**34_CONCORSO PUBBLICO, PER TITOLI ED ESAMI, PER LA COPERTURA A TEMPO DETERMINATO, DELLA DURATA DI CINQUE ANNI PER N. 1 POSTO DI RICERCATORE SANITARIO, CAT. D, LIVELLO D SUPER DA ASSEGNARE ALLA SC SERVIZIO DI MEDICINA DI LABORATORIO – GENETICA MEDICA E NEUROGENETICA**

**PROVA 1**

1. La candidata/il candidato descriva vantaggi/svantaggi nell'uso di modelli in vivo rispetto a modelli in vitro nello studio delle malattie genetiche

2. A cosa serve il programma Microsoft Excel?
   a) realizzare fogli elettronici per analisi di dati
   b) realizzare presentazioni
   c) gestire spool di stampa

3. Leggere e tradurre il testo allegato

Allegato PROVA 1

# The potential of mitochondrial genome engineering

*Pedro Silva-Pinheiro and Michal Minczuk*

Abstract | Mitochondria are subject to unique genetic control by both nuclear DNA and their own genome, mitochondrial DNA (mtDNA), of which each mitochondrion contains multiple copies. In humans, mutations in mtDNA can lead to devastating, heritable, multi-system diseases that display different tissue-specific presentation at any stage of life. Despite rapid advances in nuclear genome engineering, for years, mammalian mtDNA has remained resistant to genetic manipulation, hampering our ability to understand the mechanisms that underpin mitochondrial disease. Recent developments in the genetic modification of mammalian mtDNA raise the possibility of using genome editing technologies, such as programmable nucleases and base editors, for the treatment of hereditary mitochondrial disease.

**Mitochondrial replacement therapy**
A form of reproductive in vitro fertilization technique, based upon replacing a mother's mitochondria that harbour pathogenic mutant mitochondrial DNA with the donor's healthy mitochondria.

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Human mitochondria contain their own genome, mitochondrial DNA (mtDNA), a multi-copy, circular, double-stranded DNA molecule that encodes 13 proteins essential for oxidative phosphorylation (OXPHOS)[1] (FIG. 1). In addition, more than 1,000 proteins encoded by the nuclear genome contribute to mitochondrial biogenesis and function[2,3]; the remaining protein components of the OXPHOS system and other proteins necessary to ensure proper mitochondrial function are synthesized from nuclear genes on cytosolic ribosomes and later delivered to mitochondria using dedicated import machinery[4]. Mitochondrial maintenance and homeostasis are thus uniquely under the dual genetic control of both nuclear and mtDNA. As a consequence, pathogenic variants in both the nuclear and mitochondrial genome can result in mitochondrial disease.

Mitochondrial diseases are categorized as a subset of genetic disorders of dysfunctional mitochondria mainly owing to defects in the respiratory chain, affecting ATP production[5]. Although considered rare individually, together, mitochondrial diseases are among the most prevalent groups of inherited neurological disorders, with a prevalence of ~1 in 4,300 (22.9 in 100,000) in adults[6]. Mitochondrial diseases are clinically and genetically heterogeneous and can manifest at any age, ranging from severe early childhood-onset syndromes to milder late-onset conditions, even in patients who harbour the same underlying mutation[7]. These disorders show striking and unexplained tissue selectivity, are currently incurable and cause substantial ill-health and premature death[8]. Most patients present multi-organ involvement, especially organs with high energetic demand, such as the brain and heart. Rarely, a single organ is affected, with the most prominent example being isolated optic nerve atrophy in Leber hereditary optic neuropathy (LHON)[9]. Mutations in mtDNA also exist in healthy humans, and have been associated with common multifactorial diseases that are increasing in our ageing population, including neurodegenerative disorders such as Parkinson disease, metabolic disease, heart failure and cancer[9–11]. There is a pressing need to develop new approaches to investigate and, eventually, to treat or prevent this diverse set of conditions, in which mitochondrial dysfunction has a central role.

