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CKD in Bardet-Biedl Syndrome: Evidence Supporting Multifactorial Etiology

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Introduction: Chronic kidney disease (CKD) is a critical prognostic factor in Bardet-Biedl syndrome (BBS). Early diagnosis and intervention are essential for improving patient outcomes. The present study analyzed kidney function in patients with BBS, with the aim to explore the impact of genetic variants and common risk factors for kidney disease.

Methods: A monocentric cross-sectional study was conducted. Patients underwent genetic analysis via next generation sequencing; renal function was assessed and the relationship with the following: (i) age, obesity, hypertension and (ii) genetic mutations was analyzed.

Results: A total of 65 patients with BBS were enrolled in the study; renal function was variable, with 25% of patients showing an estimated glomerular filtration rate (eGFR) < 60 ml/min per 1.73 m^2. Patients' age was inversely correlated with the eGFR ($P = 0.002$). Reduced eGFR significantly correlated with truncating mutations in any BBS gene and hypertension; moreover, multivariate analysis using eGFR as an objective variable and multiple risk factors as explanatory variables, showed that body mass index (BMI) was independently associated with eGFR decline ($\beta = -2.45$, $P < 0.0001$), in addition to age. Interestingly, significant discordance in renal phenotype was revealed in 50% of subgroups of consanguineous or nonconsanguineous patients sharing the same pathogenic variants, indicating clinical variability even in this setting.

Conclusion: The present study suggests that BBS is a condition of vulnerability to develop kidney disease, and that age, hypertension and obesity are associated with eGFR decline in adult patients with BBS. Whether effective interventions to treat modifiable factors will reduce CKD risk requires further studies.

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BBS is a rare genetic disorder known as a model of ciliopathy.[1] In fact, its genetic background includes numerous genes related to the structure and function of the primary cilium.[2] Clinical features are variable; retinal dystrophy, learning disabilities, polydactyly, obesity, and kidney disease are the most common morbidities.[3] Despite intensive investigations, several critical key issues remain to be solved, including the reasons underlying the wide degree of variability in clinical manifestations, such as kidney disease, making risk prediction challenging.

Based on reports from the literature, kidney is variably affected in BBS and genetic heterogeneity explains only in part this variability.[4–6] Signs of renal defects may be evident as early as in fetal ultrasound, identifiable as hyperechoic and/or cystic kidneys; however, some reports indicate the absence of abnormalities on either pediatric or adult patients.[7] Structural kidney abnormalities are common in major observational studies, with variable degrees of kidney dysfunction up to kidney failure (KF). Given the pivotal role of kidney disease in determining the prognosis and quality of life in BBS, a comprehensive analysis of kidney function and its relationship with

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age, hypertension, obesity, and genetics is of high relevance. The present study aimed to address this issue, by assessing kidney function in a monocentric cohort of patients with BBS and analyzing possible correlations with known risk factors of kidney disease and with the underlying genetic variants.

METHODS

Subjects

Patients referred to the Ophthalmological Unit of University of Campania L. Vanvitelli (Naples) were recruited into the study. Patients with a diagnosis of retinal dystrophy and at least 2 additional clinical signs among the following: (i) polydactyly/syndactyly/clinodactyly, (ii) learning disability, (iii) kidney disease, and (iv) cryptorchidism in males, were referred to the multidisciplinary eye care team, including geneticists and nephrologists, for molecular diagnosis and clinical assessment, respectively.

To be included in the study, clinical criteria for the diagnosis of BBS according to Beales were fulfilled.[8] Four features among major criteria or 3 major features plus 2 minor features were required. The patients or their caregivers signed a written consent to the study. All procedures were conducted according to international guidelines and to the tenets of the Helsinki Declaration 2008 and 2013. Each patient (or parent or legal guardian) gave written consent to undergo DNA analysis, which was performed according to the guidelines for genetic testing approved by the Health Minister, Rome, Italy (G.U. n. 224, 23th September 2004). Approval for the study was obtained from the Ethics Committee of the University of Campania "Luigi Vanvitelli" (#0017030/i-13/07/2020).

Clinical and Laboratory Assessment

Patient assessment included general physical examination. Blood samples were taken after overnight fasting. Renal function was evaluated by measuring standard plasma creatinine levels and the glomerular filtration rate was estimated using the CKD Epidemiology Collaboration 2021 formula in adults, whereas Schwartz formula was used for children.[9-11] Retinal dystrophy was defined based on standard electroretinogram analysis.

In adults, obesity was defined as BMI $>$ 30 kg/m^2, whereas overweight patients were defined as BMI between 24 and 29.9 kg/m^2. In children, obesity was defined as a BMI z-score $\geq$ 2 SD and overweight as a BMI z-score $\geq$ 1 SD above the average value for sex and age according to the World Health Organization 2007 BMI reference for children aged 5 to 19 years.[12]

Fasting plasma glucose levels $>$ 126 mg/dl ($>$7.0 mmol/l) or glycosylated hemoglobin levels $>$ 6.5% (48 mmol/mol) of total hemoglobin indicated diabetes mellitus if confirmed twice.[11] Hypertension was defined as arterial pressure $>$ 140/90 mm Hg in adults, $\geq$ 130/80 mm Hg in those aged 13 to 18 years and above the 95th percentile for age, sex, and height in patients aged $<$13 years, on 2 different days.[13-15]

eGFR categories were defined according to Kidney Disease Improving Global Outcomes.[16,17] KF was defined as an eGFR $<$ 15 ml/min per 1.73 m^2 for 3 months or more. Urine albumin-to-creatinine ratio was measured by a central laboratory with an immunological method (radioimmunoasay). Albuminuria was defined when urine albumin-to-creatinine ratio was $>$ 30 mg/g. Evaluation of renal disease severity in patients with the same genetic background was assessed after stratification of patients into 3 categories, based on patients' eGFR: severe, intermediate, and mild disease if the eGFR was $<$ 30, between 30 and 90, and $>$ 90, respectively.

