



ORIGINAL ARTICLE

The ANTENATAL multicentre study to predict postnatal renal outcome in fetuses with posterior urethral valves: objectives and design

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ABSTRACT

Background. Posterior urethral valves (PUV) account for 17% of paediatric end-stage renal disease. A major issue in the management of PUV is prenatal prediction of postnatal renal function. Fetal ultrasound and fetal urine biochemistry are currently employed for this prediction, but clearly lack precision. We previously developed a fetal urine peptide signature that predicted *in utero* with high precision postnatal renal function in fetuses with PUV. We describe here the objectives and design of the prospective international multicentre ANTENATAL (multicentre validation of a fetal urine peptidome-based classifier to predict postnatal renal function in posterior urethral valves) study, set up to validate this fetal urine peptide signature.

Methods. Participants will be PUV pregnancies enrolled from 2017 to 2021 and followed up until 2023 in >30 European centres endorsed and supported by European reference networks for rare urological disorders (ERN eUROGEN) and rare kidney diseases (ERN ERKNet). The endpoint will be renal/patient survival at 2 years postnatally. Assuming $\alpha = 0.05$, $1 - \beta = 0.8$ and a mean prevalence of severe renal outcome in PUV individuals of 0.35, 400 patients need to be enrolled to validate the previously reported sensitivity and specificity of the peptide signature.

Results. In this largest multicentre study of antenatally detected PUV, we anticipate bringing a novel tool to the clinic. Based on urinary peptides and potentially amended in the future with additional omics traits, this tool will be able to precisely quantify postnatal renal survival in PUV pregnancies. The main limitation of the employed approach is the need for specialized equipment.

Conclusions. Accurate risk assessment in the prenatal period should strongly improve the management of fetuses with PUV.

Keywords: development, kidney disease, obstructive uropathy, prediction, prenatal biomarkers

INTRODUCTION

Posterior urethral valves (PUV) are a congenital anomaly associated with a wide spectrum of outcomes ranging from extremely severe phenotypes with prenatal death to live born children with normal renal function. Exclusively found in males, PUV is the major cause of severe obstructive nephropathy in children. Although a rare disease (1 in 5000–8000 children [1]), it accounts for 17% of paediatric end-stage renal disease (ESRD) [2–4].

The management of PUV is hampered by the absence of tools for adequate antenatal screening of PUV fetuses at a high risk for developing ESRD. Current clinical practice is based on prenatal ultrasound and fetal urine biochemistry, but this is clearly insufficient to predict postnatal renal outcome with any accuracy in PUV patients as shown in several (meta) studies [5–7]. This lack of efficient prognostic methods leads to many parents opting for termination of pregnancy (TOP) [6–8], and also compromises adequate selection of fetuses that would potentially benefit from prenatal intervention (vesicoamniotic shunting or laser ablation of valves). Hence, new predictive strategies are necessary to allow optimal diagnostic and therapeutic management of the disease.

In a small-scale proof-of-concept study, we recently identified a fetal urinary peptide signature that predicted *in utero* the postnatal renal function of PUV fetuses with high accuracy [9], outperforming routine ultrasound and fetal urine biochemistry. These results sparked the interest of many obstetricians, paediatric urologists and nephrologists in using this fetal signature in routine clinical care. On this background, we initiated an international investigator-driven prospective large-scale study, named ANTENATAL (clinicaltrials.gov NCT03116217; ‘multicentre vAlidationN of a fetal urine pepTidome-based classifiEr to predict postnatal reNAl function in posterior ureThral vAlves’), aiming to validate peptide signature in a much larger multicentre setting. The ANTENATAL trial will enrol 400 PUV pregnancies screened in >30 European centres with involvement of two large European Reference Networks on rare diseases over a period of 4 years and with a patient follow-up until the child’s age of 2 years. The ANTENATAL study will also be an excellent opportunity to evaluate the potential of adding other fetal urinary omics traits to peptide markers for kidney disease stratification, including proteins, metabolites and miRNAs, as we suggested in a recent study [10]. In addition, the ANTENATAL trial will allow exploration of the possibility of extending the omics analysis to amniotic fluid for a less

invasive prediction of postnatal renal function in PUV pregnancies. The present report introduces an overview, the design and objectives of the ANTENATAL trial.