Despite the present genome-engineering revolution enabled by CRISPR–Cas technologies, animal mtDNA has been resistant to transgenic manipulation owing to inefficient nucleic acid import into mitochondria[12]. The inability to manipulate or modify mtDNA sequences in mammalian mitochondria has hindered investigations into normal mtDNA processes and the development of in vivo models and therapies for mtDNA diseases. As a consequence, only five mouse models for mtDNA disease have been characterized thus far, all of which have provided important new information on mitochondrial dysfunction or aided preclinical experimental therapies[13]. Although mitochondrial replacement therapy has been developed to prevent the inheritance of mtDNA mutations through pronuclear or chromosome spindle transfer in oocytes or embryos, this method cannot cure existing or de novo cases[14]. Despite some progress in preclinical mtDNA gene manipulation[15], the field requires novel developments in the genetic modification of mtDNA in human cells and animal models to better understand the pathogenesis of mtDNA-linked diseases and to pave the way for future clinical applications.

With a focus on mammalian systems, here we first provide a brief overview of mtDNA maintenance and the concepts of heteroplasmy and homoplasmy before

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**PROVA 2**

1. Differenze tra modelli animali di malattie genetiche knock-out, knock-in e condizionali: la candidata/il candidato spieghi in cosa consistono le differenze, evidenziando vantaggi e svantaggi

2. Per URL si intende una sequenza di caratteri che:
   a) identifica univocamente l'indirizzo di una risorsa web
   b) un componente del sistema operativo
   c) un linguaggio di programmazione

3. Leggere e tradurre il testo allegato

Allegato PROVA 2

REVIEWS

HGT

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# Advances and Challenges in the Development of Gene Therapy Medicinal Products for Rare Diseases

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The development of viral vectors and recombinant DNA technology since the 1960s has enabled gene therapy to become a real therapeutic option for several inherited and acquired diseases. After several ups and downs in the gene therapy field, we are currently living a new era in the history of medicine in which several *ex vivo* and *in vivo* gene therapies have reached maturity. This is testified by the recent marketing authorization of several gene therapy medicinal products. In addition, many others are currently under evaluation after exhaustive investigation in human clinical trials. In this review, we summarize some of the most significant milestones in the development of gene therapy medicinal products that have already facilitated the treatment of a significant number of rare diseases. Despite progresses in the gene therapy field, the transfer of these innovative therapies to clinical practice is also finding important restrictions. Advances and also challenges in the progress of gene therapy for rare diseases are discussed in this opening review of a *Human Gene Therapy* issue dedicated to the 30th annual Congress of the European Society for Gene and Cell Therapy.

**Keywords:** ATMPs, gene therapy, rare diseases

## INTRODUCTION

In 1972, Friedmann and Roblin anticipated that gene therapy might ameliorate human genetic disorders.[1] Since then, several integrative and nonintegrative vectors have been generated. In parallel with this, hundreds of clinical trials have been initiated using *ex vivo* and *in vivo* gene therapy approaches with the aim of providing curative treatments for inherited and acquired diseases, many of which are life-threatening diseases affecting pediatric patients.[2,3] In addition to approaches based on the use of viral vectors, the generation of new nonviral vectors and gene editing tools has facilitated the design of gene targeting approaches not only in preclinical models but also in human clinical trials.[4,5]

In this study, we present a historic view in the development of *ex vivo* and *in vivo* gene therapy approaches that have resulted in the approval of innovative gene therapy medicinal products.

## PROGRESS OF *EX VIVO* GENE THERAPIES FOR MONOGENIC DISORDERS

*Ex vivo* gene therapy approaches are based on the collection of target cells from the body of affected patients, followed by their *ex vivo* genetic correction and reinfusion in the patient. *Ex vivo* gene therapy has been mainly used for the treatment of diseases that had been successfully treated by allogeneic cell transplantation.

