Meta-analysis of genotype-phenotype associations in Bardet-Biedl Syndrome uncovers differences among causative genes

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Abstract

Bardet-Biedl Syndrome (BBS) is a recessive genetic disease causing multiple organ anomalies. Most patients carry mutations in genes encoding for the subunits of the BBSome, an octameric ciliary transport complex, or accessory proteins involved in the BBSome assembly or function.
BBS proteins have been extensively studied using in vitro, cellular, and animal models. However, the molecular functions of particular BBS proteins and the etiology of the BBS symptoms are still largely elusive.
In this study, we applied a meta-analysis approach to study the genotype-phenotype association in humans using our database of all reported BBS patients. The analysis revealed that the identity of the causative gene and the character of the mutation partially predict the clinical outcome of the disease. Besides their potential use for clinical prognosis, our analysis revealed functional differences of particular BBS genes in humans. Core BBSome subunits BBS2, BBS7, and BBS9 manifest as more critical for the function and development of kidneys than peripheral subunits BBS1, BBS4, and BBS8/TTC8, suggesting that incomplete BBSome retains residual function at least in the kidney.

Keywords

Bardet-Biedl syndrome, BBSome, BBS, ciliopathy, genotype-phenotype, meta-analysis, rare disease, kidney disease

Structured summary

The structured summary is required by Prisma guidelines for conducting meta-analyses.

Background: Bardet-Biedl syndrome is a rare ciliopathy caused by recessive loss-of-function mutations in any of the BBS genes. Both the functions of BBS genes in humans and the links between their disruption and particular phenotypes of the disease are incompletely understood.

Methods: Using the meta-analytic approach, we collected all published records of BBS patients with available genotype and phenotype data. Within this cohort, the genotype phenotype associations were analyzed using frequentist and Bayesian statistics. The protocol for this meta-analysis was pre-registered with PROSPERO (CRD42018096099).

Results: From 85 studies, we assembled a database of genotype and phenotype data of 899 BBS patients that can be accessed online at http://bardet-biedl.img.cas.cz. The results of the genotype-phenotype correlation analyses showed that patients with mutations in *BBS3/ARL6* typically present with less major symptoms of the disease than other patients. Patients with mutations in *BBS*2 or *BBS10* have higher frequency of polydactyly and renal anomalies than patients with mutations in *BBS1*. Strikingly, patients with mutations in genes encoding for different subunits of a unitary complex BBSome show differences in the penetrance of renal anomalies, the major life-threatening BBS symptom.

Conclusions: Our results suggest that the causative gene and the character of the mutation partially predict the clinical outcome of the BBS. Moreover, our data provide new insights into the biological function of BBS proteins in human tissues. Our approach is suitable for genotype-phenotype analysis in rare genetic diseases.

Introduction

Bardet-Biedl Syndrome (BBS) belongs to a large group of ciliopathic diseases. These genetic disorders are caused by defective functions of cilia, which are cellular protrusions homologous to the eukaryotic flagellum. Mammals have two types of cilia; motile and primary cilia that have mechanical and signaling functions, respectively. BBS patients have defects in the primary cilia, but the motile cilia seem to be largely unaffected with the exception of sperm flagellum (Ansley et al., 2003; Shoemark, Dixon, Beales, & Hogg, 2015).

BBS is a multiorgan disease diagnosed based on the presence of at least 4 out of 6 primary features (retinal dystrophy, polydactyly, obesity, genital abnormalities, renal anomalies, learning difficulties/cognitive impairment) or presence of 3 major features and 2 secondary features (speech delay, developmental delay, diabetes mellitus, dental anomalies, congenital heart disease, brachydactyly/syndactyly, ataxia/poor coordination, anosmia/hyposmia) (Forsythe & Beales, 2013).

BBS is an autosomal recessive disorder caused by loss-of-function (LOF) mutations in at least 24 genes (BBS1-21 (Forsythe, Kenny, Bacchelli, & Beales, 2018), NPHP1 (Lindstrand et al., 2014), IFT74 (Lindstrand et al., 2014) and SCAPE*R* (Wormser et al., 2019)), whose function is closely-related to the primary cilium. Most of the BBS patients have mutations in one of the 8 genes encoding for the subunits of the octameric protein complex, BBSome (*BBS1,2,4,5,7,8,9,18*) (Nachury et al., 2007). The second largest group of BBS patients have mutations in genes encoding chaperonins assisting the assembly of the BBSome (BBS6/MKKS,10,12) (Seo et al., 2010). The third most common group of patients has a mutation in BBS3/ARL6, a GTPase assisting the BBSome function (Jin et al., 2010). Mutations in other BBS genes are relatively rare and/or are primarily causing other types of ciliopathies such as Joubert syndrome (BBS14/CEP290), Meckel syndrome (BBS13/MKS1) or Senior-Loken Syndrome (BBS14/CEP290, BBS16/SDCCAG8) (Reiter & Leroux, 2017). Thus, BBS is strongly associated with the dysfunction of the BBSome.

The BBSome is an ancient protein complex with a high level of evolutionary conservation in most ciliated organisms (van Dam et al., 2013). The BBSome sorts ciliary proteins into and/or out of the cilium. The canonical BBSome cargoes are ciliary G-protein coupled receptors (GPCRs), including Smo and GPR161 involved in the Sonic hedgehog signaling, and neuronal receptors SSTR3, MCHR1, NPY2R, and D1R (Berbari, Lewis, Bishop, Askwith, & Mykytyn, 2008; Jin et al., 2010; Klink et al., 2017; Loktev & Jackson, 2013; Zhang et al., 2013; Zhang, Seo, Bugge, Stone, & Sheffield, 2012). Moreover, it has been proposed that the BBSome is involved in the trafficking of the leptin receptor to the plasma membrane (D. F. Guo et al., 2016; Seo et al., 2009). Experiments done in lower eukaryotes suggest that the BBSome is able to transport cargoes lacking transmembrane domains as well (Liu & Lechtreck, 2018).

The mechanisms of how the BBSome deficiency leads to the pathologies of particular organs are only partially understood. Retinal dystrophy might be caused by photoreceptor degeneration due to the mislocalization of a photosensitive GPCR rhodopsin (Abd-El-Barr et al., 2007; Nishimura et al., 2004). However, rhodopsin was not a bona fide BBSome cargo in a screen performed in vitro (Klink et al.,

2017). Another proposed cause of the photoreceptor degeneration in the BBS patients and animal models is the accumulation of inner-segment proteins in the outer-segments of photoreceptors (Datta et al., 2015). Polydactyly and dental anomalies are most likely caused by defective Sonic hedgehog signaling during development (Zhang, Seo, et al., 2012). Cognitive impairment and developmental delay might be caused by defective signaling by neuronal GPCRs (McIntyre, Hege, & Berbari, 2016). Obesity is caused by defects in the neurological control of the appetite, although it is unclear whether defective leptin signaling (Liu & Lechtreck, 2018) or signaling by anorexigenic GPCR Neuropeptide Y family receptors is the primary cause (Loktev & Jackson, 2013).

It remains to be resolved, how the BBSome deficiency induces the renal anomalies that eventually lead to the development of a life-threatening kidney failure. Some experiments propose that the BBSome facilitates the ciliary localization and proper function of polycystin-1 and polycystin-2 proteins (PC-1 and PC-2, respectively) (Su et al., 2014; Xu et al., 2015). Mislocalization of PC-1 and/or PC-2 in BBS patients could eventually trigger the development of the kidney disease (Tobin & Beales, 2007). However, the mislocalization of PC-1 and/or PC-2 was not observed in *BBS4*-, *BBS5*-, *BBS7*-, and *BBS8/TTC8*-deficient cells (Su et al., 2014; Xu et al., 2015; Zhang et al., 2013). Non-canonical Wnt signaling pathway might be also involved in the development of the kidney symptoms (Tobin & Beales, 2007). However, two studies on animal models suggested that the renal defects are not completely intrinsic to the kidney, as caloric restriction prevented the renal defects (D. F. Guo et al., 2011) and tissue specific *Bbs10* knock-out restricted to the kidney epithelium did not develop any renal anomalies (Cognard et al., 2015). Little is known about the etiology of liver, heart, and reproductive system defects in the BBS.

The biology and function of the BBSome is still incompletely understood. Patients with mutations in any of the BBSome-encoding genes might develop any BBS symptoms. Accordingly, the reported phenotypes of mice deficient in the BBSome-encoding subunits recapitulate several aspects of the human disease (Berbari et al., 2008; Cognard et al., 2015; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Tadenev et al., 2011; Zhang et al., 2013). This evidence suggests that all the BBSome subunits are essential for the function of the whole complex and have no independent roles. However, all major BBS symptoms appear in other ciliopathies as well (Reiter & Leroux, 2017) and all the BBS symptoms have incomplete penetrance leading to a substantial phenotypic variability among BBS patients (Forsythe & Beales, 2013). These facts allow for the possibility that the individual BBSome subunits might have different roles, besides functioning as a part of the octameric BBSome complex.

Structural similarities between some BBSome subunits (Jin et al., 2010), reported functional redundancies between BBS4 and BBS5 subunits (Xu et al., 2015), multiple differences between particular BBSome deficient cellular or mouse models (Berbari et al., 2008; Cognard et al., 2015; D. F. Guo et al., 2011; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Su et al., 2014; Tadenev et al., 2011; Xu et al., 2015; Zhang et al., 2011; Zhang et al., 2013), and partially differential effects of knock-downs of particular BBSome-encoding genes in mouse embryonic brain development (J. M. Guo et al., 2015) suggest that particular BBSome subunits might have unique functions. Moreover, it is possible that the BBSome maintains a residual function even in the absence of particular subunits. The analysis of the genotype and phenotype associations in BBS patients has a strong potential to address the biology of the BBS proteins in human tissues. However, the reported genotype-phenotype studies on small cohorts addressed selected clinical aspects of this correlation (Castro-Sanchez et al., 2015; Deveault et al., 2011; Forsythe et al., 2017; Forsythe et al., 2015), but not the functional comparison of the BBS proteins. Moreover, these primary studies generally lack the statistical power to detect most differences.