ANTENATAL RATIONALE

Body fluid peptide-based biomarker panels display clear promise for the management of kidney disease because of their improved efficacy over single markers and the relative proximity of peptides to the phenotype compared with other omics traits [11, 12]. In the first attempt to show that peptides present in fetal urine could serve as biomarkers to predict postnatal renal function in PUV fetuses, we prospectively recruited 66 PUV patients from 26 different French centres. Using 28 PUV fetuses, we identified 26 fetal urinary peptides strongly associated with postnatal renal survival [9]. Twelve of those were combined in a signature that we called the '12PUV classifier'. This signature showed excellent performance in predicting the postnatal onset of early ESRD in the 38 blindly assessed additional PUV fetuses with a sensitivity of 88% [95% confidence interval (95% CI) 66–98%], a specificity of 95% (95% CI 80–100%) and an area under the receiver operator characteristic curve (AUC) of 0.94 (95% CI 0.82–0.99) (Figure 1A). The predictive efficacy of the 12PUV classifier was compared with routine clinical parameters, including ultrasound-measured characteristics of the fetal kidneys (hyperechogenicity, dysplasia, presence of cysts or aspect of renal cortex) and amniotic fluid volume (oligohydramnios or anhydramnios) as well as fetal urine biochemistry

[sodium, β 2-microglobulin (β 2M)]. Based on accuracy (Figure 1B), the 12PUV classifier correctly classified 35/38 patients [92% (95% CI 84–100%)] compared with the best ultrasound (multicystic dysplastic kidneys) and biochemical (β 2M) parameters, which correctly predicted 27/38 [71% (95% CI 57–85%)] and 26/38 patients [68% (95% CI 54–83%)], respectively. This clearly demonstrating that the 12PUV classifier outperformed the conventional approaches.

ANTENATAL OBJECTIVES

The primary objective of the ANTENATAL study is to prospectively validate the use of the fetal urine peptidome-based 12PUV classifier [9] to predict *in utero* postnatal renal function in fetuses with PUV in a large international multicentre cohort.

The secondary objectives are to: (i) evaluate whether adding additional omics traits (miRNAs, proteins and metabolites) to the 12 peptide markers further improves the stratification; (ii) explore the predictive biomarker content of amniotic fluid as a less invasive biological fluid; (iii) compare the predictive efficacy of the 12PUV classifier for renal/patient survival at 2 years postnatally with conventional methods (fetal ultrasound and urine biochemistry); (iv) assess whether the accuracy of the 12PUV-mediated prediction is modified by intervention (e.g. vesico amniotic shunting or laser cystoscopy ablation of the valves according to local clinical practice); (v) determine the stability and reproducibility of the 12PUV-mediated prediction if multiple sequential fetal urine samples of a patient are

