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Pressing diseases that represent promising targets for gene therapy

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Abstract

Over time, there has been a growing interest in the application of gene therapy within the healthcare industry as demonstrated by the nearly 3000 clinical trials associated with gene therapy that are listed in clinicaltrials.gov. However, there are various difficulties associated with gene therapy that have limited the realization of licensed gene therapies to only a handful of treatments. Furthermore, efforts to develop gene therapeutics have been narrowly focused and most clinical trials have sought to develop treatments for cancer (64.6%), monogenic diseases (10.5%), infectious diseases (7.4%), and cardiovascular diseases (7.4%). In addition, nearly 70% of clinical trials have utilized viral-based delivery systems, despite various concerns associated with this strategy. Each of these factors highlights the lack of diversity in the development of gene therapeutics that should be addressed. In recent years, developments in gene manipulation and delivery such as CRISPR and non-viral vectors (e.g., liposomes) demonstrate promise for improving outcomes for gene therapy. The increased fidelity and capacity afforded by these technologies provides the potential to improve upon contemporary gene therapy approaches and enable the development of treatments for less-emphasized disorders. In this review, we provide a summary of gene delivery technology and discuss various developments in gene therapy technology. We conclude by proposing several genetic conditions that represent promising targets for gene therapy given recent developments in gene delivery and manipulation.

Introduction

Gene delivery is a therapeutic approach in which genetic abnormalities are corrected either by restoring native functional activity or by removing a detrimental function through the process of delivering exogenous genetic material to the host's target cells (Nayerossadat et al., 2012). This strategy consequently possesses the potential to provide a direct solution to a

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disorder rather than treating its symptoms. In the case of sickle cell disease (SCD), treatment strategies revolve around preventing severe complications by treatment with antibiotics, pain killers, hydroxyurea, or bone marrow transplants in younger patients. However, SCD arises due to a mutation in hemoglobin that impairs its activity. Consequently, developing a therapeutic strategy that corrects this mutation has the potential to dramatically improves patient outcomes.

Since the advent of gene therapy, the repertoire of delivery modalities has expanded substantially beyond viral vectors (e.g., retroviral) to non-viral vectors such as biomaterials (e.g. liposomes), other biological entities (e.g., bacteria), and the combination thereof (Hill et al., 2016). In addition, there have been several critical developments in the genetic manipulation technology utilized in gene therapy. These include major advances such as RNA interference (RNAi) and clustered regularly interspaced short palindromic repeats (CRISPR), which facilitate highly specific gene repression and editing, respectively. Combined, these developments have the potential to dramatically improve gene therapy outcomes and facilitate the treatment of genetic disorders that have been largely neglected to date.

Improved understanding of the link between various diseases with diverse patterns of genetic inheritance has broadened the target scope of gene therapy modalities. In fact, clinical trials using gene therapy have been conducted for oncogenic, monogenic, infectious, cardiovascular, neurological, inflammatory, and ocular diseases. The first gene therapy study (performed in 1993) attempted to treat glioblastoma multiforme (a progressively fatal brain tumor ‘GM’) through the introduction of antisense complementary DNA (cDNA) targeting insulin-like growth factor I (IGF-I) (Trojan et al., 1993). The antisense DNA blocked IGF-1 expression of GM, leading to a loss of tumorgenicity and regression of previously established glioblastomas. However, to date only one gene therapy, an acute lymphoblastic leukemia (ALL) drug called Kymriah, has been approved by the US FDA (Andrea Fischer, 2017). Furthermore, roughly 95% of gene delivery clinical trials are pre-Phase III, demonstrating that few gene therapies progress to pivotal trials (Hill et al., 2016).

