
Universiteit
Leiden
The Netherlands

AQP1 promoter variant, water transport, and outcomes in peritoneal dialysis

Morelle, J.; Marechal, C.; Yu, Z.Z.; Debaix, H.; Corre, T.; Lambie, M.; ... ; Devuyst, O.

Citation
Morelle, J., Marechal, C., Yu, Z. Z., Debaix, H., Corre, T., Lambie, M., ... Devuyst, O. (2021). AQP1 promoter variant, water transport, and outcomes in peritoneal dialysis. *New England Journal Of Medicine*, *385*(17), 1570-1580. doi:10.1056/NEJMoa2034279

Version:	Publisher's Version
License:	Licensed under Article 25fa Copyright Act/Law (Amendment Taverne)
Downloaded from:	https://hdl.handle.net/1887/3276442

Note: To cite this publication please use the final published version (if applicable).

ORIGINAL ARTICLE

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

Johann Morelle, M.D., Ph.D., Céline Marechal, Ph.D., Zanzhe Yu, M.D., Ph.D., Huguette Debaix, M.Sc., Tanguy Corre, Ph.D., Mark Lambie, M.D., Ph.D., Marion Verduijn, Ph.D., Friedo Dekker, Ph.D., Philippe Bovy, M.D., Pieter Evenepoel, M.D., Ph.D., Bert Bammens, M.D., Ph.D., Rafael Selgas, M.D., Ph.D., Maria A. Bajo, M.D., Ph.D., Annemieke M. Coester, M.D., Ph.D., Amadou Sow, M.D., Ph.D., Nicolas Hautem, Ph.D., Dirk G. Struijk, M.D., Ph.D., Raymond T. Krediet, M.D., Ph.D., Jean-Luc Balligand, M.D., Ph.D., Eric Goffin, M.D., Ralph Crott, Ph.D., Pierre Ripoche, Ph.D., Simon Davies, M.D., and Olivier Devuyst, M.D., Ph.D.

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 (±SD) net ultrafiltration level than carriers of the CC genotype (35 to 47% of patients), both in the discovery phase (506±237 ml vs. 626±283 ml, P=0.007) and in the validation phase (368±603 ml vs. 563±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.)

The authors' affiliations are listed in the Appendix. Address reprint requests to Dr. Devuyst at Mechanisms of Inherited Kidney Disorders, Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland, or at olivier.devuyst@uzh.ch, or to Dr. Morelle at johann.morelle@uclouvain.be, or to Dr. Davies at simonj.davies55@gmail.com.

Drs. Morelle, Marechal, and Yu contributed equally to this article.

N Engl J Med 2021;385:1570-80.
DOI: 10.1056/NEJMoa2034279
Copyright © 2021 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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 erythrocytes[12] 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.

A Quick Take is available at NEJM.org

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-

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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 (±SD) age of the patients was 54±16 years, and 38%

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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 corresponds 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.

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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 3.86% glucose–based PET — ml	611±280	623±309	563±292	621±228	—	—	—	—
Daily net ultrafiltration — ml	488±633	—	—	—	—	210±788	627±635	401±521
AQP1 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.

Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
The New England Journal of Medicine
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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