Meta-analyses of individual patient data have been considered the most reliable source of information for human medicine for decades (Riley, Lambert, & Abo-Zaid, 2010; Stewart & Clarke, 1995). However, to our knowledge, this approach has not been used to assess the genotype-phenotype correlation in a rare genetic disease such as BBS. In this study, we assembled all available BBS cases published in the literature so far into a single publicly available database (http://bardet-biedl.img.cas.cz) to address the association between the genotype (causative BBS mutation) and phenotype (development of particular symptoms). Our analysis revealed that the outcome of the disease and the penetrance of some BBS symptoms are partially determined by the genotype of the patients and by the character of the mutation (missense vs. truncation). Moreover, we used our database to address the role of the BBSome in humans. Whereas the intact BBSome complex is required for some physiological functions, the penetrance of some symptoms, in particular kidney anomalies, depend on the causative BBS gene mutated.

Results

Assembling the data of all published BBS patients for whom the genotype (homozygous or compound-heterozygous mutation in a specific BBS gene) and the phenotype (presence or absence of at least two BBS symptoms) was reported enabled us to create the largest cohort of BBS cases published to date. The literature search was performed according to the protocol pre-registered in Prospero (CRD42018096099) and it followed the PRISMA guidelines whenever applicable (Supp. Table S1, Supp. Figure S1). In total, we identified 85 relevant studies (Abu-Safieh et al., 2012; Abu Safieh et al., 2010; Agha et al., 2013; Ajmal et al., 2013; Al-Hamed et al., 2014; Alazami et al., 2012; Aldahmesh et al., 2014; Azari et al., 2006; Badano, 2003; Baker et al., 2011; Bee, Chawla, & Zhao, 2015; Bennouna-Greene et al., 2011; Billingsley et al., 2010; Billingsley, Vincent, Deveault, & Heon, 2012; Branfield Day et al., 2016; Braun et al., 2014; Bujakowska et al., 2015; Castro-Sanchez et al., 2015; Castro-Sanchez et al., 2017; Chaki et al., 2011; Chul Yoon et al., 2014; Cox et al., 2012; Davies, 2018; Deveault et al., 2011; Ece Solmaz et al., 2015; Esposito et al., 2017; Estrada-Cuzcano, Koenekoop, Senechal, & et al., 2012; Estrada-Cuzcano, Neveling, et al., 2012; Fan et al., 2004; Fattahi et al., 2014; Fedick et al., 2013; Frank et al., 2007; Gerth, Zawadzki, Werner, & Heon, 2008; Ghadami et al., 2000; González-del Pozo et al., 2013; Harville et al., 2010; Heon et al., 2016; Hjortshoj, Gronskov, Brondum-Nielsen, & Rosenberg, 2009; Hjortshoj et al., 2010; Hulleman et al., 2016; Iannaccone et al., 2005; Innes et al., 2010; Iurian, Arts, Brunner, & Fintina, 2015; Janssen et al., 2011; Kamme, Mayer, Strom, Andréasson, & Weisschuh, 2017; Katsanis et al., 2001; Katsanis et al., 2000; Kerr, Bhan, & Héon, 2015; A. O. Khan, Decker, Bachmann, Bolz, & Bergmann, 2016; S. Khan et al., 2013; S. A. Khan et al., 2016; Laurier et al., 2006; Leitch et al., 2008; Lim et al., 2014; Lindstrand et al., 2014; Lindstrand et al., 2016; M'Hamdi et al., 2014; Maria et al., 2016; Marion et al., 2012; Otto et al., 2010; Pawlik et al., 2010; Pereiro et al., 2010; Phelps et al., 2017; Qi et al., 2017; Rahner, Nuernberg, Finis, Nuernberg, & Royer-Pokora, 2016; Reiner et al., 2018; Riazuddin et al., 2010; Riise, Tornqvist, Wright, Mykytyn, & Sheffield, 2002; Sathya Priya et al., 2015; Schaefer et al., 2011; Schaefer et al., 2014; Schaefer et al., 2016; Schaefer et al., 2010; Sophie Scheidecker et al., 2014; S. Scheidecker et al., 2015; Shaheen et al., 2016; Shin et al., 2015; Suspitsin et al., 2015; Suzuki et al., 2016; Ullah et al., 2017; van Huet et al., 2013; White et al., 2006; Yamamura et al., 2017; Yang et al., 2008; Young et al., 1998) from which we extracted data of 899 patients (Supp. Table S2). The database includes data about the causative BBS mutation, the presence/absence of most common BBS symptoms (retinal dystrophy, obesity, polydactyly, cognitive impairment, reproductive system anomalies, renal anomalies, heart anomalies, liver anomalies, and developmental delay), sex, age, intra-familial relations, and ethnicity for each patient, when available (Supp. Table S3). The mutations occurring in our dataset are listed in Supp. Table S4. The ethnicities of all the patients are listed in Supp. Table S5. Moreover, we established a curated online database, called "dataBBaSe", of the published anonymous BBS cases. The database is accessible through a simple interactive on-line interface (http://bardet-biedl.img.cas.cz).

It should be noted, that some of the included patients do not fulfill the up-to-date clinical criteria for the diagnosis of the BBS, but these patients were included in our dataset because they carry a causative BBS mutation.

We divided the causative BBS genes into four functional classes: (i) genes encoding for subunits of the BBSome (*BBS1,2,4,5,7,8,9,18*), (ii) *BBS3/ARL6* encoding a GTPase cooperating with the BBSsome, (iii) genes encoding for chaperonins, and (iv) other, i.e., non-canonical, BBS genes (Figure 1A). We observed that patients with causative mutations in the BBSome-encoding genes represent slightly more than half of all patients, followed by patients with causative mutations in chaperonins (28%), non-canonical BBS genes (15%), and *BBS3/ARL6* (5 %) (Supp. Figure S2A). The three most commonly mutated genes are *BBS1*, *BBS10*, and *BBS2* (Supp. Figure S2A). The sex and age characteristics of all patients grouped according to their causative genes are shown in Supp. Figure S2B-D.

Major BBS symptoms have variable penetrance

The frequency of particular symptoms among the BBS patients varies between 94.4% (retinal dystrophy) and 29.8% (heart anomalies), indicating that the BBS symptoms have very variable penetrance (Supp. Figure S2E). However, the presence or absence of all these symptoms was not reported for some BBS patients (Supp. Table S3). To compare the disease outcome in a defined group of patients, we selected a set of all 426 BBS patients with the reported presence or absence of five major BBS symptoms (retinal dystrophy, obesity, polydactyly, cognitive impairment, renal anomalies) and we called this selection *'set* (Supp. Table S6). The abnormalities in the reproductive system were not included because of the differences between the

male and female physiology. The *'set* represents a database of the most completely clinically characterized BBS patients. The frequency of causative BBS genes in the *'set* was representative of all the patients (Figure 1B, Supp. Figure S2A). Within the *'set*, we observed significant differences of the frequency of particular symptoms. Whereas 97 % of patients suffered from retinal dystrophy, only 37 % of patients did suffer from renal anomalies (Figure 1C).

To get a preliminary insight into a possible correlation between the genotype (causative BBS gene) and the outcome of the disease, we stratified the patients in the *'set* using a *syndromic score*. The *syndromic score* was calculated as the number of major symptoms present in the patient divided by 5 (all major symptoms excluding reproductive system anomalies). Theoretically, the *syndromic score* can be 0 (no major symptom present), 0.2, 0.4, 0.6, 0.8, or 1 (5 major symptoms present). The usage of the *syndromic score* enabled us to score each patient with a single number and to perform a straightforward initial comparison of the patients based on their genotype. The distribution of the *syndromic score* in all *'set* patients is shown in Figure 1D. The mean *syndromic score* was 0.73. Interestingly, the *syndromic score* did not differ between male and female BBS patients (Supp. Figure S3A).

Patients with mutations in *BBS3/ARL6* have typically a relatively low *syndromic score*

We observed statistically significant differences in the *syndromic score* among patients with mutations in particular functional classes of the BBS genes (Figure 1E). First, we realized that patients with mutations in the non-canonical BBS genes constitute a very heterogeneous group and we excluded these patients from further analysis. More importantly, patients with mutations in *BBS3/ARL6* showed significantly lower *syndromic score* than patients with mutations in the BBSome or chaperonin BBS genes. This suggested that BBS patients with mutations in *BBS3/ARL6* exhibit typically fewer of the five included symptoms than patients with mutations in other genes. We did not observe any difference between patients with causative mutations in the BBSome and chaperonin BBS genes.

The *syndromic score* was not significantly different among patients with causative mutations in three BBS genes encoding for chaperonins, i.e., *BBS6/MKKS*, *BBS10*, and *BBS12* (Figure 1F). This suggested that BBS6/MKKS, BBS10, and BBS12 chaperonins have similar and non-redundant functions.

Patients with mutations in *BBS1* or *BBS8/TTC8* have typically a lower *syndromic score* than patients with mutations in *BBS2* or *BBS7*

In the next step, we compared the *syndromic score* among patients with causative mutations in particular genes encoding for BBSome subunits (Figure 1G). We observed statistically significant differences among these groups of patients. Patients with the mutation in the *BBS1* and *BBS8/TTC8* showed the lowest mean *syndromic score* whereas patients with mutations in *BBS2* and *BBS7* showed the highest mean *syndromic score*. This suggested that patients with different BBSome mutations might have different prognosis and that particular BBSome subunits might have unique, independent, and/or redundant functions. The post hoc tests showed that typical patients with causative mutations in *BBS1* have significantly lower *syndromic score* than typical patients with mutations in *BBS2* or *BBS7* (Figure 1G).