In this review, we will enumerate the current delivery platforms for gene therapy, including the commonly viral vectors as well as non-viral vectors currently being developed and summarize their advantages and disadvantages. We will then discuss the various technologies that are implemented for genetic modification in these therapies and alternatives that are being developed that can improve their fidelity. Lastly, we will discuss the trends in research within gene therapy regarding disease focus and propose new target diseases that can be addressed more readily given the developments within the field.0

Developments in gene delivery approaches

Delivery Vectors

In order to advance the field of gene delivery, research has focused on improving the technology utilized for genetic manipulation under physiological conditions. The most commonly used vectors for gene delivery are virus-based (~70% of clinical studies), which include retroviral (RV), adenoviral (AV), adeno-associated viral (AAV), herpes simplex viral

(HSV), lentiviral (LV), poxvirus (PV), and Epstein-Bar viral (EBV) vectors (Nayerossadat et al., 2012; Yin et al., 2014). Virus-based vectors are commonly used in gene therapy due to their high transfection efficiency and their ability to promote high expression of the therapeutic gene (Walther & Stein, 2000).

AV vectors are the most commonly employed class of viral vectors, as they do not integrate their cargo into the host genome (Vorburger & Hunt, 2002). For example, AV gene delivery vectors are commonly implemented in cancer treatments to transduce tumor cells with an enzyme capable of converting a systemic pro-drug to its active form (Mizuno et al., 1998). However, use of AV vectors in clinical trials have demonstrated significant toxicity (Trask et al., 2000). To overcome this setback, AAV vectors have been developed, including variants that demonstrate decreased pathogenicity and only integrate into a single loci on chromosome 19 (Nayerossadat et al., 2012). However, one disadvantage of AAVs is their reduced genetic cargo size (4.8 kb) relative to AV vectors (8 kb). Consequently, approaches using helper vectors containing the required replicative sequences have been developed to expand the cargo size to 32 kb (Nayerossadat et al., 2012). This has resulted in researchers applying AAV systems to develop gene therapeutics for cystic fibrosis (Flotte et al., 1996), hemophilia B (Kay et al., 2000), and Leber's congenital amaurosis (Simonelli et al., 2010).

Another commonly employed class of vector for gene therapy are RV vectors and their LV subclass. Unlike other viral vectors (e.g., AVs) RVs are able to integrate their cargo into dividing cells and create permanent genetic modifications (Anson, 2004). However, this trait increases the risk of insertional mutagenesis (Bushman, 2007; Li et al., 2002) and RV vectors demonstrate low *in vivo* efficiency (Nayerossadat et al., 2012). Despite these disadvantages, RV- and lentiviral-based gene therapy strategies have proven useful in treating X-linked severe combined immunodeficiency (X-SCID) (Hacein-Bey-Abina et al., 2002), and neurological disorders (e.g., Parkinson disease, Alzheimer, Huntington's disease, etc) (Cockrell & Kafri, 2007; Wong et al., 2006). In addition to the aforementioned viruses, PV, HSV, and EBV vectors have also been employed in gene therapy, albeit to a lesser extent. Regardless, a broad limitation of viral delivery vectors is that they possess limited DNA packaging and are difficult to produce, further complicating their utility and promoting the development of alternative vectors (Hill et al., 2016; Yin et al., 2014).

Due to the concerns associated with viral vectors, several non-viral vectors have been evaluated for gene therapy. Several alternatives include gold nanoshells, polymers, liposomes, and biological vectors (e.g., engineered *Escherichia coli*) (Hill et al., 2016). These vectors can offer advantages over non-viral vectors, such as the laser-mediated cargo release that can be achieved with gold nanoshell systems (Huschka et al., 2012). Alternatively, materials-based vectors such as liposomes and polymers can be employed to encapsulate the genetic cargo for gene delivery (Yin et al., 2014). One advantage of these materials is that their compositions can be varied greatly to tailor their properties and tune gene delivery outcomes (e.g., transfection efficiency and circulation time) (Kim, 2012; Yin et al., 2014). However, these systems are substantially underrepresented in clinical trials compared to viral vectors and require further development (Schlenk et al., 2013).