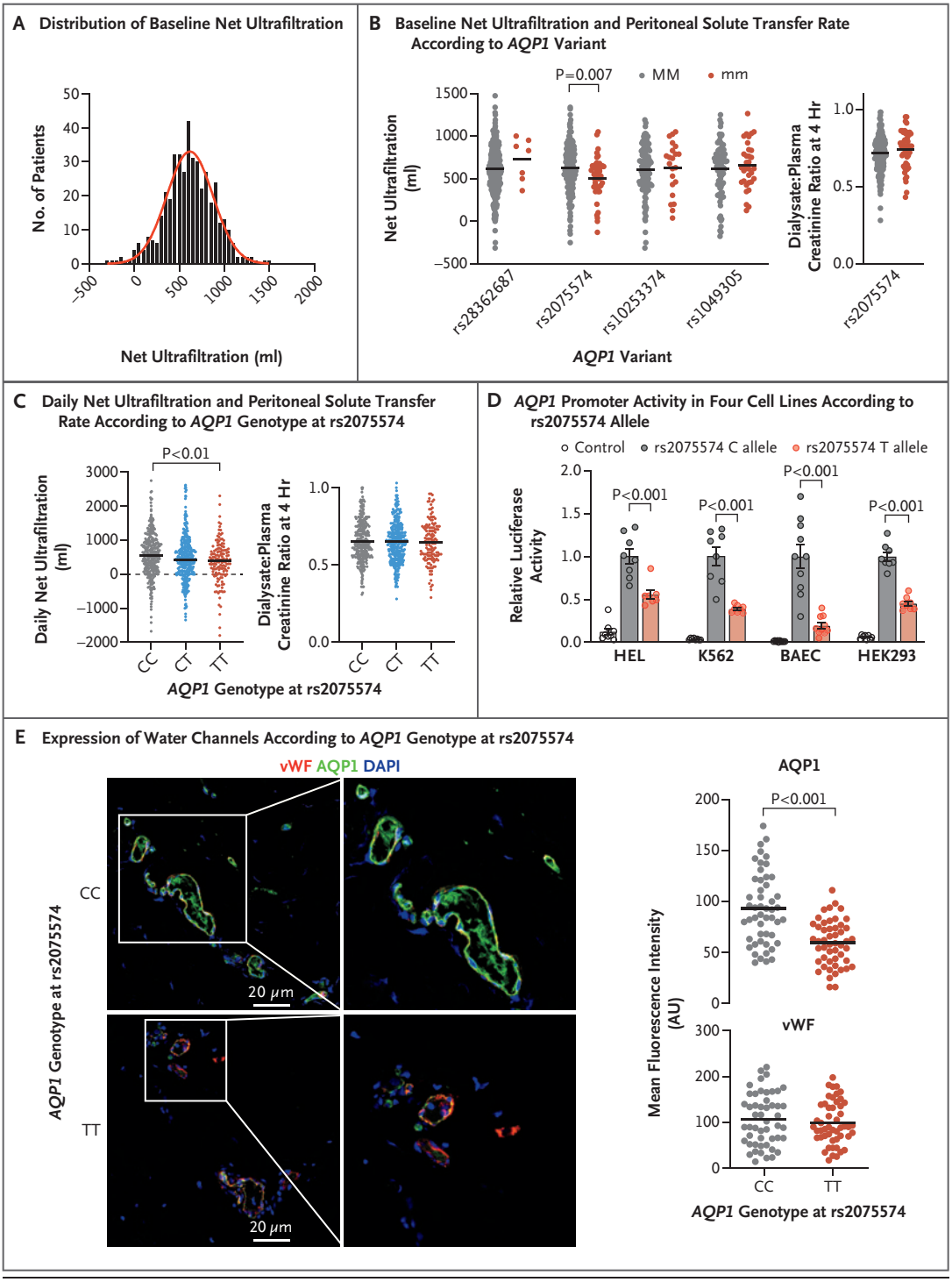
In contrast to glucose-driven crystalloid osmosis, water flow generated by the colloid osmotic agent icodextrin is independent of aquaporins.[24] We tested whether the effect of the rs2075574 variant on net ultrafiltration was dependent on the type of osmotic agent used in a subgroup of 144 patients 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 frequency 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

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.



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

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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 transport[31] 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-

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

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.

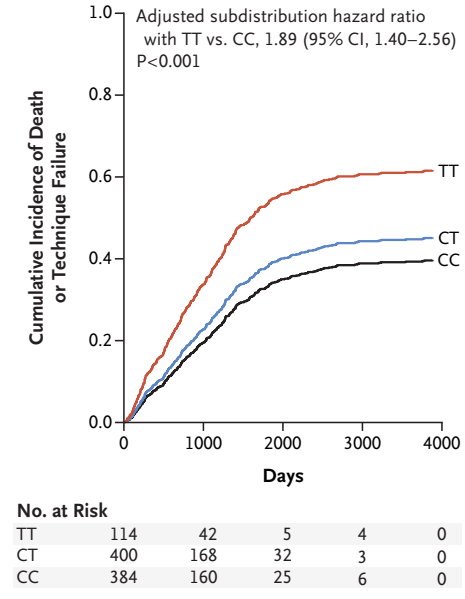
Analysis and Genotype	Cox Regression Model		Fine and Gray Regression Model for Competing Risks	
	Hazard Ratio vs. CC (95% CI)	P Value vs. CC	Subdistribution Hazard Ratio vs. CC (95% CI)	P Value vs. CC
Unadjusted analysis (N=898)				
CC	1.00	—	1.00	—
CT	1.14 (0.93–1.41)	0.21	1.18 (0.96–1.46)	0.11
TT	1.51 (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.

motic 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 standardized 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-

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.



