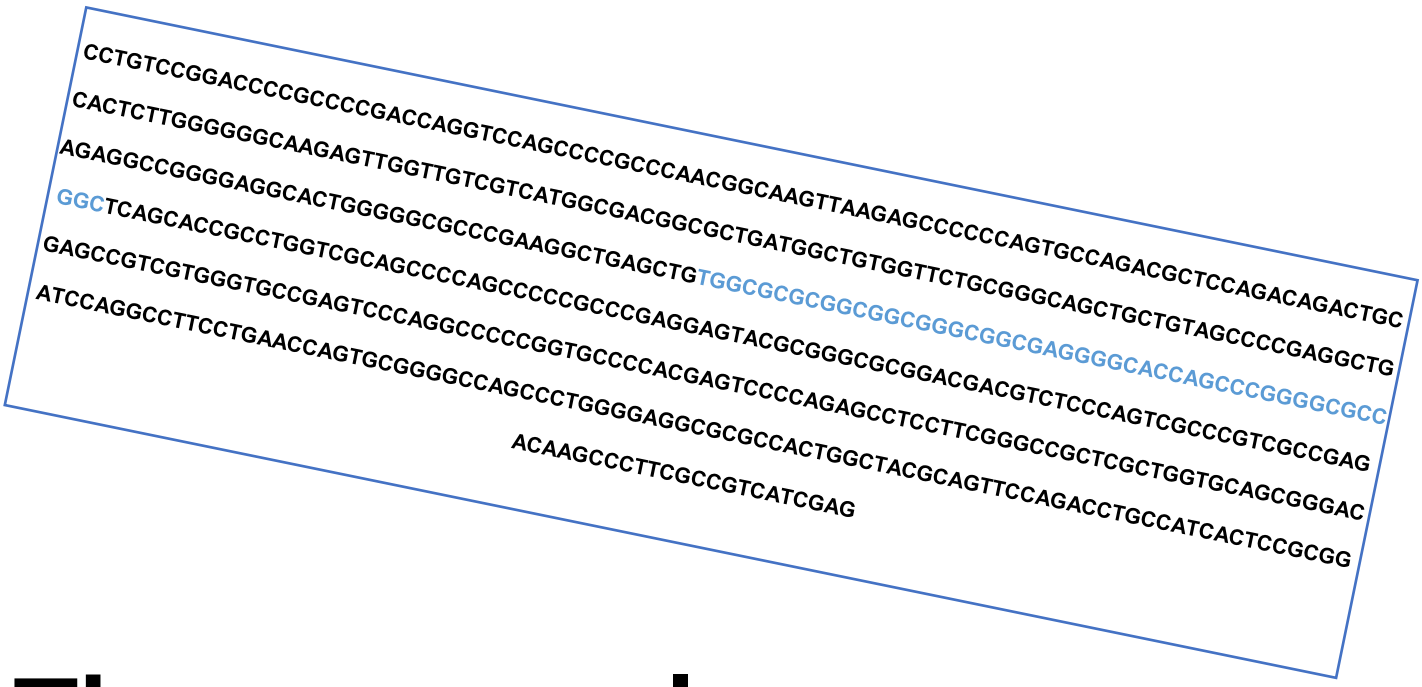

The urea cycle and the *de novo* human mutome

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All corrections determined by the jury, and only those, were incorporated.

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Nota introdutória

Este projeto está dividido em duas partes:

Parte 1 - “Molecular analyses of *NAGS* and *SIRT5* genes”;
Parte 2 - “A comprehensive analysis of *de novo* mutations in disease-associated genes”.
A escolha de dividir o projeto em duas partes prende-se com o facto de a sobreposição de temas poder não ser entendida como total do ponto de vista científico. No entanto, ambas as partes focam o tópico principal que é a Genética associada às mutações humanas e ao diagnóstico molecular.

Abstract

Part I

The urea cycle occurs in the liver, and constitutes an important metabolic pathway for elimination of ammonia through the biosynthesis of urea. The disorders of the urea cycle (UCD) are the result of the enzyme deficiency in one of the six steps that comprise the metabolic pathway of nitrogen excretion. All disorders are transmitted as autosomal recessive, except for the lack of the ornithine carbamoyltransferase (OTC), which is linked to the X-chromosome. Hyperammonemia, urinary and plasmatic amino acids and organic acids changes, constitute the main biochemical markers of the urea cycle impairment. The carbamoyl-phosphate synthase 1 (CPS1), catalyzes the first step on the urea cycle and requires deacetylation by sirtuin 5 (*SIRT5)* to be active. The N-acetylglutamate (*NAGS*) deficiency is the only UCD with specific treatment, being essential the confirmation/validation of the diagnosis using molecular studies so that the specific therapeutic strategy can be outlined. The goal of this project is to present a molecular diagnostic technique that can be applied in the routine analyses of *NAGS* and *SIRT5* genes in individuals which can now be used in molecular laboratories.

Keywords: urea cycle; disorders of the urea cycle (UCD); N-acetylglutamate synthase (*NAGS*); Sirtuin 5 (*SIRT5*); molecular diagnosis.

Part II

Each one of us, carries a small number of mutations that were not transmitted by our parents. These mutations, known as *de novo* mutations, may occur during gametogenesis or after fertilization. Recent studies have demonstrated that the higher incidence of these mutations is associated with advanced paternal age. In this project, I reviewed the literature regarding this type of mutations and compiled a total of 4220 variants were complied. When the type of mutation, the phenotype and the nucleotide position and the amino-acid were analyzed the results indicated that the type of mutation more common are replacements of a single nucleotides mainly associated with CpG dinucleotides. Concerning the type of disease, I observed that apart from the well known phenotypes associated with neurodevelopmental diseases (NDD; e.g. autism, schizophrenia and intellectual disability), many other phenotypes are causes by these type of mutations (e.g. congenital heart disease, amyotrophic lateral sclerosis and familial adenomatous polyposis).

Keywords: *de novo* mutations; advanced paternal age; human disease; neurodevelopmental diseases (NDD).

Resumo

Parte I

O ciclo da ureia ocorre no fígado, e constitui uma importante via metabólica para eliminação da amónia através da biossíntese da ureia. As doenças do ciclo da ureia (DCU) são o resultado do défice enzimático numa das seis enzimas que compõem a via metabólica de excreção do azoto. Todas as doenças têm transmissão autossómica recessiva, à exceção do défice da enzima ornitina transcarbamilase (OTC), o qual está ligado ao cromossoma X. A hiperamonemia, as alterações urinárias e plasmáticas dos aminoácidos e ácidos orgânicos, constituem os principais marcadores bioquímicos do ciclo da ureia. A carbamoil fosfato sintetase (CPS1) catalisa a primeira etapa do ciclo da ureia. A ativação da CPS1 ocorre quando ocorre deacetilação pela *SIRT5*. O défice em N-acetilglutamato sintase (*NAGS*) é o único DCU com tratamento específico, sendo por isso fundamental a confirmação/validação do diagnóstico por estudos moleculares para que possa ser delineada a estratégia terapêutica. Este projeto tem como objetivo apresentar uma técnica de diagnóstico molecular que possa ser aplicada na análise dos genes *NAGS* e *SIRT5* que pode agora ser usada nos laboratórios de genética molecular.

Palavras-Chave: ciclo da ureia; doenças do ciclo da ureia (DCU); N-acetilglutamato sintetase (*NAGS*); Sirtuína 5 (*SIRT5)*; diagnóstico molecular.

Parte II

Cada um de nós carrega um pequeno número de mutações que não são transmitidas pelos nossos pais. Estas mutações, mutações *de novo*, podem ocorrer ou durante a gametogénese ou após a fertilização. Estudos recentes têm demonstrado que a elevada incidência destas mutações estão associadas à idade paterna avançada. Neste trabalho, fiz uma revisão da literatura deste tipo de mutações e foram compiladas um total de 4220 variantes. Quando o tipo de mutação, fenótipo, cromossoma, posição no nucleótido e no aminoácido foram analisados os resultados indicaram que o tipo de mutação mais frequente são as substituições de um único nucleótido estando principalmente associadas a locais CpG. No que diz respeito ao tipo de doença, observamos que para além dos conhecidos fenótipos associados a doenças do desenvolvimento (NDD; por exemplo, autismo, esquizofrenia e deficiência intelectual), muitos outros fenótipos são consequência deste tipo de mutações (por exemplo, doença cardíaca congênita, esclerose lateral amiotrófica e polipose adenomatosa familiar).

Palavras-Chave: mutações *de novo;* idade avançada do pai*;* doenças humanas; doenças do desenvolvimento (NDD).

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List of Acronyms

- **DNA:** Deoxyribonucleic Acid
- **ARG1:** Arginase 1
- **ASL:** Argininosuccinate lyase
- **ASS1:** Argininosuccinate synthase 1
- **CPS1:** Carbamoyl-phosphate synthase 1
- **CPSD:** CPS1 deficiency
- **DMSO:** Dimethyl sulfoxide
- **EVS:** Exome Variant Server
- **GP:** Genomes Project
- **HGMD:** Human Gene Mutation Database
- **NAGS:** N-acetylglutamate synthase
- **NAGSD:** N-acetylglutamate synthase deficiency
- **NDD:** Neurodevelopmental diseases
- **NGS:** Next-Generation Sequencing
- **PCR:** Polymerase Chain Reaction
- **PGCs**: Primordial Germ Cells
- **OTC:** Ornithine carbamoyltransferase
- **OTCD:** OTC Deficiency
- **SIRT:** Sirtuins
- **SIRT3:** Sirtuin 3
- **SIRT4:** Sirtuin 4
- **SIRT5:** Sirtuin 5
- **SLC25A13:** Solute carrier family 25 member 13
- **SLC25A15:** Solute carrier family 25 member 15
- **UCDs:** Urea Cycle Disorders

PART I

Molecular analyses of *NAGS* and *SIRT5* genes

1. Introduction

1.1. Urea cycle

In living organisms, nitrogen exists mainly as ammonia, which is the main component of nucleic acids and proteins (Natesan et al., 2016). Ammonia, although essential for growth and maintenance of cellular processes, when it exceeds the normal level can quickly lead to toxicity or even death (Natesan et al., 2016). The urea cycle is essential for nitrogen homeostasis and is used by mammals to the elimination of ammonia through the biosynthesis of urea (Sun et al., 2016). The biosynthesis of urea occurs in four stages: 1) transamination, 2) oxidative deamination of glutamate, 3) transport of ammonia and 4) reactions of the urea cycle (Phenome and Consortium, 2013; Rodwell et al., 2016) that occur between the mitochondria and cytoplasm (Rocha et al., 2009). The **urea cycle** (Figure 1) occurs in the liver where the ammonia is initially converted into carbamoyl-phosphate which is catalyzed by carbamoyl-phosphate synthase 1 (CPS1), which requires an allosteric activator, N-acetylglutamate. The latter is the result of the condensation of acetyl-CoA and glutamate, in a reaction catalyzed by N-acetylglutamate synthase (NAGS). The carbamoyl-phosphate is transferred to ornithine to form citrulline, in a reaction catalyzed by ornithine carbamoyltransferase (OTC). Then citrulline is transported to the cytoplasm where it condenses with aspartate. The synthesis of argininosuccinate is catalyzed by argininosuccinate synthase (ASS1). Argininosuccinate is then hydrolyzed to arginine and fumarate, by the action of the argininosuccinate lyase (ASL) (Kong et al.). The final step of the cycle relates to the cleavage of arginine by arginase 1 (ARG1) (Raghuveer et al.), releasing urea and regenerating ornithine (Berg et al., 2002).