The use of plasmid vectors is perhaps the simplest non-viral method of delivering DNA into the cell nucleus. These vectors, which are composed of circular double-stranded plasmid DNA (pDNA), are simple to construct, elicit little to no immune response, can deliver large amounts of DNA (Williams & Kingston, 2011), and have demonstrated potential in gene therapy applications. Such applications include the treatment of critical limb ischemia (CLI), a disease in which restricts adequate blood flow to certain parts of the body, and non-healing diabetic foot ulcers through the use of hepatocyte growth factor (HGF) VM202 (Kessler et al., 2015; Kibbe et al., 2016; Perin et al., 2011). Despite these successes, pDNA vectors typically suffer from low gene transfer efficiency and are susceptible to nuclease degradation (Kawabata et al., 1995; Williams & Kingston, 2011). However, the inclusion of carrier vehicles, such as those described above, can help to overcome such limitations (Williams & Kingston, 2011).

Approaches for Genetic Manipulation

In addition to developments for gene delivery vectors, there have been significant improvement in the tools employed for genetic modifications utilized in gene therapy. One commonly used approach for gene therapy is RNA interference (RNAi), in which short, sequence-specific double-stranded RNA (i.e., short interfering RNA; ‘siRNA’) are used to impair gene expression. The application of RNAi has provided an approach for reducing gene expression without requiring permanent genetic changes. RNAi consequently can be applied to treat genetic diseases by creating a “knockdown” of a target gene that is of therapeutic interest (Grimm & Kay, 2007). In recent years, this technology has been used to target a variety of oncogenic (Mansoori et al., 2014), neurodegenerative (Boudreau & Davidson, 2006), and ocular diseases (Guzman-Aranguez et al., 2013). Furthermore, RNAi has also been adapted as a treatment for viral infections (Tan & Yin, 2004). Several clinical trials employing RNAi therapies have occurred recently, primarily for ocular diseases based in genetic abnormalities (Davidson & McCray, 2011). One notable example is a therapy for Angle closure glaucoma targeting the gene *CASP2* which is currently undergoing a Phase II/III clinical trial (NCT02341560).

The outlook for gene therapy has also been improved by the developments in genome editing strategies over the last decade. Prior to 2013, the most commonly employed techniques utilized zinc-finger nucelases (ZNFs) and transcription activator-like effector nucleases (TALENs), to introduce transgenes through the creation of double-strand breaks (DSBs). These systems had proven effective for many gene therapy applications (Gaj et al., 2013) such as the correction of the mutation associated with X-linked severe combined immune deficiency (SCID) using ZNFs (Urnov et al., 2005) and elevating expression of *globin* in sickle-cell disease (SCD) models through mutations introduced via TALENs (Wienert et al., 2015). However, both systems were hindered by need to engineer new site-specific nucleases for every target site, which can be cumbersome and time consuming. This limitation was resolved by the development of approaches using clustered regularly interspaced short palindromic repeats (CRISPR). Genome editing using CRISPR represents a paradigm-shifting innovation which was derived from bacterial immune defense systems (Barrangou et al., 2007). One major advantage associated with CRISPR is that the nuclease utilized (Cas 9), introduces site-specific DSBs using the aid of singly guide RNA (sgRNA).

Consequently, CRISPR does not require the engineering of new proteins for each desired genetic editing target (Mali et al., 2013). Shortly after CRISPR's introduction, researchers discovered that the addition of multiple sgRNAs allowed for the modification of multiple genes at once, paving the way for CRISPR-Cas9 gene therapy (Wang et al., 2013).

Following this discovery, three studies were published simultaneously that demonstrated the application of CRISPR-Cas9 to treat adult and neonatal mice with Duchenne muscular dystrophy (DMD). This genetic disorder is caused by a mutation in exon 23 of the *DMD* gene, which was corrected in muscle cells vis CRISPR-Cas9 gene therapy, thus restoring partial function of muscle cells within the mice (Long et al., 2016; Nelson et al., 2016; Tabebordbar et al., 2016). However, CRISPR-Cas9 gene editing suffers from low *in vivo* delivery efficacy (Xue et al., 2016), and off-target genome editing that can lead to malignancies (Xue et al., 2016).

