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ORIGINAL ARTICLE

AQP1 Promoter Variant, Water Transport, and Outcomes in Peritoneal Dialysis

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ABSTRACT

BACKGROUND

Variability in ultrafiltration influences prescriptions and outcomes in patients with kidney failure who are treated with peritoneal dialysis. Variants in *AQP1*, the gene that encodes the archetypal water channel aquaporin-1, may contribute to that variability.

METHODS

We gathered clinical and genetic data from 1851 patients treated with peritoneal dialysis in seven cohorts to determine whether *AQP1* variants were associated with peritoneal ultrafiltration and with a risk of the composite of death or technique failure (i.e., transfer to hemodialysis). We performed studies in cells, mouse models, and samples obtained from humans to characterize an *AQP1* variant and investigate mitigation strategies.

RESULTS

The common *AQP1* promoter variant rs2075574 was associated with peritoneal ultrafiltration. Carriers of the TT genotype at rs2075574 (10 to 16% of patients) had a lower mean (\pm SD) net ultrafiltration level than carriers of the CC genotype (35 to 47% of patients), both in the discovery phase (506 \pm 237 ml vs. 626 \pm 283 ml, $P=0.007$) and in the validation phase (368 \pm 603 ml vs. 563 \pm 641 ml, $P=0.003$). After a mean follow-up of 944 days, 139 of 898 patients (15%) had died and 280 (31%) had been transferred to hemodialysis. TT carriers had a higher risk of the composite of death or technique failure than CC carriers (adjusted hazard ratio, 1.70; 95% confidence interval [CI], 1.24 to 2.33; $P=0.001$), as well as a higher risk of death from any cause (24% vs. 15%, $P=0.03$). In mechanistic studies, the rs2075574 risk variant was associated with decreases in *AQP1* promoter activity, aquaporin-1 expression, and glucose-driven osmotic water transport. The use of a colloid osmotic agent mitigated the effects of the risk variant.

CONCLUSIONS

A common variant in *AQP1* was associated with decreased ultrafiltration and an increased risk of death or technique failure among patients treated with peritoneal dialysis. (Funded by the Swiss National Science Foundation and others.)

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PERITONEAL DIALYSIS IS THE LEADING form of home-based dialysis therapy for patients with kidney failure. The advantages of peritoneal dialysis, as compared with hemodialysis, include ease of use, a decreased need for trained medical staff and technical support, and accessibility in remote and rural locations. Over the past decade, the use of peritoneal dialysis increased dramatically in many parts of the world, including the United States and developing countries.^{1,2} The efficiency of peritoneal dialysis depends on ultrafiltration, which is the ability to remove excess water to restore normal body-fluid status, as well as to clear waste substances.¹ Among patients starting treatment with peritoneal dialysis, there is broad variability in water and solute transport across the peritoneal membrane, which influences dialysis prescriptions and outcomes.³⁻⁶ The factors involved in this baseline variability are poorly defined,^{7,8} and thus strategies to reduce complications and improve outcomes in patients treated with peritoneal dialysis are limited. A recent genomewide association study showed that the peritoneal small-solute transport rate was associated with a polygenic risk score and with 17% heritability; these findings support a genetic influence on solute transport across the peritoneal membrane.⁹ The translation of genetic and molecular insights to precision medicine would help to fill an important need in the understanding of dialysis and dialysis care.^{9,10}

Aquaporin-1 is the archetype of a family of water channels that facilitate water transport across cell membranes.¹¹ It was first identified in erythrocytes¹² and is abundantly expressed in endothelial cells that line peritoneal capillaries.¹³ Studies in *Aqp1*-knockout mice showed that aquaporin-1 is the transcellular pore located in the microvascular endothelium, mediating fast osmotic water transport and up to half the ultrafiltration during peritoneal dialysis performed with hypertonic glucose dialysate.¹⁴⁻¹⁶ Studies involving persons with the extremely rare null variant in *AQP1* (the gene that encodes aquaporin-1), who lack the Colton blood group, showed decreased fluid transport in the lung and defective urinary concentrating ability that was attributed to impaired water transport in the vasa recta of the kidney.^{17,18} Together, these studies suggest that variation in *AQP1* may influence water transport and outcomes in patients treated with peritoneal dialysis.

We gathered clinical and genetic information from 1851 patients in seven cohorts to determine whether variants in *AQP1* were associated with ultrafiltration and outcomes in peritoneal dialysis. Studies in cells, mouse models, and samples obtained from humans were performed to substantiate the functional relevance of the variants and to develop strategies that may ultimately mitigate the deleterious effects of *AQP1* variation in patients treated with peritoneal dialysis.