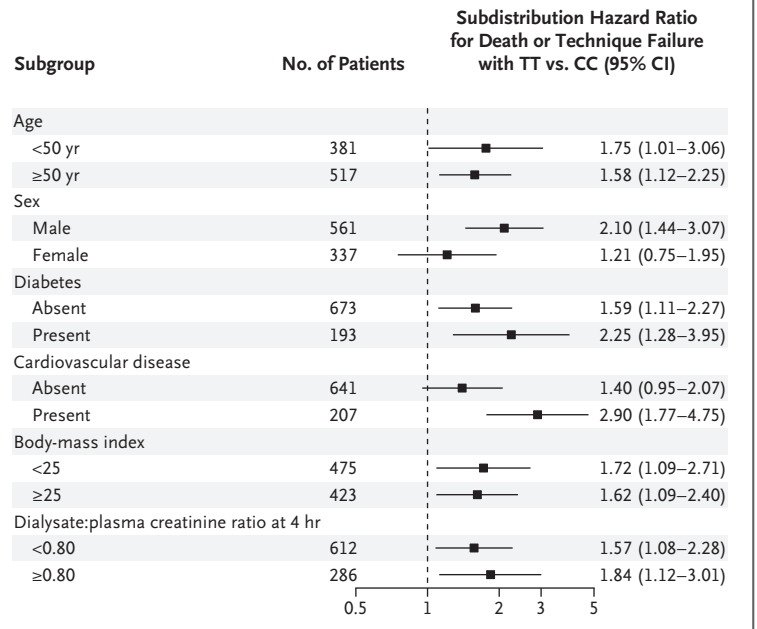


Figure 2. Association of the *AQP1* Genotype at rs2075574 with Outcomes in Patients Treated with Peritoneal Dialysis.

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.

Supported in part by grants from the Swiss National Science Foundation (310030_189044), the University Research Priority Program Innovative Therapies in Rare Diseases of the University of Zurich, and the Swiss National Centers of Competence in Research Kidney Control of Homeostasis (all to Dr. Devuyst); grants from Fonds de la Recherche Scientifique (40000157 and 4003771), Fondation Saint-Luc, and Fonds de Recherche Clinique des Cliniques Universitaires Saint-Luc (all to Dr. Morelle); a Concerted Research Action grant (ARC16-21/074, to Drs. Balligand and Devuyst); and a grant from the Walloon Excellence in Lifesciences and Biotechnology (WELBIO) Institute (to Dr. Balligand). Dr. Balligand is a WELBIO established investigator. Dr. Sow is supported by a Research Fund of the UCLouvain Medical School. Dr. Yu is supported by a Dorothy Hodgkin Postgraduate Award and an award from the Shanghai Pujiang Program (16PJ1405900). Drs. Morelle, Davies, and Devuyst are beneficiaries of the IMPROVE-PD (Identification and Management of Patients at Risk — Outcome and Vascular Events in Peritoneal Dialysis) project funded by the European Union Horizon 2020 program under a Marie Skłodowska-Curie grant agreement (812699). The PD-CRAFT study was supported by a grant from the National Institute for Health Research (PB-PG-0610-22456). The Stoke-on-Trent study was supported in part by the North Staffordshire Medical Institute. NECOSAD was supported by the Dutch Kidney Foundation.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the patients, nurses, and staff members of home dialysis units who participated in this study; Yvette Cnops, Sebastien Druart, and Henriette de Loor for technical assistance; Murielle Bochud (University of Lausanne, Lausanne, Switzerland) and Karin Dahan (Institute of Pathology and Genetics, Gosselies, Belgium) for genotyping information; Caroline Bouzin and the staff of Institut de Recherche Expérimentale et Clinique Imaging Platform (UCLouvain, Brussels); Gulgielmo Schiano for bioinformatics (University of Zurich, Zurich, Switzerland); Zhaohui Ni, Leyi Gu, and Wei Fang for help with the China cohort; and the staff of NECOSAD for assistance with that cohort.