To combat off-target mutations introduced by CRISPR-Cas9, an aspartate-to-alanine substitution (D10A) mutation was introduced into Cas9 to convert the enzyme into a Cas9 nickase (Cas9n) (Cong et al., 2013). This system employs two offset sgRNAs that bind both strands of the target sequence with Cas9s, a nuclease which introduces only single-stranded nicks (Ran et al., 2013). Since the nicked DNA is repaired via the endogenous base-excision repair pathway, these nucleases are less likely to cause damage to the human genome, even in the event of off-target effects (Shen et al., 2014). For example, one study showed that Cas9ns reduced the off-target activity by 50-1,500-fold relative to CRISPR-Cas9 without sacrificing on-target cleavage efficiency (Ran et al., 2013).

Additional innovations which were developed to reduce off-target effects include CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). Both of these approaches utilize a catalytically inactive version of Cas9 (dCas9) that doesn't create DSBs. In contrast to the systems using Cas9, these systems require only one protein a sequence-specific sgRNA (Qi et al., 2013) and have a high gene specificity with minimal off-target effects (Gilbert et al., 2013). Since dCas9 doesn't insert DSBs, when dCas9 is fused with repressive or activating effectors, gene expression can be reversibly repressed or activated respectively (Gilbert et al., 2013).

Promising targets for gene therapy:

While cancer treatment has been the main focus of gene therapy, with approximately 65% of clinical trials addressing oncogenic diseases (Edelstein, 2017), it has also been applied to a wide variety of genetic diseases that lack traditional treatments. Leber's Congenital Amaurosis (LCA), a genetic retinal disease, was one of the first diseases treated in humans (Beltran et al., 2012) and there is currently one gene therapeutic in Phase III clinical trial (Boye et al., 2013) (NCT00999609; clinicaltrials.gov). To date, no gene therapy for genetic diseases has obtained FDA approval; however, in a historic decision, the US FDA recently approved the cancer therapy, CTL019, making it the first gene therapy approved in the USA (Andrea Fischer, 2017). Despite such successes, there are several challenges impairing the widespread development of gene therapeutics. Gene therapy is often still limited to diseases associated with single genes (i.e., monogenic diseases), such as Sickle Cell Disease

(Table 1), due to the complexities associated with targeting multiple genes simultaneously. However, progress has been made in treating complex genetic diseases by targeting genes that alleviate or improve symptoms rather than treating the condition, as seen in therapies targeting Parkinson's disease (Schapira et al., 2014). In addition, there are a number of potential disease targets for gene therapy that have been largely ignored by current efforts discussed below.

Hemoglobin-linked diseases

Over 270 million people worldwide are classified as carriers for thalassaemia, a group of inherited diseases affecting the function of hemoglobin (Quek & Thein, 2007). Currently, researchers have identified over 200 mutations of the β globin (*HBB*) gene that are associated with diseases such as β-thalassaemia and sickle cell disease (SCD) (Cao & Galanello, 2010). The most common genotype for SCD, is a single amino acid substitution in the *HBB* gene from glutamic acid to valine (G6V) (Rees et al., 2010), which makes SCD a promising candidate for gene therapy.

In the case of SCD, gene therapy efforts have centered around the introduction of a functional *HBB* transgene into hematopoietic stem cells (HSCs) (Cavazzana et al.) that are used to repopulate the haematopoietic system (Baum et al., 1992). Unlike LCA treatments detailed above, gene therapy strategies for SCD have traditionally utilized RV vectors to deliver their genetic cargo. However, recent studies have transitioned to LV vectors due to their increased DNA cargo capacity and ability to transduce non-dividing HSCs (Hoban et al., 2016). For example, the delivery of a *HBB* triple mutant with a high affinity for α-globin subunits via a lentivirus containing an anti-sickling β-globin gene was capable of treating SCD in mice (Levasseur et al., 2003). Such studies have resulted in several clinical trials, including a study utilizing the LentiGlobin BB305 vector expressing an antisickling β-globin. Following this study, one of the three patients treated no longer required monthly blood transfusions that had been necessary since the patient's childhood (Cavazzana-Calvo et al., 2010). In addition, genome engineering techniques have advanced the potential for SCD gene therapy significantly in recent years. For example, CRISPR cas9, has been employed to correct the G6V mutation in human haematopoietic stem and progenitor cells, derived from patients with SCD, *in vitro* (Dever et al., 2016), demonstrating gene therapy's potential to treat SCD in the future.