METHODS

STUDY POPULATION

The study included persons with kidney failure who were treated with peritoneal dialysis. Patients were recruited from seven cohorts in Belgium, the Netherlands (the AMC [Amsterdam University Medical Center] cohort and the NECOSAD [Netherlands Cooperative Study on the Adequacy of Dialysis] cohort), Spain, the United Kingdom (the Stoke-on-Trent cohort and the PD-CRAFT [Peritoneal Dialysis Competitive Risk Analysis for Long-Term Outcomes] study cohort), and China. Patients were required to have available DNA samples and information about peritoneal water transport or outcomes or both; details are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. Demographic and clinical characteristics were obtained from patient charts, and primary kidney diseases were classified according to the European Renal Association code. Residual urine volume was defined as a daily urine output of 200 ml or more. Patients were followed until they died, were transferred to hemodialysis, underwent kidney transplantation, or were withdrawn from dialysis or until the end of the follow-up period. Information about missing data is provided in Table S1 in the Supplementary Appendix. All patients provided written informed consent.

STUDY DESIGN AND OVERSIGHT

The study was conducted in accordance with the Declaration of Helsinki and the appropriate laws related to experiments in humans and data protection. The study was approved by the institutional review board at Cliniques Universitaires Saint-Luc, Brussels; the National Research Ethics Service Committee of North West Central Liverpool, Liverpool, United Kingdom; the Clinical Research Ethical Committee, Hospital Universi-



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tari Arnau de Vilanova, Lleida, Catalonia, Spain; NECOSAD, Amsterdam; and the institutional review board at the Shanghai Jiao Tong University School of Medicine and Renji Hospital, Shanghai, China.

The study was designed by the last author, in consultation with other authors. All the authors contributed to the collection and analysis of the data and vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol. The first, second, and last authors wrote the manuscript, which was reviewed and approved by all the authors.

GENOTYPING

Variants with a minor allele frequency greater than 10% (rs28362687, rs2075574, rs10253374, and rs1049305), covering the major linkage disequilibrium blocks over *AQP1*, were genotyped (Fig. S1). Genomic DNA was extracted from peripheral-blood leukocytes (Puregene, Gentra Systems) and analyzed with a competitive allele-specific polymerase-chain-reaction assay (LGC Group). Estimates of linkage disequilibrium between the *AQP1* variants were obtained in the CoLaus study cohort, Lausanne, Switzerland (not included in the study population).¹⁹

PERITONEAL TRANSPORT TESTING

Peritoneal transport was assessed with the use of a standardized 2.27% or 3.86% glucose (i.e., 2.5% or 4.25% dextrose)-based peritoneal equilibration test.^{3,7,20} Data from 3.86% glucose-based peritoneal equilibration tests were used to assess for an association with *AQP1* variants, because this test accurately evaluates net ultrafiltration and sodium sieving, which is a reliable surrogate for free-water transport.²¹ Net ultrafiltration was defined as the difference between the volume drained at 4 hours and the volume infused at the start of the test. Sodium sieving was defined as the ratio of the difference between the sodium level in dialysate at the beginning of the dwell and the level at 1 hour to the sodium level in plasma with a correction for sodium diffusion.²² The peritoneal solute transfer rate was defined as the ratio of the creatinine level in dialysate to the creatinine level in plasma at 4 hours. In a subgroup of patients from the Belgian cohort, the association between the rs2075574 variant and ultrafiltration induced by 3.86% glucose dialysate (crystalloid agent) was compared with the association between the

rs2075574 variant and ultrafiltration induced by 7.5% icodextrin (colloid agent).

STATISTICAL ANALYSIS

Comparisons of means between groups that were defined according to genotype were performed with the use of unpaired t-tests, chi-square tests, or one-way analysis of variance followed by testing for multiple comparisons, as appropriate. Binary dependent variables were analyzed by means of multivariate logistic-regression analyses, and continuous dependent variables by means of ordinary least-squares regression analyses. Multivariate analyses were adjusted for the following prespecified covariates at study baseline: peritoneal solute transfer rate and diabetes status (in the analysis of net ultrafiltration during the baseline 3.86% glucose-based peritoneal equilibration test); peritoneal solute transfer rate, residual urine volume, and duration of dialysis treatment (in the analysis of daily net ultrafiltration); and age, sex, cardiovascular disease status, diabetes status, and peritoneal solute transfer rate (in the survival analysis). Time to death or technique failure (i.e., transfer to hemodialysis) was used as a composite outcome in order to provide adequate statistical power. This composite outcome was the primary outcome of the study. Time-to-event analyses were performed with Cox regression models and with Fine and Gray regression models to account for competing risks.²³ The association between risk variant and icodextrin-induced ultrafiltration was analyzed by means of a random-effect generalized linear mixed model, which accounted for up to three icodextrin dwells for each patient. All analyses were performed with GraphPad Prism software, version 8.0.0 (GraphPad Software), or Stata software, version 16.0 (StataCorp). A P value of less than 0.05 was considered to indicate significance. Additional information about the experimental methods is provided in the Supplementary Appendix.

