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EDITORIAL COMMENTARY

# A breakthrough in readthrough? Could geneticin lead the way to effective treatment for cystinosis nonsense mutations?

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The report by Brasell et al. in this issue of *Pediatric Nephrology* [1] raises the possibility that individuals with cystinosis who have a nonsense mutation that is transcribed into a premature termination codon (PTC) can have functional cystinosin by taking a medication that allows translation of the complete *CTNS* gene. This is achieved by reducing translational fidelity which allows a change in one nucleotide of a PTC, turning it into a sense codon. This complete translation, rather than termination of translation due to the PTC, permits readthrough (RT) of the mutated allele. Treatment with an RT agent could not only correct the lysosomal accumulation of cysteine but also correct other defects due to cystinosis [2, 3]. PTC suppression therapy allowing translational RT has the potential to treat disease due to in-frame nonsense mutations.

There are two reasons that a PTC in the *CTNS* gene, as one of two mutated alleles, can cause infantile cystinosis. First, faulty mRNA is dealt with by various mechanisms that reduce the quantity of truncated proteins that might otherwise arise and interfere with normal cellular function. Thus, mRNA containing PTCs, either due to somatic mutations or transcriptional error, is dealt with by nonsense-mediated mRNA decay (NMD); PTCs reduce the stability of mRNAs, which are subject to degradation prior to translation. This reduces the ability of PTC-RT agents to promote translation since there may be little or no mRNA available. Second, shortened mRNA is translated into a truncated cystinosis protein that has reduced or no activity.

NMD inhibitors have been suggested as an adjunct to RT agents to improve the efficiency of the treatment, which is well illustrated by Fig. 1. But such treatment must be approached with caution as it could increase many potentially toxic truncated proteins [4].

Despite a proof of concept in a mouse model of Duchene muscular dystrophy (DMD) in 1999 that aminoglycosides improve the amount and function of dystrophin in skeletal muscles with lower creatinine kinase levels [5], PTC suppression therapy has not yet made a significant impact for patients with genetic disease [6–9]. Correction of the cystinosis phenotype was first reported in abstract in 2000 [10] and fully in 2002 [11]. This leads to the question of what barriers need to be overcome to translate these laboratory findings into a successful treatment for individuals who have cystinosis.

Of the various types of DNA mutations, only a subset (mostly missense mutations) give rise to a stop in translation [12]. Brasell et al. note that only the 15% of individuals with cystinosis that have a nonsense mutation are potentially treatable with PTC-RT therapy [1].

Both the actual triplet codon of the PTC and sequences surrounding a PTC—its context—affect the ability of RT agents to effect translation and thus have a significant influence in determining effective drug-induced suppression of a PTC [13]. This context effect explains some of the variability in RT efficiencies of gentamicin in cultured cells from muscular dystrophy patients, with not all likely amenable to pharmacological treatment [14].

The specific chemical structure of RT agents may also affect their efficacy. For example, not all preparations of gentamicin contain the same proportions of isoforms that may have a differential contribution to RT efficacy. Various gentamicin C isoforms have the most antibacterial activity compared to A, B, or X isoforms but their side chain differences affect their ability to effect RT [6].

Suppression of PTCs raises the question as to whether, under the influence of an RT agent, normal stop codons might fail to terminate translation of RNA leading to an extension of the amino acid string affecting the structure and function of

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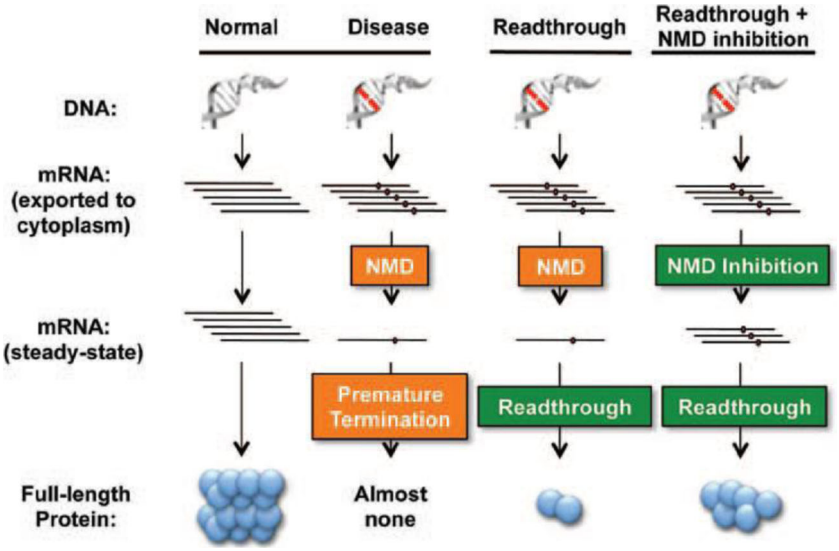