Parkinson disease

Parkinson's disease (PD) is a neurological disorder that affects more than 4 million people worldwide and is characterized by the loss of dopaminergic neurons in the striatum and substantia nigra (Kalburgi et al., 2013). Neurological conditions such as PD represent some of the most challenging conditions to treat using conventional therapeutics due to the blood-brain barrier (BBB) that prevents the diffusion of drugs into the central nervous system (CNS). Much progress has been made to overcome this challenge by utilizing vector-mediated gene delivery (Kalburgi et al., 2013) due in part to the discovery that AAV9 could cross the BBB (Foust et al., 2009). However, unlike the diseases detailed above, multiple genes are involved in PD, which complicates treatment via gene therapy (Gray et al., 2010).

For example, autosomal recessive PD (ARPD) involves mutations in parkin, PINK1, DJ-1, ATP13A2, FBXO7, DNAJC6, SYNJ1, and PLA2G6 (Scott et al., 2017).

Due to the complexity of PD, many efforts to develop gene therapies have focused on alleviating symptoms or halting disease progression to prevent further damage by utilizing viral delivery vectors. To ease PD symptoms, studies frequently attempt to deliver genes for the enzymes and / or cofactors necessary for the production of dopamine from tyrosine (Coune et al., 2012). One of the first attempts delivered genes encoding for aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase, and GTP cyclohydrolase using an equine infectious anemia virus (EIAV) as the vector. EIAV was selected as the vector due it its large transgene capacity (>8 kb) which enabled it to deliver all three genes at once, and was shown to reverse functional deficits in rodent and primate models of PD (Azzouz et al., 2002; Jarraya et al., 2009). Due to promising results in preclinical studies, this strategy advanced into Phase I/II clinical trials as ProSavin (NCT00627588) and is currently undergoing a safety and efficacy study (NCT01856439). This strategy can be enhanced by employing recent advances in gene delivery that have enabled novel approaches targeting PD. For example, one study combined the use of non-viral delivery vectors with gene silencing to alleviate symptoms of PD. The non-viral peptide vectors used in this study would be able to cross the BBB and specifically target neuronal cells to protect and deliver siRNA against the α-synuclein gene, thus effectively alleviating PD-like symptoms in mice (Javed et al., 2016).

Additional gene therapy targets

The improvements in gene delivery offer the opportunity to target rare or untreatable diseases that often lack conventionally therapies (Table 2). In fact, approximately 70% of rare diseases are monogenic and only 8% of these diseases are currently treatable by an FDA-approved drug (Chen & Altman, 2017). Consequently, there is a significant opportunity to address these currently unmet needs using gene therapy. Hutchinson–Gilford progeria syndrome (HGPS), for example, is a disease associated with rapid aging in children and affects 1 in 8 million live births. This condition is typically linked to a mutation within exon 11 of the LMNA gene (Pollex & Hegele, 2004). To date, only one attempt has been made to correct this disease using a combination of stem-cell engineering and gene therapy. In this study pluripotent stem cells from HGPS patents were modified using a helper-dependent AV vector to induce homologous recombination-based mutation correction, thus restoring the wild-type phenotype (Liu et al., 2011). While the use of CRISPR Cas9 has been suggested (Arancio et al., 2015), no further studies have been attempted. In addition, Noonan syndrome, which has a prevalence of 1 in 1000 people, is another neglected disease in gene therapy studies. It is the most common cause of congenital heart disease, and has been linked to mutations in four genes: *PTPN11, SOS1, RAF1* and *KRAS*. While this disease is not monogenic, up to 50% of Noonan cases are caused by the *PTPN11* mutation, making it a promising target for gene therapy (Roberts et al., 2007).