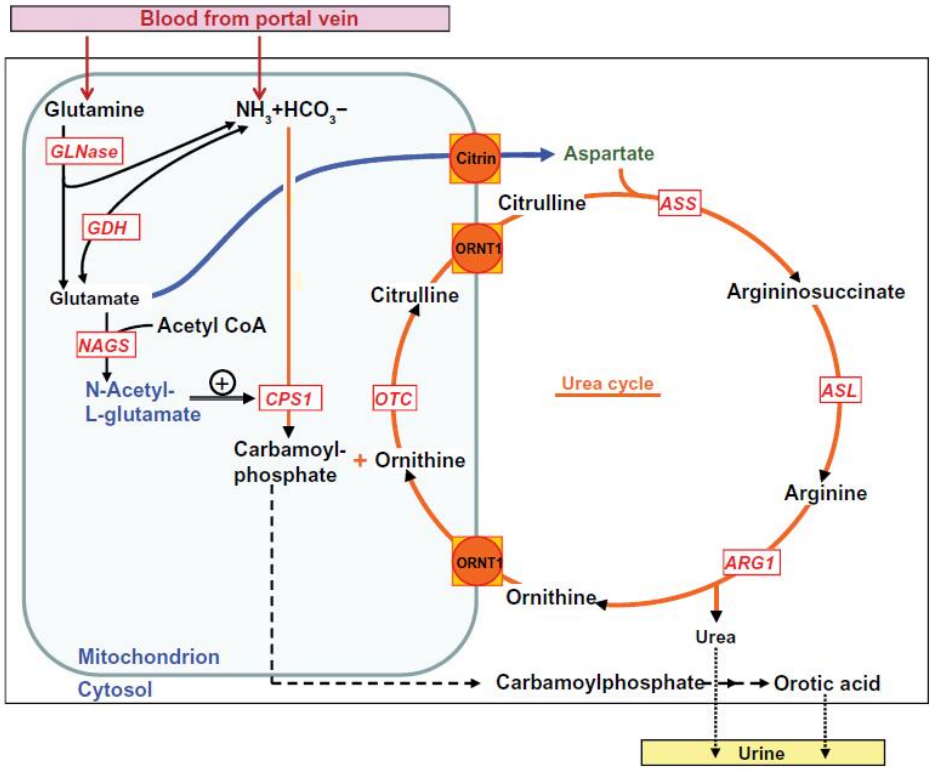


Figure 1: The urea cycle and associated pathways (modified from Häberle 2012).

1.2. Urea cycle enzymes

The urea cycle is composed of one cofactor synthesizing enzyme: the NAGS, five catalytic enzymes: CPS1, OTC, ASS1, ASL and ARG1 (Raghuveer et al.) and two transporters that are the mitochondrial aspartate/ glutamate carrier SLC25A13, and the mitochondrial ornithine transporter SLC25A15 (Waisbren et al., 2016). The NAGS, CPS1 and OTC are mitochondrial enzymes whereas the ASS1, ASL, and ARG1 are cytoplasmic enzymes (Leonard, 2006).

1.3. Disorders of urea cycle (UCDs)

The **urea cycle disorders (UCDs)** are a set of inherited disorders of the metabolism caused by the impair of one of the six enzymes or transporters involved in the urea cycle (Rocha et al., 2016) (Table 1).

Table 1: Urea cycle deficiencies (modified from Haberle 2011).

Gene	Disease Name	Protein
NAGS	NAGS deficiency	N-acetylglutamate synthase
CPS1	CPS1 deficiency	Carbamoylphosphate synthase 1
OTC	OTC deficiency	Ornithine carbamoyltransferase
ASS1	ASS1 deficiency (Citrullinemia type 1)	Argininosuccinate synthase 1
ASL	ASL deficiency (Argininosuccinic aciduria)	Argininosuccinate lyase
ARG1	ARG1 deficiency (Hyperargininemia)	Aginase 1
SLC25A15	HHH syndrome (Hyperornithinemia-hyperammonemia-homocitrullinuria)	Mitochondrial ornithine transporter
SLC25A13	Citrullinemia type 2	Citrin (aspartate/glutamate carrier)

1.3.1. Genetics

The UCDs show an autosomal recessive mode of transmission, with the exception of the OTC deficiency (OTCD) which is X-linked inherited (Waisbren et al., 2016). Among all the enzymatic deficits causing an UCD, the OTCD is the most common error of the urea cycle (Table 2).

Table 2: Global incidence of each UCDs (modified from Rocha 2009).

Disease	Incidence
NAGS deficiency	Not available
CPS1 deficiency	1:62000
OTC deficiency	1:14000
ASS1 deficiency	1:57000
ASL deficiency	1:70000
ARG1 deficiency	1:363000

1.4. Hyperammonemia

All the UCDs lead to **hyperammonemia**. This is translated by an increase in the levels of ammonia in the blood (Häberle, 2013).
From the biochemical point of view, two types of hyperammonemia can be considered:

1. ***Primary hyperammonemia when*** the urea cycle is directly affected by a defect in one of the enzymes or transporters involved in the cycle.

2. ***Secondary hyperammonemia when*** the function of the urea cycle may be inhibited by the accumulation of metabolites or by deficits of the substrate.

In infants, hyperammonemia usually displays irritability, vomiting and lethargy. If not treated, often leads to rapid development of encephalopathy with coma, seizures, and possible death. In older children and adults, hyperammonemia may result in nausea, vomiting, a decline in the level of consciousness, abnormal motor function and gastro-intestinal distress (Dutoit et al., 2010). The high levels of ammonia can lead to liver failure, including liver cirrhosis and acute liver failure (Kosenko et al., 2003).

1.5. CPS1 and NAGS deficiency

The **CPS1 deficiency (CPS1D)** is characterized by an increase in alanine and glutamine and a decrease in citrulline and arginine, without increase of orotic acid (Diez-Fernandez and Häberle, 2017). The **NAGS deficiency (NAGSD)** is characterized by an increase in glutamine and alanine, without an increase in the levels of orotic acid (Fernandes et al., 2006). The NAGSD and CPS1D are indistinct from both the biochemical and clinic points of view (Sancho-Vaello et al., 2016). The most direct way is the validation of the clinical diagnosis by molecular studies that result in genes sequencing.

1.6. OTC deficiency

The **OTC deficiency (OTCD)** is a common defect of the urea cycle disorder (Raghuveer et al., 2006) that manifests through vomiting, respiratory distress, headache, lethargy, decrease in the consciousness level, hypotonia, ataxia, seizures, coma, and hyperammonemia (Bellido et al., 2016; Dutoit et al., 2010). When there is an accumulation of carbamoyl-phosphate, the activation of the synthesis of pyrimidines occurs, which leads to an increase of orotic acid urinary excretion. This is a biochemical marker of the OTCD.

Table 3: Biochemical markers of diagnosis of urea cycle (modified from Rocha 2016).

Disease Name	Citrulline	Argininosuccinic Acid	Arginine	Ornithine	Orotic acid
NAGS deficiency	⇓	Not detectable	⇓	Normal	⇓
CPS1 deficiency	⇓	Not detectable	⇓	Normal	⇓
OTC deficiency	⇓	Not detectable	⇓	Normal	⇑⇑⇑
ASS1 deficiency	⇑⇑⇑	Not detectable	⇓	Normal	⇑⇑
ASL deficiency	⇑	⇑⇑⇑	⇓	Normal	⇑
ARG1 deficiency	⇑	Not detectable	⇑⇑⇑	Normal	⇑
HHH syndrome	Normal	Not detectable	Normal	⇑⇑	⇑

1.7. Clinical presentation

The clinical manifestations of UCDs can occur during **the neonatal period**, **during late infancy** or in **puberty** (Fernandes et al., 2006). The neonatal period correspond to the first 24h after birth and the most common symptoms are poor feeding, vomiting and lethargy and/or irritability (Fernandes et al., 2006). During late infancy, the symptoms are less acute and include vomiting or recurrent gastroenteritis, lethargy, anorexia, and growth retardation (Martín-Hernández, 2005). During puberty, neurological illness is more frequent, acute encephalopathy, lethargy, and episodes of disorientation (Martín-Hernández, 2005).

1.8. Diagnosis

The UCDs diagnosis must be done through the determination of **biochemical markers** (Table 3), **determination of residual enzyme activity** and **molecular studies**. Validation of the clinical/biochemical suspect of UCDs is done by the molecular studies, which routinely use PCR and direct sequencing of DNA samples to find the disease-associated mutation. In CPS1D, the large size of the gene (38 exons) difficults the molecular diagnosis by Sanger sequencing.

Next-Generation Sequencing (NGS) (Frans et al.) technologies have been demonstrated to be clinically applicable for the molecular diagnosis of various UCDs [CPS1 (Choi et al., 2017) and ASL (Wen et al., 2016)] allowing a faster, more efficient and cost-effective molecular diagnosis when compared with Sanger sequencing. The molecular diagnosis by Sanger sequencing of *NAGS* gene was technically difficult due to the fact that many exons have repetitive sequences of nucleotides that flank the exons. The *NAGS* coding sequence is GC-rich; its GC content is 67%, compared to the overall 41% GC content of the human genome (Caldovic et al., 2007).

1.9. Treatment

Early treatment of the UCDs is fundamental and includes dietary protein restriction, medications (Smith et al., 2005), replacement of deficient nutrients, and alternative pathways for nitrogen excretion (Fernandes et al., 2006). In the case of NAGSD there is a specific treatment for affected patients. Although the NAG itself cannot be used as a drug because it is degraded by plasma enzymes there is an 'orphan medicine' (a medicine used in rare diseases) which is used in the treatment of these patients: the CARBAGLU. Therefore, the confirmation of the disease by molecular diagnosis is crucial for the implementation of the adequate therapy (Abacan and Boneh, 2013).

1.10. Post-translational modifications of urea cycle enzymes

The sirtuins (SIRT) are a family of NAD+ dependent proteins, with the deacetylase and ADP-ribosiltransferase activity, that regulate various cellular processes and several diseases (Soares, 2014). In mammals there are seven isoforms (SIRT 1-7) distributed in three cellular compartments (Soares, 2014). *SIRT3*, *SIRT4* and *SIRT5* have activity of deacetylase (Nakagawa and Guarente, 2009). *SIRT5*, located in the mitochondrial matrix, is a CPS1 deacetylase (Nakagawa et al., 2009) and the deacetylation results in the activation of CPS1 enzymatic activity (Nakagawa and Guarente, 2009).