APPENDIX

The authors' affiliations are as follows: the Division of Nephrology, Cliniques Universitaires Saint-Luc (J.M., E.G., O.D.), and Institut de Recherche Expérimentale et Clinique (J.M., C.M., H.D., A.S., N.H., J.-L.B., E.G., O.D.) and Institut de Recherche Santé et Société, Faculty of Public Health (R.C.), UCLouvain, Brussels, the Division of Nephrology, Clinique Saint-Joseph, Liege (P.B.), and the Department of Microbiology, Immunology, and Transplantation, Nephrology and Renal Transplantation Research Group, KU Leuven (P.E.,

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.

B.B.), and the Department of Nephrology, Dialysis, and Renal Transplantation, University Hospitals Leuven (P.E., B.B.), Leuven — all in Belgium; the Department of Nephrology, Shanghai Jiao Tong University School of Medicine and Renji Hospital, Shanghai, China (Z.Y.); the Faculty of Medicine and Health Sciences, Keele University, Keele, United Kingdom (Z.Y., M.L., S.D.); the Institute of Physiology, University of Zurich, Zurich (H.D., O.D.), and the Center for Primary Care and Public Health (Unisanté), University of Lausanne, Lausanne (T.C.) — both in Switzerland; the Department of Clinical Epidemiology, Leiden University Medical Center, Leiden (M.V., F.D.), the Division of Nephrology, Department of Medicine, Amsterdam University Medical Center, University of Amsterdam, Amsterdam (A.M.C., D.G.S., R.T.K.), and the Department of Surgery, University Medical Center Groningen, Groningen (A.M.C.) — all in the Netherlands; the Division of Nephrology, Hospital Universitario La Paz, and Instituto de Investigación Sanitaria La Paz, Red de Investigación Renal, Universidad Autonoma, Madrid (R.S., M.A.B.); and Institut National de la Transfusion Sanguine, Paris (P.R.).