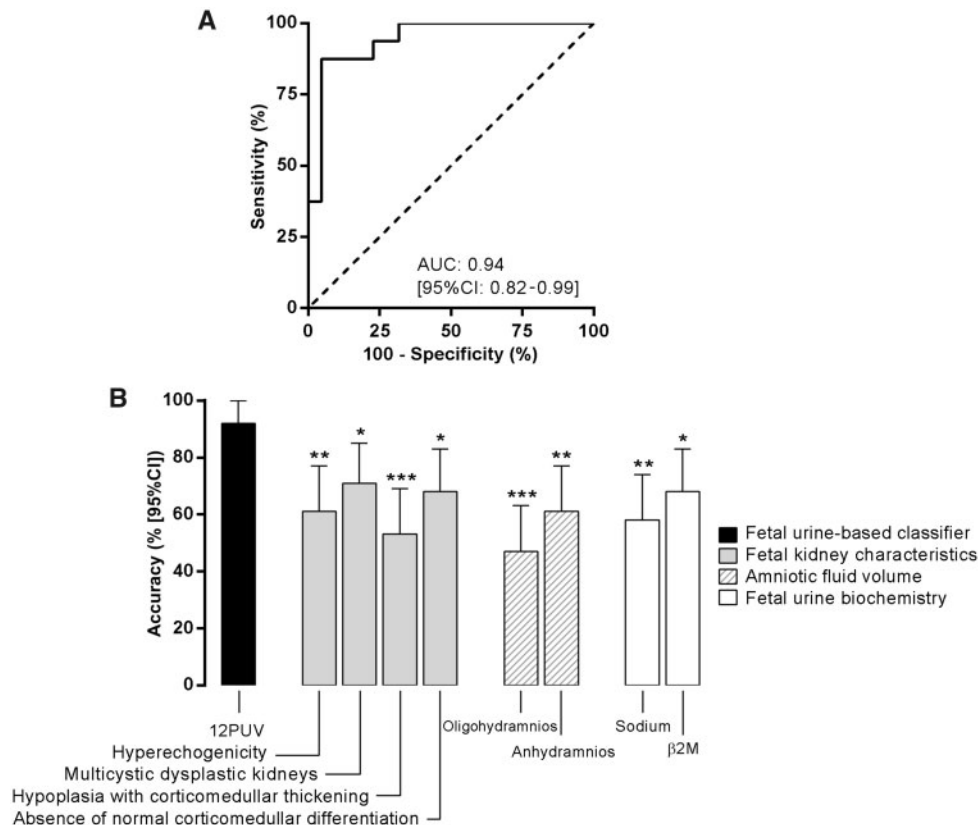


FIGURE 1: Performance of a panel of 12 fetal urinary peptides (12PUV classifier) in predicting postnatal renal outcome in fetuses with PUV (proof of concept study). (A) Receiver operator characteristic curve for prediction of postnatal renal survival using the 12PUV classifier (38 fetuses, 16 with early ESRD, 22 without ESRD at 2 years postnatally). Adapted from Klein et al. [9] with permission. (B) Predictive accuracy of the 12PUV classifier compared with the clinical parameters including routinely ultrasound-measured characteristics of the fetal kidneys and amniotic fluid volume (oligoamnios or anhydramnios) as well as fetal urine biochemistry. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the 12PUV classifier using the McNemar test for paired proportions. Adapted from Klein et al. [9] with permission.

available; and (vi) have a standardized collection of pre- and postnatal clinical and demographic data on the outcome of PUV pregnancies.

ANTENATAL IMPLEMENTATION

Design, participants and setting

ANTENATAL is an international multicentre prospective case study designed to validate the performance of the 12PUV classifier for *in utero* prediction of the postnatal renal outcome of PUV fetuses. ANTENATAL anticipates to enrol 400 PUV pregnancies in >30 multidisciplinary prenatal diagnosis centres in nine European countries (Belgium, France, Germany, Italy, Poland, Spain, The Netherlands, Switzerland and Sweden). Inclusion started in June 2017 and plans to end, under optimal conditions, in June 2021, while follow-up will continue up to December 2023. Eligible patients are mothers carrying male singleton fetus with suspected PUV according to the following inclusion criteria: (i) detection of a megabladder associated with urinary tract anomalies with or without dysplastic or hyperechogenic parenchyma in a first ultrasound; (ii) confirmation of

megabladder in the second ultrasound; (iii) collection of fetal urine taken during the routine management of the disease for biochemistry evaluation; and (iv) signature of a written informed consent. Non-inclusion criteria are: (i) persons protected by law and (ii) refusal to participate in the study. The exclusion criteria include: (i) patients lost in follow-up, leaving the study or withdrawing consent; (ii) detection of major structural or chromosomal anomalies in karyotype; and (iii) lack of PUV confirmation in the postnatal period or by anatomic-pathological analysis in case of pregnancy termination or neonatal death. The setup of the ANTENATAL study is reported in Figure 2.

Ethical aspects

The ANTENATAL study (clinicaltrials.gov NCT03116217, started on 26 June 2017) was approved by the national ethics committee for the coordinating centre (No. RCB2016-A01914-47, France). The study is carried out in accordance with the ethical principles expressed in the Declaration of Helsinki. In particular, appropriate written informed consent for fetal urine sampling and laboratory testing will be obtained from all parents of fetuses participating in the study, as previously indicated in the inclusion criteria.