It was recently reported that acetylation of Lys^{88} inhibits OTC catalytic activity during caloric restriction (Yu et al., 2009). Another study revealed that during caloric restriction the *SIRT3* catalytic activity is necessary for OTC deacetylation (Hallows et al., 2011). Apart from these studies, little is known about the post-translational regulation of the urea cycle enzymes, although this knowledge could contribute to a better understanding of the phenotype-genotype relationships.

2. Objectives

NAGSD is the only UCD with a specific treatment. Therefore, the confirmation/validation of the diagnosis by molecular diagnosis is critical to direct the therapeutic strategies.

The objective of this project is to delineate a simple strategy to the molecular analyses of *NAGS* gene that can be used in routine molecular diagnosis which are not yet provided in our lab.

In addition, I also aimed to delineate a strategy to the amplification and sequencing of the *SIRT5* gene given its relevance as a CPS1 activator.

3. Methodology

3.1. DNA samples

In this project, I used commercially available DNA samples for protocol optimization. DNA samples (healthy UK Caucasian blood donors) of the ECACC (European Collection of Cell Cultures) Human Random Control DNA Panel were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Polymerase Chain Reaction (PCR)

All reactions were prepared using 5 µM HotStarTaq® Master Mix Kit (Qiagen, Germantown, MD, USA), 0.5 µM (final concentration) of each primer (Supplementary information I, Table 7 and 8), 3 µl H_2O and 1 µM Q-solution 10% (Qiagen) or 1 µM dimethyl sulfoxide (DMSO). PCR amplification was performed as follows: 95 °C (15 min), 35 cycles at 94°C (30 sec), 58–68 °C (1.30 min) and 72°C (1 min), and final extension at 72 °C (10 min). Amplification products were separated by horizontal polyacrylamide gel electrophoresis and visualized by silver staining (Supplementary information II, Table 9). Table 4 shows the PCR conditions used for the amplification of *NAGS* and *SIRT5* genes.

Table 4: PCR conditions for the amplification of *NAGS* and *SIRT5* genes.

NAGS

Exon	Desnaturation	Annealing			Extension		Nº Cicles	Q-solution	DMSO
1	94.0 °C 15:00 min	94.0 0:30	58.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	✓
2/3	94.0 °C 5:00 min	94.0 0:30	65.0 1:30	72.0 1:00	72.0 7:00	12.0 ∞	1+35+1	X	✓
4	94.0°C 5:00 min	94.0 0:30	58.0 1:30	72.0 1:00	72.0 7:00	12.0 ∞	1+35+1	X	X
5/6	94.0 °C 5:00 min	94.0 0:30	62.0 1:30	72.0 1:00	72.0 7:00	12.0 ∞	1+35+1	X	✓
7	94.0 °C 5:00 min	94.0 0:30	62.0 1:30	72.0 1:00	72.0 7:00	12.0 ∞	1+35+1	✓	✓

SIRT5

Exon	Desnaturation	Annealing			Extension		Nº Cicles	Q-solution	DMSO
1	95.0 °C 15:00 min	94.0 0:30	65.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	X
2	94.0 °C 5:00 min	94.0 0:30	58.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	X
3	94.0 °C 5:00 min	94.0 0:30	62.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	X
4	94.0 °C 5:00 min	94.0 0:30	65.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	X
5	94.0 °C 5:00 min	94.0 0:30	68.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	X
6	95.0 °C 15:00 min	94.0 0:30	68.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	✓	X
7	94.0 °C 5:00 min	94.0 0:30	58.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	✓
8	95.0 °C 15:00 min	94.0 0:30	66.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	✓
9	95.0 °C 15:00 min	94.0 0:30	65.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	✓
10	95.0 °C 15:00 min	94.0 0:30	62.0 1:30	72.0 1:00	72.0 10:00	12.0 ∞	1+35+1	X	✓

3.3. Sequencing analysis

PCR products were purified with Sephadex and sequenced with the ABI Big Dye Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA).

Sequencing reactions were prepared using 2.5 µM purified product, 0.5 µM primer and 2 µM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®). The sequencing of reaction was performed in a thermocycler as follows: 96 °C (2 min), 35 cycles at 96 °C (15 sec), 58–68 °C (10 sec) and 72 °C (2 min), and final extension at 60 °C (10 min). The temperature used for the sequencing reaction was the same used in the PCR reaction.

The products of the sequencing reaction were purified in appropriate columns on which 750 µl of Sephadex™ G50 (GE Healthcare) was centrifuged to 4400 rpm (4 min). Next, the columns were transferred to eppendorfs and the total sample added. A new centrifugation at 4400 rpm (4 min) was performed. A total of 10 µl of Hi-Di™ formamide (Applied Biosystems) were added to each Eppendorf tube with the purified sample. Fragments were analyzed in an ABI PRISM 3130xl (Applied Biosystems). Sequences were aligned using Geneious v.10.0.5 (Biomatters, available from http://www.geneious.com).

4. Results

4.1. Amplification of *NAGS* and *SIRT5* genes

During this project, several attempts to the amplification of all exons of *NAGS* and *SIRT5* were performed. The results here presented are the ones that best fit the purpose of this project and which will be used in the future diagnosis of the diseases caused by the impairment of any of these genes.

After several attempts, the successful strategy for the amplification of the *NAGS* and *SIRT5* genes was achieved and the results are displayed in figures 2 and 3. The main challenge to overcome during the laboratory project was the high percentage of GC flanking the exonic regions of the genes. GC-rich DNA sequences complicates amplification because of generation of secondary structures that hinder denaturation and primer annealing (Strien et al., 2013). To overcome this difficulty I used Q-solution (Qiagen) and DMSO well-known additives for to successful DNA amplification GC-rich regions (Chakrabarti and Schutt, 2001; Strien et al., 2013) (Table 4).

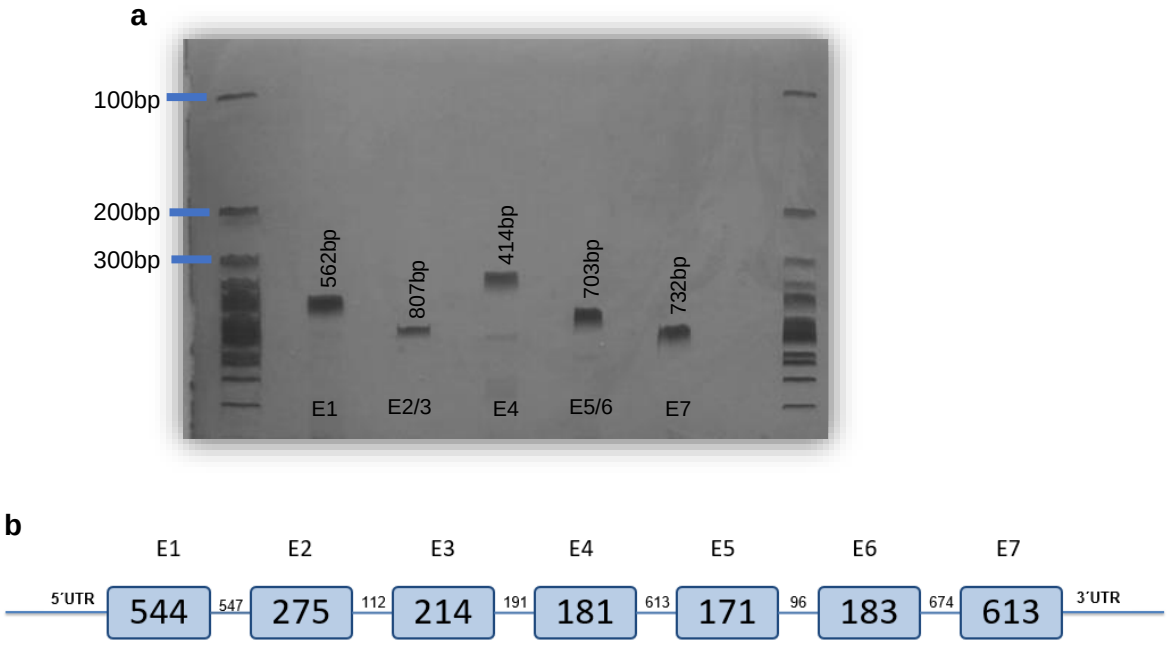


Figure 2: (a) PCR products of *NAGS. NAGS* gene is composed by seven exons. For each fragment, a negative control used to control DNA contamination was inserted in the gel immediately on the right of the corresponding fragment. (b) Graphical representation of *NAGS* gene. The size of each exon and intron is shown.

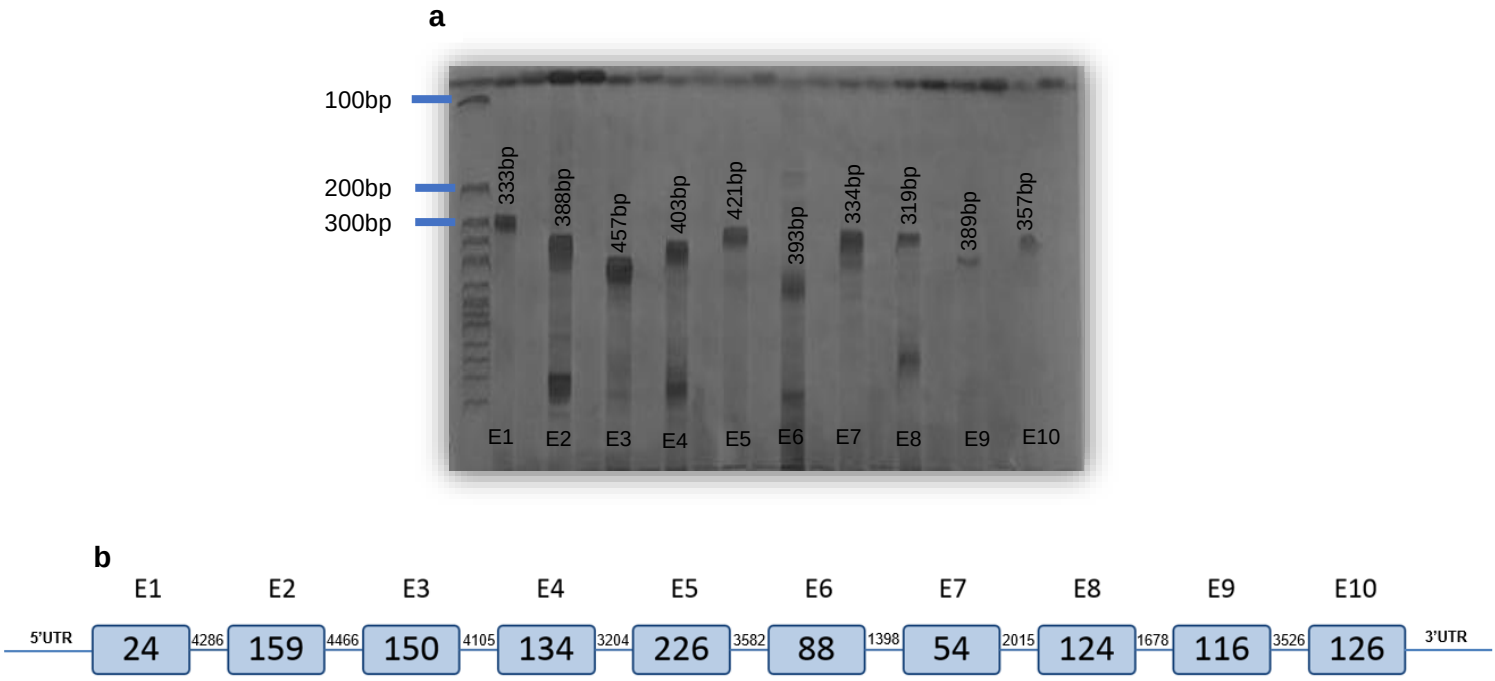


Figure 3: (a) PCR products of *SIRT5*. *SIRT5* gene is composed by ten exons. For each fragment, a negative control used to control DNA contamination was inserted in the gel immediately on the right of the corresponding fragment. (b) Graphical representation of *SIRT5* gene. The size of each exon and intron is shown.

