Towards Personalised Medicine in Bardet-Biedl Syndrome

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Abstract

Personalised medicine is becoming routine in the treatment of common diseases such as cancer, but has lagged behind in the field of rare diseases. It is currently in the early stages for the treatment of Bardet-Biedl syndrome. Advances in the understanding of ciliary biology and diagnostic techniques have opened up the prospect of treating BBS in a patient-specific manner. Owing to their structure and function, cilia provide an attractive therapeutic target and genetic therapies are being explored in ciliopathy treatment. Promising avenues include gene therapy, gene editing techniques and splice-correcting and read-through therapies. Targeted drug design has been successful in the treatment of genetic disease and research is underway in the discovery of known and novel drugs to treat BBS.

Introduction

Bardet-Biedl syndrome (BBS) is one of a family of disorders known as ciliopathies, all of which exhibit a defect in the structure or function of cilia. Cilia are evolutionarily conserved, complex organelles that project from the apical cell membranes of most cells in vertebrates. Their structure differs depending on whether they are motile or immotile. Immotile cilia, also known as primary cilia are implicated in BBS aetiology. These have roles in cell signaling pathways, including sonic hedgehog and WNT signaling[1, 2]. They have been implicated in the development of cancer and obesity as well as ciliopathies[1, 3]. The basal body, which is a specialised centriole, anchors the cilium to the apical cell membrane, and along with the BBSome, which consists of BBS1, BBS2, BBS4, BBS5, BBS7, BBS8 and BBS9 proteins, is involved in the formation and maintenance of cilia[4]. Other BBS proteins, BBS6, BBS10 and BBS12, form a chaperonin complex purported to facilitate protein folding [5]. Mutations in any of these and some other genes localizing primarily to the basal body can cause BBS syndrome

Ciliopathies share a number of features including obesity, structural brain defects, retinal degeneration, situs inversus, skeletal abnormalities, cognitive impairment and structural renal and hepatobiliary defects[6]. While most of these features can be seen in BBS, the primary features are rod-cone dystrophy resulting in blindness, postaxial polydactyly, obesity, cognitive impairment, hypogonadism or genital abnormalities and renal anomalies[7]. Minor features, such as facial dysmorphism are also considered in the diagnostic criteria. It is a multisystem disease which begins during embryonic development and involves many organs and body systems, making treatment very challenging. It is thought to affect approximately 1 in 100,000 in North America and up to 1 in 160,000 people in parts of Northern Europe, but a higher prevalence is seen in some isolated or consanguineous populations, for example 1 in 13,500 in Kuwaiti Bedouins and 1 in 18,000 in Newfoundland [7-10].

BBS is inherited in an autosomal recessive manner, though rare cases with more complex modes of inheritance have been reported[11-13]. Currently, 21 genes are known to be implicated in BBS, but mutations cannot be found in about 20% of patients, suggesting that additional genes are yet to be identified [14-16]. Mutations in *BBS1* and *BBS10* account for approximately 50% and 20% of cases in the United Kingdom[17]. There is significant phenotypic variability between patients with BBS, even between patients with identical mutations and patients in the same family. The basis for this variability is not understood, though genetic modifiers have been identified in other ciliopathies[18, 19]. It is also possible that environmental factors and epigenetic modifications play a role. Some genotype-phenotype correlations have been made but these are not always consistent. For example, patients with mutations in *BBS1* have a lower risk of developing renal disease and appear to develop eye disease later[17, 20-22]. Genes known to cause BBS have also been implicated in other ciliopathies, for example *CEP290* mutations causes Joubert syndrome, Meckel syndrome, Leber congenital amaurosis and Senior-Loken syndrome as well as BBS[22-26].

Personalised medicine is defined by the Horizon 2020 advisory group as "a medical model using characterization of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention"[27]. The identification of biomarkers is allowing early detection of disease and relapse, opening up previously unavailable avenues for treatment. However, this has proved challenging in complex, multisystem disorders, particularly those, like BBS, that manifest in utero and has yet to catch up with other fields, such as oncology. However, the potential for benefit to these patients is enormous, given the burden of morbidity and mortality.