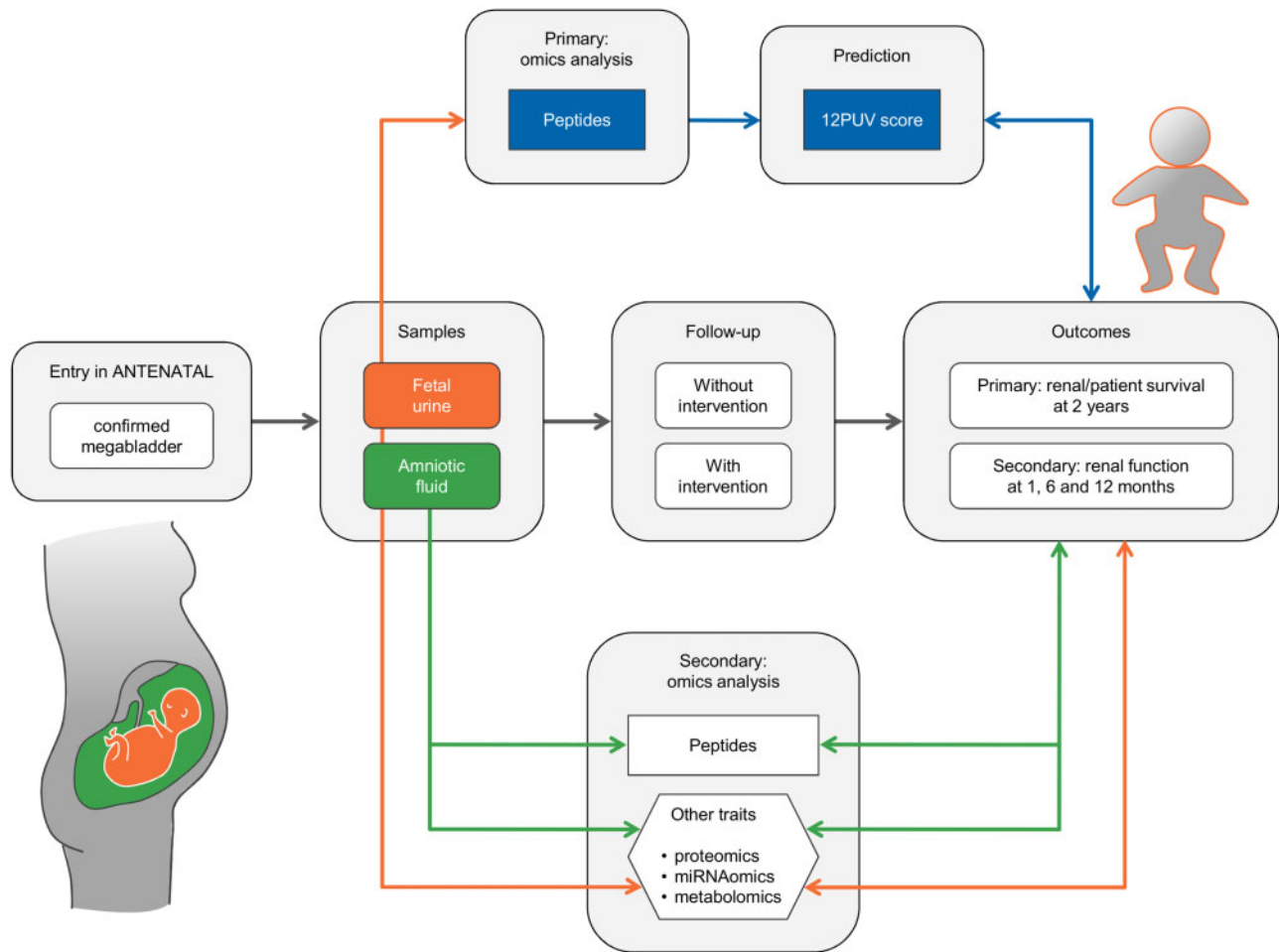


FIGURE 2: ANTENATAL study setup. Four hundred patients recruited in >30 centres will enter the ANTENATAL study with a confirmed presence of a megabladder in the prenatal period. Fetal urine and/or amniotic fluid will be sampled. Fetal urine will be scored with the previously identified peptide-based 12PUV classifier and then compared with renal outcome at 2 years, and renal function at a number of time-points after birth. Fetal urine content in additional omics traits, including proteins, miRNAs and metabolites, will be explored to determine the added value of combining those traits with the peptide-based 12PUV classifier. Amniotic fluid samples will be screened for omics biomarkers of postnatal function in PUV to eventually move the analysis into a less invasive body fluid. Follow-up of all patients will be performed according to local standard care, which can be with or without intervention (vesicoamniotic shunt/laser ablation of valves).