4.2. Sequencing analysis

The sequencing analysis of *NAGS* gene was also technically difficult due to the fact that many exons have flanking repetitive sequences of GC nucleotides. Figure 4 shows part of the electropherogram resulting from the amplification of the first exon of *NAGS*, where the high GC content can be easily observed. The fact that gene flanking sequences show a high GC content challenge the routine amplification and sequencing conditions which had be adjusted for each case.

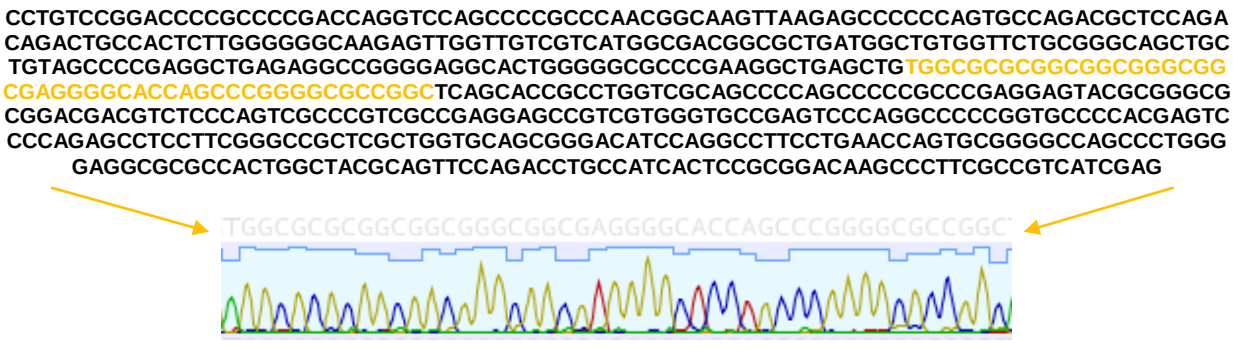


Figure 4: *Electropherogram corresponding to part the exon 1 of NAGS* gene.

To illustrate this, I analyzed 50 nucleotides upstream and downstream of each exon for the *NAGS* and *SIRT5* genes (Figure 5). A direct relationship was observed in relation to the GC content of exon-intron flanking sequences and the need to use DMSO or Q-solution for the amplification of the exons. For instance, for the *SIRT5* gene, the percentage of GC is lower for exons 1 to 5 (Figure 5), therefore there was no need to add DMSO or Q-solution during the amplification of these fragments (Table 4). On the other hand, all exons of the *NAGS* gene show a high GC percentage (Figure 5) which resulted in the need to optimize the amplification by adding Q-solution or DMSO during the amplification. This result is in agreement with previous data that show the DNA templates considered difficult to optimize to DNA sequencing are those with GC content higher than 60–65% (Kieleczawa, 2006).

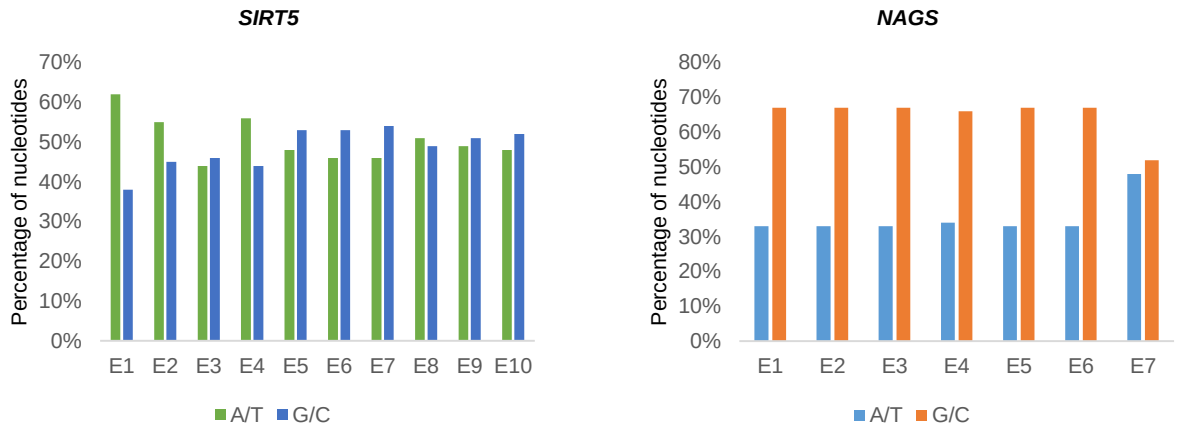


Figure 5: Percentages of A/T and G/C in *SIRT5* and *NAGS* genes.

5. Conclusions

The main aim of this project was to successfully design a PCR-based methodology for the amplification of *NAGS* and *SIRT5* genes in our laboratory. The challenge was to overcome the difficulty in amplifying the GC-rich exonic fragments of both genes but particularly of the *NAGS* gene. The strategy I present here will certainly be extremely useful in the diagnosis routine in our lab as until now no such diagnosis service could be offered. In a further note, it is also important to stress that the diagnosis of *NAGS* is of critical relevance due to the possibility of therapeutic action using the pharmaceutical "CARBAGLU".

The correct diagnosis of deficits in the urea cycle must be done through the determination of biochemical markers, determination of residual enzyme activity and molecular studies. Early detection and treatment during the neonatal period, are fundamental for preventing the progression of symptoms in this group of pathologies, enabling a treatment appropriate to each case, saving lives and avoiding frequent serious complications.

Molecular diagnosis is currently being revolutionized by the development of innovative techniques of genomics analysis. These techniques, coupled with bioinformatics analyses, exacerbate the rapid diagnosis and effective means of genetic diseases, infectious diseases and cancerous diseases, by increasingly sophisticated methods and non-invasive, even before they manifest themselves.

The NGS methodology enables the simultaneous detection of mutations in multiple genes, allowing a molecular diagnosis faster, more efficient and cost-effective than Sanger sequencing of all genes. Even when the NGS technology is applied, Sanger sequencing shall be used to confirm the putative mutation. Therefore, the laboratory routine should always include the final validation of the mutation by PCR and Sanger sequencing strategies which can now be applied our lab for *NAGS* and *SIRT5* affected genes as a result of this project.

Part II

A comprehensive analysis of *de novo* mutations in disease-associated genes

1. Introduction

Germline mutations are the source of all evolutionary adaptations and heritable diseases (Ségurel et al., 2014).

1.1. Origin

***De novo* mutations** are mutations that arise in offspring from non-carrier parents (Ku et al., 2013). These mutations can arise either in one of the gametes of the parents of the affected individual (pre-zygotic origin), or after fertilization in one of the cells of the embryo (post-zygotic origin) (Prochazkova et al., 2009).

The *de novo* mutation rate is within the range between 1.1×10^{-8} and 3.8×10^{-8} **per nucleotide** per generation (Awadalla et al., 2010; Kondrashov, 2003; Lynch, 2010; Nachman and Crowell, 2000; Project, 2011; Xue et al., 2009), corresponding to a rate of 109-145 mutations per diploid genome per generation [assuming to a rate 2.0×10^{-8} (±10−9) per nucleotide]. The mutation rate for humans is smaller than the average rate of invertebrates (*Drosophila melanogaster*: 4.65×10^{-9}; *C. elegans*: 5.60×10^{-9}) and plants (*Arabidopsis thaliana*: 6.50×10^{-9}) but greater than the rate estimated for unicellular organisms (*Saccharomyces cerevisiae*: 3.3×10^{-8} and *Escherichia coli*: 2.6×10^{-8}) (Lynch, 2010).

1.2. Timing of *de novo* mutations

Postzygotic *de novo* mutational events are likely to occur randomly to generate mosaicism (Campbell et al., 2015), and be present in many different tissues of the organism (Biesecker and Spinner, 2013). **Mosaicism** is a state in which genetically distinct cells from a single zygote co-exist in the same individual (Acuna-Hidalgo et al., 2015) and which may have several phenotypic implications in humans (Lupski, 2013).

1.3. The effect of paternal age

The mutation rate during the gametogenesis, which comprises two stages of oogenesis [Pre-primordial germ cells (PGCs) and Post-primordial germ cells] and three stages of spermatogenesis (Pre-primordial germ cells, Post-primordial germ cells and Post-puberty) appears to be similar in both male and female. Before the specification of PGCs and in post-PGC, the mutation rate is similar in both sexes, with 0,2-0,6 mutations per haploid genome per cell division and 0,5-0,7 mutations per haploid genome per cell division, respectively (Acuna-Hidalgo et al., 2016; Rahbari et al., 2015). However, after puberty, there are differences between male and female gametogenesis (Acuna-Hidalgo et al., 2016), especially in the number of germline cell divisions between the sperm and egg (Crow, 2000). The spermatogonial cells continue to divide throughout life, assuming a constant rate of 23 divisions per year (Sagi-Dain et al., 2016) while the number of cell divisions in the egg are 22 before meiosis and two during meiosis (Crow, 2000) suggesting a possible explanation for advanced paternal age effect (Crow, 2000).

It is estimated that 80% of all *de novo* germline point mutations arise on the paternal allele (Acuna-Hidalgo et al., 2016). Several studies have shown that the main factor responsible for the increase in the number of *de novo* mutations in the offspring is **advanced paternal age** (Acuna-Hidalgo et al., 2016; Bhandari et al., 2011; Gratten et al., 2016; Janecka et al., 2017; Kong et al., 2012) while the maternal age has a small effect (Goldmann et al., 2016) as demonstrated in the project realized in patients with Apert syndrome (Moloney et al., 1996).

The advanced paternal age have been associated to spontaneous dominant disorders such as congenital anomalies, childhood cancers, acute lymphoblastic leukemia, breast cancer, increased telomere length, autism, schizophrenia, bipolar disorder and reduced neurocognitive abilities in childhood and infancy (Dalman and Allebeck, 2002; Frans et al., 2008; Goriely and Wilkie, 2012; Reichenberg et al., 2006; Saha et al., 2009).