RESULTS

STUDY POPULATION AND *AQP1* VARIANTS

Demographic characteristics, information about treatment and outcomes, and genetic data for the 1851 patients treated with peritoneal dialysis who were included in the study are provided in Table 1 and Tables S1 and S2. The mean (\pm SD) age of the patients was 54 \pm 16 years, and 38%

were women. Most patients were of European descent (75%) or Asian descent (23%). The association between AQP1 variants and peritoneal ultrafiltration was investigated in discovery and validation phases. Thereafter, the association between the functional AQP1 variant rs2075574 and outcomes was analyzed (Fig. S2).

AQP1 GENOTYPE AND PERITONEAL WATER TRANSPORT

In the discovery phase, data from baseline 3.86% glucose-based peritoneal equilibration tests were used to assess for potential associations with AQP1 variants. Data were available for 433 patients, who had undergone peritoneal dialysis for a median of 58 days. At the start of peritoneal dialysis, the mean net ultrafiltration level was 611 ± 280 ml, the mean peritoneal solute transfer rate 0.72 ± 0.11 , and the mean sodium sieving level 0.05 ± 0.02 . Results for net ultrafiltration and sodium sieving, which are both measures of water transport, varied widely across individual patients (Fig. 1A and Fig. S3). Among AQP1 variants, rs2075574 (c.781C→T) had a significant association with measures of water transport, whereas other variants did not. Patients with the TT genotype at rs2075574 had a significantly lower net ultrafiltration level than patients with the CC genotype (506 ± 237 ml vs. 626 ± 283 ml, $P=0.007$), as well as a lower sodium sieving level, despite having a similar peritoneal solute transfer rate and similar clinical characteristics (Fig. 1B, Fig. S3, and Table S3). The association between rs2075574 and water transport was independent of other covariates (Tables S4 and S5).

The association between rs2075574 and ultrafiltration was validated in 985 patients from the three cohorts from the United Kingdom and China (Fig. S4). Patients with the TT genotype had a lower daily net ultrafiltration level than patients with the CC genotype (368 ± 603 ml vs. 563 ± 641 ml, $P=0.003$) (Fig. 1C, Table 2, and Tables S6, S7, and S8). The rs2075574 variant was not associated with residual urine volume (Table S9). These data indicate an independent association between the AQP1 genotype at rs2075574 and peritoneal ultrafiltration in a racially diverse cohort of patients treated with peritoneal dialysis.

FUNCTIONAL RELEVANCE OF AQP1 PROMOTER RISK VARIANT

The rs2075574 variant is located in a conserved region of the AQP1 promoter. The sequence cor-

responds to a transcription factor binding site (CTGTC) that regulates the expression of erythroid-specific genes, as well as to a significant expression quantitative trait locus in AQP1 (Fig. S5).

In endothelial cells and other cell lines, the T allele of rs2075574 was associated with a significantly lower level of AQP1 promoter activity than that observed with the C allele ($P<0.001$) (Fig. 1D). This effect resulted in a 27% lower aquaporin-1 messenger RNA level and a 37% lower aquaporin-1 protein level in the peritoneum in patients with the TT genotype than in patients with the CC genotype, in the absence of structural changes such as vascular proliferation, fibrosis, or inflammation (Fig. 1E, Fig. S6, and Table S10). There was a 47% lower level of aquaporin-1 expression in red-cell membranes in patients with the TT genotype than in patients with the CC genotype ($P<0.001$), a finding reflected by a significant decrease in the specific permeability for water (Fig. S7).

As in patients with the TT genotype at rs2075574, an approximately 50% decrease in aquaporin-1 expression was observed in the peritoneal membrane of *Aqp1^{+/-}* mice, in the absence of structural changes (Fig. S8). With the use of glucose as an osmotic agent, the *Aqp1^{+/-}* mice had a 17% lower net ultrafiltration level than *Aqp1^{+/+}* littermates ($P<0.001$), with no effect on the peritoneal solute transfer rate or osmotic gradient (Fig. S9). In contrast, with the use of icodextrin, water transport was similar in *Aqp1^{+/-}* mice and in *Aqp1^{+/+}* littermates, which suggests the potential value of colloid osmotic agents for the treatment of patients with a deleterious AQP1 genotype. These data indicate that the rs2075574 variant was associated with decreased expression of aquaporin-1 water channels in human tissues, a finding reflected by alterations in osmotic water transport.

AQP1 PROMOTER RISK VARIANT AND OUTCOMES IN PERITONEAL DIALYSIS

Because water removal is critical for dialysis efficiency and the rs2075574 variant was associated with alterations in peritoneal water transport, we tested whether there was an association between the AQP1 genotype at rs2075574 and outcomes in patients treated with peritoneal dialysis. Data were available for 898 of 988 patients (91%) in the cohorts assessed (Fig. S2 and Table S1). After a mean follow-up of 944 days, 419 patients (47%) had died or had technique failure.

