathie Diabétique" Study; GPX, glutathione peroxidase; HR, hazard ratio; MAF, minor allele frequency; MDRD, "Modification of Diet in Renal Disease" formula; OR, odds ratio; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; SURGENE, "Survival Genetic Nephropathy" Study; UAC, urinary albumin concentration; UTR, untranslated region.

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up for 9 and 5 years, respectively. Plasma concentrations of isoprostane and AOPP were measured at baseline in GENEDIAB. Hazard ratios (HR) were estimated for incidence of kidney complications.

*Results*. In SURGENE, 98 renal events (new cases of microalbuminuria or progression to more severe stage of diabetic nephropathy) occurred during follow-up. The minor T-allele of rs3448 was associated with the incidence of renal events (HR 1.81, 95% CI 1.16–2.84, p = 0.008). In GENESIS/GENEDIAB pooled study, end stage renal disease (ESRD) occurred during follow-up in 52 individuals. The same variant was associated with the incidence of ESRD (HR 3.34, 95% CI, 1.69–6.98, p = 0.0004). The variant was also associated with higher plasma isoprostane concentration in GENEDIAB cohort: 2.02 ± 0.12 (TT + CT) vs 1.75 ± 0.13 (CC) ng/mL (p = 0.009), and with higher plasma AOPP in the subset of participants with the baseline history of ESRD (TT + CT 67 ± 6 vs CC 48 ± 6 μmol/L, p = 0.006).

*Conclusions*. The minor T-allele of rs3448 was associated with kidney complications (incidences of microalbuminuria, renal events and ESRD) in patients with type 1 diabetes. The risk allele was associated with higher plasma concentrations of isoprostane and AOPP. Our results are consistent with the implication of GPX1 in the mechanism of renal protection against oxidative stress in type 1 diabetic patients.



## 1. Introduction

Diabetic nephropathy is a frequent complication in people with diabetes and is a leading cause of end stage renal disease (ESRD) [1]. Data from the Centers for Disease Control and Prevention (CDC) estimate that diabetes might be the primary cause of disease in ~40% of the patients with new-onset kidney failure in the United States [2].

Oxidative stress induced by hyperglycemia is an early molecular mechanism in the pathogenesis of diabetic kidney complications [3,4]. Oxidative stress leads to increased oxidation of proteins, lipids, carbohydrates and DNA that results in tissue and organ damage. Advanced oxidation protein products (AOPP) and isoprostane (8-iso-prostaglandin), a product of arachidonic acid metabolism derived from the oxidation of polyunsaturated fatty acids, were identified as markers of oxidative stress in patients with kidney diseases [5,6]. Circulating and tissue antioxidant enzymes play a major role in the defense mechanisms against oxidative stress [7]. The glutathione peroxidases (GPX) are a family of antioxidant enzymes that catalyze the reduction of hydrogen peroxide or of lipid hydroperoxides to water or lipid alcohols, respectively, using glutathione as an electron donor [7]. Hydrogen peroxide and lipid hydroperoxides can react with metals, such as $Fe^{2+}$ and $Cu^{2+}$, to form hydroxyl radical ($^{\cdot}HO$), the most reactive of the reactive oxygen species (ROS). Eight GPX isoforms have been described [8–12]. GPX1, the most abundant isoform, is an intracellular selenoprotein expressed in most tissues and presenting with high expression levels in erythrocytes, liver and kidneys [13].

Genetic factors modulate the susceptibility to diabetic complications [14–17]. We have previously observed associations with diabetic nephropathy of allelic variations in several genes related to cellular redox status [18–20], including the genes encoding GPX4 [21] and catalase [22], enzymes that like GPX1 catalyze the reduction of hydrogen peroxide. In the present study we investigated associations of a set of single nucleotide polymorphisms (SNPs) in the *GPX1* locus with the risk of kidney complications in three prospective cohorts of type 1 diabetic patients. Genotype correlations with circulating levels of isoprostane and AOPP were also studied.

## 2. Methods

### 2.1. Participants

We studied three cohorts designed to evaluate the genetic components of diabetic nephropathy (Table 1). The "Survival Genetic Nephropathy" (SURGENE) was a prospective study conducted in 340 people with type 1 diabetes, recruited in the outpatient clinic of the University Hospital of Angers, France [23]. The prevalence of incipient, established or advanced diabetic nephropathy at baseline was 17.6% (n = 60). Mean duration of follow-up was 10 ± 3 years. Main outcomes during follow-up were the incidence of incipient nephropathy (new cases of persistent microalbuminuria in consecutive biannual assessments) and the incidence of renal events, defined as a new case of microalbuminuria or the progression to a more severe stage of nephropathy.

The "Génétique de la Néphropathie Diabétique" (GENEDIAB) and GENESIS are multicenter binational (Belgium and France) cohorts. GENEDIAB was conducted in people with type 1 diabetes and pre-proliferative or proliferative retinopathy [24]. GENESIS was a family-based study including probands with type 1 diabetes and non-proliferative or proliferative retinopathy [25]. In the present investigation we studied 461 participants from GENEDIAB and 584 from GENESIS from whom DNA samples were available at baseline. Incipient, established or advanced diabetic nephropathy was present in 306 participants from GENEDIAB (66%) and 299 participants from GENESIS (51%) at baseline. In a prospective observational study, subsets of GENEDIAB (n = 237) and GENESIS (n = 466) participants were followed up for 9 ± 3 and 5 ± 2 years, respectively. The subsets were composed of participants who attended outpatient clinics at least once during the follow-up period. The main outcome during follow-up was the incidence of ESRD, defined as the new cases of renal replacement therapy (hemodialysis or kidney