REFERENCES

1. Mehrotra R, Devuyst O, Davies SJ, Johnson DW. The current state of peritoneal dialysis. J Am Soc Nephrol 2016;27:3238-52.
2. Li PK-T, Chow KM, Van de Luijtgaarden MWM, et al. Changes in the worldwide epidemiology of peritoneal dialysis. Nat Rev Nephrol 2017;13:90-103.
3. Twardowski ZJ, Nolph KO, Khanna R, et al. Peritoneal equilibration test. Perit Dial Int 1987;7:138.
4. Brown EA, Davies SJ, Rutherford P, et al. Survival of functionally anuric patients on automated peritoneal dialysis: the European APD Outcome Study. J Am Soc Nephrol 2003;14:2948-57.
5. Brimble KS, Walker M, Margetts PJ, Kundhal KK, Rabbat CG. Meta-analysis: peritoneal membrane transport, mortality, and technique failure in peritoneal dialysis. J Am Soc Nephrol 2006;17:2591-8.
6. Morelle J, Stachowska-Pietka J, Öberg C, et al. ISPD recommendations for the evaluation of peritoneal membrane dysfunction in adults: classification, measurement, interpretation and rationale for intervention. Perit Dial Int 2021;41:352-72.
7. Gillerot G, Goffin E, Michel C, et al. Genetic and clinical factors influence the baseline permeability of the peritoneal membrane. Kidney Int 2005;67:2477-87.
8. La Milia V, Cabiddu G, Virga G, et al. Peritoneal equilibration test reference values using a 3.86% glucose solution during the first year of peritoneal dialysis: results of a multicenter study of a large patient population. Perit Dial Int 2017;37:633-8.
9. Mehrotra R, Stanaway IB, Jarvik GP, et al. A genome-wide association study suggests correlations of common genetic variants with peritoneal solute transfer rates in patients with kidney failure receiving peritoneal dialysis. Kidney Int 2021 June 29 (Epub ahead of print).
10. Devuyst O. Assessing transport across the peritoneal membrane: precision medicine in dialysis. Perit Dial Int 2021;41:349-51.
11. Agre P. Aquaporin water channels (Nobel Lecture). Angew Chem Int Ed Engl 2004;43:4278-90.
12. Preston GM, Carroll TP, Guggino WB, Agre P. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 1992;256:385-7.
13. Devuyst O, Nielsen S, Cosyns JP, et al. Aquaporin-1 and endothelial nitric oxide synthase expression in capillary endothelia of human peritoneum. Am J Physiol 1998;275(1):H234-H242.
14. Yang B, Folkesson HG, Yang J, Matthay MA, Ma T, Verkman AS. Reduced osmotic water permeability of the peritoneal barrier in aquaporin-1 knockout mice. Am J Physiol 1999;276(1):C76-C81.
15. Ni J, Verbavatz J-M, Rippe A, et al. Aquaporin-1 plays an essential role in water permeability and ultrafiltration during peritoneal dialysis. Kidney Int 2006;69:1518-25.
16. Morelle J, Sow A, Vertommen D, Jamar F, Rippe B, Devuyst O. Quantification of osmotic water transport in vivo using fluorescent albumin. Am J Physiol Renal Physiol 2014;307(8):F981-F989.
17. King LS, Nielsen S, Agre P, Brown RH. Decreased pulmonary vascular permeability in aquaporin-1-null humans. Proc Natl Acad Sci U S A 2002;99:1059-63.
18. King LS, Choi M, Fernandez PC, Cartron JP, Agre P. Defective urinary concentrating ability due to a complete deficiency of aquaporin-1. N Engl J Med 2001;345:175-9.
19. Firmann M, Mayor V, Vidal PM, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord 2008;8:6.
20. Wang AY-M, Dong J, Xu X, Davies S. Volume management as a key dimension of a high-quality PD prescription. Perit Dial Int 2020;40:282-92.
21. La Milia V, Pozzoni P, Virga G, et al. Peritoneal transport assessment by peritoneal equilibration test with 3.86% glucose: a long-term prospective evaluation. Kidney Int 2006;69:927-33.
22. Mujais S, Nolph K, Gokal R, et al. Evaluation and management of ultrafiltration problems in peritoneal dialysis. Perit Dial Int 2000;20:Suppl 4:S5-S21.
23. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-509.
24. Morelle J, Sow A, Fustin C-A, et al. Mechanisms of crystalloid versus colloid osmosis across the peritoneal membrane. J Am Soc Nephrol 2018;29:1875-86.
25. Davies SJ, Brown EA, Reigel W, et al. What is the link between poor ultrafiltration and increased mortality in anuric patients on automated peritoneal dialysis? Analysis of data from EAPOS. Perit Dial Int 2006;26:458-65.
26. Davies SJ, Phillips L, Griffiths AM, Russell LH, Naish PF, Russell GI. What really happens to people on long-term peritoneal dialysis? Kidney Int 1998;54:2207-17.
27. Kim Y-L, Van Biesen W. Fluid overload in peritoneal dialysis patients. Semin Nephrol 2017;37:43-53.
28. Veiras LC, Girardi ACC, Curry J, et al. Sexual dimorphic pattern of renal transporters and electrolyte homeostasis. J Am Soc Nephrol 2017;28:3504-17.
29. Bekri S, May A, Cotter PD, et al. A promoter mutation in the erythroid-specific 5-aminolevulinate synthase (ALAS2) gene causes X-linked sideroblastic anemia. Blood 2003;102:698-704.
30. van Wijk R, van Solinge WW, Nerlov C, et al. Disruption of a novel regulatory element in the erythroid-specific promoter of the human PKLR gene causes severe pyruvate kinase deficiency. Blood 2003;101:1596-602.
31. Rippe B, de Arteaga J, Venturoli D. Aquaporins are unlikely to be affected in marked ultrafiltration failure: results from a computer simulation. Perit Dial Int 2001;21:Suppl 3:S30-S34.
32. Stoenoiu MS, Ni J, Verkaeren C, et al. Corticosteroids induce expression of aquaporin-1 and increase transcellular water transport in rat peritoneum. J Am Soc Nephrol 2003;14:555-65.
33. Yool AJ, Morelle J, Cnops Y, et al. AqF026 is a pharmacologic agonist of the water channel aquaporin-1. J Am Soc Nephrol 2013;24:1045-52.
34. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 1998;273:4296-9.
35. Mistry CD, Mallick NP, Gokal R. Ultrafiltration with an isosmotic solution during long peritoneal dialysis exchanges. Lancet 1987;2:178-82.
36. Davies SJ, Woodrow G, Donovan K, et al. Icodextrin improves the fluid status of peritoneal dialysis patients: results of a double-blind randomized controlled trial. J Am Soc Nephrol 2003;14:2338-44.

Copyright © 2021 Massachusetts Medical Society.

The New England Journal of Medicine
Downloaded from nejm.org at Walaeus Library - Leiden University Medical Center on September 8, 2023. For personal use only. No other uses without permission.
Copyright © 2021 Massachusetts Medical Society. All rights reserved.