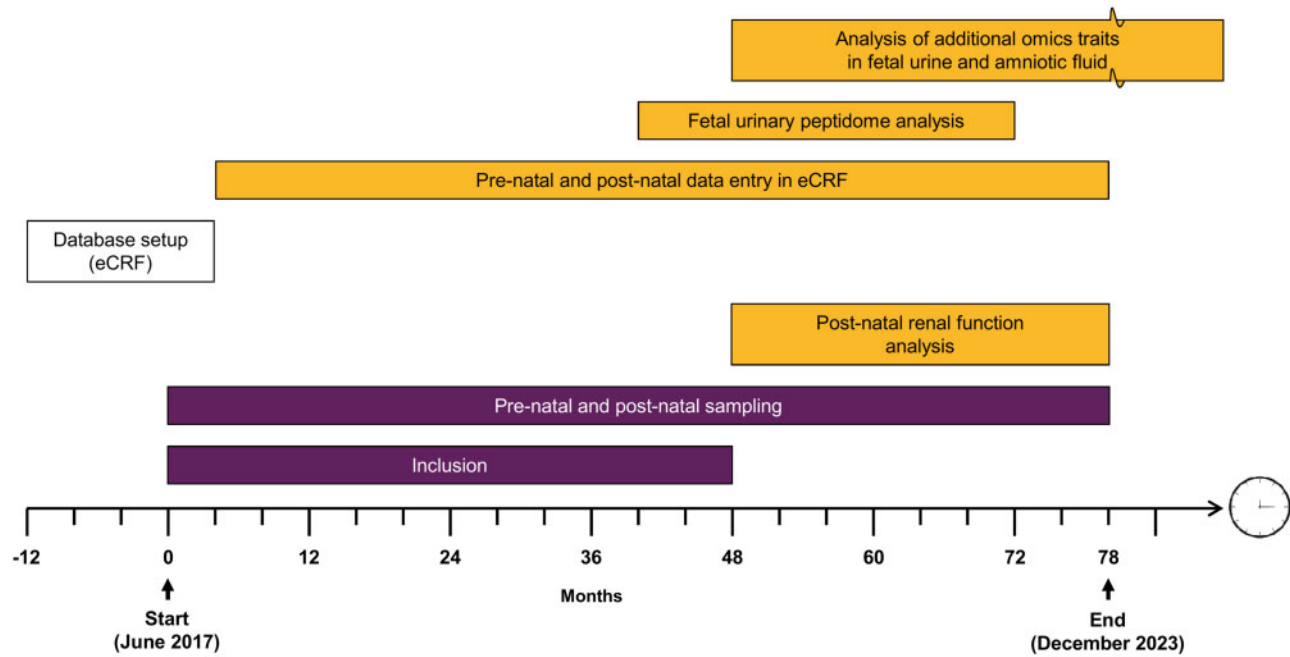


FIGURE 3: ANTENATAL study flow chart. The inclusion period will last for 48 months. The participation of each patient will carry on for 30 months, thereby resulting in a total duration of 78 months for the ANTENATAL trial.

Outcomes

The primary judgment criterion (endpoint) is renal/patient survival at 2 years postnatally, defined by the need for dialysis or death. The secondary judgment criterion is renal function of the live born at 1 month, 6 months and 1 year postnatally.

Procedure and assessment

Study organization. The study organization has been schematized in [Figure 3](#). An in-house-developed electronic case report form (eCRF, [Supplementary data](#)) has been established. According to local clinical practice to limit additional burden on the patients, fetal urine, amniotic fluid (if still present in a certain amount) and postnatal urine samples, as well as pre- and post-clinical data, will be collected. Clinical data can be anonymously entered in the centralized eCRF at any time during follow-up. It is anticipated that recruitment will last for 4 years to reach the 400 patients to be screened. An additional follow-up period of at least 2 years will be required to obtain endpoint data for the last included patients. This period is paralleled by fetal urinary peptidome analysis and initiation of the analysis of additional omics traits in both fetal urine and amniotic fluid.