Conclusion

Current approaches to gene therapy frequently suffer from cellular and physiological toxicity, off-target effects that can result in mutagenesis, and limited cargo capacity. Taken together, these limitations have prevented successes in clinical trials in the past (Griesenbach, 2007). However, recent innovations in gene therapy, such as non-viral gene delivery and the development of CRISPR-mediated genome engineering, have dramatically improved the potential for discovery of gene therapeutics. For example, there has been some evidence that combining CRISPR technology with both viral and non-viral vectors could improve therapeutic efforts. By separately delivering sgRNA and Cas9 using AAV vectors and liposomal nanoparticles, respectively, off-target effects were reduced by making use of the long-term circulation of sgRNA via AAV vectors, in addition to the short-term circulation of Cas9 via liposomal vectors (Yin et al., 2016).

To date, most gene therapy research has focused on cancer, monogenic disorders, and infectious diseases. However, gene therapy has the potential to treat a number of underrepresented conditions, such as inflammatory and autoimmune diseases, which only represent 0.6% of all gene therapy clinical trials (Edelstein, 2017). These diseases are often complex and involve interactions between a large number of genes, making them difficult to target under the previous gene therapy paradigm. Advances in meta-data analysis, however, have already enhanced our understanding of the genetic bases of these complex diseases (e.g., Crohn's disease and ulcerative colitis) (Jostins et al., 2012) and will play a critical role for future gene therapy studies.

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Table 1.

Current Gene Therapy Clinical Trials for Monomeric Diseases

Disorder	*Target Gene*	Drug	Sponsor	Clinical Trial No.	Vector	Phase
Ocular Disorders						
Stargardt Disease	*ATP-binding cassette, sub-family A, member 4 (ABCA4)*	SAR422459	Sanofi	NCT01736592	Lentivirus	I/II
Usher Syndrome	*Myosin VIIA (MYO7A)*	UshStat	Sanofi	NCT01505062	lentivirus	I/II
Choroideremia	*Rab escort protein-1 (REP1)*	AAV2-REP1	Byron Lam, University of Miami	NCT02553135	AAV	II
		AAV2-hCHM	Spark Therapeutics	NCT02341807	AAV	I/II
Achromatopsia	*Cyclic Nucleotide-Gated Channel, Beta-3 (CNGB3)*	AAV - CNGB3	MeiraGTx UK II Ltd	NCT03001310	AAV	I/II
		AGTC-402	Applied Genetic Technologies Corp	NCT02935517	AAV	I/II
X-Linked Retinoschisis	*Retinoschisin 1 (RS1)*	RS1 AAV Vector	National Eye Institute	NCT02317887	AAV	I/II
		rAAV-hRS1	Applied Genetic Technologies Corp	NCT02416622	AAV	I/II
Blood Disorders						
β-thalassemia	*Hemoglobin Subunit Beta (HBB)*	LentiGlobin BB305	bluebird bio	NCT02906202	lentivirus	III
Sickle Cell Disease	*HBB*	LentiGlobin BB305	bluebird bio	NCT02140554	Lentivirus	I
Hemophilia	*Factor IX (f9)*	AskBio009	Shire	NCT01687608	AAV	I/II
		SPK-9001	Spark Therapeutics	NCT02484092	AAV	I/II
Immune Disorders						
Wiskott-Aldrich Syndrome	*Wiskott-Aldrich syndrome (WAS)*	N/A	GlaxoSmithKline	NCT01515462	Lentivirus	II
X-linked Chronic Granulomatous Disease	*Cytochrome B-245 Beta Chain (CYBB)*	N/A	Genethon	NCT01855685	Lentivirus	I/II
Neurological Disorders						
Mucopolysaccharidosis IIIB	*Alpha-N-acetylglucosaminidase (NAGLU)*	rAAV9.CMV. hNAGLU	Kevin Flanigan	NCT03315182	AAV	I/II
Aromatic L-amino Acid Decarboxylase deficiency	*Dopa Decarboxylase (DDC)*	AAV2-hAADC	Krystof Bankiewicz	NCT02852213	AAV	II
Skin Disorders						
Recessive Dystrophic Epidermolysis Bullosa	*Collagen Type VII Alpha 1 Chain (COL7A1)*	LZRSE-Col7A1 Engineered Autologous Epidermal Sheets	Stanford University	NCT01263379	Gamma-retroviral	I/II
Misc. Disorders						
Mucopolysaccharidosis type 1 (Hurler Syndrome)	*Iduronidase, Alpha-L-(IDUA)*	SB-318	Sangamo Therapeutics	NCT02702115	AAV	I