Genetics

Patients with BBS underwent genetic analysis via next generation sequencing. DNA was extracted from blood using the QIAmp DNA Blood Kit by Qiagen and its quality and quantity were assessed with spectrophotometry (Nanodrop ND 1000, Thermo Scientific Inc., Rockford, IL) and fluorometry (Qubit 2.0 Fluorometer, Life Technologies, Carlsbad, CA). Clinical exome sequencing, containing all disease-causing genes (approximately 5000 genes), was used for genetic screening. Enrichment was conducted using the HaloPlex Target Enrichment System (Agilent) and sequencing using the HiSeq1000 system (Illumina inc., San Diego, CA). Variants were filtered for quality and number of reads (at least 5). Population databases (ExAC, gnomAD, and an internal database) were used to estimate population frequency: only rare alleles (minor allele frequency $<$ 1%) were included. Pathogenicity was predicted with *in silico* tools such as SIFT, FATHMM, MutationAssessor, Polyphen-2, MutationTaster and Provean, MuPRO, PANTHER, PhD-SNP, and SNP n GO. Detected mutations were searched in the Clinvar database. Familial segregation was performed whenever possible. Variant pathogenicity was interpreted according to The American College of Medical Genetics and Genomics guidelines. The supposed causative mutations were finally confirmed by Sanger sequencing.

Statistical Analysis

Student *t* test was used for the comparison between continuous variables, whereas Fisher exact test was used for the comparison between categorical variables. Given the strong tendency of the relationship between our data toward 1 direction, to increase the power of our test, 1-tailed Fisher test was applied. *P*-values were considered significant when $<$ 0.05. These statistical

analyses were performed using MedCalc statistical software.

Spearman's correlation was used to assess linear dependence between eGFR and age and BMI.

Correlation was judged very strong for results included between 1 and 0.9, strong between 0.9 and 0.7, moderate between 0.7 and 0.5, low between 0.5 and 0.3, and poor below 0.3. The alpha risk was set to 0.05. A multivariate linear regression analysis was performed to assess the relation between eGFR and the following explanatory variables: BMI, gender, and age. The low number of patients for each mutated gene made it impossible to include genetics in the analysis. A P-value < 0.05 was considered statistically significant. Patients with missing data were excluded from the analysis. Univariate and multivariate regression analyses were performed with EasyMedStat (version 3.36; www.easymedstat.com).

RESULTS

Cohort of Patients With BBS

Sixty-five patients with BBS were recruited in the study, via referral to the Ophthalmological Unit of University of Campania, L. Vanvitelli, between 2010 and 2023. Mean age was 30.3 ± 12.5 years. Relatives' consanguinity was ascertained in 13 cases. During the period of observation, 2 patients died of cardiovascular complications and sepsis with subsequent worsening of renal function. All patients were born after uneventful maternal pregnancy. Prenatal kidney and/or urinary tract abnormalities on ultrasound were reported in 4 patients. Delays in speech and motor development were present in 60% of patients. Nocturnal vision loss was reported in almost all patients (60/61). Obesity was ascertained in 34 of 60 patients (56.6%), whereas 17 of 60 (28.3%) were overweight and the remaining patients were of normal weight. Major clinical signs of patient cohort are defined in Table 1.

Kidney Dysfunction

On kidney ultrasound, major abnormalities were fetal lobulation, hyperechoic kidneys, poor corticomedullar differentiation, and renal hypoplasia. Pelvic dilation was quite common. Many patients showed hyposthenuria as early sign of kidney dysfunction[18] Only a few patients have had febrile urinary tract infections at the time of observation and/or in the personal story (Supplementary Table S1).

Table 1. Clinical features of patient cohort

Clinical signs	*n* (%)
Polydactyly	42/63 (66.6%)
Learning disabilities	36/60 (60%)
Retinal dystrophy	60/61 (98%)
Obesity	34/60 (57%)
Diabetes	4/60 (7%)
Hypertension	25/65 (38.5%)

Mean eGFR was 74.21 ml/min per 1.73 m^2, with a wide variability found among patients. The eGFR was $>$ 90 ml/min per 1.73 m^2 in 42 patients (65%) and below 90 in 23 patients (35%). Mean ages of patients with CKD1 and CKD 2 to 5 were 27 ± 9 and 37 ± 15 years, respectively ($P = 0.002$, Figure 1a). Patient stratification by age revealed a progressive reduction of eGFR, with the oldest patients showing significantly declined eGFR compared with young individuals (Figure 1b).

Ten patients reached KF (15%). Of those, 7 patients were under hemodialysis treatment and 3 patients received kidney transplant. No patient was under peritoneal dialysis. Age at KF is defined in Table 2; 2 patients at KF were pediatric whereas the remaining patients were adults. The first 2 patients showed both abnormal kidney (renal cysts) and urinary tract abnormalities (obstructive uropathy) on prenatal ultrasound.

Patients with BBS that reached KF in adult age showed a long-lasting story of CKD; KF was reached after an acute kidney disease-on-CKD in 3 patients, due to aminoglycoside toxicity, sepsis and nephrectomy for renal cancer. Interestingly, patients with CKD stage 2–5 showed a higher prevalence of obesity and hypertension compared with patients with an eGFR $>$ 90 ml/min per 1.73 m^2 (Table 3).[19,20] Mean systolic blood pressure was 112.13 ± 12.89 and 135.68 ± 12.84 mm Hg in patients with BBS with preserved renal function and patients with CKD2–5 BBS, respectively; with a statistically significant difference ($P < 0.0001$). Similar results were found for diastolic blood pressure, with mean values of 77.51 ± 9.87 mm Hg and 86.59 ± 8.92 mm Hg in CKD1 and patients with CKD2–5 BBS ($P = 0.0007$), respectively.

Ten out of 32 patients with GFR ≥ 90 ml/min per 1.73 m^2, and 17 of 22 with a lower eGFR had hypertension ($P = 0.0010$).[21] Given the low number of pediatric patients, and considering that obesity generally requires years before leading to eGFR decline, causing hyperfiltration for a while and then ending up in CKD,[19,20] we analyzed the effect of BMI in adult patients only. One-tailed Fisher exact test showed a significant higher prevalence of obese patients (BMI ≥ 30) in case of CKD2–5 ($P = 0.0478$).