Table 1. Baseline Characteristics of the Patients According to Study Cohort.*

Characteristic	Overall (N = 1851)	Belgium (N = 277)	Netherlands, AMC (N = 81)	Spain (N = 156)	Netherlands, NECOSAD (N = 344)	United Kingdom, Stoke-on-Trent (N = 130)	United Kingdom, PD-CRAFT (N = 483)	China (N = 380)
Age at start of peritoneal dialysis — yr	54±16	54±19	55±15	53±14	53±14	47±16	58±16	52±14
Female sex — no./total no. (%)	695/1851 (38)	105/277 (38)	42/81 (52)	51/156 (33)	111/344 (32)	64/130 (49)	158/480 (33)	164/380 (43)
Body-mass index†	24.5±4.9	24.0±4.2	24.7±4.2	26.7±5.3	25.0±3.8	25.8±4.5	24.8±6.5	22.9±3.4
Race — no./total no. (%)‡								
European	1372/1833 (75)	258/277 (93)	80/81 (99)	148/153 (97)	344/344 (100)	120/129 (93)	422/469 (90)	0/380
African	22/1833 (1)	7/277 (3)	1/81 (1)	0/153	0/344	3/129 (2)	11/469 (2)	0/380
Asian	421/1833 (23)	12/277 (4)	0/81	2/153 (1)	0/344	4/129 (3)	23/469 (5)	380/380 (100)
Other	18/1833 (1)	0/277	0/81	3/153 (2)	0/344	2/129 (2)	13/469 (3)	0/380
Cardiovascular disease — no./total no. (%)	276/1310 (21)	76/260 (29)	26/81 (32)	28/155 (18)	85/316 (27)	20/119 (17)	—	41/380 (11)
Diabetes — no./total no. (%)	426/1780 (24)	72/276 (26)	22/81 (27)	32/156 (21)	64/317 (20)	23/119 (19)	127/452 (28)	86/380 (23)
Daily urine volume — ml	948±771	879±715	—	—	1150±816	1134±731	1182±763	585±631
Peritoneal membrane function								
Dialysate:plasma creatinine ratio at 4 hr	0.68±0.13	0.73±0.12	0.75±0.13	0.70±0.10	0.72±0.12	0.64±0.14	0.69±0.14	0.62±0.12
Net ultrafiltration during baseline glucose-based PET — ml	611±280	623±309	563±292	621±228	—	—	—	—
Daily net ultrafiltration — ml	488±633	—	—	—	—	210±788	627±635	401±521
AQPI genotype at rs2075574 — no. (%)								
CC	758 (41)	129 (47)	37 (46)	60 (38)	147 (43)	46 (35)	203 (42)	136 (36)
CT	842 (45)	119 (43)	36 (44)	76 (49)	148 (43)	64 (49)	216 (45)	183 (48)
TT	251 (14)	29 (10)	8 (10)	20 (13)	49 (14)	20 (15)	64 (13)	61 (16)

* Plus-minus values are means ±SD. The cohorts are listed in the order in which the analyses were performed (with the discovery phase preceding the validation phase). PET denotes peritoneal equilibration test.
 † Body-mass index is the weight in kilograms divided by the square of the height in meters.
 ‡ Race was determined by the investigator. The categories shown are those used by the investigators.

In total, 139 patients (15%) died, 280 (31%) were transferred to hemodialysis, 410 (46%) underwent transplantation, 32 (4%) were withdrawn from dialysis, and 34 (4%) were lost to follow-up (Table S11). Patients with the TT genotype had a higher risk of the composite of death or technique failure than patients with the CC genotype (58% vs. 42%, $P=0.01$), as well as a higher risk of death from any cause (24% vs. 15%, $P=0.03$) (Table 2).

Time-to-event analyses confirmed the association between the *AQP1* genotype at rs2075574 and outcomes in patients treated with peritoneal dialysis. In univariate analyses, patients with the TT genotype had a significantly higher risk of the composite outcome than patients with the CC genotype (hazard ratio, 1.51; 95% confidence interval [CI], 1.13 to 2.02; $P=0.005$); a significant association was also observed in the analysis that accounted for competing risks (subdistribution hazard ratio, 1.67; 95% CI, 1.24 to 2.25; $P=0.001$) (Table 3). Adjustment for additional risk factors strengthened the association (adjusted hazard ratio, 1.70; 95% CI, 1.24 to 2.33; $P=0.001$), including in the analysis that accounted for competing risks (adjusted subdistribution hazard ratio, 1.89; 95% CI, 1.40 to 2.56; $P<0.001$) (Fig. 2A, Table 3, and Tables S12 and S13). The trend toward worse outcomes in patients with the TT genotype was consistent across subgroups and cohorts, with women being less likely than men to have poor outcomes associated with the TT genotype (Fig. 2B and Table S14). Sensitivity analyses that accounted for residual urine volume and cohort confirmed the increased risk conferred by the TT genotype (Tables S15 through S18). There was no association between the *AQP1* genotype at rs2075574 and the risk of peritonitis or death from infection.