Disorder	*Target Gene*	Drug	Sponsor	Clinical Trial No.	Vector	Phase
Alpha 1 Antitrypsin Deficiency	*Serpin Family A Member 1 (SERPINA1)*	ADVM-043	Adverum Biotechnologies, Inc.	NCT02168686	AAV	I/II
Homozygous Familial Hypercholesterolemia	*Low Density Lipoprotein Receptor (LDLR)*	N/A	University of Pennsylvania	NCT02651675	AAV	I/II

Table 2.

Examples of rare or untreatable diseases for potential gene therapy targets.

Condition	Description	Frequency	Gene	References
Monogenic Diseases				
Hutchinson–Gilford progeria syndrome (HGPS)	Rapid aging in children	1 in 8 million live births	*lamin A/C (LMNA)*	(Eriksson et al., 2003; Pollex & Hegele, 2004)
Achondroplasia	Short-limbed dwarfism	1 in 15,000 to 40,000 newborns	*fibroblast growth factor receptor 3 (FGFR3)*	(Shiang et al., 1994; Waller et al., 2008)
***MECP2* Duplication Syndrome**	Moderate to severe intellectual disability	150 described cases	*methyl-CpG Binding Protein 2 (MECP2)*	(Van Esch, 2014)
Pendred syndrome	Early hearing loss in children	1 in 2000 live births	*phytoene desaturase (PDS)*	(Reardon et al., 1999)
Multigenic Diseases				
Leber hereditary optic neuropathy	Blurring, clouding, and loss of vision	1 in 25,000 to in northeast England	*mitochondrially encoded NADH:ubiquinone oxidoreductase core subunits (MT-ND1, ND4, ND4L, ND6)*	(Man et al., 2002)
Noonan syndrome	Deformed facial features, heart defects, short stature, bleeding problems, skeletal deformities	1 in 1000 people	*1. protein tyrosine phosphatase, non-receptor type 11 (PTPN11)* *2. SOS Ras/Rac Guanine Nucleotide Exchange Factor 1 (SOS1)* *3. RAF proto-oncogene serine/threonine-protein kinase (RAF1)* *4. KRAS Proto-Oncogene, GTPase (KRAS)*	*(*Roberts et al., 2007*)*
Congenital myasthenic syndrome	Muscle weakness	Unknown	*1. receptor associated protein of the synapse (RAPSN)* *2. Choline O-Acetyltransferase (CHAT)* *3. Collagen Like Tail Subunit Of Asymmetric Acetylcholinesterase (COLQ)* *4. Docking Protein 7 (DOK7)*	(Barišić et al., 2011; Engel, 2007)
Hereditary hemorrhagic telangiectasia	Blood vessel abnormalities	1 in 10,000 in North America	*1. Activin A Receptor Like Type 1 (ACVRL1)* *2. Endoglin (ENG)* *3. SMAD Family Member 4 (SMAD4)*	(Pagon et al.)