To better address the role of age, BMI, and gender on eGFR decline in adult patients with BBS, univariate and multivariate linear regression analysis was performed in our cohort. Poor negative correlation was found between eGFR and BMI ($\rho = -0.21$; $r^2 = 0.106$; $P = 0.117$) in univariate analysis, whereas a moderate

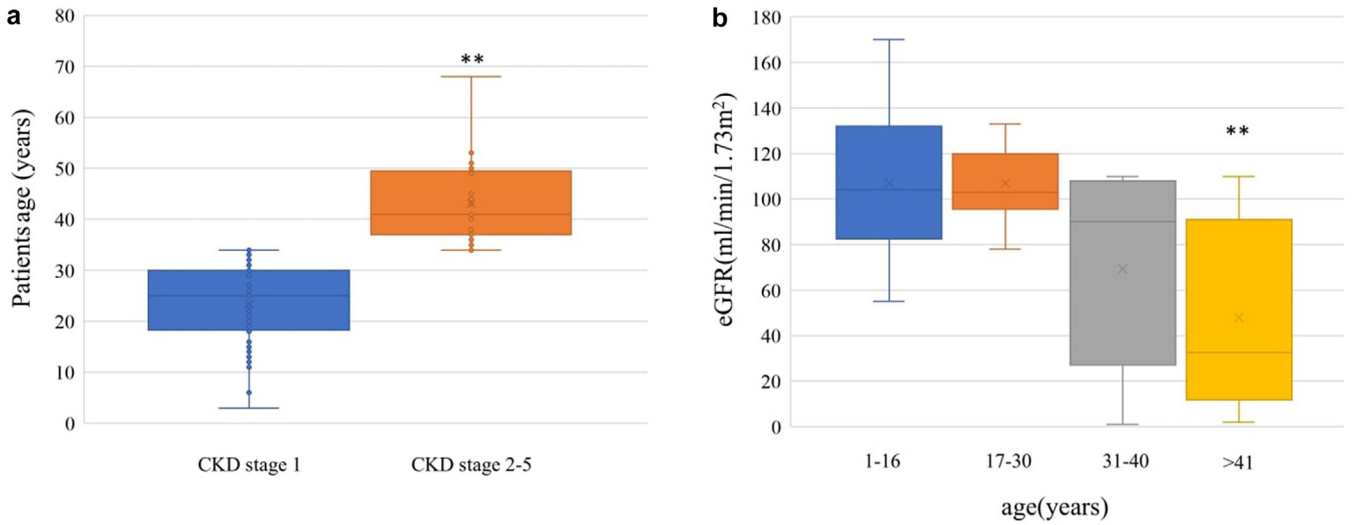

Figure 1. (a) Age distribution of patients with BBS with CKD1 and CKD2-5. Median age is significant different between the 2 groups. (b) eGFR distribution according to age groups. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

negative correlation was found between eGFR and age ($\rho = -0.61$; $r^2 = 0.381$; $P < 0.001$). In Supplementary Figure S1, we show the correlation between BMI and eGFR.

Conversely, in multivariate analysis, age ($\beta = -2.45$, [−3.39 to −1.5], $P < 0.0001$) and BMI ($\beta = -1.71$, [−3.03 to −0.38], $P = 0.0126$) were both significantly associated with lower eGFR values. Gender ($\beta = 7.61$, [−9.66 to 24.88], $P = 0.38$) had no impact effect on eGFR.

Albuminuria was uncommon, as expected in primary tubulointerstitial disorders; when present, it was accompanied by eGFR decline in major cases. In fact, only 3 of 28 patients (10.7%) with normal eGFR presented with albuminuria; conversely, higher albumin-to creatinine ration was ascertained in 8 of 18 patients with declined eGFR (44.4%). The association between albuminuria and eGFR decline was statistically significant ($P = 0.0122$). Interestingly, 8 of 12 patients (66.7%) with albuminuria versus 10 of 30 patients (33.3%) without albuminuria were obese ($P = 0.05$). These results are in line with those of the general population, in which obesity has been demonstrated to cause proteinuria and glomerulopathy.[21]

Genetics and Genotype to Renal Phenotype Correlation

Fifty-eight of 65 patients under study (89%) underwent genetic analysis with next generation sequencing. Forty-nine patients showed biallelic pathogenic variants in known BBS genes, whereas 9 patients (15.5%) were negative on genetic analysis.

In Table 4, we show the molecular variants of all patients and the main associated clinical features. Clinical features of patients with negative, missing, or pending genetic analysis are detailed in Supplementary Table S2.

In Figure 2a, we show gene frequency in our patients cohort. Although in Western countries the major reported BBS diagnosis are *BBS1* and *BBS10*, in our cohort the most prevalent genes were *BBS10* (14 of 58 patients, 24.1%), followed by *BBS12* (10 of 58, 17.2%). *BBS4* accounted for almost 14% of patients ($n = 8$), whereas *BBS1* and *BBS9* caused 12% and 11% of cases,

Table 2. List of patients with KF, with clinical and genetic information

Patient	Age at KF	Age at last presentation	Gender	Prenatal kidney and /or UT abnormalities on ultrasound	Polyuria as presenting sign	BMI	Gene	Type of mutation
P1	9	35	F	yes	yes	35	*BBS9*	2 frameshift
P2	50	50	M	no	yes	42	*BBS12*	2 stopgain
P3	35	45	F	no	Unknown	40	*BBS4*	2 splicing
P4	44	51	F	no	unknown	24	unknown	-
P5	22	35	M	yes	yes	29	*BBS10*	2 frameshift
P6	8	32	M	yes	yes	35	unknown	-
P7	37	38	F	no	no	35	*BBS9*	2 frameshift
P8	68	68	M	unknown	no	29	unknown	-
P9	51	51	M	unknown	no	33	unknown	-
P10	50	53	M	unknown	no	30	unknown	-

BMI, body mass index; F, female; KF, kidney failure; M, male; UT, urinary tract.