Figure 1

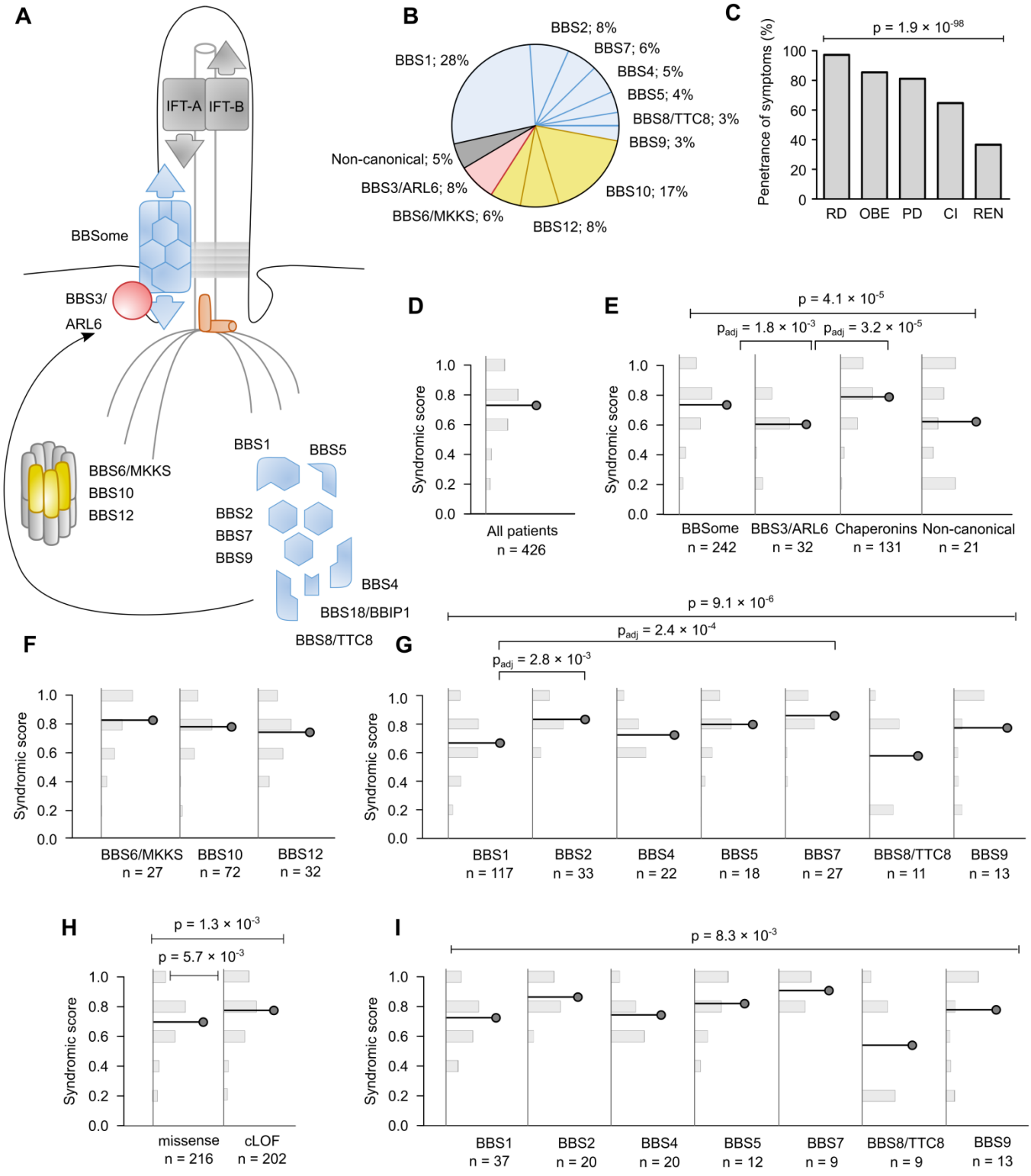
A
IFT-A
IFT-B
BBSome
BBS3/
ARL6
BBS6/MKKS
BBS10
BBS12
BBS1
BBS5
BBS2
BBS7
BBS9
BBS4
BBS18/BBIP1
BBS8/TTC8
B
BBS2; 8%
BBS7; 6%
BBS4; 5%
BBS5; 4%
BBS8/TTC8; 3%
BBS9; 3%
BBS10; 17%
BBS12; 8%
BBS6/MKKS; 6%
BBS3/ARL6; 8%
Non-canonical; 5%
BBS1; 28%
C
p = 1.9 × 10-98
Penetrance of symptoms (%)
RD
OBE
PD
CI
REN
D
Syndromic score
All patients
n = 426
E
p = 4.1 × 10-5
padj = 1.8 × 10-3
padj = 3.2 × 10-5
BBSome
n = 242
BBS3/ARL6
n = 32
Chaperonins
n = 131
Non-canonical
n = 21
F
BBS6/MKKS
n = 27
BBS10
n = 72
BBS12
n = 32
G
p = 9.1 × 10-6
padj = 2.4 × 10-4
padj = 2.8 × 10-3
BBS1
n = 117
BBS2
n = 33
BBS4
n = 22
BBS5
n = 18
BBS7
n = 27
BBS8/TTC8
n = 11
BBS9
n = 13
H
p = 1.3 × 10-3
p = 5.7 × 10-3
missense
n = 216
cLOF
n = 202
I
p = 8.3 × 10-3
BBS1
n = 37
BBS2
n = 20
BBS4
n = 20
BBS5
n = 12
BBS7
n = 9
BBS8/TTC8
n = 9
BBS9
n = 13

Figure 1. The disease outcome in patients with mutations in different functional groups of BBS genes. (A) Schematic representation of the functions of the BBS proteins in the primary cilium. Eight of the BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8/TTC8, BBS9, BBS18/BBIP1) form a transport complex called BBSome (blue). Chaperonin-like proteins BBS6/MKKS, BBS10, and BBS12 (yellow) facilitate the BBSome assembly and BBS3/ARL6 (red) is a GTPase regulating the BBSome entry to (and exit from) the cilium. **(B)** Pie chart showing the distribution of the BBS genes mutated in the *'set* of BBS patients. The *'set* represents a subset of patients with reported presence or absence for all 5 major symptoms (n = 426). Blue – mutations in the BBSome-encoding genes, red – mutations in *BBS3/ARL6*, yellow – mutations in the chaperonin-encoding genes. **(C)** Frequency of symptoms in the *'set* of BBS patients. RD – retinal dystrophy, OBE – obesity, PD – polydactyly, CI – cognitive impairment, REN – renal anomalies. **(D-I)** *Syndromic score* was calculated as a fraction of present symptoms out of the main 5 phenotype categories (retinal dystrophy, obesity, polydactyly, cognitive impairment, renal anomalies). **(D)** *Syndromic score* among the whole *'set* of BBS patients. **(E)** *Syndromic score* among BBS patients with mutations in the BBSome-encoding genes, in *BBS3/ARL6*, in chaperonin-encoding genes, or in non-canonical BBS genes. **(F)** *Syndromic score* in BBS patients with mutations in the indicated chaperonin-encoding genes. **(G)** Syndromic score in BBS patients with mutations in the indicated BBSome-encoding genes. **(H)** *Syndromic score* in BBS patients with missense mutations (mono- or biallelic single amino acid substitutions or short in-frame deletions) and in patients with assumed complete loss of function (cLOF) mutations (large deletions, frameshift mutations, splicing defects). **(I)** *Syndromic score* in BBS patients with assumed complete loss of function mutations in the indicated BBSome-encoding genes.

Data information: (C) P-value was calculated using chi-square test. **(D-I)** Black lines with dots represent the mean. Histograms showing the data distribution were normalized to max. Statistical significance of differences among all groups of patients was determined by Kruskal-Wallis test. Post hoc testing of differences between individual groups was determined by Dunn's Multiple comparison test. P-values higher than 0.05 are not indicated in any graphs. **(H)** Statistical significance of difference between the two groups of patients was determined by Mann-Whitney test. Statistical significance of difference between the percentages of patients presenting with the maximal *syndromic score* was calculated using the Fisher's exact test.

Some of the causative BBS mutations are hypomorphic

BBS is a recessive monogenic disease caused by LOF mutations in any of the BBS genes. However, it is not known whether a particular causative BBS mutation cause a partial or complete disruption of the respective protein. Typical patients with hypomorphic (i.e., only partial LOF) mutations would be expected to have a milder disease than typical patients with complete LOF mutations (cLOF). We assumed that mutations causing a substantial structural disruption of the gene product (such as truncation via frameshifts or splicing defects) are cLOF mutations, whereas some missense mutations leading typically to single amino acid substitutions might be hypomorphic (Hirayama et al., 2008; Xu et al., 2015; Zaghloul et al., 2010). Interestingly, patients with assumed cLOF mutations in both alleles exhibit higher average *syndromic score* than patients with one or two missense mutations in the causative BBS gene (Figure 1H). We carried out a similar comparison among patients with mutations in canonical BBS genes other than *BBS1* (Supp. Figure S4A), patients with mutations in *BBS1* (Supp. Figure S4B), and patients with mutations in the BBSome encoding genes (Supp. Figure S4C). For all these groups, we observed the tendency that patients with assumed cLOF mutations have higher *syndromic score* than patients with monoallelic or biallelic milder mutations (Supp. Figure S4A-C). Moreover, patients with assumed cLOF mutations have higher frequency of the most severe form the disease (*syndromic score* = 1) than patients with mono- or biallelic missense mutations in each group of BBSome-deficient patients (Supp. Figure S4D).

These results indicate that some causative missense mutations in the BBSome encoding genes are hypomorphic. Unfortunately, it is not possible to identify the specific hypomorphic mutations because of the high number of causative BBS mutations and insufficient sample size. To address the possibility that the frequency of hypomorphic BBS mutations might differ between individual BBS genes and influence the previous analysis (Figure 1G), we compared the disease outcome among patients with causative mutations in particular BBSome-encoding genes including only patients with biallelic assumed

cLOF mutations (Figure 1I). Despite the substantially smaller sample size and statistical power, the differences largely recapitulated the results from the previous analysis that included both types of causative mutations (Figure 1G). Overall, these results suggest that the lower average *syndromic score* in patients with mutations in *BBS1* and *BBS8/TTC8* than in patients with causative mutations in other BBSome-encoding genes could be explained by intrinsic differences in the respective genes/proteins.

Assessing the differences in particular symptoms

The analysis of the disease outcome based on the *syndromic score* suggested possible differences in the disease manifestation among patients with different affected genes. This encouraged us to carry out a more complete analysis based on the frequency of individual symptoms. In this analysis, we calculated frequency of each particular symptom from all patients with reported presence/absence of the symptom (i.e., the analysis was not limited to the *'set*). We focused on the major BBS symptoms (retinal dystrophy, obesity, polydactyly, cognitive impairment, reproductive system anomalies, renal anomalies) and three frequently reported common minor symptoms (heart anomalies, liver anomalies, and developmental delay). The sporadic reporting on the presence/absence of other symptoms in the literature did not allow us to cover more than nine symptoms in our meta-analysis.

We used two different statistical approaches to assess the differences between the groups of BBS patients. The first one is a frequentist comparison of the frequency of particular symptoms among different patient groups, which provides a straightforward interpretation, but assumes no effect of the variability between the studies on the result. We complemented the frequentist statistics with a more complex Bayesian analysis with a hierarchical model. The Bayesian statistical model considered the between-study variability and the type of mutation, i.e., missense or assumed cLOF. Moreover, we generated additional variants of the Bayesian model that took into account possible roles of other parameters such as sex, age, pedigree, and ethnicity (described in detail in Supplemental Statistical Analysis). Because we wanted to have high level of confidence in our overall conclusions, we were seeking for potential genotype-phenotype links identified by both statistical approaches.