Routine monitoring. Prenatal visits ([Table 1](#)) aim to obtain a set of ultrasound-measured parameters of kidneys and urinary tract of PUV fetuses. A minimum of one prenatal visit is required and optimally data from four prenatal visits are expected. This will provide information on the antenatal evolution of the disease and will allow comparison of the efficacy of the omics-based prognosis tools with the routine clinical parameters. Postnatal visits ([Table 1](#)) are scheduled to perform anthropometric examination, postnatal biochemistry, and kidney and urinary tract ultrasound. The first postnatal visit further aims to confirm the presence of PUV (or confirm the correct removal of the valves in case of prenatal laser intervention); it will also serve to record the outcome of the pregnancy. In case

of TOP or neonatal death, a standardized anatomic-pathological analysis will be proposed to the parents for the determination of the severity of renal lesions (oligomeganephronia, dysplasia, presence of immature glomeruli and tubules). The final visit includes recording of documentation of surgery, any postnatal complications, admissions to the hospital, number of urinary tract infections and medication during the first 2 years of life. Four postnatal visits are ideally planned during the 2-year postnatal follow-up, the minimum being one visit. The end of study visit equals the 24-month visit (M24), except for TOP or neonatal death, where the last visit will serve as the end of study visit.

Biosamples and omics analysis. Details can be found in [Supplementary data](#).

Statistics

To reach the primary objective, which is the validation of the fetal urine peptidome-based 12PUV classifier to predict *in utero* postnatal renal function in fetuses with PUV, we need to confirm its both sensitivity (88%) and specificity (95%). The 12PUV classifier comprises a scoring scheme, which includes positive and negative predictive values (PPV and NPV, respectively). Power and sample size calculation for this specific setting and the validation of both PPV and NPV have been addressed by Steinberg *et al.*, developed especially for this type of diagnostic tests [13]. Hence, according to Steinberg's method, focusing on the test's specificity without compromising sensitivity, using a range of prevalence of ESRD in PUV individuals from 0.24–0.45 (mean: 0.35) [14, 15] and assuming $\alpha = 0.05$ (significance level) and $1 - \beta = 0.8$ (power), we calculated that the sample size estimation to reach the primary objective is 400 patients to be enrolled.

DISCUSSION

It is essential to confirm that any developed diagnostic or prognostic method also predicts well in, and thus is generalizable to,

Table 1. Synopsis of clinical data obtained in the ANTENATAL study

Data	Prenatal visits				Postnatal visits				
	Screening	Inclusion	Prenatal 14-23 WA ^{a,b}	Prenatal 23-32 WA ^{a,b}	Prenatal 32-41 WA ^{a,b}	Birth-M1 ^{a,g}	Postnatal M5-M7 ^{a,g}	Postnatal M11-M13 ^{a,g}	Postnatal M23-M25 ^{a,g}
Ultrasound of kidneys and urinary tract									
Morphologic data ^c	X	X	X	X	X				
Doppler analysis ^d		X	X	X	X				
Amniotic fluid volume		X	X	X	X				
Keyhole sign		X	X	X	X				
Cervical length		X	X	X	X				
Confirmation of PUV		X	X	X	X				
Outcome of pregnancy miscarriage, perinatal mortality, neonatal mortality or survival						X			
Anthropometric examination ^e						X	X	X	X
Ultrasound of kidneys and urinary tract morphologic data ^c						X	X	X	X
Documentation of any postnatal surgery									X
Documentation of any postnatal complications including start of dialysis									X
Admissions to hospital during first two year-number and length									X
Number of acute renal tract infections during first two years lower urinary tract infection or ascending infection									X
Medication during first two years									X
Fetopathology/anatomopathology if TOP ^f or neonatal death						X			X

^aRange accepted.^bWeeks of amenorrhea.^cKidney size and volume, absence or presence of renal hyperechogenicity, corticomedullary differentiation or renal cysts, kidney cortex thickness, pelvis and ureter maximum diameter if pelvic or ureter dilatation is present, respectively, bladder volume and wall thickness.^dRenal artery pulsatility index (PI) and peak systolic velocity (PSV) on color and pulsed wave doppler.^eWeight, length, head circumference, systolic and diastolic blood pressure.^fTermination of pregnancy.^gMonths.