Table 3. Main clinical features of patients with CK1 versus CKD2–5

Characteristics	CKD1	CKD2–5	*P*-values
Male gender (%)	22/42 (52.4%)	12/23 (52.2%)	
Age, mean	26.90 ± 9.54	36.65 ± 14.82	*t*-Student: $P = 0.0020$
Hypertension	10/32 (31.25%)	17/22 (77.3%)	One-tailed Fisher's test: $P = 0.0010$
Obesity	15/5 adult patients (42.8%)	14/20 adult patients (70.0%)	One-tailed Fisher's test: $P = 0.0478$

respectively. *BBS2* and *BBIP1* mutations were found in 3 patients (5.1%) and 1 patient, respectively.

In Figure 2b**,** we show the genetics of patients with CKD2–5. The analysis revealed that majority of these patients carried mutations in *BBS10*. *BBS12*, *BBS4*, and *BBS9* were slightly overrepresented in this subgroup compared with the entire cohort, whereas *BBS1* and *BBS2* mutations were absent. The high prevalence of BBS10 and BBS12 in patients with declined eGFR may depend on the high prevalence of these genotypes in the entire cohort. Thus, we divided patients based on genotype, showing the percentage of individuals with normal eGFR versus declined eGFR for each genotype. Interestingly, the analysis revealed that *BBS1* mutations are absent in BBS with kidney dysfunction, whereas *BBS10* and *BBS12* showed CKD2–5 in 36% and 50% of cases, respectively; and *BBS4* and *BBS9* in 50% and 40%, respectively (Figure 3).

The type of genetic variants was variable in the population (Figure 4); the most prevalent mutations in patients with CKD 2–5 were frameshift mutations (41% of cases).

Among patients with detected pathogenic variants, 88.9 % with CKD2–5 presented with at least 1 truncating mutation; conversely, at least 1 truncating mutation was present in 18 of 29 patients (62.0 %) with preserved kidney function. The difference was statistically significant ($P = 0.0448$).

Discordance in Kidney Disease Severity in Patients Sharing the Same Pathogenic BBS variants

Although all affected family members are carriers of the same pathogenic variants, intrafamilial clinical variability has been described.[3,22] For a deeper analysis of renal phenotype among individuals with the same pathogenic variants, a total of 26 patients with BBS were selected from the cohort. This study included 6 groups of siblings (named groups 1–6) and 4 groups of nonconsanguineous patients sharing the same genetic mutation (groups 7–10).

In Table 5, we show the clinical features of these patients, demonstrating that kidney disease may be highly variable even in the presence of the same pathogenic genotype. In 50% of patients' groups, there was discordance in kidney disease severity. Interestingly, in patients' group 1, a significant variability of kidney function was ascertained: the oldest brother was transplanted during infancy, due to structural abnormalities of kidney and urinary tract diagnosed at the birth; his brother was born after an uneventful maternal pregnancy and grew up as an adult age with nearly normal renal function. Of note, in all groups, women had a lower eGFR than men, with the exception of group 4, which consisted of 2 siblings with unknown genetic mutations where the oldest brother was obese and had a slight decline in renal function; because in the entire cohort, all patients with declined eGFR were more often obese and had hypertension. In particular, 5 of 5 patients with severe kidney disease and available data were obese and 4 of 5 were hypertensive; whereas only 5 of 11 with mild kidney disease presented with obesity and 1 of 11 with hypertension (1-tailed Fisher test for obesity, $P = 0.0577$; 1-tailed Fisher test for hypertension, $P = 0.0128$).

DISCUSSION

CKD has been recognized as a public health problem due to its association with cardiovascular mortality, poor outcomes, and severe complications. These universal observations have been confirmed in patients with BBS, where kidney disease is a known cause of morbidity and mortality; however, discordance in kidney disease severity is a known characteristic of patients with BBS.[6,18,23]

The present study describes renal function in an Italian cohort of 65 patients with BBS. The study demonstrates that: (i) kidney dysfunction correlates with the type of genetic mutation; (ii) age, obesity and hypertension, common risk factors for CKD, correlate with declined eGFR; and (iii) the severity of kidney dysfunction is highly variable even among patients with the same pathogenic variants. Overall, these data suggest that a combination of genetic and systemic factors contribute to determining kidney outcome in BBS.

Studies describing structural kidney and urinary tract abnormalities demonstrated a high frequency of defects such as cortical cysts, pelvic dilation, V-U reflux, and renal hypodysplasia.[8,24] Functional abnormalities include a progressive decline of the eGFR often preceded by polyuria due to possible tubulointerstitial fibrosis.[25] Glomerulopathies are sometimes described.