Deep phenotyping, diagnostics and patient stratification

Deep phenotyping in Bardet-Biedl syndrome

Peter Robinson has defined deep phenotyping as "the precise and comprehensive analysis of phenotypic abnormalities in which the individual components of the phenotype are observed and described" and is

a vital tool in personalised medicine for rare disease [28]. Accurate and detailed phenotypic information about patients enables stratification for research, clinical trials and treatment. One of the ways to ensure that such data are routinely collected is for patients to attend a specialised multidisciplinary clinic. The National Health Service England has commissioned a specialist BBS clinic, where patients are seen by clinicians including a clinical geneticist, endocrinologist, ophthalmologist, renal physician, psychologist, dietician, speech and language therapist and a family advocate. Detailed clinical data is recorded and a standardized set of investigations including comprehensive genetic testing is performed at each visit. These data allow patients involved in research to be accurately stratified, essential in the search for biomarkers and treatment targets. The use of Human Phenotype Ontology (HPO) terms aids in the standardisation and accurate recording of data[29]. The recording of data in a standardised format enables the integration of clinical data with other datasets.

Diagnostics

Until recently, diagnostics for diseases such as BBS was done on a gene by gene basis, with the most likely gene, in this case *BBS1*, being sequenced first. If no mutation was found, the next most likely gene would be sequenced and so on. However, in the last few years, diagnostics for conditions with significant locus heterogeneity has moved to panel-based next generation sequencing (NGS)[30-32]. Currently the approach to BBS testing in the UK is to perform exome sequencing and analyse a panel of 21 BBS genes. This has replaced a panel of 11 BBS-related genes. The diagnostic yield is approximately 80%[33]. This approach is faster and more cost effective than previous strategies. The remaining 20% are sent for whole genome sequencing.

As the cost of next-generation sequencing decreases, the utility of whole genome sequencing (WGS) is beginning to be evaluated in the diagnosis of ciliopathies including BBS. There are both technical and diagnostic advantages to using WGS over WES[34]. One advantage is that intronic regions can be examined for areas of interest. Mutations in regulatory regions have occasionally been reported in disease[35]. In the case of BBS, mutations outside coding regions have been reported[26, 36]. WGS is superior to WES in that copy number variants can be detected across the genome thus providing a better chance of an individual molecular diagnosis. An accurate molecular diagnosis is important even in the absence of confirmed genotype-phenotype correlations. Firstly, it allows advice to be given about recurrence risk. Secondly, it allows the stratification of patients for research. Thirdly, in the age of personalised medicine, this knowledge will be required for tailoring treatments to the patient. The diagnostic yield of WGS has yet to be evaluated in this patient population.

Understanding variability in Bardet-Biedl syndrome

One of the major goals in BBS and ciliopathy research is to understand the variability between patients. Both genetic and environmental factors are likely to influence this[4, 37]. The search for genetic and epigenetic modifiers is ongoing. This is particularly challenging in rare diseases, especially those exhibiting significant phenotypic variability because of the difficulty of assembling large, homogenous

cohorts. In smaller or phenotypically varied cohorts only genetic modifiers with a very strong effect will be identified.

One of the ways of overcoming this difficulty is the use of multiomics. Multiomics is a term which describes the co-analysis and integration of multiple “omics” datasets from a sample or collection of samples, for example genomics, proteomics, transcriptomics, metabolomics, microbiomics, epigenomics and so on. This approach generates large volumes of data, which can then be interrogated for possible biomarkers, modifiers, novel pathways and treatment targets, but also has other applications in the longitudinal study of disease such as in the stratification of patients and identification and monitoring of treatment response or relapse. Multiomics is currently a research tool in the study of BBS. A pilot project at the UCL Great Ormond Street Institute of Child Health, The HIGH5 project, is looking at multiomics in BBS and other rare genetic disorders and aims to identify biomarkers and treatment targets. The identification of targets for therapy is of enormous importance in reaching the potential of personalised medicine in BBS. It is hoped that pilot projects such as HIGH5, as well as enhancing understanding of conditions such as BBS and identifying biomarkers, modifiers and treatment targets, will provide a framework for future multiomics studies in rare disease.

Patient stratification

Patient stratification is very important in the applications of personalised medicine. Given the expense of bringing a new drug to market, estimated by the Tuft's Centre for the Study of Drug Development at over $2.5 billion, errors in patient selection for clinical trials are extremely costly. In depth multiomics studies and detailed phenotypic information can aid in the selection of patients for trials and can also be used to select appropriate therapies for patients after the trial stage. Currently, stratification in BBS depends mainly on clinical data. Evidence from the UK patient cohort demonstrates that adults with missense mutations in *BBS1*, normal renal function and structure are very unlikely to develop renal disease provided that they maintain adequate blood pressure and glycaemic control [17]. The development of gene therapy for rod-cone dystrophy has progressed to animal studies [51]and will benefit from the longitudinal data available from almost 500 patients attending national multidisciplinary specialist clinics including extensive annual ophthalmological investigation.