The major caveat of our analysis was the insufficient number of patients with certain genotypes and imperfect reporting as the presence/absence of the BBS symptoms was recorded only for a fraction of patients, reducing the power of the statistical analysis. However, our analysis of the complete reported patients' data was still substantially more robust than any previous study focusing on the genotype-phenotype association in the BBS.

Patients with mutations in *BBS3/ARL6* show low penetrance of cognitive impairment and renal anomalies

First, we compared the frequency of the BBS symptoms among patients with mutations in genes encoding for BBSome, chaperonins, or *BBS3/ARL6* (Figure 2, Supp. Figure S5A-C). The frequentist analysis showed that patients with causative mutations in *BBS3/ARL6* exhibited significantly lower frequency of cognitive impairment, reproductive system anomalies, renal anomalies, and heart anomalies than patients with causative mutations in other BBS genes (Figure 2A, Supp. Figure S5A). Interestingly, the penetrance of retinal dystrophy, obesity, polydactyly, and liver anomalies was not significantly different between patients with mutated *BBS3/ARL6* and other patients (Figure 2A, Supp. Figure S5A). To account for the potential influence of hypomorphic mutations, we compared the penetrance of particular mutations only in patients with truncating mutations using the frequentist statistics (Figure 2B, Supp. Figure S5B). This analysis shows significant differences only in the penetrance of cognitive impairment and renal anomalies among the three groups of patients (Figure 2B). We complemented our frequentist statistical analysis with a Bayesian statistical model that took into account the variability between the studies (Figure 2C, Supp. Figure S5C). The results of the Bayesian statistics showed a relatively low penetrance of cognitive impairment and renal anomalies, and to a lower extent also reproductive system anomalies and heart disease in patients with mutated *BBS3/ARL6* in comparison to other BBS patients (Figure 2C, Supp. Figure S5C).

Figure 2

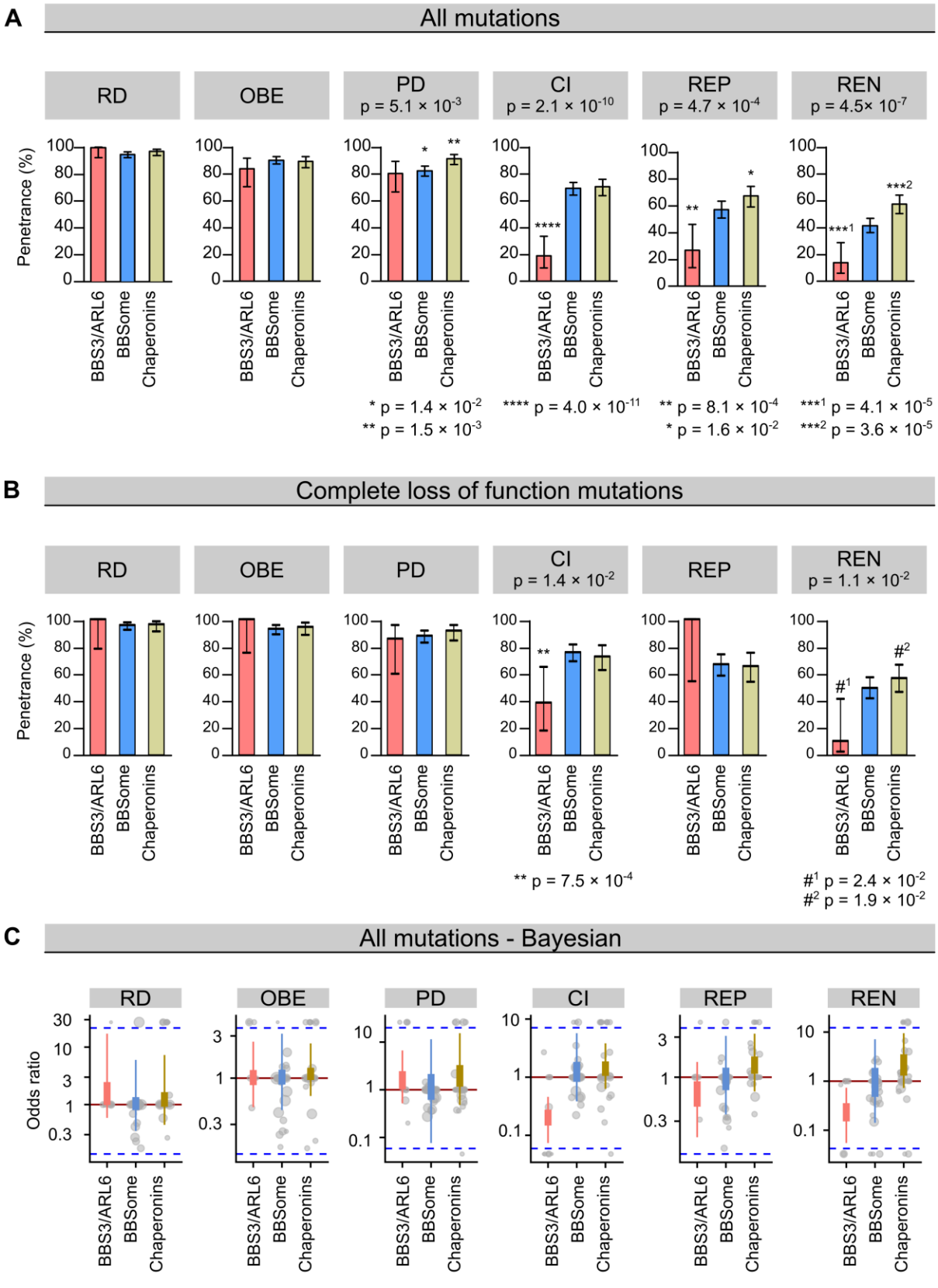
A
All mutations
RD
OBE
PD
p = 5.1 × 10-3
CI
p = 2.1 × 10-10
REP
p = 4.7 × 10-4
REN
p = 4.5× 10-7
Penetrance (%)
BBS3/ARL6
BBSome
Chaperonins
* p = 1.4 × 10-2
** p = 1.5 × 10-3
**** p = 4.0 × 10-11
** p = 8.1 × 10-4
* p = 1.6 × 10-2
***1 p = 4.1 × 10-5
***2 p = 3.6 × 10-5
B
Complete loss of function mutations
RD
OBE
PD
CI
p = 1.4 × 10-2
REP
REN
p = 1.1 × 10-2
** p = 7.5 × 10-4
#1 p = 2.4 × 10-2
#2 p = 1.9 × 10-2
C
All mutations - Bayesian
Odds ratio

Figure 2. Penetrance of major BBS symptoms in patients with mutations in different functional groups of genes. (A) The frequency of the indicated symptoms in BBS patients with mutations in the indicated functional groups of BBS genes. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. RD – retinal dystrophy, OBE – obesity, PD – polydactyly, CI – cognitive impairment, REP – reproductive system anomalies, REN – renal anomalies. Numbers of patients (BBS3/ARL6, BBSome, Chaperonins): RD – 45, 438, 233; OBE – 44, 420, 230; PD – 46, 403, 204; CI – 42, 352, 192; REP – 26, 254, 141; REN – 36, 316, 193. **(B)** The frequency of the indicated symptoms in BBS patients with assumed complete loss of function mutations in the indicated functional groups of BBS genes. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. RD – retinal dystrophy, OBE – obesity, PD – polydactyly, CI – cognitive impairment, REP – reproductive system anomalies, REN – renal anomalies. Numbers of patients (BBS3/ARL6, BBSome, Chaperonins): RD – 14, 220, 107; OBE – 12, 222, 105; PD – 14, 218, 92; CI – 13, 175, 88; REP – 5, 140, 71; REN – 10, 157, 88. **(C)** Bayesian model: Posterior 95% (thin) and 50% (thick) credible intervals for ratio of odds for a phenotype given a mutation within the indicated functional group to odds for the phenotype given a mutation across all groups shown. Numbers of patients are the same as in A. Gray dots show the odds ratio calculated similarly for individual studies included in the meta-analysis. Dots outside of the dashed lines correspond to studies where the empirical odds ratio is 0 or infinity. Dot size corresponds to the number of relevant cases in the study. The model assumes odds ratios (but not the absolute odds) are the same regardless of whether the mutation is assumed cLOF.

Data information: (A, B) Statistical significance of differences among all groups of patients was determined by Fisher's exact test. Statistical significance of differences between individual groups was determined post hoc using Fisher's exact test (one group vs. all other groups taken together) with the Sidak correction for multiple comparions. #, *, **, ***, and **** represent the significance of p-values corresponding to $p < 0.1$, $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, after the Sidak correction. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. P-values higher than 0.1 are not indicated in any graphs. **(C)** Detailed description of the Bayesian model can be found in the Supplemental Statistical Analysis.

All three types of analyses consistently show that patients with mutated *BBS3/ARL6* exhibit lower penetrance of cognitive impairment and renal anomalies than patients with mutations in the BBSome- or chaperonin-encoding genes. The analyses of reproductive system anomalies and heart disease suggested possible differences in the same direction but were not entirely conclusive, possibly because of the low number of patients with truncating BBS3 mutations. The penetrance of retinal dystrophy, obesity, polydactyly, liver disease, and developmental delay was comparable in all three groups. Thus, the differences in the *syndromic score* between patients with mutations in *BBS3/ARL6* and other patients (Figure 1E) can be largely explained by the differences in the cognitive impairment and renal anomalies. Overall, these data suggest that the function of the BBSome is partially independent of *BBS3/ARL6*, which is prominent in the development of the cognitive impairment and the renal disease.

Mutations in particular BBSome-encoding genes show different penetrance of polydactyly and renal anomalies

The differences in the average *syndromic score* among patients with causative mutations in the particular BBSome-encoding genes motivated us to address the link between the causative BBSome-encoding genes and the penetrance of particular symptoms. Again, we performed three basic types of statistical analyses: the frequentist analysis of all patients with known presence/absence of the symptom (Figure 3A, Supp. Figure S5D), the frequentist analysis limited to patients having biallelic truncating mutations (assuming cLOF mutations in these patients) which suffered from a relatively low number of patients in some of the groups (Figure 3B, Supp. Figure S5E), and the Bayesian analysis taking into the account the variability between studies (Figure 3C, Supp. Figure S5F).