1.4. Causes of *de novo* mutations

Many factors influence the rate of *de novo* mutations such as chromosomal location, length of coding sequence, number and length of introns, nucleotide composition (for example, GC level) and/or repetitive sequence both within and flanking the gene, errors during DNA replication and efficiency of DNA repair mechanisms (Ku et al., 2013). In the human genome most of the *de novo* mutations occur in CpG sites (Francioli et al., 2015), being that the rate of deamination in these regions lower in females than males (Wong et al., 2016). In CpG sites, transitions result from C to T replacements are more common molecular event (Acuna-Hidalgo et al., 2016).

2. Objectives

Many studies have been become available concerning *de novo* mutations in several human genetic diseases. In this project, I reviewed the literature on the topic of *de novo* mutations in order to compile these from the available literature and to use the database to infer about some mechanistic features of those that are in the origin of all mutations.

3. Methodology

I searched the literature for published studies where human *de novo* variants had been identified. This literature search resulted in 801 variants collected from 120 studies. From these studies I gathered essential information on each new variant, including mutation type, phenotype, nucleotide change, aminoacid change, chromosome, reference variant and alternative variant.

I also included in my database *de novo* variants from a previous published database (denovo-db, available at http://denovo-db.gs.washington.edu). Denovo-db consists of 32,991 variants collected from 40 genome wide studies. I removed the variants present in controls; variants that validation was unknown; duplicated variants; cases the affected gene was not identified; variants without pubmed ID identification, and variants without information about aminoacid change. Table 5 shows the number of genes, phenotypes, variants and references retrieved manually from the literature and from the Denovo-db.

Table 5: Statistics for the *de novo* mutations used in this project.

	Number of genes	**Distinct phenotypes**	**Variants**	**Number of references**
Literature (this project)	566	124	801	120
Denovo-db	2020	14	3419	40

4. Results

A total of 4220 variants were identified in 2586 genes (Supplementary information III, Table 10).

The mutation type, shown in figure 6, is here divided in: single nucleotide substitutions (missense, nonsense, synonymous, splicing and stop-lost); small deletions and insertions (up to 20 nucleotides including) and large deletions and insertions (more than 20 nucleotides). As observed in figure 6a, 88% (3723/4220) of the *de novo* mutations collected in this project are represented by single nucleotide replacements. This scenario matched the one observed for the total number of mutations found in human genetic disease. In fact, in the Human Gene Mutation Database (HGMD), about 65% (133,089/203,885) of the inherited mutations are represented by single nucleotide replacements. The percentage of small deletions and insertions is very similar and both represent the second and third category of *de novo* mutations. As expected, large deletions and insertions correspond to the lowest number of mutations found in our dataset.

Concerning the type of replacements, the proportion of C/T and G/A replacement (45%) is much higher than other replacements (Figure 6b). This might be related with the fact that most of these mutations result from the deamination of citosines at CpG sites.

Concerning the category of small insertions, our data shows that 65% (150/230) of small insertions are represented by the insertion of a single nucleotide (Figure 6d). A similar scenario was observed for small deletions, where most of the deleted events correspond to a single nucleotide, although in this case two and four nucleotide deletions are also frequently found among *de novo* mutations (Figure 6c).

Figure 7 shows the distribution of *de novo* mutations per chromosome which revealed a widespread distribution of these mutations in the human genome. About 3% (122/4220) of all *de novo* mutations in our dataset occur in the X-chromosome.

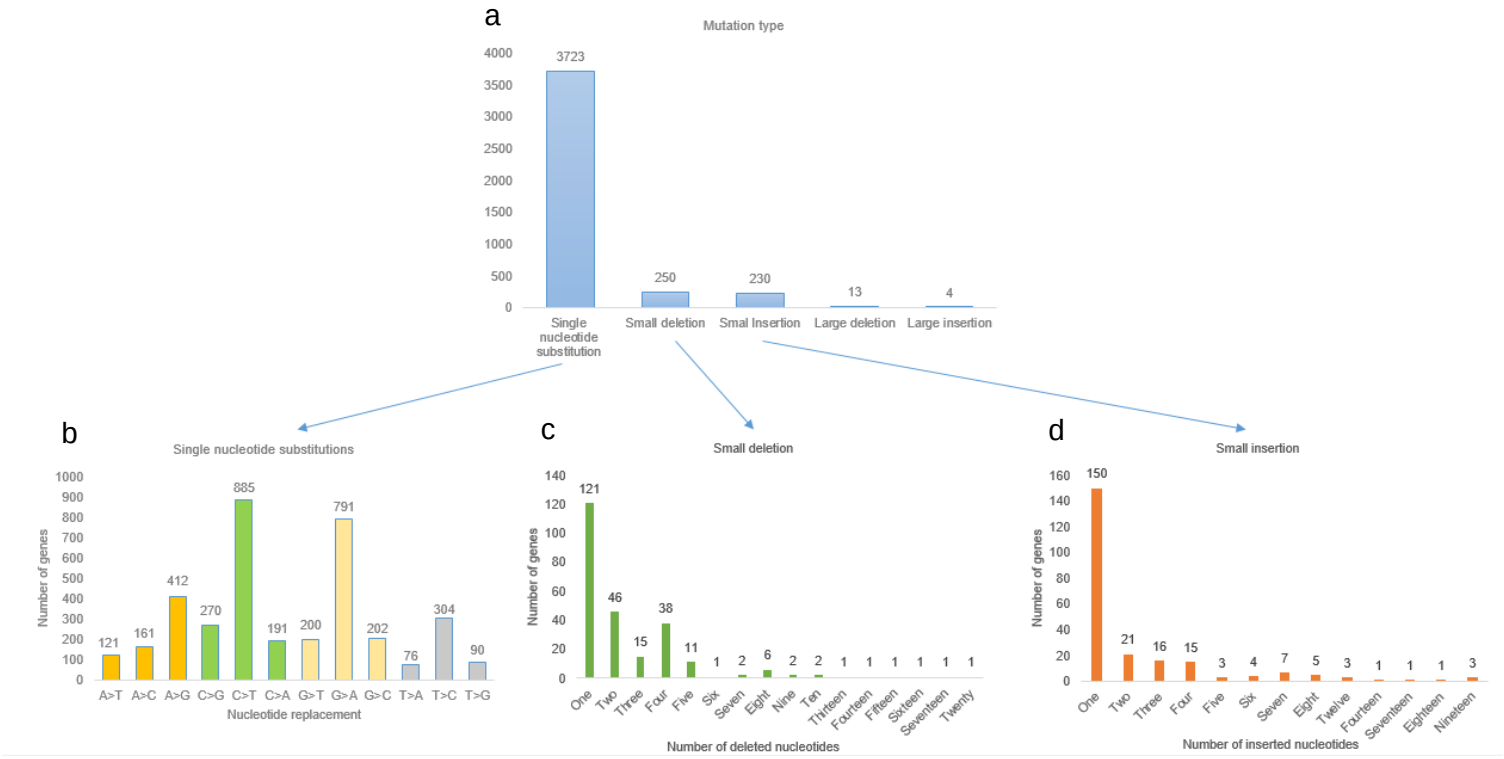


Figure 6: (a) Number of different mutations by mutation type present. (b) Frequency of single nucleotide substitutions. (c) Frequency of number of deletions. (d) Frequency of number of insertions.

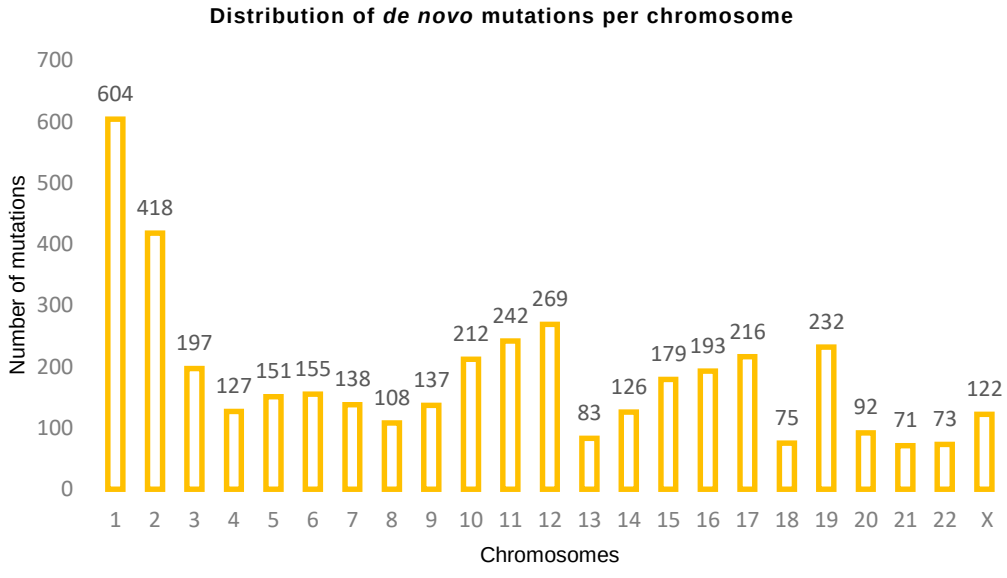


Figure 7: Number of the *de novo* mutations that occur in each chromosome.

A total of 138 phenotypes associated with the 4220 variants that constitute our dataset (Table 6). The majority of *de novo mutations* (41%; 1743/4220) are related to autism associated disorders. These mutations were found among 1095 genes. The second and third class of disorder is the schizophrenia and congenital heart disease with (16%; 685/4220) e (13%; 576/4220), respectively.

Noteworthy, most of the literature refers that *de novo* mutations are highly associated with neurodevelopmental diseases (NDD). Our collection of these mutations allowed us to detect many mutations in non-NDD diseases like cardiac-associated phenotypes, amyotrophic lateral sclerosis and familial adenomatous polyposis. Moreover, I was able to gather all the phenotypes reported to date in the literature and future studies may rely in this database for understanding the molecular mechanisms associated with these type of mutations.

Table 6: Phenotypes associated with *de novo* mutations.