Treatments in Bardet-Biedl syndrome

Currently, there is no curative treatment for Bardet –Biedl syndrome. Treatments are targeted at the effects of the condition and are supportive. They include aggressive management of cardiovascular risk factors such as raised cholesterol and blood pressure, standard diabetes treatments, dialysis or transplant for end-stage renal failure, diet and exercise for obesity and surgical management of polydactyly. Despite significant research efforts, there is no consensus treatment for rod-cone dystrophy such as that seen in BBS and no preventative treatments for that or renal cyst development[38-45].

One of the major challenges in treating BBS is that it is a multisystem disorder with many different organs affected. As BBS is caused by mutations in a number of genes, gene therapy and related

treatments are one of the main foci of research at present. Figure 1 shows how many patients with BBS will benefit from personalised treatments.

Traditional gene therapy for Bardet Biedl Syndrome

Gene therapy involves correcting a deficient or malfunctioning protein by replacing the mutated copy of a gene with a normal copy. Theoretically, gene therapy can be germ-line, which involves modifying an egg or sperm and thereby eliminating the genetic defect in offspring and future generations, or somatic, in which case a particular organ or tissue is targeted and the genetic defect is corrected in a limited area. However, because of ethical concerns, all current research is in the area of somatic gene therapy.

Genes are introduced using viral or non-viral vectors. Viral methods involve inserting DNA into an altered virus and infecting a patient or tissue with the virus. The virus integrates into the host genome and the normal gene product is produced in the patient cells. Viral vectors currently in use include Adeno-associated virus (AAV) and Lentivirus[46, 47]. Viral gene therapy is ideal for targeting a single organ, particularly one that is easy to reach, such as the eye, and has been attempted in animal models of retinitis pigmentosa, including in Bardet Biedl syndrome [48-52]. Wert *et al* showed that some rescue is possible even after neurodegeneration has occurred[53]. This is very important as for many people, a diagnosis of BBS may be made only after visual symptoms develop. Much of the gene therapy work carried out so far has been in animal models, although trials of gene therapy have been initiated in other similar eye conditions and found to result in an improvement in visual function[54]. Recently, the Food and Drug Administration (FDA) granted orphan drug designation to an AAV potential gene therapy treatment for X-linked retinitis pigmentosa, a phenotype similar to that seen in BBS. It is thought that trials will begin in the UK in 2017[55].

The eye is not the only organ affected in BBS that may be amenable to treatment with gene therapy. Another possible target is the kidney, although as not all patients develop renal problems and those that do develop them early in life, it may be difficult to target this appropriately. As yet, little work has been done, even in pre-clinical models, with gene therapy for inherited renal disorders.

Gene Editing Therapies

Another important novel therapeutic approach is the use of gene editing therapies. The development of CRISPR/Cas9 gene editing strategies in particular, which are more accurate than previous gene editing approaches, has raised the possibility of direct gene-editing in patients, especially if off-target effects can be avoided [56, 57]. CRISPR/Cas9 is a method of defense used by bacteria. Cas9 is an endonuclease that introduces double-stranded breaks into DNA at a specific site. The resultant break can be repaired, thus providing the means of correcting a mutation or introducing a gene[58]. The technology is already being widely used to develop animal models of disease [59, 60]. In vitro work has established that gene editing can correct defects in certain diseases, such as sickle cell disease and recently, the NIH granted permission for early trials [61, 62]. Other gene editing techniques such as TALENs and zinc finger have already been used in patients[63, 64]. Such gene editing technologies have already been used to treat

blindness in a rat model of retinitis pigmentosa, although the application of these technologies to humans may be problematic, as in this case, direct injection of rat visual cortex was required[65].

Applying these technologies to Bardet Biedl syndrome is a strong possibility. Again, retinal degeneration is the obvious target, as the eye is relative accessible compared to other organs. However, what is not yet certain is when treatment would need to be started in order for useful vision to be retained and whether this would need to be done before the onset of symptoms, meaning it would be unsuitable for patients diagnosed only after the onset of visual symptoms.