We observed statistically significant differences between patients with mutations in the particular

BBSome genes (Figure 3A-C, Supp. Figure 5D-F). Some of the differences were not supported by all types of statistical analysis, which could be potentially caused by the low number of reported patients or by the different nature of the statistical analyses (see Supplemental Statistical Analysis). Although we cannot exclude relatively large differences in the odds ratios, the high penetrance of retinal dystrophy seems to be universal among all the BBS genes tested. Obesity, cognitive impairment, reproductive system anomalies, heart anomalies, liver anomalies, and developmental delay suggested some genotype-phenotype links, but the different analytical approaches were not entirely conclusive.

However, the genotype-phenotype associations were strongly supported by all statistical models in the case of polydactyly and renal anomalies (Figure 3A-C).

First, all the analyses indicate that patients with mutations in *BBS2* have higher penetrance of polydactyly than other patients and some of the analyses point to a relatively low penetrance of polydactyly in patients with mutations in *BBS1* (Figure 3A-C).

Second, the penetrance of renal anomalies is highly determined by the particular affected gene (Figure 3A-C). Whereas patients with mutations in *BBS1*, *BBS4*, or *BBS8/TTC8* show low frequency of renal anomalies, patients with mutations in *BBS2*, *BBS7*, or *BBS9* have relatively high frequency of renal anomalies. *BBS5* is similar to *BBS2*, *BBS7*, and *BBS9* in the frequentist analyses, but intermediate in the Bayesian analysis. The convincing differences in the penetrance of kidney anomalies among the patients make a biological sense as the causative genes linked to high frequency of kidney anomalies, i.e., *BBS2*, *BBS7*, and *BBS9*, encode for structurally similar proteins forming the core of the BBSome (Zhang, Yu, Seo, Stone, & Sheffield, 2012).

The revealed patterns for polydactyly and renal anomalies were recapitulated also by the Bayesian pairwise comparisons analysis (Figure 4A)

Overall, we identified variable relationship between the penetrance of particular symptoms and the BBSome gene mutated. As some specific, sometimes contradictory, statements about the presence or absence of the genotype-phenotype relationships were previously proposed in smaller cohorts of BBS patients (Abu Safieh et al., 2010; Billingsley et al., 2010; Brinckman et al., 2013; Castro-Sanchez et al., 2015; Deveault et al., 2011; Esposito et al., 2017; Estrada-Cuzcano, Koenekoop, et al., 2012; Forsythe et al., 2017; Forsythe et al., 2015; Hjortshoj et al., 2010; Kerr et al., 2015; Moore et al., 2005; Pawlik et al., 2010; Schaefer et al., 2010), we addressed these statements using the frequentist statistics applied on the data from our complete patient database (Supp. Table S7).

Figure 3

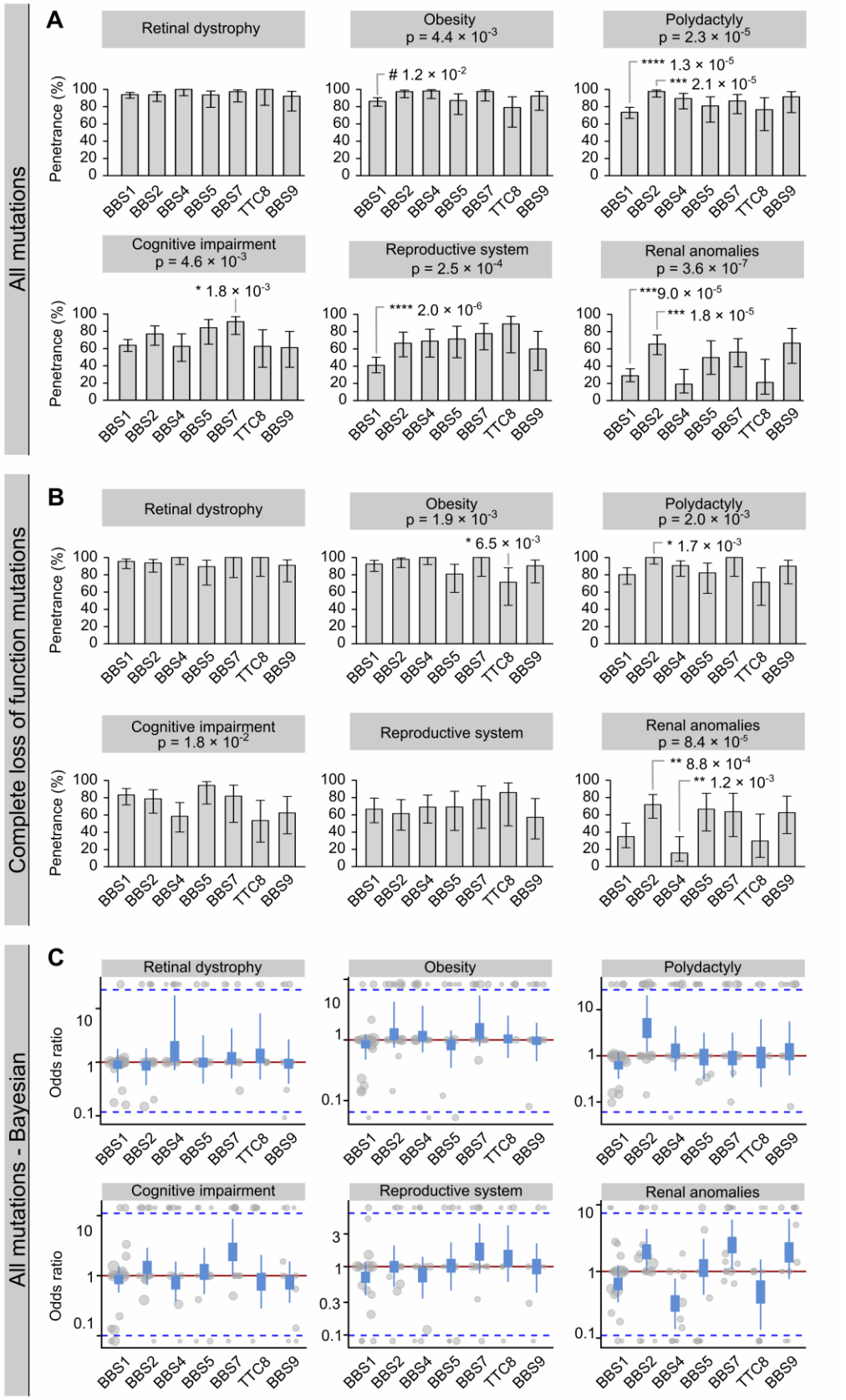
All mutations
A
Retinal dystrophy
Obesity
p = 4.4 × 10-3
# 1.2 × 10-2
Polydactyly
p = 2.3 × 10-5
**** 1.3 × 10-5
*** 2.1 × 10-5
Cognitive impairment
p = 4.6 × 10-3
* 1.8 × 10-3
Reproductive system
p = 2.5 × 10-4
**** 2.0 × 10-6
Renal anomalies
p = 3.6 × 10-7
***9.0 × 10-5
*** 1.8 × 10-5
Penetrance (%)
BBS1 BBS2 BBS4 BBS5 BBS7 TTC8 BBS9
Complete loss of function mutations
B
Retinal dystrophy
Obesity
p = 1.9 × 10-3
* 6.5 × 10-3
Polydactyly
p = 2.0 × 10-3
* 1.7 × 10-3
Cognitive impairment
p = 1.8 × 10-2
Reproductive system
Renal anomalies
p = 8.4 × 10-5
** 8.8 × 10-4
** 1.2 × 10-3
All mutations - Bayesian
C
Retinal dystrophy
Obesity
Polydactyly
Cognitive impairment
Reproductive system
Renal anomalies
Odds ratio

Figure 3. Penetrance of major BBS symptoms in patients with mutations in particular BBSome-encoding genes. (A) The frequency of the indicated symptoms in BBS patients with mutations in the indicated BBSome subunits. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. Numbers of patients (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8/TTC8, BBS9): retinal dystrophy – 208, 79, 45, 31, 34, 15, 25; obesity – 190, 71, 47, 31, 37, 17, 26; polydactyly – 180, 77, 45, 26, 36, 15, 23; cognitive impairment – 181, 51, 30, 25, 32, 14, 18; reproductive system anomalies – 112, 40, 29, 21, 27, 9, 15; renal anomalies 135, 65, 29, 22, 32, 14, 18. **(B)** The penetrance of the indicated symptoms in BBS patients with assumed complete loss of function mutations in the indicated BBSome-encoding genes. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. Numbers of patients (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8/TTC8, BBS9): retinal dystrophy – 66, 48, 40, 19, 12, 12, 22; obesity – 69, 45, 40, 21, 13, 12, 21; polydactyly – 67, 47, 41, 17, 13, 12, 20; cognitive impairment – 61, 32, 27, 17, 10, 11, 16; reproductive system anomalies – 40, 27, 29, 13, 9, 7, 14; renal anomalies – 41, 40, 23, 15, 11, 10, 16. **(C)** Bayesian model: Posterior 95% (thin) and 50% (thick) credible intervals for ratio of odds for a phenotype given a mutation within the indicated functional group to odds for the phenotype given a mutation across all groups shown. Numbers of patients are the same as in A. Gray dots show the odds ratio calculated similarly for individual studies included in the meta-analysis. Dots outside of the dashed lines correspond to studies where the empirical odds ratio is 0 or infinity. Dot size corresponds to the number of relevant cases in the study. The model assumes odds ratios (but not the absolute odds) are the same regardless of whether the mutation is assumed cLOF.

Data information: (A, B) Statistical significance of differences among all groups of patients was determined by Fisher's exact test. Statistical significance of differences between individual groups was determined post hoc using Fisher's exact test (one group vs all other groups taken together) with the Sidak correction for multiple comparions. #, *, **, ***, and **** represent the significance of p-values corresponding to $p < 0.1$, $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively, after the Sidak correction. The error bars represent 95% posterior credible intervals assuming uniform prior on the proportions. P-values higher than 0.1 are not indicated in any graphs. **(C)** Detailed description of the Bayesian model can be found in the Supplemental Statistical Analysis.