Phenotypes
o Acetazolamide-Responsive Episodic Ataxia
o Acute lymphoblastic leukemia (ALL)
o Acute Myeloid Leukemia
o Adult-onset Autosomal-dominant progressive external ophthalmoplegia with multiple mitochondrial DNA deletions;
o Allan–Herndon– Dudley syndrome (AHDS)
o Alternating hemiplegia of childhood (AHC)
o Amyotrophic lateral sclerosis (ALS)
o Angelman syndrome
o Arthrogryposis multiplex congenita & bilateral perisylvian polymicrogyria
o Autism Spectrum Disorder (ASD)
o Autism Spectrum Disorder; Epilepsy
o Autism Spectrum Disorder; Rett Syndrome
o Autosomal dominant hypocalcemia (ADH)
o Autosomal Dominant Nonsyndromic Hearing loss (DFNA4B)
o Autosomal dominant polycystic kidney disease (ADPKD)
o Autosomal recessive juvenile parkinsonism
o Autosomal-Dominant Penttinen Syndrome
o BENTA disease (B cell Expansion with NF-κB and T cell Anergy)
o Betapropeller protein-associated neurodegeneration' (BPAN)
o Bipolar disorder (BD)
o Breast and/or ovarian cancer
o Cantu syndrome
o Cardio-facio-cutaneous (CFC) syndrome
o Cerebellar ataxia
o Cerebellar ataxia associated with intellectual instability and arthrogryposis

- Cerebral palsy (spastic diplegia)
- Challenging Parathyroid Tumor Diagnoses
- Charcot-Marie-Tooth disease 1a
- CHARGE syndrome
- Childhood-onset mitochondrial myopathy and cardiomyopathy
- Childhood-onset Schizophrenia (Akcurin et al.)
- Chronic myelomonocytic leukemia (CMML1)
- Chronic myelomonocytic leukemia (CMML2)
- Congenital diaphragmatic hernia
- Congenital disorders of glycosylation (CDG)
- Congenital heart disease (CHD)
- Congenital long-QT syndrome (LQTS) is a familial disorder
- Cornelia de Lange syndrome (CdLS)
- Cryopyrinassociated periodic syndromes (CAPS)
- Cushing's disease in children with multiple endocrine neoplasia type 1 (MEN1)
- Developmental and intellectual impairment with hypotonia
- Dravet syndrome (DS)
- Dystrophic epidermolysis bullosa (DEB)
- Early infantile epileptic encephalopathy
- Early myoclonic encephalopathy (EME)
- Early onset alzheimer
- Early onset parkinson
- Early-onset multiorgan autoimmune
- Ectrodactyly-ectodermal dysplasia-clefting (EEC)
- Epilepsy
- Epilepsy (Infantile spasms, controlled)
- Epilepsy (Focal seizures as infant, now myoclonic, tonic and occasional tonic-clonic)
- Epilepsy (Head drops, then infantile spasms. Continued head drops and atypical absences)
- Epilepsy (Multiple daily seizures, intractable)
- Epilepsy (Seizures)
- Epilepsy of infancy with migrating focal seizures (EIFMS)
- Epileptic encephalopathy
- Exudative Vitreoreretinopathy
- Familial Adenomatous Polyposis (FAP)
- Familial hypocalciuric hypercalcemia (FHH)
- Gaucher disease (Janecka et al.)
- Global developmental delay, prominent speech delay, microcephaly, short stature and discrete facial dysmorphisms.
- Gnathodiaphyseal dysplasia (GDD)
- Gross motor delay, and Autism spectrum disorder (ASD)
- Group 1: Focal cortical dysplasia (FCD) type 2a
- Group 2: Asymmetric megalencephaly (MEG) and pigmentary mosaicism in skin that resembles hypomelanosis of Ito
- Group 3: Diffuse MEG and intellectual disability
- Hartsfield syndrome
- Hereditary motor and sensory neuropathy type I (HMSN I);

○ Hereditary spastic paraplegias (HSPs)
○ Holoprosencephaly
○ Holt-Oram syndrome (HOS) is an autosomal dominant
○ Homo sapiens mutL homolog 1 (MLH1)-associated Lynch syndrome
○ Hyperparathyroidism (FIHP) - jaw tumour syndrome (HPT-JT)
○ ID and ASD
○ Idiopathic hypoparathyroidism (IHP)
○ Incontinentia pigmenti (IP)
○ Intellectual disability (ID)
○ Intellectual disability and postnatal overgrowth
○ Intellectual Disability; Mental Retardation
○ Interstitial lung
○ Kabuki syndrome
○ Li–Fraumeni Syndrome
○ Malignant migrating partial seizures of infancy (MMPSI)
○ Megalencephaly-polymicrogyriapolydactyly-hydrocephalus syndrome
○ Mental Retardation
○ MÖbius syndrome (MBS)
○ Multiple Endocrine Neoplasia Type I (MEN I)
○ Multiple neurological and developmental lesions
○ Myelodysplastic syndromes (MDS) and their association with secondary acute myeloid leukemia (AML)
○ Neural tube defects
○ Nicolaides-Baraitser syndrome
○ Non-syndromic and intellectual disability
○ Noonan syndrome (NS)
○ Osteopathia striata congenita with cranial sclerosis (OSCS)
○ Otosclerosis (OTSC)
○ Overgrowth syndromes
○ Pancreatic agenesis
○ Pendred syndrome (PS) and DFNB4 hearing loss
○ Peutz–Jeghers syndrome (PJS)
○ Poikiloderma
○ Primrose syndrome
○ Profound mental retardation, Severe spastic quadriplegia
○ Progressive myoclonus epilepsies (PMEs)
○ Pseudohypoaldosteronism, type 2E
○ PTEN hamartoma tumor syndrome
○ Purpose Immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is an extremely rare autosomal recessive
○ Rare autosomal dominant inheritable disease called microcephaly (-2 SDs)
○ Rare autosomal dominant inheritable disease called microcephaly (-2 SDs) with mild mental retardation, microphthalmia in the right eye
○ Rare autosomal dominant inheritable disease called microcephaly (-6 SDs) with Chorioretinopathy,
○ Rare autosomal dominant inheritable disease called microcephaly with Microphthalmia in the left eye

○ Rubinstein–Taybi syndrome (RSTS)
○ Schizophrenia
○ Severe achondroplasia with developmental delay and acanthosis nigricans
○ Severe central nervous system phenotype characterized by microcephaly and severe intellectual disability
○ Severe non-syndromic developmental delay
○ Severe obesity and developmental delay
○ Severe Persistent Hyperinsulinemic Hypoglycemia
○ Severe static encephalopathy, intellectual disability, and generalized epilepsy
○ Severe, early-onset epilepsies
○ Sotos syndrome (SS)
○ Spinocerebellar
○ Sporadic infantile spasm syndrome
○ Stimulator of interferon genes (STING) - associated vasculopathy with onset in infancy
○ Syndromic developmental delay with persistent gastro-esophageal reflux
○ Syndromic form of Autism and ID
○ Tuberous sclerosis complex (Dreßen et al.)
○ Type 2A Von Willebrand disease (VWD)
○ Type 2B Von Willebrand disease (VWD)
○ Type 2M Von Willebrand disease (VWD)
○ Ullrich congenital muscular dystrophy
○ Von Hippel-Lindau (VHL)
○ West syndrome
○ Wolfram syndrome (WS)
○ X-linked chronic granulomatous disease (X-CGD) is a primary immunodeficiency
○ X-linked hypophosphatemic rickets
○ X-linked recessive intellectual disability syndrome
○ Zimmermann-Laband syndrome

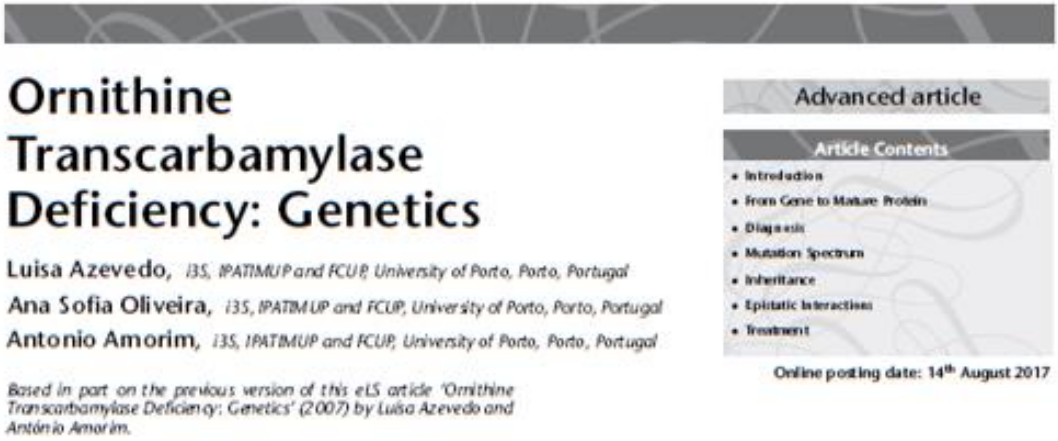
5. Conclusions/future perspectives

I successfully gathered virtually all *de novo* mutations reported to date in the literature.

This database can be used to study a series of features that have biological relevance. In this sense, I plan to compare the mutational spectrum of *de novo mutations* with non-pathological mutations from the 1000 Genomes Project (GP) (http://www.1000genomes.org) or Exome Variant Server (EVS) (http://snp.gs.washington.edu/EVS). Another analysis that may be important is the Gene Ontology in order to know if there are any genes enriched for *de novo* mutations and if there is any relationship with the genetic redundancy exhibited by the gene in question. Also important, would be to establish a conservation score of the residue mutated in the case of missense *de novo* mutations. These and other studies are now possible the collection of *de novo* mutations here presented.

Review article

OTC deficiency (OTCD) is the most common urea cycle disorder. The OTCD is X-linked inherited and the majority of female patients show *de novo* mutations. Male patients can also carry *de novo* mutations but much less frequently. During the time I spent in the project of this thesis, I had the opportunity to collaborate in a review article focused in genetics OTCD. As expected, my contribution was related to the topic *de novo* mutations in the *OTC* gene.



Ornithine Transcarbamylase Deficiency: Genetics

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Ana Sofia Oliveira, *i3S, IPATIMUP and FCUP, University of Porto, Porto, Portugal*
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Based in part on the previous version of this eLS article 'Ornithine Transcarbamylase Deficiency: Genetics' (2007) by Luisa Azevedo and António Amorim.

Advanced article

Article Contents

- Introduction
- From Gene to Mature Protein
- Diagnosis
- Mutation Spectrum
- Inheritance
- Epistatic Interactions
- Treatment

Online posting date: 14th August 2017

ABSTRACT

Ornithine transcarbamylase deficiency, the most common urea cycle disorder, is an X-linked trait displaying large phenotypic heterogeneity, which includes cases of symptomatic heterozygotes and cases of very mildhemizygous individuals. A wide mutational spectrum, which includes more than 500 disease-associated mutations, is currently known. More than 2/3 of the mutations are nucleotide replacements at the coding region of the gene. De novo mutations are commonbeing responsible for as much as three-fourth of female abnormal chromosomes. Recurrent mutations found in different human populations are associated with hypermutable CpG dinucleotides. and these features render diagnosis andgenetic counselling particularly challenging.The responsible gene is, however, an excellent model for research on human mutation, due to (a) the mode of transmission (allowing direct haplotype ascertainment and evaluation of the role of recombination, which occurs only in females) and (b) the abundance and diversity of mutations. Treatment strategies involve reduction of protein level intake and stimulation of alternative metabolic pathways or liver transplantation.