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## PROVA 3

1. La candidata/il candidato descriva quali strategie conosce per dimostrare la patogenicità di una variante genica sconosciuta identificata in un individuo affetto da una malattia

2. Il termine "Open Source" indica:
   a) un software i cui autori ne permettono e favoriscono il libero studio e l'apporto di modifiche da parte di altri programmatori
   b) un software che può essere modificato da chiunque a patto di corrispondere all'autore una offerta libera
   c) un software protetto da diritti d'autore che non può essere modificato da nessuno tranne da chi ne detiene i diritti

3. Leggere e tradurre il testo allegato

Allegato PROVA 3

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Original Article

# Mitochondrial aminoacyl-tRNA synthetases trigger unique compensatory mechanisms in neurons

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## Abstract

Mitochondrial aminoacyl-tRNA synthetase (mt-ARS) mutations cause severe, progressive, and often lethal diseases with highly heterogeneous and tissue-specific clinical manifestations. This study investigates the molecular mechanisms triggered by three different mt-ARS defects caused by biallelic mutations in AARS2, EARS2, and RARS2, using an in vitro model of human neuronal cells. We report distinct molecular mechanisms of mitochondrial dysfunction among the mt-ARS defects studied. Our findings highlight the ability of proliferating neuronal progenitor cells (iNPCs) to compensate for mitochondrial translation defects and maintain balanced levels of oxidative phosphorylation (OXPHOS) components, which becomes more challenging in mature neurons. Mutant iNPCs exhibit unique compensatory mechanisms, involving specific branches of the integrated stress response, which may be gene-specific or related to the severity of the mitochondrial translation defect. RNA sequencing revealed distinct transcriptomic profiles showing dysregulation of neuronal differentiation and protein translation. This study provides valuable insights into the tissue-specific compensatory mechanisms potentially underlying the phenotypes of patients with mt-ARS defects. Our novel in vitro model may more accurately represent the neurological presentation of patients and offer an improved platform for future investigations and therapeutic development.

Keywords: aminoacyl-tRNA synthetase; neurological disease; protein synthesis; mitochondrial biology

## Introduction

Healthy mitochondria are essential for maintaining metabolic homeostasis and ATP production via oxidative phosphorylation (OXPHOS). Disruption to mitochondrial biogenesis or energy production underlies a broad range of metabolic diseases, primarily affecting skeletal muscle and the nervous system. Mitochondrial diseases are often progressive and fatal, representing the most common group of inherited metabolic disorders with no effective therapy [1, 2]. Mitochondrial disorders are caused by mutations in either the 16.5 kb mitochondrial DNA (mtDNA) encoding 13 OXPHOS proteins, or in over 300 nuclear-encoded mitochondrial proteins. Mitochondrial protein translation requires a separate apparatus for synthesizing mtDNA-encoded proteins, separate from eukaryotic (cytosolic) translation. Aminoacyl tRNA synthetase (ARS) enzymes catalyze the charging of amino acids to their cognate tRNA molecules to facilitate their delivery to the ribosome or mitoribosome, the site of protein translation. There are 17 mt ARS enzymes, which are present in all cells, as they charge mt-tRNAs with the corresponding (cognate) amino acid to enable synthesis of the 13 mtDNA-encoded proteins [1, 2]. They are distinct from the 18 cytosolic ARS, which aminoacylate tRNAs in the cytosol. Two ARS are present both in the cytosol and mitochondria (glycyl-tRNA synthetase (GARS1) & lysyl-tRNA synthetase (KARS1)). Mutations in both mitochondrial and cytosolic ARS cause tissue-specific phenotypes frequently involving neurodegeneration [3]. Mutant mt-ARS may cause abnormal amino acid composition of mtDNA-encoded proteins, leading to combined respiratory chain deficiency [1, 2].

Originally thought to be ultra-rare, mt-ARS mutations are major causes of mitochondrial diseases in children [3, 4]. Paradoxically, all mt-ARS are present in each cell, but their mutations affect different cells and organs, most frequently the brain, leading to leukoencephalopathy with thalamus and brainstem involvement and high level of lactate (mitochondrial glutamyl-tRNA synthetase, EARS2), leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (mitochondrial aspartyl-tRNA synthetase, DARS2), leukoencephalopathy with ovarian failure (mitochondrial alanyl-tRNA synthetase, AARS2), pontocerebellar hypoplasia (mitochondrial arginyl-tRNA synthetase, RARS2) or epileptic encephalopathy (mitochondrial phenylalanyl-tRNA synthetase, FARS2, and

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