Figure 4

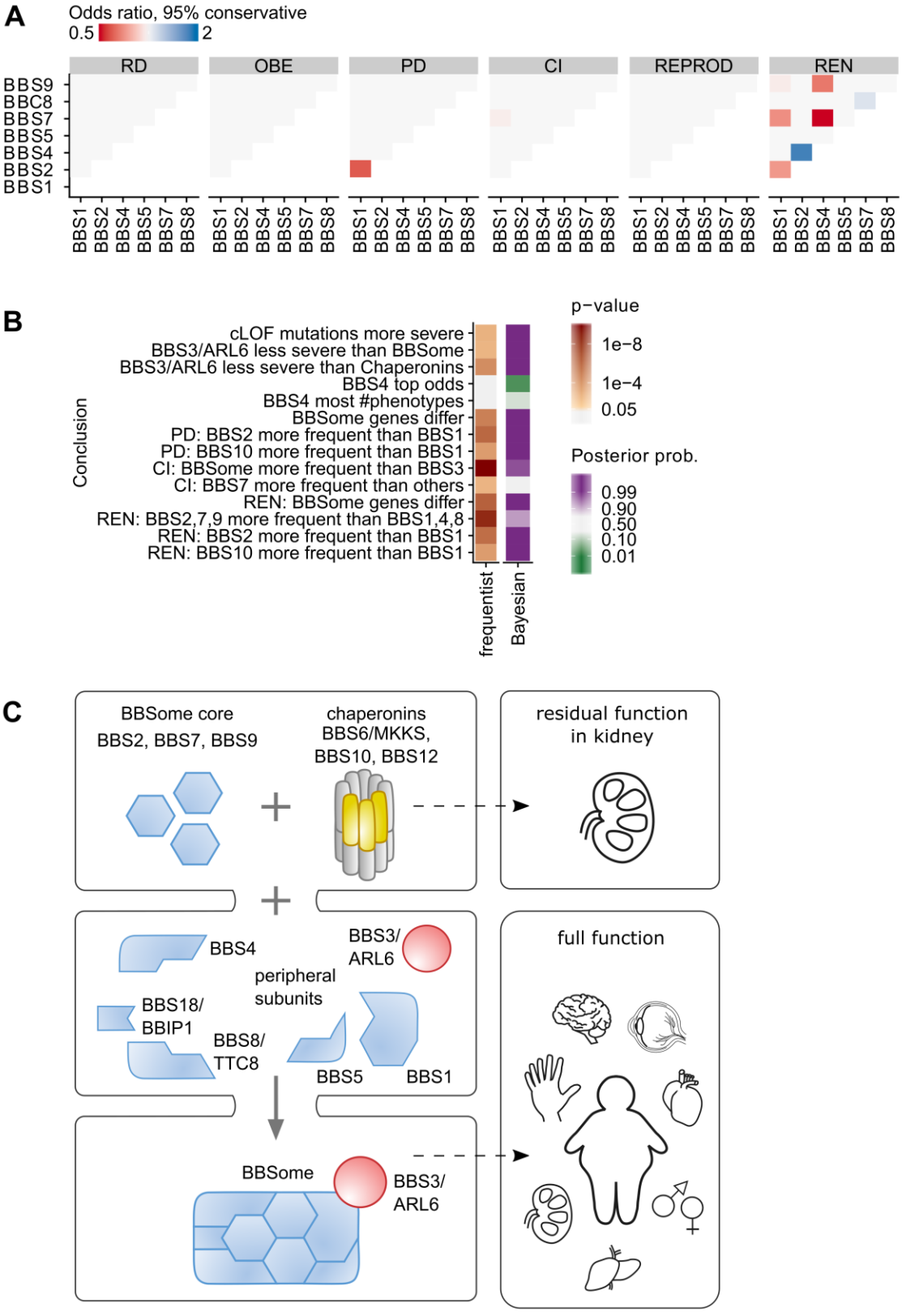
A
Odds ratio, 95% conservative
0.5
2
RD
OBE
PD
CI
REPROD
REN
BBS9
BBC8
BBS7
BBS5
BBS4
BBS2
BBS1
BBS1 BBS2 BBS4 BBS5 BBS7 BBS8
B
Conclusion
cLOF mutations more severe
BBS3/ARL6 less severe than BBSome
BBS3/ARL6 less severe than Chaperonins
BBS4 top odds
BBS4 most #phenotypes
BBSome genes differ
PD: BBS2 more frequent than BBS1
PD: BBS10 more frequent than BBS1
CI: BBSome more frequent than BBS3
CI: BBS7 more frequent than others
REN: BBSome genes differ
REN: BBS2,7,9 more frequent than BBS1,4,8
REN: BBS2 more frequent than BBS1
REN: BBS10 more frequent than BBS1
frequentist
Bayesian
p−value
1e−8
1e−4
0.05
Posterior prob.
0.99
0.90
0.50
0.10
0.01
C
BBSome core
BBS2, BBS7, BBS9
chaperonins
BBS6/MKKS,
BBS10, BBS12
residual function
in kidney
BBS4
BBS3/
ARL6
peripheral
subunits
BBS18/
BBIP1
BBS8/
TTC8
BBS5
BBS1
full function
BBSome
BBS3/
ARL6

Figure 4. Main conclusions. (A) For each phenotype, the heatmap shows the most conservative pairwise odds ratios within 95% posterior credible intervals for gene on the horizontal axis against the gene on the vertical axis. **(B)**The heatmap shows statistical evaluation of selected statements using Bayesian model taking into account the variability between studies and the character of the mutation (posterior probability) and the frequentist statistics (p value). PD – polydactyly, CI – cognitive impairment, REN – renal anomalies. **(C)** Schematic representation showing the importance of the intact BBSome for most organs and the residual function of BBS2, BBS7, and BBS9 core in the kidney development and function as suggested by our analysis.
Data information: The p-values not specified in previous figures were calculated using one-tailed Fisher's exact test. See Supplemental Statistical Analysis for the exact formulation of the statements in the Bayesian analysis. See Supplemental Statistical Analysis for a detailed description of all models and the imputation procedure as well as for assessments of model fit.

Rigorous testing of the genotype-phenotype relationships among BBS patients

As the final step of our analysis, we carried out a thorough testing of the selected genotype-phenotype links. We focused on the genotype-phenotype associations revealed by our analysis, the differences among patients with mutations in the most common causative genes *BBS1*, *BBS2*, and *BBS10*, and a hypothetical high disease severity caused by mutations in *BBS4*, which can be concluded from the reported models of the BBS (Berbari et al., 2008; Cognard et al., 2015; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Tadenev et al., 2011; Zhang et al., 2011; Zhang et al., 2013). We summarized the frequentist analysis and performed extensive Bayesian tests taking into account additional parameters including the possible variability among studies, sex, age, type of mutation, ethnicity or pedigree structure (Supplemental Statistical Analysis). Based on these analyses (Figure 4B, Supp. Figure S6), we concluded that: (I) Patients with mutations in *BBS3/ARL6* have typically milder phenotype than patients with mutations in BBSome- or chaperonin-encoding BBS genes. (II) Patients with mutations in *BBS4* do not suffer from a more severe disease than other BBS patients. (III) Patients with mutations in *BBS*2 or *BBS10* have higher frequency of polydactyly and renal anomalies than patients with mutations in *BBS1*. (IV) Patients with mutations in *BBS1*, *BBS4*, or *BBS8/TTC8* have low penetrance of kidney anomalies, whereas patients with mutations in *BBS2*, *BBS7*, or *BBS9* have high penetrance of kidney anomalies (Figure 4C).

Discussion

The links between the phenotype and the genotype among the patients suffering from the BBS have been addressed by several previous studies (Castro-Sanchez et al., 2015; Deveault et al., 2011; Forsythe et al., 2017; Forsythe et al., 2015). The lack of a sufficient number of patients and analysis of data limited to a certain aspect of the multiorgan disease were the major caveats of these studies. To circumvent such issues, we established a strategy to perform phenotype-genotype association study across all published patients in different studies. Our approach applies the principles usually used for the meta-analyses of the treatment-outcome clinical studies to assess the genotype-phenotype relationship in rare genetic diseases, such as the BBS.
In this study, we assembled a complete database of the reported BBS patients with their genotype and phenotype records. We analyzed these data to evaluate the association between the genotype and the phenotype of the patients with two major aims. First objective was to address a possible link between the genotype and the phenotype of BBS patients to assess the prognosis based on the causative mutation. The second objective was to get the insight into the biology of the BBSome and the role of the individual BBS genes in humans.
Altogether, our database contains more than 350 reported causative BBS mutations. The effects of the individual mutations depend on the BBS gene mutated as well as on how much the mutation impairs the function of the gene product. We show that BBS patients with structurally more disrupting mutations (frameshift mutations, splicing mutations) typically have more severe disease than patients with more subtle mutations (single amino acid substitutions or short in-frame deletions) indicating that some of the causative BBS mutations are hypomorphic. The hypomorphic nature of several BBS mutations was shown previously by evaluating the effect of disease-causing mutations in the zebrafish (Zaghloul et al., 2010), Caenorhabditis elegans (Xu et al., 2015) and human cell line (Hirayama et al., 2008) models. Unfortunately, our data are not robust enough to

identify which particular missense mutations induce cLOF and which do not.

Mutations in *BBS1*, *BBS2*, and *BBS10* are the most common ones among BBS patients (making around 50% of all cases). Some clinical studies analyzed small cohorts of BBS patients to propose that the outcome of the disease and the presence of specific symptoms differs among BBS patients with different causative BBS genes mutated (Castro-Sanchez et al., 2015; Forsythe et al., 2017; Forsythe et al., 2015; Hjortshoj et al., 2010), whereas such a correlation was not observed by other studies (Deveault et al., 2011; Moore et al., 2005). Our approach revealed that the disease is typically milder in patients with mutations in *BBS1* than in patients with mutations in *BBS2* or *BBS10*. Specifically, patients with mutations in *BBS2* or *BBS10* have higher penetrance of renal anomalies and polydactyly than patients with mutations in *BBS1*. One possible explanation of this observation is the hypothetical hypomorphic nature of the most common mutation of *BBS1*, manifesting as missense M390R mutation (Forsythe et al., 2017; Hjortshoj et al., 2010). However, our analysis of patients with assumed cLOF causative mutations suggests that the low penetrance of some symptoms in BBS1 patients is intrinsically connected to the *BBS1* gene, not to a particular hypomorphic mutation.