Bibliographic References

- Abacan, M., and Boneh, A. (2013). Use of carglumic acid in the treatment of hyperammonaemia during metabolic decompensation of patients with propionic acidaemia. *Molecular genetics and metabolism 109*, 397-401.
- Acuna-Hidalgo, R., Bo, T., Kwint, M.P., van de Vorst, M., Pinelli, M., Veltman, J.A., Hoischen, A., Vissers, L.E., and Gilissen, C. (2015). Post-zygotic point mutations are an underrecognized source of de novo genomic variation. *The American Journal of Human Genetics 97*, 67-74.
- Acuna-Hidalgo, R., Veltman, J.A., and Hoischen, A. (2016). New insights into the generation and role of de novo mutations in health and disease. Genome Biology *17*, 241.
- Akcurin, S., Turkkahraman, D., Tysoe, C., Ellard, S., De Leener, A., Vassart, G., and Costagliola, S. (2008). A family with a novel TSH receptor activating germline mutation (p. Ala485Val). European Journal of Pediatrics *167*, 1231-1237.
- Awadalla, P., Gauthier, J., Myers, R.A., Casals, F., Hamdan, F.F., Griffing, A.R., Côté, M., Henrion, E., Spiegelman, D., and Tarabeux, J. (2010). Direct measure of the de novo mutation rate in autism and schizophrenia cohorts. The American Journal of Human Genetics *87*, 316-324.
- Bellido, M., Perez, M.G., Ruiz, A.M., and Barcelo, I. (2016). Considerations on urea cycle disorders: a child with ornithine transcarbamylase deficiency. Pediatric Anesthesia and Critical Care JournaL *4*, 28-29.
- Berg, J.M.T., John, L., Stryer, L., Berg, J.M., Tymoczko, J.L., and Stryer, L. (2002). Biochemistry. In (W H Freeman).
- Bhandari, A., Sandlow, J.I., and Brannigan, R.E. (2011). Risks to offspring associated with advanced paternal age. Journal of Andrology *32*, 121-122.
- Biesecker, L.G., and Spinner, N.B. (2013). A genomic view of mosaicism and human disease. Nature Reviews Genetics *14*, 307-320.
- Caldovic, L., Morizono, H., and Tuchman, M. (2007). Mutations and polymorphisms in the human N-acetylglutamate synthase (NAGS) gene. Human Mutation *28*, 754-759.
- Campbell, I.M., Shaw, C.A., Stankiewicz, P., and Lupski, J.R. (2015). Somatic mosaicism: implications for disease and transmission genetics. Trends in Genetics *31*, 382-392.

- Chakrabarti, R., and Schutt, C.E. (2001). The enhancement of PCR amplification by low molecular-weight sulfones. Gene *274*, 293-298.
- Choi, R., Park, H.-D., Yang, M., Ki, C.-S., Lee, S.-Y., Kim, J.-W., Song, J., Chang, Y.S., and Park, W.S. (2017). Novel Pathogenic Variant (c. 580C> T) in the CPS1 Gene in a Newborn With Carbamoyl Phosphate Synthetase 1 Deficiency Identified by Whole Exome Sequencing. Annals of Laboratory Medicine *37*, 58-62.
- Crow, J.F. (2000). The origins, patterns and implications of human spontaneous mutation. Nature Reviews Genetics *1*, 40-47.
- Dalman, C., and Allebeck, P. (2002). Paternal age and schizophrenia: further support for an association. American Journal of Psychiatry *159*, 1591-1592.
- De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., and Walker, S. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. Nature *515*, 209-215.
- Diez-Fernandez, C., and Häberle, J. (2017). Targeting CPS1 in the treatment of Carbamoyl phosphate synthetase 1 (CPS1) deficiency, a urea cycle disorder. Expert Opinion on Therapeutic Targets *21*, 391-399.
- Dreßen, M., Lahm, H., Lahm, A., Wolf, K., Doppler, S., Deutsch, M.A., Cleuziou, J., Pabst von Ohain, J., Schön, P., and Ewert, P. (2016). A novel de novo TBX5 mutation in a patient with Holt–Oram syndrome leading to a dramatically reduced biological function. Molecular Genetics & Genomic Medicine *4*, 557-567.
- Dutoit, A.P., Flick, R.R., Sprung, J., BABOVIC-VUKSANOVIC, D., and Weingarten, T.N. (2010). Anesthetic implications of ornithine transcarbamylase deficiency. Pediatric Anesthesia *20*, 666-673.
- Fernandes, J., Saudubray, J.-M., Van den Berghe, G., and Walter, J.H. (2006). Inborn metabolic diseases: diagnosis and treatment (Springer Science & Business Media).
- Francioli, L.C., Polak, P.P., Koren, A., Menelaou, A., Chun, S., Renkens, I., Van Duijn, C.M., Swertz, M., Wijmenga, C., and Van Ommen, G. (2015). Genome-wide patterns and properties of de novo mutations in humans. Nature genetics *47*, 822-826.
- Frans, E.M., Sandin, S., Reichenberg, A., Lichtenstein, P., Långström, N., and Hultman, C.M. (2008). Advancing paternal age and bipolar disorder. Archives of General Psychiatry *65*, 1034-1040.
- Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P., Georgieva, L., Rees, E., Palta, P., and Ruderfer, D.M. (2014). De

novo mutations in schizophrenia implicate synaptic networks. Nature *506*, 179-184.

- Goldmann, J.M., Wong, W.S., Pinelli, M., Farrah, T., Bodian, D., Stittrich, A.B., Glusman, G., Vissers, L.E., Hoischen, A., and Roach, J.C. (2016). Parent-of-origin-specific signatures of de novo mutations. Nature Genetics.
- Goriely, A., and Wilkie, A.O. (2012). Paternal age effect mutations and selfish spermatogonial selection: causes and consequences for human disease. The American Journal of Human Genetics *90*, 175-200.
- Gratten, J., Wray, N.R., Peyrot, W.J., McGrath, J.J., Visscher, P.M., and Goddard, M.E. (2016). Risk of psychiatric illness from advanced paternal age is not predominantly from de novo mutations. Nature genetics.
- HABERLE, J. (2011). Diagnosis and treatment of urea cycle disorders. Journal of Pediatric Sciences *3*.
- Häberle, J. (2013). Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. Archives of biochemistry and biophysics *536*, 101-108.
- Hallows, W.C., Yu, W., Smith, B.C., Devires, M.K., Ellinger, J.J., Someya, S., Shortreed, M.R., Prolla, T., Markley, J.L., and Smith, L.M. (2011). Sirt3 promotes the urea cycle and fatty acid oxidation during dietary restriction. Molecular cell *41*, 139-149.
- Janecka, M., Mill, J., Basson, M.A., Goriely, A., Spiers, H., Reichenberg, A., Schalkwyk, L., and Fernandes, C. (2017). Advanced paternal age effects in neurodevelopmental disorders—review of potential underlying mechanisms. Translational Psychiatry *7*, e1019.
- Kieleczawa, J. (2006). Fundamentals of sequencing of difficult templates—an overview. Journal of biomolecular techniques: JBT *17*, 207.
- Kondrashov, A.S. (2003). Direct estimates of human per nucleotide mutation rates at 20 loci causing Mendelian diseases. Human mutation *21*, 12-27.
- Kong, A., Frigge, M.L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., Gudjonsson, S.A., Sigurdsson, A., Jonasdottir, A., and Jonasdottir, A. (2012). Rate of de novo mutations and the importance of father/'s age to disease risk. Nature *488*, 471-475.
- Kosenko, E., Llansola, M., Montoliu, C., Monfort, P., Rodrigo, R., Hernandez-Viadel, M., Erceg, S., Sánchez-Perez, A.M., and Felipo, V. (2003). Glutamine synthetase activity and glutamine content in brain: modulation by NMDA receptors and nitric oxide. Neurochemistry international *43*, 493-499.

- Ku, C., Polychronakos, C., Tan, E., Naidoo, N., Pawitan, Y., Roukos, D., Mort, M., and Cooper, D.N. (2013). A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. Molecular Psychiatry *18*, 141-153.
- Leonard, J.V. (2006). Disorders of the urea cycle and related enzymes. In Inborn Metabolic Diseases (Springer), pp. 263-272.
- Lupski, J.R. (2013). Genome mosaicism—one human, multiple genomes. Science *341*, 358-359.
- Lynch, M. (2010). Rate, molecular spectrum, and consequences of human mutation. Proceedings of the National Academy of Sciences *107*, 961-968.
- Martín-Hernández, I. (2005). Una aproximación a los desórdenes hereditarios del ciclo de la urea en el hombre. Rev Biomed *16*, 193-206.
- Moloney, D.M., Slaney, S.F., Oldridge, M., Wall, S.A., Sahlin, P., Stenman, G., and Wilkie, A.O. (1996). Exclusive paternal origin of new mutations in Apert syndrome. Nature Genetics *13*, 48-53.
- Nachman, M.W., and Crowell, S.L. (2000). Estimate of the mutation rate per nucleotide in humans. Genetics *156*, 297-304.
- Nakagawa, T., and Guarente, L. (2009). Urea cycle regulation by mitochondrial sirtuin, SIRT5. Aging (Albany NY) *1*, 578-581.
- Nakagawa, T., Lomb, D.J., Haigis, M.C., and Guarente, L. (2009). SIRT5 Deacetylates carbamoyl phosphate synthetase 1 and regulates the urea cycle. Cell *137*, 560-570.
- Natesan, V., Mani, R., and Arumugam, R. (2016). Clinical aspects of urea cycle dysfunction and altered brain energy metabolism on modulation of glutamate receptors and transporters in acute and chronic hyperammonemia. Biomedicine & Pharmacotherapy *81*, 192-202.
- Phenome, E., and Consortium, E.K. (2013). De novo mutations in epileptic encephalopathies. Nature *501*, 217-221.
- Prochazkova, K., Pavlikova, K., Minarik, M., Sumerauer, D., Kodet, R., and Sedlacek, Z. (2009). Somatic TP53 mutation mosaicism in a patient with Li–Fraumeni syndrome. American Journal of Medical Genetics Part A *149*, 206-211.
- Project, G. (2011). Variation in genome-wide mutation rates within and between human families. Nature Genetics *43*, 712-714.
- Raghuveer, T.S., Garg, U., and Graf, W.D. (2006). Inborn errors of metabolism in infancy and early childhood: an update. Am fam physician *73*, 1981-1990.