Table 4. Molecular variants and main clinical features of patients cohort

Patient ID	Age (yrs)	eGFR (ml/min per 1.73 m^2)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	Gene	Zygosity	cDNA change	Protein change	NM
P1	12	55	30	130	80	*BBIP1*	comp het	c.38-11T>G; c.112+2T>A	splicing; splicing	NM_001195306.2
P2	19	133	27.7			*BBS1*	comp het	c.46A>T ; c.1285dup	p.Ser16Cys; p.Arg429Profs*72	NM_024649.5
P3	20	130	26	100	80	*BBS1*	hom	c.664G>C	p.Gly222Arg	NM_024649.5
P4	22	120	30	100	80	*BBS1*	hom	c.664G>C	p.Gly222Arg	NM_024649.5
P5	18	115	26	120	80	*BBS1*	hom	c.46A>T	p.Ser16Cys	NM_024649.5
P6	34	110	24	120	80	*BBS1*	comp het	c.1169T>G; c.1642del	p.Met390Arg; Leu548Trpfs*31	NM_024649.5
P7	43	94	24	110	80	*BBS1*	hom	c.1169T>G	p.Met390Arg	NM_024649.5
P8	19	90	23	90	60	*BBS1*	comp het	c.670G>A; c.1642del	p.Glu224Lys; p.Leu548Trpfs*31	NM_024649.5
P9	11	170				*BBS2*	comp het	c.1421C>A; c.944G>A	p.Ser474*; p.Arg315Gln	NM_031885.5
P10	31	108	31	135	90	*BBS2*	hom	c.1909_1910del	Met637Glufs*12	NM_031885.5
P11	33	100	31	118	78	*BBS2*	hom	c.224T>G	p.Val75Gly	NM_031885.5
P12	25	125	29	104	80	*BBS4*	comp het	c.332+8T>C; c.341del	splicing; p.Leu114Trpfs*28	NM_033028.5
P13	12	120	29	100	70	*BBS4*	comp het	c.1248+1G>T; c.883C>T	p.Arg295Thr*	NM_033028.5
P14	25	120	33	118	80	*BBS4*	unknown	unknown	unknown	
P15	26	104	29	90	70	*BBS4*	hom	c.332+2_332+3insTT	splicing	NM_033028.5
P16	34	72	29	110	85	*BBS4*	comp het	c.332+2_332+3insTT	splicing	NM_033028.5
P17	49	50	30.9	140	90	*BBS4*	comp het	c.341del; c.1091C>A	p.Leu114Trpfs*28; p.Ala364Glu	NM_033028.5
P18	32	24	40	150	90	*BBS4*	comp het	c.332+2_332+3insTT; c.1091C>A	splicing; p.Ala364Glu	NM_033028.5
P19	45	2	42	120	80	*BBS4*	hom	c.332+2_332+3insTT	splicing	NM_033028.5
P20	32	110	43	130	80	*BBS9*	hom	c.785T>C	p.Val262Ala	NM_198428.3
P21	29	99	28	105	70	*BBS9*	comp het	c.586_587del; c.2153del	p.Val196Leufs*10; Gly678Alafs*10	NM_198428.3
P22	36	52	23	140	90	*BBS9*	comp het	c.8del; c.1276_1277del	p.Leu3Tyrfs*38; Gln426Serfs*5	NM_198428.3
P23	33	30	47	140	90	*BBS9*	comp het	c.586_587del; c.51_54del	p.Val196Leufs*10; p.Glu18Asnfs*22	NM_198428.3
P24	38	5	52	160	100	*BBS9*	comp het	c.586_587del; c.51_54del	p.Val196Leufs*10; p.Glu18Asnfs*22	NM_198428.3
P25	35	1	35	140	90	*BBS9*	comp het	c.310del; c.1276_1277del	p.Cys104Valfs*20; p.Gln426Serfs*5	NM_198428.3
P26	32	110	23	120	80	*BBS10*	comp het	c.101G>C; c.962A>G	p.Arg34Pro; p.Tyr321Cys	NM_024685.4
P27	35	108	30	130	90	*BBS10*	comp het	c.2137_2140del; c.962A>G	p.Lys713Phefs*16; p.Tyr321Cys	NM_024685.4
P28	3	100		90	60	*BBS10*	hom	c.899A>C	p.His300Pro	NM_024685.4
P29	25	100	24	120	80	*BBS10*	comp het	c.101 G>C; c.962A>G	p.Arg34Pro; p.Tyr321Cys	NM_024685.4
P30	30	100	24	120	80	*BBS10*	comp het	c.101 G>C; c.962A>G	p.Arg34Pro; p.Tyr321Cys	NM_024685.4
P31	29	95	38.06			*BBS10*	hom	c.261_263 del	p.Ile88del	NM_024685.4
P32	14	90		100	80	*BBS10*	hom	c.271dup	p.Cys91Leufs*5	NM_024685.4
P33	29	90	40	120	90	*BBS10*	comp het	c.531C>A; c.273C>G	p.Tyr177*; p.Cys91Trp	NM_024685.4
P34	30	90	28	105	70	*BBS10*	hom	c.1091del	p.Asn364Thrfs*5	NM_024685.4
P35	6	80	30	135	90	*BBS10*	hom	c.271dup	p.Cys91Leufs*5	NM_024685.4
P36	50	80	33	110	70	*BBS10*	hom	c.641T>A	p.Val214Glu	NM_024685.4
P37	21	78	30	140	90	*BBS10*	comp het	c.325_328del; c.145C>T	p.Met109Valfs*25; p.Arg49Trp	NM_024685.4
P38	15	70				*BBS10*	comp het	c.271dup	p.Cys91Leufs*5	NM_024685.4
P39	34	2	26	140	90	*BBS10*	comp het	c.235dup; c.271dup	p.Thr79Asnfs*17; p.Cys91Leufs*5	NM_024685.4
P40	16	140	33	110	80	*BBS12*	hom	c.898C>T	p.Gln300*	NM_152618.3
P41	30	110	29	105	85	*BBS12*	hom	c.2023C>T	p.Arg675*	NM_152618.3

(Continued on following page)

Table 4. (Continued) Molecular variants and main clinical features of patients cohort

Patient ID	Age (yrs)	eGFR (ml/min per 1.73 m^2)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	Gene	Zygosity	cDNA change	Protein change	NM
P42	33	98	41.5	130	90	*BBS12*	comp het	c.62_64del; c.2023C>T	p.Ser21del; p.Arg675*	NM_152618.3
P43	38	91	27.34	124	84	*BBS12*	comp het	c.62_64del; c.2023C>T	p.Ser21del; p.Arg675*	NM_152618.3
P44	44	90	29.1	120	80	*BBS12*	hom	c.1577T>C	p.Leu526Ser	NM_152618.3
P45	21	80	32	135	90	*BBS12*	hom	c.2023C>T	p.Arg675*	NM_152618.3
P46	37	78	23	140	100	*BBS12*	hom	c.2023C>T	p.Arg675*	NM_152618.3
P47	40	55	35	130	90	*BBS12*	hom	c.1577T>C	p.Leu526Ser	NM_152618.3
P48	50	2	35	150	90	*BBS12*	hom	c.2023C>T	p.Arg675*	NM_152618.3
P49	13	136		104	48	*BBS12*	hom	c.1063C>T	p.Arg355*	NM_152618.3
P50	21	130	28			unknown				
P51	23	125	36,1	110	80	unknown				
P52	25	120	37	110	62	unknown				
P53	37	110	40	120	90	unknown				
P54	41	110	28	102	76	unknown				
P55	15	108	28			unknown				
P56	26	102	34	100	70	unknown				
P57	44	100	26	120	85	unknown				
P58	27	97	33	130	90	unknown				
P59	37	95	28	100	80	unknown				
P60	34	90	31	100	60	unknown				
P61	51	15	35	150	90	unknown				
P62	53	15	29	115	60	unknown				
P63	68	15	33	140	80	unknown				
P64	32	5	38	140	90	unknown				
P65	51	2	24	130	80	unknown				