'similar but different' individuals outside the setting in which the method was developed [16]. The ANTENATAL trial is expected to validate the previously identified 12PUV classifier [9] in the first and the largest prospective cohort focusing on fetuses with PUV. Here, validation will be simultaneously temporal and geographical, since some new patients will be recruited in the same institutions (France) as in the development sample, but at a later point in time, while other patients will be enrolled from eight additional countries. On 1 July 2019, the eCRF contained 55 patient entries from 15 different centres.

Since PUV is a rare disorder, international collaboration is necessary to obtain data from the patients needed for this study. The European Reference Networks (ERNs) were established to tackle rare conditions by bringing together healthcare providers across Europe. ANTENATAL is an international study that was initiated before the start of the ERNs, but after their acceptance, we approached both the centres participating in the European Rare Kidney Diseases Reference Network (ERN ERKNet, www.erknet.org; 31 July 2019, date last accessed) and the centres participating in the Rare Urogenital Diseases Network (ERN eUROGEN, <http://eurogen-ern.eu/>; 31 July 2019, date last accessed). This led to additional centres including patients for ANTENATAL, and endorsement of the ANTENATAL study by those two ERNs.

Collaboration was also established with the Radboudumc Aetiologic research into Genetic and Occupational/Environmental Risk Factors for Anomalies in Children (AGORA) data and biobank [17]. AGORA currently contains data of ~500 PUV patients. To further enrich this resource, we asked the ANTENATAL centres to collect DNA samples of children and their parents and parental questionnaires on a range of non-genetic risk factors for AGORA. This collaboration allows the AGORA researchers to include more patients, and allows us to also evaluate the added value of genomic biomarkers.

With 88% sensitivity and 95% specificity shown in the proof-of-concept study [9], we anticipate that the 12PUV classifier will display superiority over conventional methods in predicting postnatal renal outcome. Beyond its best predictive accuracy, this fetal urine peptide-based tool will have the double benefit of being objective and quantifiable. Indeed, the measurement of fetal urine peptides is not subjected to personal interpretation, as can be the case for sonographic imaging. In addition, the recent and fast evolution of techniques for the analysis of the molecular content of biological fluids opens the possibility of studying additional molecular levels, including miRNAs, proteins and metabolites. As demonstrated recently [10], the combination of fetal urinary peptide and metabolite markers extracted from the same sample generated a clear added value with respect to metabolites alone for the prediction of progression of PUV disease.

In addition to fetal urine, omics analysis in amniotic fluid displays promise for the clinic, since it has emerged as a reliable source of biomarkers for fetal diseases [18, 19]. In this context, we recently identified a novel amniotic fluid peptide signature that predicts postnatal renal function in bilateral congenital anomalies of kidney and urinary tract, including but not limited to PUV, thereby potentially strongly improving the prenatal diagnostic workup of the disease (clinicaltrials.gov NCT02675686, 178 fetuses, unpublished results). Hence, the expansion of biomarker research to amniotic fluid in the ANTENATAL project opens the possibility of predicting postnatal renal function in fetuses with PUV using a less invasive sample. Of note, the omics analysis of markers of PUV severity can most likely not be extended to

maternal plasma or urine even if such body fluids are easier to collect than amniotic fluid [18]. Furthermore, it appears that the risk of miscarriage and stillbirth in women undergoing amniocentesis is mostly increased in high-risk pregnancies [20, 21].

Announcement of anomalies such as PUV is a traumatic and emotive event for families anticipating an unsecured long-term health outcome for their future newborn. In addition, the limited value of ultrasound-based analysis and fetal urinary biochemistry in predicting postnatal ESRD led in the past to unnecessary fetal interventions, TOPs, sometimes contradicted by fetopathology, or continuation of pregnancies resulting in early ESRD [5–8]. The introduction of an ANTENATAL-validated prognostic panel of fetal urinary peptides in the prenatal care of patients with PUV can guide clinicians in the counselling process by delivering unbiased and unambiguous prognostic information. This will improve disease management in many ways, impacting both affected patients and their family by: (i) reducing stress of parents related to the uncertainty of well-being of their unborn infant; (ii) alleviating the enormous psychological burden of parents associated with their decision to continue or terminate pregnancy; (iii) potentially avoiding unnecessary interventions, stressful for the mother and risky even if performed by fetoscopic surgery; (iv) potentially avoiding unnecessary pregnancy termination; and (v) anticipating renal replacement therapy or palliative care of newborns if continuation of the pregnancy is decided in fetuses predicted in the high-risk stratum.