Renal anomalies represent one of the major BBS symptoms. The clinical data show that the penetrance of the renal anomalies is highly dependent on the particular causative gene even among the BBSome-encoding genes. Whereas the patients with causative mutations in *BBS1*, *BBS4*, or *BBS8/TTC8* collectively suffer from renal anomalies only in less than 30% cases, patients with mutations in the core BBSome subunits *BBS2*, *BBS7*, or *BBS9* collectively manifest renal anomalies in more than 60% cases. Because the renal failure is the major life-threatening symptom in BBS patients, we believe that our data might have a prognostic value.

The fact that patients with causative mutations in any BBS gene might develop similar symptoms suggests a functional connection among the BBS genes. However, the BBS symptoms overlap with the symptoms of other ciliopathies (reviewed in (Lee & Gleeson, 2011; Reiter & Leroux, 2017)), suggesting that relatively general disturbances in the physiology of the cilium might lead to the development of the symptoms. Moreover, the penetrance of the BBS symptoms is incomplete and variable among the patients. These facts allow for the possibility that some of the BBS genes might have specific functions, which are at least partially independent of other BBS genes.

The second aim of our study was to reconcile how are the BBSome, BBS chaperonins, and BBS3/ARL6 functionally interlinked and whether the BBSome subunits work invariably as a single functional unit. Our analysis revealed that *BBS3/ARL6* deficiency leads to lower penetrance of cognitive impairment, renal anomalies and heart anomalies than deficiency in the BBSome subunits. This indicates that the BBSome function is at least partially independent of BBS3/ARL6. On the contrary, patients with mutations in the BBSome-encoding and chaperonin-encoding genes did not show substantial differences in the disease progression, indicating that the function of the BBSome might be completely dependent on the chaperonin genes. These conclusions are in a good agreement with the reported mouse models which suggest that the phenotype of the *Bbs3/Arl6*-deficient mice is milder than the phenotype of mice deficient in *Bbs1*, *Bbs2*, *Bbs4*, *Bbs5*, *Bbs6*/*Mkks*, *Bbs7*, *Bbs10*, or *Bbs18*/*Bbip1* (Berbari et al., 2008; Cognard et al., 2015; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Tadenev et al., 2011; Zhang et al., 2011; Zhang et al., 2013).

Comparison of reported phenotypes in cell line or mouse models suggests that deficiencies in the particular BBSome subunits might cause different outcomes (Berbari et al., 2008; Cognard et al., 2015; D. F. Guo et al., 2011; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Su et al., 2014; Tadenev et al., 2011; Xu et al., 2015; Zhang et al., 2011; Zhang et al., 2013). This can be explained by the technical differences among the studies (genetic background, housing conditions etc.) or by the fact that not all the individual BBSome subunits work only as a single functional unit. A large-scale study of BBS patients from multiple sources might be more resistant to some technical challenges arising upon comparison of animal strains originating from a single founder or upon comparison of two different cellular and/or mouse models generated and characterized by two different research groups. Moreover, the analysis of the clinical data overcomes the issue that cell line and mouse models do not completely recapitulate BBS symptoms (e.g., polydactyly).

Patients with *BBS1* and *BBS8/TTC8* deficiency showed less severe phenotype measured by the *syndromic score* than patients with causative mutations in other BBSome-encoding genes. The phenotype of *BBS1* deficiency resembles the phenotype of *BBS3/ARL6* deficiency in the patients. As BBS1 is the BBSome subunit directly interacting with BBS3/ARL6 (Mourao, Nager, Nachury, & Lorentzen, 2014), it is possible that the major function of BBS1 is to provide the connection of the BBSome to BBS3/ARL6. When this axis is impaired

in patients with mutations in *BBS1* or *BBS3/ARL6*, the rest of the BBSome subunits retain some residual function. We observed that this residual function of core BBSome subunits takes place in the kidney development and function.

Renal anomalies were the major BBS symptom manifesting clear differences between particular patient groups. The mutations in *BBS2*, *BBS7*, or *BBS9* induced a very high incidence of renal anomalies. Because these three genes encode structurally similar subunits that were previously proposed to establish the core of the BBSome (Zhang, Yu, et al., 2012), it is plausible that the BBSome core plays a specific role in the kidney disease. In contrast, the renal anomalies had relatively low penetrance in patients with causative mutations in *BBS1*, *BBS4*, and *BBS8*/*TTC8*. The genes encode rather peripheral BBSome subunits (Katoh, Nozaki, Hartanto, Miyano, & Nakayama, 2015; Klink et al., 2017; Woodsmith et al., 2017) that might be at least partially dispensable for the formation of the BBSome core and its function. The example of renal anomalies shows that the BBSome operates as a single entity, but even in the absence of one peripheral subunit, the BBSome can retain some partial function at least in some process and/or tissues. This model is consistent with the observation that two core subunits BBS2 and BBS7 stabilize each other on the protein level in testis (Zhang et al., 2013), predicting that patients with causative mutations in either of these genes should manifest with a similar phenotype. Accordingly, our analysis shows that patients with mutations in *BBS2* and those with mutations in *BBS7* showed comparable disease outcome and penetrance of major BBS symptoms. However, we cannot exclude that the peripheral BBSome subunits have unique roles in other tissues or physiological processes.

Analysis of the phenotype-genotype links in other symptoms than renal anomalies was less conclusive, with the exception that patients with *BBS1* mutations had generally milder disease manifestation than patients with *BBS2* mutations. Our analysis documents that previously suggested functional redundancy between BBS4 and BBS5 described in human cell lines, Caenorhabditis elegans and zebrafish (Xu et al., 2015) and the most severe phenotype of *Bbs4*-deficient mouse among the available BBS mouse models (Berbari et al., 2008; Cognard et al., 2015; Davis et al., 2007; D. F. Guo et al., 2011; Kulaga et al., 2004; Loktev & Jackson, 2013; Nishimura et al., 2004; Rahmouni et al., 2008; Tadenev et al., 2011; Zhang et al., 2013) do not apply in humans.

The BBS is a rare disease and the number of reported patients is limited. Moreover, the presence/absence of particular symptoms was not reported for each patients. We are aware that the low number of patients in some genetic groups was the major limiting factor in our study. Because of the sporadic reporting on the secondary symptoms, our analysis was limited to 6 major symptoms and 3 other most frequently reported symptoms. We cannot excluded that some other minor secondary symptoms show different genotype-phenotype correlation patterns than the symptoms covered by our meta-analysis. Another caveat is that the data allowed us to assess the presence/absence of the BBS symptom, but not grading the severity of individual symptoms. This might explain, why we did not observe differences in retinal dystrophy between patients with causative mutations in *BBS1* and other patients as was previously reported in small cohorts of patients using a quantitative test of the visual acuity (Daniels et al., 2012; Esposito et al., 2017). It is possible that the penetrance of renal anomalies showed the clearest link to the genotype only because the overall penetrance of renal anomalies was the lowest of all major BBS symptoms, allowing us to observe the stratification. However, we managed to analyze the complete set of currently available data, which is the major advance over the primary studies on small cohorts of patients. Despite all the limitations, we made several conclusions which are relevant for the clinical prognosis of BBS patients and for the understanding of the function of the BBSome. A prospective study focusing on our retrospective analysis would be helpful in this respect, but it is not feasible to carry out such a study in a reasonable timeframe because of the rarity of the patients. However, we recommend addressing our conclusion in any prospective cohort that will be analyzed.

To our knowledge, a similar study addressing the link between a genotype and phenotype based on a complete set of previously reported patients' data has not been carried out previously for any rare human disease. Our strategy can be used to get relevant information for the clinical prognostic criteria as well as an insight into the biology of various human genetic diseases.

Methods

PRISMA guidelines

The meta-analysis was performed in accordance with the guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, Altman, & Group, 2009). Although PRISMA guidelines were primarily designed for studies with different objectives, we followed the guidelines wherever applicable. Our

study was designed to address the following PICO question: Do BBS patients with mutations in different genes have different phenotypic outcome of the syndrome? The protocol for this meta-analysis was pre-registered with PROSPERO (CRD42018096099).

Search strategy

PubMed and Google Scholar databases were searched in May 2018 for the following keywords: [bardet-biedl syndrome AND (genotype phenotype OR cohort)]. The titles and abstracts of the identified studies were screened independently by two researchers (VN, OT) and evaluated for relevance. All studies considered relevant at least by one of the researchers were further examined by reading the full text. Other suitable records were identified by snowball searching, in particular, by retrieving relevant articles from the references of the studied full-texts. In addition, all the references included in the publicly available Euro-Wabb database (https://lovd.euro-wabb.org/home.php) (Farmer et al., 2013) were covered. Our search was limited to the literature published in English language and covered the period from the inception of each database to the 21st of May 2018.

Study selection

The full-texts of potentially relevant articles were studied independently by two researchers (VN, OT) and evaluated against the following inclusion criterium: contains information about at least one patient with BBS whose individual genotype and phenotype are reported. Studies that were considered eligible only by one of the two researchers were included/excluded following discussion and consultation with an independent reviewer (OS).

Next, all the included studies were studied in detail and individual patients from each of the studies were included in the database if they met the following criteria: (i) at least two different phenotypes are reported for the patient, (ii) the patient was born alive, (iii) the gene was mutated in both alleles (i.e., the patient was not a heterozygote).

Individual patients or cohorts of patients that have been described by the same research group in multiple articles were included only once. If possible, phenotypes of the same patient reported in two different studies were merged to obtain the most complete data. In several cases, we found discrepancies between the phenotypic outcome in the same patient reported in multiple studies. As it is likely that the phenotype was present at least in some time-point, we considered the patient positive for the phenotype and these cases were marked by an exclamation mark in the database as '1!'.