BMI, body mass index; comp het, composed heterozygous; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; hom, homozygous; SBP, systolic blood pressure.

Previous studies have analyzed the frequency of kidney dysfunction in patients with BBS. In 1996, O'Dea *et al.*[24] described the natural history of BBS in a cohort of 38 patients compared with 58 siblings. The paper showed that 25% of patients, by the age of 48 years, had renal impairment. Webb *et al.*[26] examined clinical features of 46 patients with BBS from Newfoundland. The authors showed that there was 15-fold increased risk of CKD in patients with BBS compared with noncarriers.

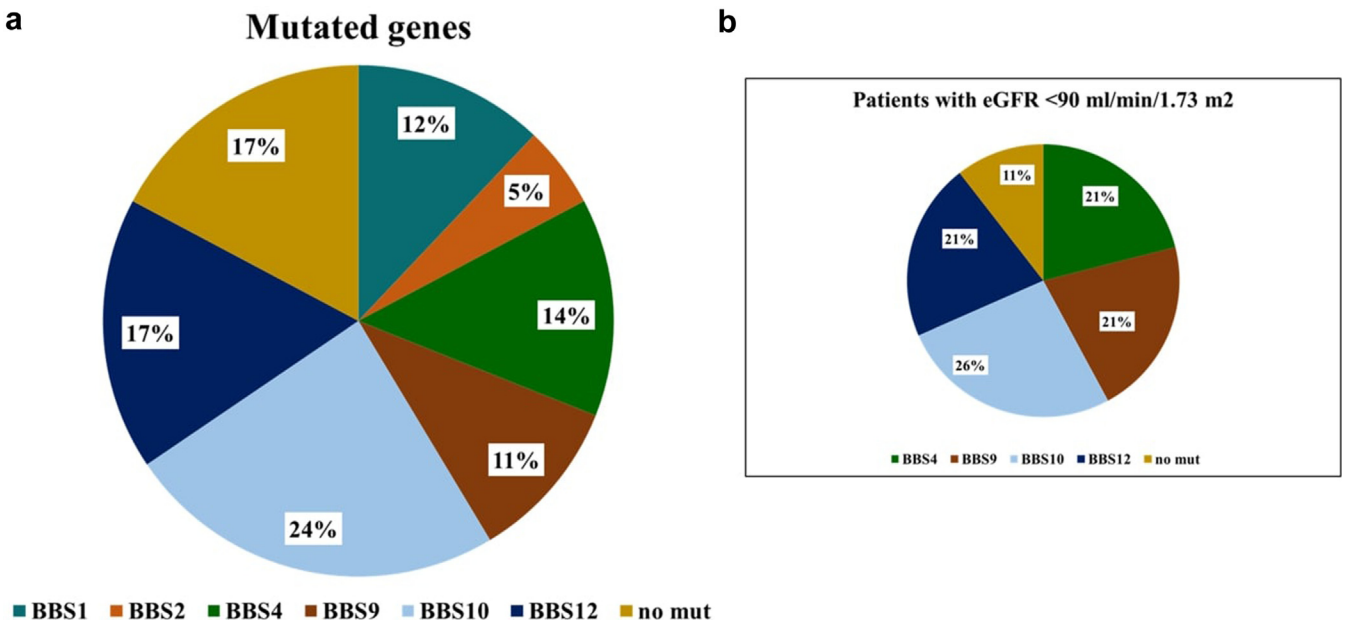

Figure 2. (a)Frequency of genetic variants in the entire BBS cohort and (b) in patients with stage 2–5 CKD. eGFR, estimated glomerular filtration rate.