**Fig. 1** The process of DNA-to-protein synthesis in "Normal," "Disease," "Readthrough" (RT), and "RT with nonsense-mediated mRNA decay (NMD) inhibition" scenarios. The figure illustrates that cells have a mechanism (NMD) which reduces the amount of mRNA in the presence of premature termination codons (PTC) that contributes to the absence of functional protein. RT therapy, with the low steady state of mRNA, may only result in a small amount of functional protein. The combination of NMD inhibition and RT therapy could allow for synthesis of larger amounts of protein. (Reproduced from Keeling et al. 2012 with permission [4])

the protein [4]. The evidence is that PTCs are different to natural stop codons, with RT of end of gene stop codons occurring about tenfold less than at PTCs [13, 15, 16]. Reassuringly, RT polypeptides could not be detected in various tissues from rats, dogs, and humans treated with ataluren, formerly known as PTC124, a PTC-RT drug without antibiotic properties [17].

While PTC-RT agents may allow the synthesis of functional protein, the amount of protein produced may be crucial in determining the success of therapy [18]. Individuals who have only one *CTNS* allele mutated, such as parents of a child with cystinosis, have 50% transport capacity in their lysosomes [19] and somewhat increased WBC cystine levels, but do not manifest disease. In the paper by Brasell et al., lysosomal transport was restored equally with one missense mutation as with two missense mutations [1]. Whether the efficiency of PTC suppression therapy in possible clinical trials of individuals with cystinosis will be similar is yet to be determined.

Not all missense *CTNS* mutations may be equally treated by RT agents, as in some cases the restored full-length protein may not be functional. Function may depend on which amino acid is inserted into the final protein by the PTC-RT agent. Insertion of a different amino acid than usual into a part of the protein that is crucial for that protein's function may render the protein less than fully functional or even non-functional. Thus, not all cystinosin PTCs or nonsense mutations may be amenable to PTC-RT treatment [4].

There is evidence that peak of PTC-RT drug levels rather than steady levels may be more efficacious [5]. This combined with a short half-life may mean that suppression induced by a PTC-RT agent is not a prolonged effect, and so suppression therapy may require several doses every day [17], something that is all too well known in cysteamine therapy for cystinosis.

For individuals with cystinosis, clinical trials will be needed to determine whether combining a PTC-RT agent with an NMD inhibitor will induce more efficient and broad PTC-RT. It may even be that NMD inhibition without a PTC-RT agent could enable reduction in lysosomal cystine truncated proteins in increased amounts sufficient for partial function.

Experiences in other diseases illustrate the difficulty of bringing lab findings to clinical practice. PTC-RT agent efficacy may be disease specific and not recapitulate the promise from laboratory studies. Ataluren has been trialed in DMD and cystic fibrosis (CF). Although the initial clinical trials of PTC-RT agents in DMD patients were not all promising [14], further trials led to ataluren being licensed in Europe for use in muscular dystrophy, but the FDA has not approved this treatment. Ataluren has not been shown to be effective in CF [8, 20].

Which PTC-RT agents should be used in possible clinical trials in individuals with cystinosis? Several approaches to developing potential therapeutic RT agents for diseases caused by PTC mutations appear to exist. The first is design of aminoglycoside derivatives with lower or no toxicity [21]. The second is screening of new compounds for RT activity [22]. The existence of animal models for cystinosis, such as a zebrafish model with a homozygous nonsense mutation, may facilitate this [23]. Third is to repurpose an existing medication, which is attractive in terms of access and possibly cost. Intriguingly, macrolides are capable of inducing PTC-RT [18].

Combining the second and third approaches may be an efficient way of identifying readily available medications that are not prohibitively expensive, with acceptable, known adverse

effects. An herbal anti-inflammatory drug escin was identified as a potential PTC-RT agent, demonstrating efficacy in cells from patients with CF [24]. Escin appears to have a dual potential action as it is an NMD inhibitor and was shown to increase the level of functional cellular protein. Given a promising adverse effect profile, escin may be a potential PTC-RT agent.

Cystinosis is a lifelong disease that is currently only partially successfully treated with the cystine-depleting agent cysteamine. A genetic cure may be possible by rescue with hematopoietic stem cell transplant, for which there is enthusiasm within the cystinosis community [25]. Genetic manipulation of the genome by CRISPR-Cas9 raises future therapeutic possibilities [26]. Restoration of functional cystinosin would provide a cure although not reverse kidney damage. Whether PTC-RT agents could successfully treat some individuals with cystinosis is intriguing. To explore this, a clinical trial might be planned. The identification of a suitable trial medication could be done with fibroblast cell lines from individuals with cystinosis. However a wide selection of nonsense mutations should be tested, since different nonsense mutations may require different agents or different doses depending on the PTC context [27]. If a medication was found to be efficacious, lifelong treatment would be needed. Individuals with cystinosis are ordinarily taking medications at least daily, either because of a proximal tubulopathy or because of a kidney transplant, and so the addition of an effective, non-toxic PTC-RT medication might not be too onerous, especially if it allowed a reduction or discontinuation of cysteamine therapy.

## Compliance with ethical standards

**Conflict of interest** The author declares that he has no conflict of interest.



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