EFFECT OF AQP1 PROMOTER RISK VARIANT AND DIALYSATE TYPE

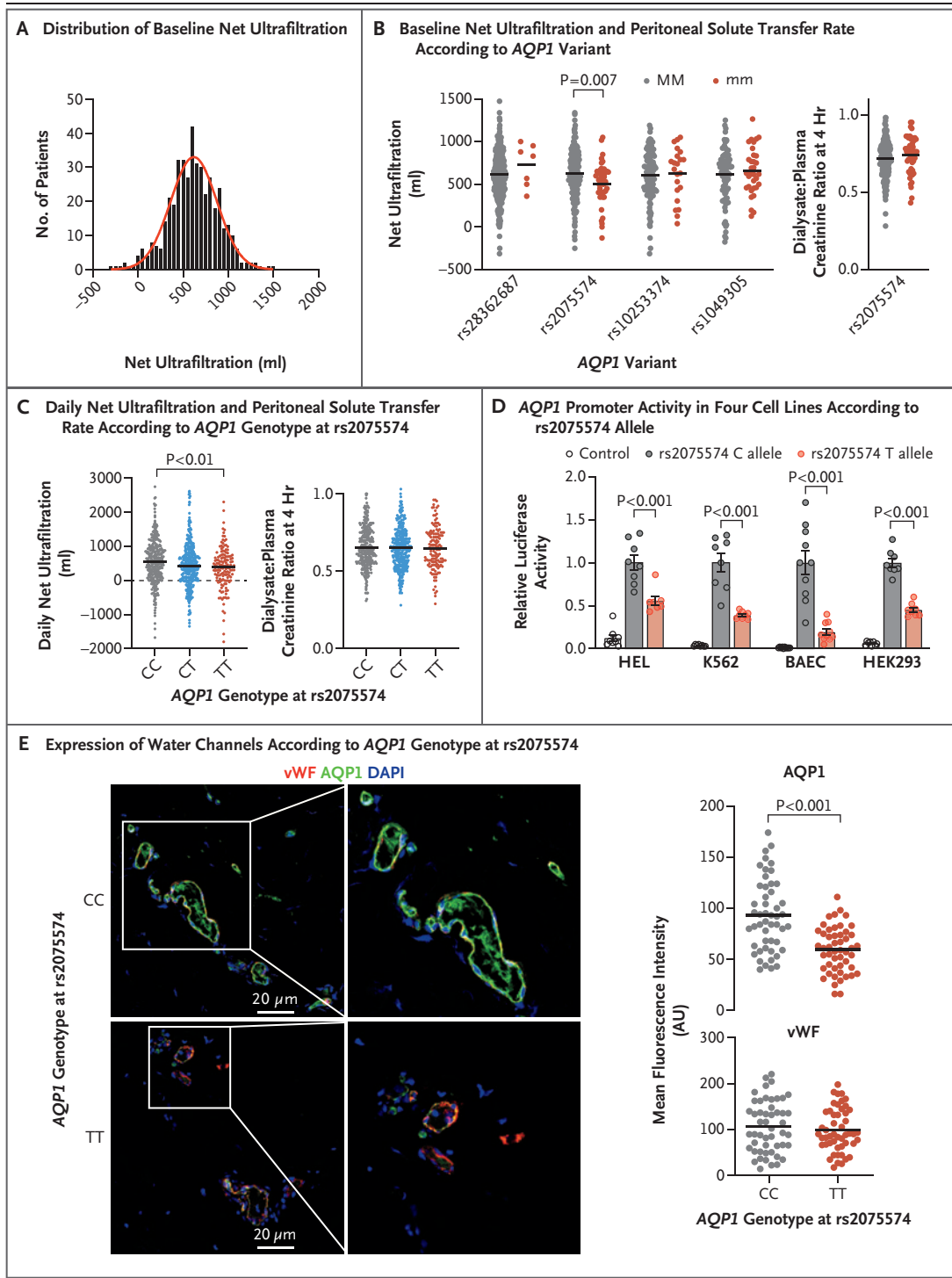
In contrast to glucose-driven crystalloid osmosis, water flow generated by the colloid osmotic agent icodextrin is independent of aquaporins.²⁴ We tested whether the effect of the rs2075574 variant on net ultrafiltration was dependent on the type of osmotic agent used in a subgroup of 144 patients treated with peritoneal dialysis who had available data from both a baseline 3.86% glucose-based peritoneal equilibration test and icodextrin dwells. In this subgroup, the fre-

quency of the TT genotype (11%) was similar to that in other cohorts, and patients with the TT genotype had a significantly lower net ultrafiltration level with the use of hypertonic glucose than patients with the CC genotype (Table S19). The association between the *AQP1* genotype at rs2075574 and glucose-induced water flow remained significant after adjustment (Table S20). In contrast, there was no association between the rs2075574 variant and osmosis induced by icodextrin. These data suggest that the use of a colloid osmotic agent mitigated the water-transport defect associated with the *AQP1* risk variant.