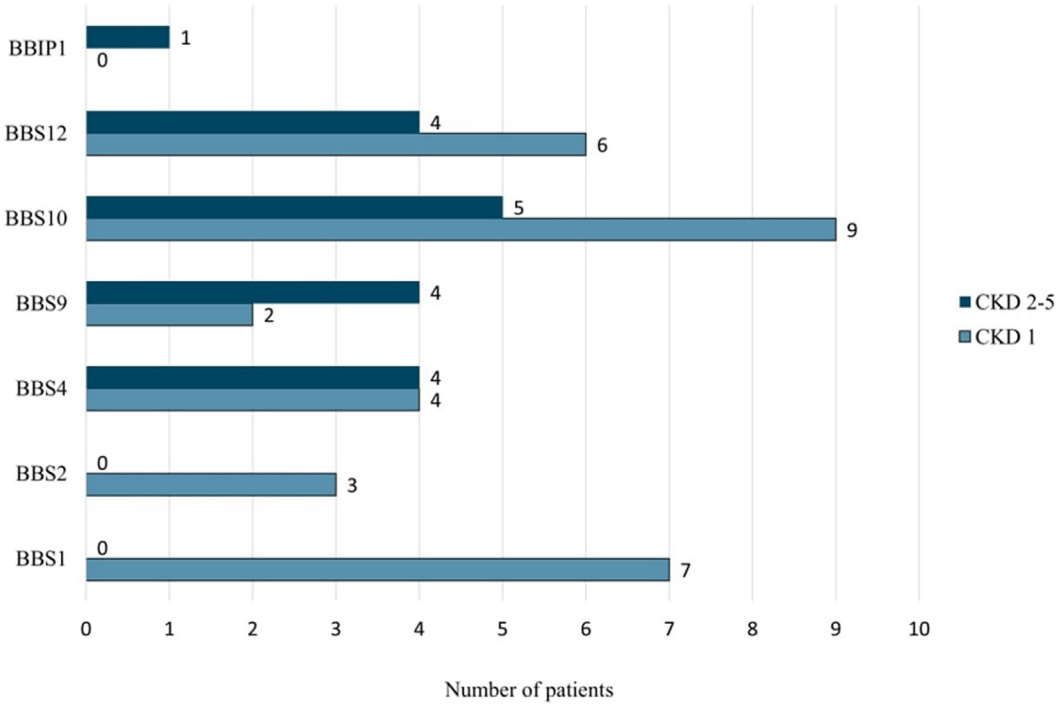

Figure 3. Major mutated genes in the cohort and frequency of patients with CKD1 and those with CKD2–5 for each genotype. CKD, chronic kidney disease.

Twenty-one percent of patients developed KF by the age of 60 years.

Imhoff *et al.*[4] in 2011, described renal and cardiovascular phenotypes in 33 French patients with BBS aged >16-years. Renal involvement was reported in 82% of cases, confirming that kidney disease is a hallmark of BBS. Thirty-three percent of patients had CKD stage 1, suggesting that the majority of young adult patients with BBS have normal eGFR, as observed in our study; 9% of patients had an eGFR < 60 ml/min per 1.73 m^2 (Modification of Diet in Renal Disease formula).

Forsythe *et al.*[6] in 2017, described the prevalence of CKD in 350 patients referred to the United Kingdom national BBS clinic. Data on eGFR were available for 189 adult and 133 pediatric patients. Truncating mutations in any BBS gene and mutations in *BBS2*, *BBS10*, and *BBS12* were associated with worse renal phenotype compared with *BBS1* mutations. The prevalence of KF was 5% and 6% in adult and pediatric patients, respectively. The authors concluded that patients who do not reach KF in young age preserve renal function in adulthood. Meyer *et al.*[23] analyzed the presence of CKD in patients with BBS from the international registry of patients with BBS, CRIBBS registry, to establish the incidence of KF (eGFR < 15 ml/min per 1.73 m^2) and to establish correlations with genetics. Forty-four patients out of 607 (7.2%) had KF; the authors highlighted that 77.3% of patients with kidney disease were females. The study confirmed that truncating BBS variants correlated with kidney disease. The median age of KF was 12.5 years. Major deaths in infancy occurred especially in patients with KF, suggesting that KF is a high risk of mortality. Unfortunately, the study does not provide data on eGFR based on age, thus the cumulative prevalence of CKD in adult individuals and the risk to develop CKD cannot be estimated.

The present study confirms that structural renal abnormalities are diffuse in patients with BBS and shows that reduced eGFR is not uncommon in adult patients with BBS. Genetics strongly affect renal phenotype: in our cohort, frameshift mutations correlated with severe renal phenotype, confirming that truncating mutations predict poor renal outcome.[5] However, variability in kidney phenotype is a well-known feature of patients with BBS, and sometimes intrafamilial variability has been described. Thus, we studied the influence of common risk factors for kidney disease on eGFR in our cohort of patients.

Our results revealed that age, BMI, and blood pressure were inversely correlated with eGFR, suggesting that common cardiovascular factors impact kidney function in this setting. However, though hypertension may be either the cause and the consequence of renal dysfunction, the correlation with obesity requires attention and opens possible avenues to prevent kidney disease progression. For a deeper analysis of variability in renal phenotype within the same genotype, renal dysfunction has been analyzed in subgroups of patients sharing the same pathogenic