Read-through therapy

Read-through therapy is a therapy targeted at genetic disease caused by premature termination codons (PTC). PTCs, also known as nonsense mutations, result in a premature termination of translation and the resultant protein is particularly susceptible to nonsense-mediated decay[66]. PTCs are thought to account for approximately one third of all genetic diseases, although the proportion varies significantly by disease[67]. In BBS, the proportion of disease caused by PTCs has been estimated at approximately 12%[68].

Compounds such as aminoglycosides and Ataluren (PTC124) have been identified as potential therapies[67]. As aminoglycosides have significant side effects at the dose required for PTC read-through and have only been moderately effective in clinical trials, recent focus has been on alternative compounds such as Ataluren, a compound that was found to increase read-through at PTCs without affecting termination at normal stop codons and without major side effects. Clinical trials in patients with cystic fibrosis and Duchenne muscular dystrophy (DMD) have not yet proved to significantly benefit patients [69, 70]. Read-through therapy results in the insertion of a non-native amino acid, the effect being similar to that of a missense mutation, which should result in a milder phenotype, and efficacy is affected by both the specific PTC and the surrounding nucleotides, so will not be beneficial in every case. Current therapies may not be efficient enough to restore sufficient normal protein to give clinically significant benefits efficient compounds are sought[71]. It is likely that only a small number of patients would benefit from this approach and work is ongoing to assess its potential.

Splice-correcting and exon-skipping therapies

A small proportion of BBS mutations result in aberrant splicing[7]. These may be amenable to splice-correcting therapy approaches. These include the use of antisense oligonucleotides, snRNAs and RNA interference among others[72]. These bind to the RNA and can silence the aberrant splice site. Antisense oligonucleotides have been trialed in the treatment of DMD and found to have few side effects[73]. However, unlike read-through therapy compounds where any nonsense mutation can be targeted, these therapies require targeting to the precise mutation seen in a patient which at present is not cost effective as only small numbers of patients are affected by recurrent splice-affecting mutations. However, this approach has been used in vitro to show potential therapeutic effects in fibroblasts with a *BBS1* mutation and other ciliopathies may also be amenable to this approach[74, 75]. If the costs for the

design and clinical implementation of splice-correcting therapies can be significantly reduced, this would usher in an era of truly individualised medicine.

Targeted drug therapies

One of the earliest targeted therapies was Imatinib (Glivec) for the treatment of *BCR-ABL*-associated chronic myeloid leukaemia which was licensed in 2001. Imatinib is a tyrosine kinase inhibitor which inhibits the constitutively active tyrosine kinase formed by the bcr-abl fusion protein and it was developed using a drug and resulted in a significantly improved prognosis for patients[76]. More recently, the development of Ivacaftor (Kalydeco), has changed the course of cystic fibrosis (CF) treatments for patients with G551D mutations in *CFTR,* approximately 4-5% of patients with CF[77]. The G551D mutation results in the correct formation and localization of the cystic fibrosis transmembrane regulator but it is not regulated normally and cannot effectively transport chloride ions. Ivacaftor, identified by high throughput drug screening and optimisation, is a potentiator of CFTR and increases the probability of chloride ions being transported. When combined with a second drug, Lumacaftor, it can be used in the treatment of CF caused by F508del, the commonest CF-causing mutation[78]. These targeted therapies for CF underline the importance of obtaining an accurate genetic diagnosis for patients.

The cilium provides an excellent potential novel therapy target. Recently it has been shown that primary cilia are lost in some cancers and that cilia may be involved in common conditions such as obesity and osteoarthritis which have increased interest in potential ciliary drugs[79, 80]. Theoretically, drugs could be developed which restore the cilia function. This could be achieved either by screening novel compounds or screening previously licensed drugs (reprofiling) for ciliary effects. The latter approach is particularly helpful as drugs already licensed in humans are more likely to be approved for clinical trials as safety data is already available. Again, drug delivery may be an issue with both the eye and kidney being good potential targets. Other symptoms such as obesity might also be amenable to treatment with this approach as drugs can be taken systemically.

Symptomatic treatments and pharmacogenomics

Currently, as there is no curative therapy for BBS, patients are treated with drugs designed to prevent or treat the symptoms and complications of the disease. These include hypertension, diabetes, hypercholesterolaemia, obesity and renal failure, the control or treatment of which are very important for long term outcomes. However, their treatment may result in significant polypharmacy for some patients and increases the risk of adverse drug effects. One of the drivers for adverse drug reactions is an individual's pharmacogenomics profile; genetic variations in genes involved in the distribution, targets or metabolism of drugs[81]. Pharmacogenomic variants are seen at differing frequencies in different populations and can be used to predict how patients will respond to drugs. There is evidence to suggest that clinical testing for these variants and resulting alterations in prescriptions may not only reduce adverse reactions but also be cost effective[82].