DISCUSSION

The analysis of our combined genetic, translational, and clinical data showed that the *AQP1* promoter variant rs2075574 influenced osmotic water transport and ultrafiltration and was independently associated with an increased risk of the composite of death or technique failure in patients with kidney failure who were treated with peritoneal dialysis. The higher risk of the composite outcome in patients with the TT genotype than in patients with the CC genotype was driven by a significantly higher risk of death from any cause with the TT genotype. The rs2075574 variant influenced *AQP1* promoter activity, the expression of aquaporin-1 in peritoneal microvessels, and osmotic water transport. The use of colloid osmotic agents may mitigate the risk associated with the rs2075574 variant. These results substantiate the influence of genetic factors on the efficiency of peritoneal dialysis and provide a perspective for precision medicine in dialysis treatment.

The common *AQP1* variant rs2075574 was associated with peritoneal ultrafiltration in this racially diverse cohort of patients starting treatment with peritoneal dialysis. The frequency of the minor T allele in the study population was consistent with the frequency in populations of East Asian descent and of European descent. The net ultrafiltration level during baseline peritoneal equilibration testing was approximately 120 ml lower and the daily net ultrafiltration level was approximately 200 ml lower with the deleterious TT genotype than with the CC genotype, and these findings were independent of race, peritoneal dialysis practices, and changes in solute transport. These effects on ultrafiltration are



clinically relevant, given that similar differences in baseline ultrafiltration levels have been reported to be associated with increased mortality and decreased survival in several cohorts.^{4,6,25} Furthermore, in patients who are anuric or oliguric and rely on peritoneal ultrafiltration to

attain fluid balance, such differences may be critical with regard to whether the patient can maintain adequate nutrition.²⁰ Patients with the TT genotype had a 70% higher risk of the composite of death or technique failure than patients with the CC genotype, with a significantly

Figure 1 (facing page). Association of the AQP1 Risk Variant with Peritoneal Transport, AQP1 Promoter Activity, and Expression of Water Channels in Patients Treated with Peritoneal Dialysis.

Panel A shows the distribution of the net ultrafiltration level at the start of peritoneal dialysis in 433 patients with data from a baseline 3.86% glucose–based peritoneal equilibration test (PET) (discovery phase). The relative contribution to the baseline net ultrafiltration level, estimated by means of ordinary least-squares regression, of diabetes status and peritoneal solute transfer rate (defined as the ratio of the creatinine level in dialysate to the creatinine level in plasma at 4 hours) was 1.4 and 14.6, respectively. **Panel B** shows the net ultrafiltration level and peritoneal solute transfer rate at the start of peritoneal dialysis in 433 patients with data from a baseline 3.86% glucose–based PET, according to homozygosity for either major (MM) or minor (mm) alleles of AQP1 variants (discovery phase). The P value was calculated by means of the unpaired t-test. **Panel C** shows the daily net ultrafiltration level in 985 patients and the peritoneal solute transfer rate in 933 patients, according to AQP1 genotype at rs2075574 (validation phase). The P value was calculated by means of one-way analysis of variance and Sidak’s multiple-comparisons test. **Panel D** shows a quantitative analysis of the relative effects of the major (C) and minor (T) alleles of rs2075574 on the transcriptional activity of the AQP1 promoter, as assessed by means of a luciferase reporter assay in two types of human erythroleukemia cells (HEL and K562), bovine aortic endothelial cells (BAEC), and human embryonic kidney 293 (HEK293) cells. Control corresponds to the promoterless vector. Data from four independent experiments are expressed as means, with I bars indicating standard errors. The P values were calculated by means of one-way analysis of variance and Dunnett’s multiple-comparisons test. **Panel E** shows aquaporin-1 (AQP1) expression in endothelial cells, quantified as mean fluorescence intensity (arbitrary units [AU]) in 50 peritoneal vessels (10 vessels per patient in 5 patients) on confocal microscopic examination, according to AQP1 genotype at rs2075574. Representative images are provided of double immunostaining with anti-AQP1 antibodies (in green) and anti-von Willebrand factor (vWF) antibodies (in red), viewed under confocal fluorescence microscopy, in peritoneal sections from patients treated with peritoneal dialysis who had the CC genotype or the TT genotype at rs2075574. Nuclei are counterstained in blue with DAPI (4’,6-diamidino-2-phenylindole). The P value was calculated by means of the unpaired t-test. In Panels B, C, and E, the scatter plots show individual patient values and means.

higher risk of death from any cause, after adjustment for all relevant covariates.