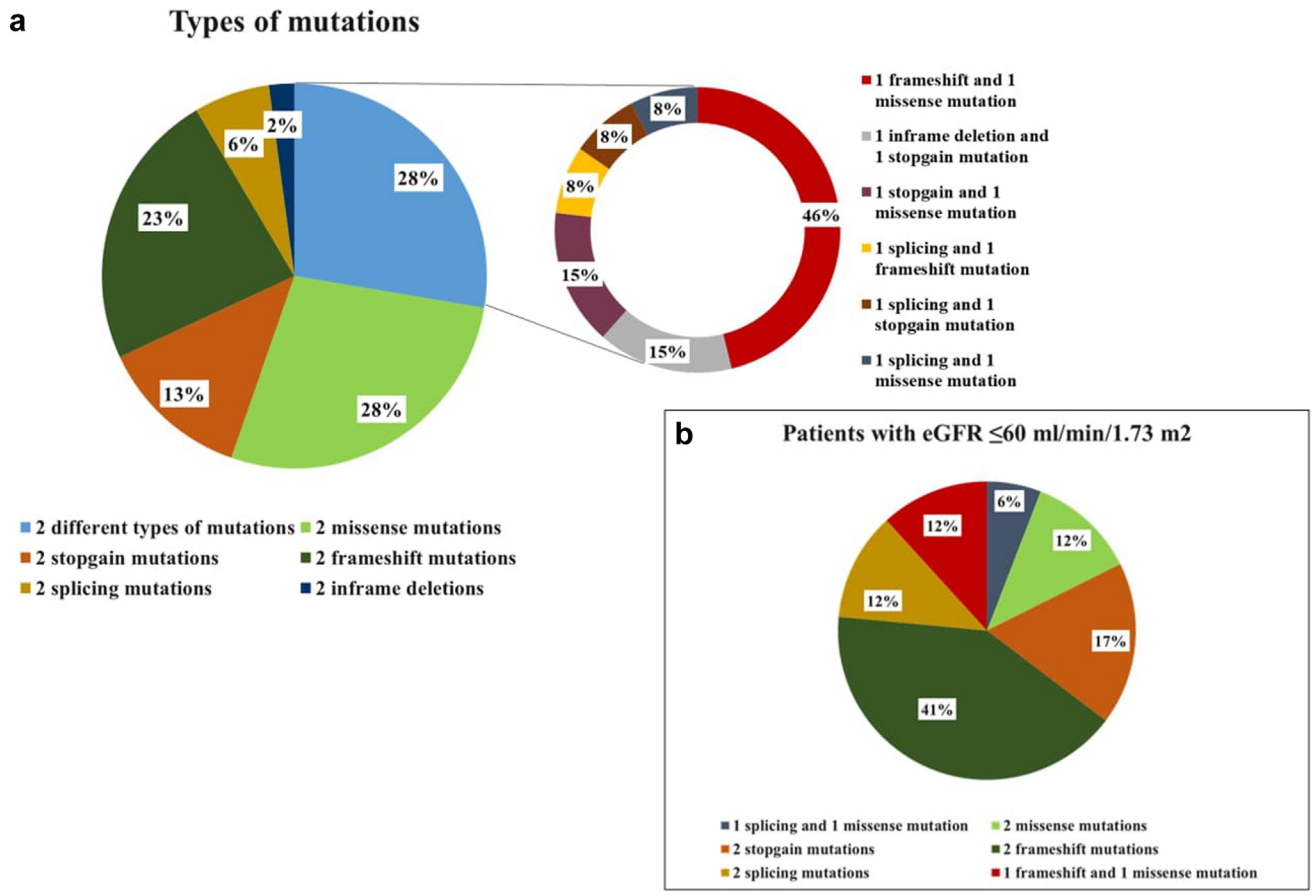

Figure 4. (a) Type of genetic variants in the entire population and (b) in patients with CKD2-5 BBS. eGFR, estimated glomerular filtration rate.

Table 5. Patients sharing the same pathogenic variants renal dysfunction

	Gender	Consanguinity	Age	eGFR	Severity of kidney disease	Obesity	Hypertension	High plasma UA	High plasma cholesterol	Gene	Type of mutation
Group 1	M	yes		1	S	yes	yes	yes		unknown	-
	M			90	M	yes	no	no			
Group 2	F	yes	38	5	S	yes	Yes			*BBS9*	Frameshift/frameshift
	F		33	30	S	Yes	No				
Group 3	F	yes	38	91	M					*BBS12*	Missense/stopcodon
	M		33	98	M						
Group 4	F	yes		120	M	Yes	No			Unknown	
	M			90	M	No	No				
Group 5	F	yes	40	55	I	Yes	Yes			*BBS12*	Missense/missense
	M		44	90	M	No	Yes				
Group 6	M	yes	32	110	M	No	No			*BBS10*	Missense/missense
	F		30	100	M	No	No				
	F		25	100	M	No	No				
Group 7	M	No	22	120	M	Yes	No	no	no	*BBS1*	Missense/missense
	M		20	130	M	Yes	No	no	no		
Group 8	M	No	37	78	I	No	No	no	no	*BBS12*	Stop-codon/stop-codon
	M		50	2	S	Yes	Yes				
	M		30	110	M	No	No				
	F		21	78	I	Yes	Yes				
Group 9	F	No	45	2	S	Yes	Yes			*BBS4*	Splicing/splicing
	F		34	72	I	No	No				
	F		26	104	M	Yes	No				
Group 10	M	No	14	90	M					*BBS10*	Truncating/truncating
	M		15	70	I						
	F		6	80	I	Yes	Yes				

eGFR, estimated glomerular filtration rate; gender (F, female; M, male); severity (I, intermediate; M, medium; S, severe).
The table shows the presence of variable eGFR even in patients with the same pathogenic variants, indicating the importance of additional factors in determining renal dysfunction.

variants. The study included 26 individuals divided into 10 groups. Our study shows a variability in kidney phenotype in 50% of the groups analyzed, thereby supporting the multifactorial origin of kidney dysfunction in this setting.

Our data suggest that BBS is a condition of vulnerability to CKD. A combination of genetics and systemic factors could determine progressive kidney disease. The proven role of BBS proteins in signaling pathways involved in embryonic development and response to kidney injury, including glucose, lipid metabolism and mitochondrial function may be determinant in causing and/or amplifying tubular damage and possibly renal fibrosis,[27,28] thus providing a fascinating hypothesis that impaired kidney repair after an injury determines CKD.

The study has strengths and limitations. We highlight the nature of data collection: the entire patient cohort has been evaluated by a reference center, with all reported features analyzed in a standardized manner, thus limiting referral bias. The limitations are the low number of individuals for each genotype, the presence of patients with unknown genetic mutation and the low number of pediatric patients, limiting genotype-phenotype correlation analysis and comparison between pediatric and adult patients. Moreover, the study includes patients referred to the Eye Unit of University of Campania, in the presence of suspicion of syndromic retinal degeneration. Patients with low syndromic score, patients with still preserved vision and patients referred to other centers for rare disorders may be excluded from the study. Thus, the present study does not provide data on disease prevalence and on genotype distribution among patients with BBS in a defined geographic area.

In conclusion, the present study emphasizes the contribution of genetics plus common risk factors in determining renal phenotype in adult patients with BBS. Age, hypertension, and obesity are known independent risk factors of kidney disease progression in the general population[21,29]; thus, our study demonstrates the role of these conditions in contributing to kidney disease in a genetic model of kidney disease, the BBS. Whether a strict control of blood pressure and BMI may[30] improve renal outcomes in those patients requires further studies. The analysis of the effects of antihypertensive and antiobesity drugs on kidney disease in patients with BBS could give new insights into hypertension and obesity-mediated kidney damage.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Relationship between eGFR and BMI. When BMI increases, eGFR decreases. Even if the correlation is weak in univariate analysis, it becomes statistically significant in multivariate model (see text)

Table S1. Kidney and urinary tract ultrasound appearance and other urinary signs in patients' cohort.

Table S2. Clinical features of patients without a molecular diagnosis. The presence of Beales' criteria was verified in all patients.

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