One project currently underway at the UCL Great Ormond Street Institute of Child Health in conjunction with the nationally commissioned BBS clinical service is detailed phenotyping and pharmacogenomic profiling of a small cohort of BBS patients. Adult patients were found to be taking an average of three medications. When pharmacogenomics variants were analysed all patients were found to have pharmacogenomic variants that would potentially alter prescribing advice, in some cases for drugs they were already taking[83].

Knowledge of pharmacogenomic variation is very important in the discovery and development of novel pharmaceutical compounds and the repurposing of previously licensed drugs, as potential side effects in certain groups of patients can be predicted and dosing can be adjusted. Eliglustat, recently licensed for the treatment of Gaucher disease, is prescribed in accordance with CYP2D6 haplotype information, with a reduced dose being prescribed to patients who are predicted to be poor metabolisers of the drug[84]. This approach may be important in the development of drugs to treat BBS.

Future Perspective

Personalised medicine is the practice of treating every patient as an individual and tailoring their treatment as much as possible to them and their disease. Many novel therapies are being developed in the field of rare genetic diseases. Many of these diseases are multi-system in their manifestation, and so are likely to require multiple therapies. Even in the absence of a specific personalised curative therapy, personalised medicine can be practiced in the phenotyping, diagnosis and stratification of patients and in the prescription of supportive therapies. In this sense, BBS is beginning to provide a paradigm for manner in which other rare diseases are managed and treated. It is very likely that in future patients with BBS will have many treatment options, including gene therapy and drugs targeted to the cilium and that the first clinical trials will begin in the next five years. In the interim, patients are benefitting from the use of advanced diagnostic techniques, detailed phenotyping and pharmacogenomic analysis so that they are well placed to take advantage of therapeutic developments.

Executive summary

Bardet-Biedl Syndrome

- Bardet-Biedl syndrome (BBS) is an inherited multisystem ciliopathy
- The features include retinal dystrophy, obesity, hypogonadism, polydactyly, cognitive impairment and renal anomalies
- It affects approximately one in 160,000 people in Northern Europe
- 21 causative genes have been identified so far

Deep phenotyping, diagnostics and patient stratification

- Deep phenotyping is a vital tool in the study or rare disease and specialist clinics can aid in the collection of these data

- Next-generation sequencing has led to advances in the diagnosis of rare diseases such as BBS. The introduction of whole exome and whole genome sequencing is likely to increase diagnostic yield
- Patient stratification depends on accurate phenotyping and diagnosis and is very important in the provision of personalised treatments for rare disease

Personalised therapies for Bardet-Biedl syndrome

- Gene therapy is a promising treatment for BBS, particularly for retinal and renal manifestations. Trials are underway in the treatment of other inherited retinal dystrophies
- Gene editing therapies, which have been used in the treatment of diseases such as leukaemia, represent an important area of research in the treatment of BBS
- Therapies such as exon-skipping, read-through and splice-correcting therapies will be suitable for a proportion of patients with BBS
- The cilium represents an important target for drug design and reprofiling, and drugs targeting the cilium may be useful in the treatment of common as well as rare disease
- Symptomatic treatments are currently the mainstay of treating BBS. Pharmacogenomic profiling can reduce the morbidity associated with this and there is evidence to suggest that this approach is cost-effective

Future perspectives

- Multisystem diseases such as BBS are likely to require multiple therapies to treat the various manifestations
- Clinical trials in the treatment of BBS are likely to begin in the next 5 years

Figure 1: Prospective personalised therapeutics for Bardet-Biedl syndrome. Several genetic therapeutic modalities will likely be employed. Percentages based on mutations observed in UK BBS patients (n=265). 1a. 70% of patients could benefit from gene therapy for patients with mutations in *BBS1* and *BBS10*. Currently animal trials are underway for *BBS1* gene therapy. 1B. 11% of patients could benefit from premature termination codon therapy. The sequence of the premature stop codon determines the likely substituted amino acid (presented here in order of frequency) [85]. The most frequently amino acid replacements are reported here. 1C. 9% of patients could benefit from exon skipping therapy. D. The remaining 16% of patients have primarily private mutations which are not amenable to any established genetic therapeutic approaches. A promising approach includes genome editing. *Some patients in this group may also benefit from other genetic therapeutic interventions.

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