The consistency of the effects of the AQP1 genotype supports the potential role of fluid overload in driving the results for the composite outcome in this cohort. Fluid overload is a substantial contributor to death among patients

treated with peritoneal dialysis, regardless of whether death occurs suddenly or after a debilitation period characterized by frequent hospital admissions.²⁶ Death among patients receiving dialysis may involve several mechanisms linked to poor fluid removal and overhydration, such as cardiovascular events, malnutrition, systemic inflammation, transfer to hemodialysis, and hospitalizations.²⁷ These many issues may explain why there was no significant difference in the risk of death from cardiovascular or infectious causes between groups defined according to AQP1 genotype. The effect of sex on outcomes may be related to the higher level of aquaporin-1 expression in males than in females, which has been observed in various species and in various organs, including the peritoneal membrane and the kidney.^{15,28} Altogether, these findings underscore the importance of genotype-driven differences in water transport as a critical factor in peritoneal dialysis adequacy and outcomes.

The biologic relevance of the rs2075574 variant is supported by its effect on AQP1 promoter activity, which is possibly due to disruption of a motif that regulates erythroid-specific genes.^{29,30} The expression of aquaporin-1 in the peritoneal microvasculature was approximately 50% lower with the TT genotype than with the CC genotype, in the absence of other structural changes. Studies in *Aqp1*^{+/-} mice showed that such a decrease in aquaporin-1 expression led to an approximately 15% decrease in osmotic water transport, a finding similar to that observed in patients treated with peritoneal dialysis who had the TT genotype. These data are consistent with findings seen in models of the effect of water channels on transport³¹ and seen with pharmacologic modulation of aquaporin-1.^{32,33}

The association between the AQP1 genotype at rs2075574 and peritoneal ultrafiltration was observed in patients starting dialysis and in patients undergoing maintenance dialysis. In the latter group, the association was independent of the duration of treatment; longer treatment duration has been associated with decreased osmotic water transport.^{21,22} The rs2075574 variant was not associated with residual urine volume, a finding consistent with the observation of normal urinary concentrating ability in *Aqp1*^{+/-} mice.³⁴

In patients with the TT genotype and in *Aqp1*^{+/-} mice, the effect of decreased aquaporin-1 expression on peritoneal water transport was observed only with the use of glucose as an os-

Table 2. Association of the AQP1 Genotype at rs2075574 with Peritoneal Water Transport and Outcomes in Patients Treated with Peritoneal Dialysis.*

Variable	Overall	CC	CT	TT	P Value†
Peritoneal water transport					
Discovery phase					
No. of patients	433	184	199	50	—
Net ultrafiltration during baseline 3.86% glucose-based PET — ml	611±280	626±283	625±282	506±237	0.02
Validation phase					
No. of patients	985	383	459	143	—
Daily net ultrafiltration — ml	488±633	563±641	463±629	368±603	0.003
Outcomes					
No. of patients	898	384	400	114	—
Death or technique failure — no. (%)	419 (47)	162 (42)	191 (48)	66 (58)	0.01
Technique failure — no. (%)	280 (31)	105 (27)	136 (34)	39 (34)	0.10
Death from any cause — no. (%)	139 (15)	57 (15)	55 (14)	27 (24)	0.03

* Plus–minus values are means ±SD.

† P values are based on one-way analysis of variance that compared the three AQP1 genotypes.

Table 3. Hazard Ratios for Time to the Composite of Death or Technique Failure According to the AQP1 Genotype at rs2075574.

Analysis and Genotype	Cox Regression Model		Fine and Gray Regression Model for Competing Risks	
	Hazard Ratio vs. CC (95% CI)	P Value vs. CC	Subdistribution Hazard Ratio vs. CC (95% CI)	P Value vs. CC
Unadjusted analysis (N=898)				
CC	1.00	—	1.00	—
CT	1.14 (0.93–1.41)	0.21	1.18 (0.96–1.46)	0.11
TT	1.51 (1.13–2.02)	0.005	1.67 (1.24–2.25)	0.001
Adjusted analysis (N=767)*				
CC	1.00	—	1.00	—
CT	1.19 (0.95–1.50)	0.13	1.19 (0.95–1.49)	0.13
TT	1.70 (1.24–2.33)	0.001	1.89 (1.40–2.56)	<0.001

* Analyses were adjusted for age, sex, cardiovascular disease status, diabetes status, and peritoneal solute transfer rate at baseline.

motric agent and not with the use of icodextrin. These data confirm that the colloidal fractions of icodextrin induce osmosis independently of aquaporins^{24,35,36} and suggest that the use of colloid osmotic agents may mitigate the deleterious effect of the TT genotype on water transport.