SUPPLEMENTARY DATA

Supplementary data are available at [ckj](http://ckj.oup.com/ckj/advance-article-abstract/doi/10.1093/ckj/sfz107/5574386) online.

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CONFLICT OF INTEREST STATEMENT

No other disclosures are reported.

REFERENCES

1. Thakkar D, Deshpande AV, Kennedy SE. Epidemiology and demography of recently diagnosed cases of posterior urethral valves. *Pediatr Res* 2014; 76: 560-563
2. Agarwal S. Urethral valves. *BJU Int* 1999; 84: 570-578
3. Hodges SJ, Patel B, McLorie G et al. Posterior urethral valves. *Sci World J* 2009; 9: 1119-1126
4. Krishnan A, de Souza A, Konijeti R et al. The anatomy and embryology of posterior urethral valves. *J Urol* 2006; 175: 1214-1220
5. Morris RK, Ruano R, Kilby MD. Effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. *Ultrasound Obstet Gynecol* 2011; 37: 629-637
6. Morris RK, Quinlan-Jones E, Kilby MD et al. Systematic review of accuracy of fetal urine analysis to predict poor postnatal renal function in cases of congenital urinary tract obstruction. *Prenat Diagn* 2007; 27: 900-911
7. Hogan J, Dourthe ME, Blondiaux E et al. Renal outcome in children with antenatal diagnosis of severe CAKUT. *Pediatr Nephrol* 2012; 27: 497-502
8. Morris RK, Malin GL, Khan KS et al. Antenatal ultrasound to predict postnatal renal function in congenital lower urinary tract obstruction: systematic review of test accuracy. *BJOG* 2009; 116: 1290-1299
9. Klein J, Lacroix C, Caubet C et al. Fetal urinary peptides to predict postnatal outcome of renal disease in fetuses with posterior urethral valves (PUV). *Sci Transl Med* 2013; 5: 198ra106
10. Buffin-Meyer B, Klein J, Breuil B et al. Combination of the fetal urinary metabolome and peptidome for the prediction of postnatal renal outcome in fetuses with PUV. *J Proteomics* 2018; 184: 1-9
11. Mischak H, Delles C, Vlahou A, Vanholder R. Proteomic biomarkers in kidney disease: issues in development and implementation. *Nat Rev Nephrol* 2015; 11: 221-232
12. Decramer S, Wittke S, Mischak H et al. Predicting the clinical outcome of congenital unilateral ureteropelvic junction obstruction in newborn by urinary proteome analysis. *Nat Med* 2006; 12: 398-400
13. Steinberg DM, Fine J, Chappell R. Sample size for positive and negative predictive value in diagnostic research using case-control designs. *Biostatistics* 2009; 10: 94-105
14. Yohannes P, Hanna M. Current trends in the management of posterior urethral valves in the pediatric population. *Urology* 2002; 60: 947-953
15. Casella DP, Tomaszewski JJ, Ost MC. Posterior urethral valves: renal failure and prenatal treatment. *Int J Nephrol* 2012; 2012: 1
16. Moons KG, Kengne AP, Grobbee DE et al. Risk prediction models: II. External validation, model updating, and impact assessment. *Heart* 2012; 98: 691-698
17. van Rooij IA, van der Zanden LF, Bongers EM et al. AGORA, a data- and bio-bank for birth defects and childhood cancer. *Birth Defects Res A Clin Mol Teratol* 2016; 106: 675-684
18. Klein J, Buffin-Meyer B, Mullen W et al. Clinical proteomics in obstetrics and neonatology. *Expert Rev Proteomics* 2014; 11: 75-89
19. Desveaux C, Klein J, Leruez-Ville M et al. Identification of asymptomatic fetuses infected with cytomegalovirus using amniotic fluid peptide biomarkers. *PLoS Pathog* 2016; 12: e1005395
20. Malan V, Bussieres L, Winer N et al. Effect of cell-free DNA screening vs direct invasive diagnosis on miscarriage rates in women with pregnancies at high risk of trisomy 21. A Randomized Clinical Trial. *JAMA* 2018; 320: 557-565
21. Wulff CB, Gerds TA, Rode L et al. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147,987 singleton pregnancies. *Ultrasound Obstet Gynecol* 2016; 47: 38-44