Extraction of the data

The following information were extracted (if available) from each of the selected studies: (i) general information about the study (first author, year, origin of patients such as geographical location/clinic, number of participants), (ii) methodology of study (method of collecting phenotypic information, method of genotyping), (iii) individual patient data (patient ID, causative BBS gene, principal mutation – nucleotide change, principal mutation – protein change, additional mutation, sex, age, intra-familial relations with other patients, ethnicity, and the presence of the phenotypic features involving retinal dystrophy, obesity, polydactyly, cognitive impairment, reproductive system anomalies, renal anomalies, heart anomalies, liver anomalies, developmental delay). The following conditions were considered positive for each phenotype: retinal dystrophy – rod-cone/cone-rod dystrophy/degeneration, retinitis pigmentosa, Leber congenital amaurosis, night blindness, pigmentary retinopathy, tapetoretinal degeneration, ocular nerve atrophy, poor night vision; obesity – BMI > 25 for adults and children older than 15 years of age, weight > 95 quantile for children up to 15 years of age, medical history of obesity; polydactyly – pre-, meso-, post-axial polydactyly; cognitive impairment – IQ < 70, learning difficulties, mental retardation, reduced intelligence; reproductive system anomalies – males: micropenis, hypospadias, cryptorchidism, hypogonadism, females: primary amenorrhea, vaginal atresia/agenesis, urigenital sinus malforation, vaginal agenesis, malformed/infantile/bicornate/abnormally positioned uterus, uterine leiomyoma, hydrometrocolpos, polycystic ovary syndrome, ovarian cysts/tumour, both: abnormal/ambigous genitalia; renal anomalies – chronic kidney disease, polycystic kidney disease/cystic kidney/renal cysts, renal failure, renal dysplasia, renal transplant, renal cortical atrophy, dilatation of renal pelvis, horseshoe kidney, enlarged/small kidneys, hydronephrosis, impaired renal function, kidney agenesis with milder renal symptoms, kidney stone, nephrolithiasis, nephronophthisis, fetal lobulation; heart anomalies – congenital cardiac anomaly, congenital heart disease, atrial septal defect, heart anomalies; liver anomalies – non-alcoholic fatty liver, hepatomegaly, polycystic liver/liver cysts, liver steatosis, liver impairment, abnormal liver structure, abnormal liver function; developmental delay – motor delay, growth delay, general developmental delay, psychomotor delay,

delayed sexual development. The following conditions were not scored as positive: retinal dystrophy – ocular phenotypes unrelated to retina; polydactyly – brachydactyly, syndactyly, clinodactyly; reproductive system anomalies – gynaecomastia, recurrent urinary tract infections, irregular menstruation.

The notation of causative mutations was unified according to the standard nomenclature of genetic variations wherever possible (den Dunnen et al., 2016). Mutations leading to one to three amino acid substitutions (missense substitutions, deletions of 3, 6 or 9 nucleotides) were considered missense, while frameshift mutations, nonsense substitutions, large deletions and splicing defects were assumed to lead to cLOF of the protein.

The information about intra-family relations between included patients was used to allocate a unique randomly generated Family ID code to each included family, so that the members of one family share the same Family ID.

The information about the ethnic origin of the individual patients or the whole cohorts of patients was extracted from the original studies and used for subsetting the patients into eight different ethnic groups labeled EG-A to EG-H according to the geographic location. The list of particular ethnicities/countries of origin and the associated ethnic groups can be found in the Supp. Table S5.

Data were extracted by one of the researchers (VN, OT) and after extraction underwent control by the other researcher. After completing the extraction, an independent control of 20 random records was carried out by the third independent researcher (OS) to confirm the reliability of the extraction process. Also, additional post hoc control was carried out to prevent multiple involvement of the same patient.

Risk of bias

As our meta-analysis was focused on the extraction of the primary data of individual patients from original sources, the most commonly used methods of assessment of the risk of bias (such as the Cochrane Risk of Bias Tool) were not applicable. Included studies were therefore evaluated across three domains: reporting bias risk, risk due to incoherence, and risk due to the used method of genotyping/phenotyping. Reporting bias risk was considered high in cases where it was not possible to determine whether a particular syndrome was absent in some patients or not assessed. Risk due to incoherence was considered high in pairs of studies that reported the same patient, but with different phenotypic outcome. Risk due to the method of genotyping/phenotyping was considered unknown if there were no information about the methods used in the original article. Identification of high or unknown risk did not influence the inclusion of the patients in our dataset.

Frequentist statistical analysis

To assess the phenotypic outcome of the disease, we used a set of BBS patients with reported presence or absence for 5 major symptoms: retinal dystrophy, obesity, polydactyly, cognitive impairment, and renal anomalies. The reproductive system anomalies were not included as their etiology might substantially differ between male and female patients. This set of patients is further referred to as *'set*. The differences in the outcome of the disease across different groups within the *'set* of patients were assessed using Kruskal-Wallis test with Dunn's Multiple Comparisons Test. The differences in the *syndromic score* between two groups of patients, e. g. for testing differences between patients with missense vs. cLOF mutations, were tested using the nonparametric Mann-Whitney test.

For the analysis of the penetrance of symptoms in different groups of patients, we assembled contingency tables showing the number of positive/negative cases within each group of patients. The differences in the penetrance of phenotypes throughout multiple groups of patients were assessed using Fisher's exact test. Statistical significance of differences between individual groups was determined post hoc using Fisher's exact test (one group vs. all other groups taken together) with the Sidak correction for multiple comparisons. 95% posterior credible intervals used for the visualization of the error bars in the contingency table bar graphs were calculated as 0.975 and 0.025 quantile of the Beta distribution with parameters $(x + 1, y + 1)$, where x and y represent the number of patients positive and negative for the corresponding phenotype, respectively.

P-value < 0.05 was considered statistically significant in all tests except for multiple comparison tests with Sidak correction, where the significance level was adjusted to the number of tests carried out. Statistical analyses were performed in RStudio (V1.0.136, RStudio, Inc.). The full code for the reported frequentist analysis is reposited at Zenodo (DOI: 10.5281/zenodo.3243400) and can be also found at https://github.com/vercanie/bbs-metaanalysis-freq.

Bayesian statistical analysis

A fully Bayesian analysis was performed with a hierarchical logit model using the brms package (Bürkner, 2017). Unlike the frequentist case, the

Bayesian approach does not require filtering of infrequently occurring mutations and our models were fit with the complete dataset and all of the 9 reported phenotypes. Each phenotype was treated separately (no combined score was used). All of the results reported here were produced by a model with one fixed effect for the interaction of cLOF and phenotype and two varying intercepts for each phenotype – one grouped by gene with explicitly modelled global phenotype-phenotype correlations which is the main effect of interest, and other grouped by study to account for between-study variability (without correlation). Most notably the model assumes that for all phenotypes, choosing any study and either only cLOF or only other mutations, the odds ratios between different genes are the same. On the other hand, the absolute odds are allowed to vary between studies and between cLOF and other mutations. An accessible explanation of the complete assumption of the models and its exact mathematical form is given in Supplemental Statistical Analysis. Mildly skeptical *N(0,2)* priors were put on the intercept, fixed effects and hierarchical variance parameters.

We also performed a multiverse analysis (Steegen, Tuerlinckx, Gelman, & Vanpaemel, 2016) running multiple model variants to ensure robustness of the results. The results remain almost identical when omitting cLOF as covariate, using wider or narrower priors, modelling different covariance structures for the per-gene varying intercepts and when sex and/or age is included in the model, using multiple imputation with the mice package (van Buuren & Groothuis-Oudshoorn, 2011) to account for missing values. The direction of the effects of interest is also unchanged when only patients with cLOF mutations are included, a varying intercept for cLOF grouped by gene is included, missingness in age and sex is handled by removing the respective cases, the family structure or the ethnicity is taken into account and the between-study variability is ignored. However, the magnitude and associated uncertainty of the effects changes noticeably in those cases, which would, in some cases, alter some of the conclusions. However, we believe that the most of the alternative models are not well justified, since ignoring the between-study variability leads to a poor fit, filtering for age/sex/cLOF reduces the dataset by over 40% while controlling for age and sex or cLOF by gene shows only minor improvement in model fit.

The Bayesian analysis reported here, including full code, is described in detail in the Supplemental Statistical Analysis – Part 1, all the details of the alternative models and model selection are presented in the Supplemental Statistical Analysis – Part 2 and the robustness of our main conclusions to model choice is discussed in the Supplemental Statistical Analysis – Part 3. The complete code is archived at Zenodo, DOI: 10.5281/zenodo.3243264 and also available at https://github.com/martinmodrak/bbs-metaanalysis-bayes.

All the quantities reported for the Bayesian model are derived from 95% and 50% posterior credible intervals for expected odds of the phenotypes in a hypothetical new study drawn at random from the same population of studies as those included in our meta-analysis for a patient with a cLOF mutation. Since the observed between-study variability is large, we mostly report ratios of odds within the hypothetical study, which the model assumes to be the same for all studies and mutation types.

Acknowledgement

We thank Petr Divina and the IT department of the Institute of Molecular Genetics for the development of the online interface for the curated database of anonymized BBS patients.

This study was supported by the Czech Science Foundation (17-20613Y to MH). The contribution by MM was supported by C4Sys research infrastructure project (MEYS project No: LM20150055). Group of Adaptive Immunity at the Institute of Molecular Genetics in Prague is supported by an EMBO Installation Grant (3259 to OS) and the Institute of Molecular Genetics of the Czech Academy of Sciences core funding (RVO 68378050). OS is supported by the Purkyne Fellowship provided by the Czech Academy of Sciences. OT is a student of the Faculty of Science, Charles University, Prague.

Author contribution

VN designed the database of BBS patients and performed the frequentist statistical analysis. MM designed and performed the Bayesian statistical analysis and contributed to the frequentist analysis. VN and OT screened the primary reports for BBS patient data and extracted the data from the primary sources. VN, MH, and OS conceived the study and were in charge of the overall direction and planning. OS randomly checked the extracted data from the primary sources. VN, MM, OT, MH, and OS wrote the manuscript.

Conflict of interest

All authors declare that they have no conflict of interest.

Supplemental Figures

Supp. Figure S1. Flow diagram of the literature search for the meta-analysis.

Supp. Figure S2. Characteristics of all reported BBS patients.

Supp. Figure S3. Characteristics of the subset of BBS patients used for the *syndromic score* analysis.

Supp. Figure S4. The disease outcome in patients with missense mutations and assumed complete loss of function mutations.

Supp. Figure S5. Penetrance of heart and liver anomalies and developmental delay in patients with mutations in particular BBS genes.

Supp. Figure S6. Robustness of main conclusions to analytical decisions.

Supplemental Tables

Supp. Table S1. PRISMA Checklist.

Supp. Table S2. Summary of the included studies and evaluation of the risk of bias.

Supp. Table S3. Database of all reported BBS patients.

Supp. Table S4. List of the BBS-causing mutations occurring in our dataset.

Supp. Table S5. List of the ethnicities/countries of origin and the matching ethnic groups of the included patients.

Supp. Table S6. Data of the *'set* of BBS patients used for the *syndromic score* analysis.

Supp. Table S7. Previously published observations about BBS genotype-phenotype correlations.

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