The strengths of this study include the enrollment of a large number of patients treated with peritoneal dialysis who had available genetic and clinical information, the diverse racial backgrounds of the patients, the availability of stan-

dardized measures of peritoneal transport, the performance of sensitivity analyses that accounted for cohort, and the performance of translational studies. The study also has certain limitations, including its retrospective design, the absence of evaluation of volume status, and the heterogeneity in available clinical information. Further studies may substantiate the link between the AQP1 variant-associated decrease in ultrafiltration, fluid overload, and death from any cause, with the potential for genetic pleiot-

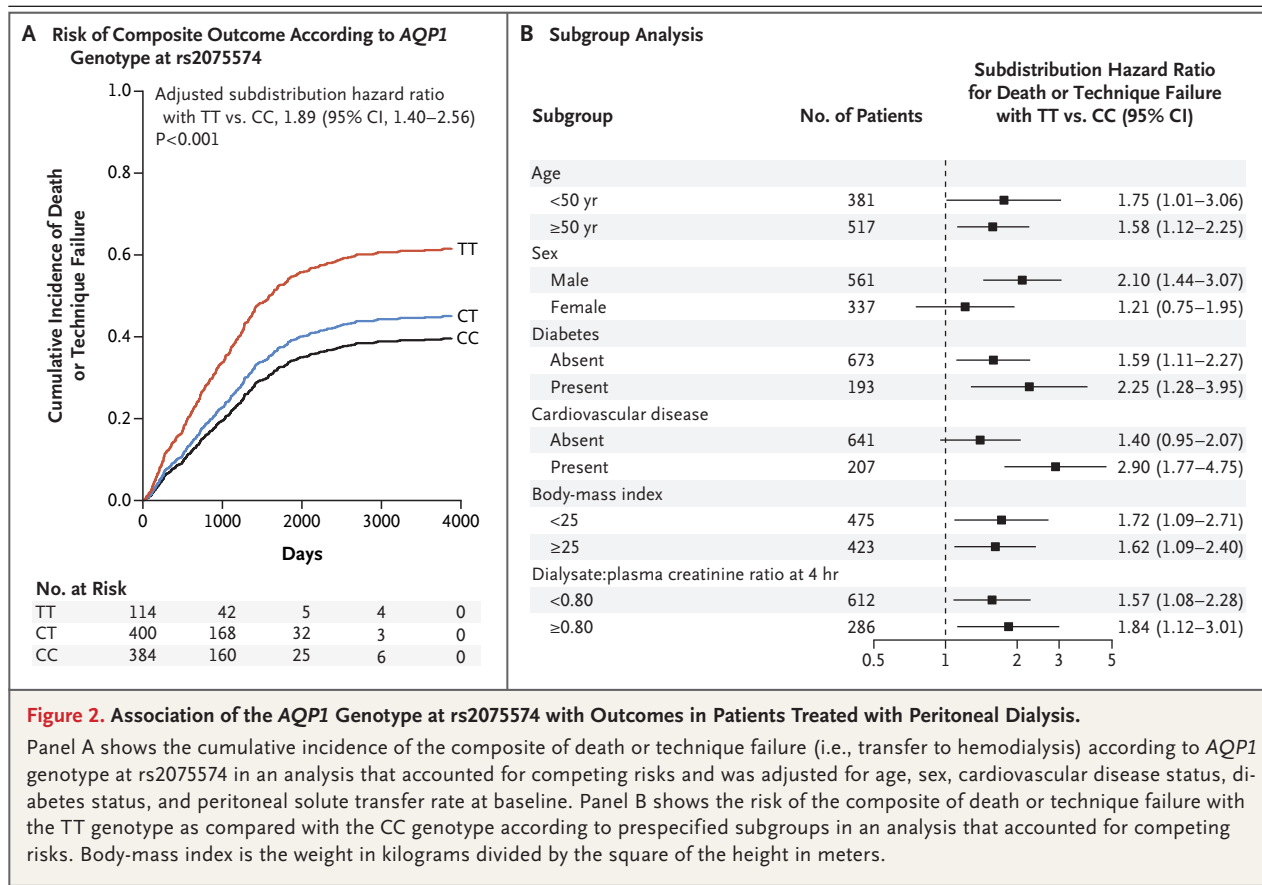


Figure 2. Association of the AQP1 Genotype at rs2075574 with Outcomes in Patients Treated with Peritoneal Dialysis.

Panel A shows the cumulative incidence of the composite of death or technique failure (i.e., transfer to hemodialysis) according to AQP1 genotype at rs2075574 in an analysis that accounted for competing risks and was adjusted for age, sex, cardiovascular disease status, diabetes status, and peritoneal solute transfer rate at baseline. Panel B shows the risk of the composite of death or technique failure with the TT genotype as compared with the CC genotype according to prespecified subgroups in an analysis that accounted for competing risks. Body-mass index is the weight in kilograms divided by the square of the height in meters.

ropy. They may also test whether adaptation of prescriptions for peritoneal dialysis may mitigate the risk conferred by the AQP1 variant.

In this study, a common AQP1 promoter variant influenced the expression of water channels in the peritoneal membrane, affecting water transport, ultrafiltration, and outcomes in patients with kidney failure who were treated with peritoneal dialysis.

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APPENDIX

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