- Rahbari, R., Wuster, A., Lindsay, S.J., Hardwick, R.J., Alexandrov, L.B., Al Turki, S., Dominiczak, A., Morris, A., Porteous, D., and Smith, B. (2015). Timing, rates and spectra of human germline mutation. Nature Genetics.
- Reichenberg, A., Gross, R., Weiser, M., Bresnahan, M., Silverman, J., Harlap, S., Rabinowitz, J., Shulman, C., Malaspina, D., and Lubin, G. (2006). Advancing paternal age and autism. Archives of General Psychiatry *63*, 1026-1032.
- Rocha, H., Sousa, C., Fonseca, H., Marcão, A., Lopes, L., Carvalho, I., and Vilarinho, L. (2016). Rastreio neonatal dos défices do ciclo da ureia em Portugal. Boletim Epidemiológico Observações *5*, 12-15.
- Rocha, J.C., Sequeira, S., Cabral, A., and Ferreira Almeida, M. (2009). Consenso para o tratamento nutricional das Doenças do Ciclo da Uréia.
- Rodwell, V.W., Bender, D.A., Botham, K.M., Kennelly, P.J., and Weil, P.A. (2016). Bioquímica Ilustrada de Harper. In (McGraw Hill Brasil).
- Sagi-Dain, L., Sagi, S., and Dirnfeld, M. (2016). The Effect of Paternal Age on Oocyte Donation Outcomes. Obstetrical & gynecological survey *71*, 301-306.
- Saha, S., Barnett, A.G., Foldi, C., Burne, T.H., Eyles, D.W., Buka, S.L., and McGrath, J.J. (2009). Advanced paternal age is associated with impaired neurocognitive outcomes during infancy and childhood. PLoS Med *6*, e1000040.
- Sancho-Vaello, E., Marco-Marín, C., Gougeard, N., Fernández-Murga, L., Rüfenacht, V., Mustedanagic, M., Rubio, V., and Häberle, J. (2016). Understanding N-Acetyl-L-Glutamate Synthase Deficiency: Mutational Spectrum, Impact of Clinical Mutations on Enzyme Functionality, and Structural Considerations. Human Mutation *37*, 679-694.
- Ségurel, L., Wyman, M.J., and Przeworski, M. (2014). Determinants of mutation rate variation in the human germline. Annual Review of Genomics and Human Genetics *15*, 47-70.
- Smith, W., Kishnani, P.S., Lee, B., Singh, R.H., Rhead, W.J., King, L.S., Smith, M., and Summar, M. (2005). Urea cycle disorders: clinical presentation outside the newborn period. Critical Care Clinics *21*, S9-S17.
- Soares, T.J.C. (2014). Sirtuínas-Artigo de Revisão Bibliográfica.
- Strien, J., Sanft, J., and Mall, G. (2013). Enhancement of PCR amplification of moderate GC-containing and highly GC-rich DNA sequences. Molecular Biotechnology *54*, 1048-1054.
- Sun, R.-P., Xi, Q.-Y., Sun, J.-J., Cheng, X., Zhu, Y.-L., Ye, D.-Z., Chen, T., Wei, L.-M., Ye, R.-S., and Jiang, Q.-Y. (2016). In low protein diets, microRNA-19b regulates urea synthesis by targeting SIRT5. Scientific Reports *6*.

- Waisbren, S.E., Gropman, A.L., Batshaw, M.L., and Consortium, U.C.D. (2016). Improving long term outcomes in urea cycle disorders-report from the Urea Cycle Disorders Consortium. Journal of Inherited Metabolic Disease *39*, 573-584.
- Wen, W., Yin, D., Huang, F., Guo, M., Tian, T., Zhu, H., and Yang, Y. (2016). NGS in argininosuccinic aciduria detects a mutation (D145G) which drives alternative splicing of ASL: a case report study. BMC Medical Genetics *17*, 9.
- Wong, W.S., Solomon, B.D., Bodian, D.L., Kothiyal, P., Eley, G., Huddleston, K.C., Baker, R., Thach, D.C., Iyer, R.K., and Vockley, J.G. (2016). New observations on maternal age effect on germline de novo mutations. Nature Communications *7*.
- Xue, Y., Wang, Q., Long, Q., Ng, B.L., Swerdlow, H., Burton, J., Skuce, C., Taylor, R., Abdellah, Z., and Zhao, Y. (2009). Human Y chromosome base-substitution mutation rate measured by direct sequencing in a deep-rooting pedigree. Current Biology *19*, 1453-1457.
- Yu, W., Lin, Y., Yao, J., Huang, W., Lei, Q., Xiong, Y., Zhao, S., and Guan, K.-L. (2009). Lysine 88 acetylation negatively regulates ornithine carbamoyltransferase activity in response to nutrient signals. Journal of Biological Chemistry *284*, 13669-13675.

Supplementary information

Supplementary information I – Primers used in PCR

Table 7: Primers used in PCR for the amplification of *NAGS* gene.

Exon	Primer forward (5´- 3´)	Primer Reverse (5´- 3´)
1	CAACGGCAAGTTAAGAGCCCCCCAG	AGGGGATGGGGTTGTGCGGCCA
2/3	AAGGGTAGGGTCACCGAGACGG	CTCACTAGCAAGCCGGGTGGGTAGA
4	GGGCGGAAGTAAGGATAAAGGGGTC	GTAGGGGGAGGCGGGGGGTGTCA
5/6	GACGGAAATTGTCCCACCAGCGCC	GGCAGAACACACAGAAAGCCTGAGAT
7	GTGAGTAATGAACACTGGCCTTGCCC	GAAGTGACCCCGTGGGAGCAAGA

Table 8: Primers used in PCR for the amplification of *SIRT5* gene.

Exon	Primer Forward (5´- 3´)	Primer Reverse (5´- 3´)
1	GCTGGTGACTGGCTAGCGGTTG	GTTGGAAAGTAATCCCACCCGAGC
2	GATCTTGGGAATAATTCCAGGTG	GGCAGTATTCAGAGTTTTGATCTTC
3	GCCAGTTCAGAGGATGATCTGTTAC	GGGCAAGGTTTTGGAGAAAGCC
4	CACTCACCCAGCAGGTCACATG	CCCCGAAACCCCAGGGAAATG
5	GCCATCTCCAGCCTGCACCTTC	CACCTGTCCTGCTGTTTCAGCATCA
6	TACACTCCAGCCTGGGTAACTGG	GCTCATGCCTGTAATCCCAGCACT
7	GTTTTATACCACATACTGCCAGGC	GTGAAAGGACAAAGGATGAACCAA
8	ATCTGGATGTACTAGGTTTGGGGC	CCTCCCTCCCTTGCTTCTTTCC
9	ACCACAGACCTGCCTGAGTTTG	GTAGACTAAGGTA
10	CAGAAAACTCGAAGAGTGGGACAGG	CCAGTTCAGGAGAGATTCTTGGC

Supplementary information II - Protocol for silver staining

Table 9: Protocol for silver staining used in this project.

1. Ethanol (10%)	10 min
2. Nitric Acid (1%)	5 min
3. Washing with distilled water	2x10 sec
4. Silver nitrate (0,2%)	20 min
5. Washing with distilled water	2x10 sec
6. Sodium carbonate (0,28M) + Formaldehyde (0,02%)	
7. Acetic Acid	2 min
8. Washing with distilled water	

Supplementary information III – Table of genes

Table 10: List of genes associated with *de novo* mutations*.*

○ *A2M*	○ *ABCC9*	○ *ADAMTS10*
○ *A2ML1*	○ *ABCF1*	○ *ADAMTS13*
○ *A2ML2*	○ *ABI3BP*	○ *ADAMTS14*
○ *AAAS*	○ *ABL2*	○ *ADAMTS17*
○ *AAGAB*	○ *ABR*	○ *ADAMTS18*
○ *AASDH*	○ *ACACA*	○ *ADAMTS2*
○ *AASS*	○ *ACACB*	○ *ADAMTS3*
○ *ABCA10*	○ *ACCSL*	○ *ADAMTS6*
○ *ABCA12*	○ *ACE*	○ *ADAMTS9*
○ *ABCA13*	○ *ACKR2*	○ *ADAMTSL1*
○ *ABCA2*	○ *ACLY*	○ *ADAMTSL4*
○ *ABCA3*	○ *ACN9*	○ *ADCY4*
○ *ABCA7*	○ *ACSL5*	○ *ADCY5*
○ *ABCB1*	○ *ACSS1*	○ *ADCY7*
○ *ABCB6*	○ *ACTB*	○ *ADCY9*
○ *ABCC5*	○ *ACTL7B*	○ *ADH1B*
○ *ABCC8*	○ *ACTRT3*	○ *ADH7*

○ *ADNP*	○ *ANKRD17*	○ *ATAD3B*
○ *ADPRM*	○ *ANKRD35*	○ *ATAD5*
○ *ADRBK2*	○ *ANKRD50*	○ *ATCAY*
○ *ADSL*	○ *ANKRD66*	○ *ATF4*
○ *AFAP1L2*	○ *ANKS1B*	○ *ATG12*
○ *AFF2*	○ *ANO3*	○ *ATG2B*
○ *AFF4*	○ *ANO5*	○ *ATG4C*
○ *AFM*	○ *ANO9*	○ *ATIC*
○ *AFTPH*	○ *ANP32A*	○ *ATM*
○ *AGAP1*	○ *ANXA6*	○ *ATP10A*
○ *AGAP2*	○ *AOX1*	○ *ATP10D*
○ *AGO1*	○ *AP3B1*	○ *ATP11A*
○ *AGO4*	○ *AP3B2*	○ *ATP11C*
○ *AGTRAP*	○ *AP3D1*	○ *ATP1A3*
○ *AHCY*	○ *AP3M1*	○ *ATP1A4*
○ *AHDC1*	○ *AP3S2*	○ *ATP1B1*
○ *AHI1*	○ *APAF1*	○ *ATP2A1*
○ *AHNAK*	○ *APBA1*	○ *ATP2B2*
○ *AHR*	○ *APBB1*	○ *ATP2B4*
○ *AIM1L*	○ *APC*	○ *ATP4A*
○ *AIPL1*	○ *APCS*	○ ATP6V1B2
○ *AK5*	○ *APLP1*	○ *ATP7B*
○ *AK9*	○ *APLP2*	○ *ATP8B2*
○ *AKAP12*	○ *APOA1BP*	○ *ATRNL1*
○ *AKAP4*	○ *APOA5*	○ *ATRX*
○ *AKAP6*	○ *APOB*	○ *ATXN2L*
○ *AKAP9*	○ *ARAP1*	○ *AUTS2*
○ *AKR1C2*	○ *AREL1*	○ *AXL*
○ *AKR1C4*	○ *ARFGAP3*	○ *AZIN2*
○ *AKTIP*	○ *ARFGEF2*	○ *B3GNT4*
○ *ALCAM*	○ *ARHGAP19*	○ *B3GNT6*
○ *ALDH16A1*	○ *ARHGAP30*	○ *B4GALNT1*
○ *ALDH18A1*	○ *ARHGAP33*	○ *BACH2*
○ *ALDH1A2*	○ *ARHGAP44*	○ *BAI1*
○ *ALDH1A3*	○ *ARHGAP5*	○ *BAI2*
○ *ALDH1L1*	○ *ARHGEF10L*	○ *BAIAP2*
○ *ALDH4A1*	○ *ARHGEF11*	○ *BAIAP3*
○ *ALDH9A1*	○ *ARHGEF15*	○ *BAZ2B*
○ *ALG13*	○ *ARHGEF17*	○ *BCAR3*
○ *ALK*	○ *ARHGEF5*	○ *BCAT1*
○ *ALMS1*	○ *ARID1B*	○ *BCL11A*
○ *ALOX15*	○ *ARID2*	○ *BCL2L11*
○ *ALOX15B*	○ *ARID5A*	○ *BCL9*
○ *ALPI*	○ *ARIH1*	○ *BCL9L*
○ *ALPK3*	○ *ARL16*	○ *BEST3*
○ *ALPL*	○ *ARL6IP6*	○ *BHLHE40*
○ *ALPPL2*	○ *ARNT*	○ *BICC1*
○ *ALS2*	○ *ARRDC1*	○ *BICD1*
○ *ALS2CL*	○ *ARSA*	○ *BICD2*
○ *ALS2CR11*	○ *ARSE*	○ *BIRC6*
○ *AMOTL1*	○ *ARVCF*	○ *BLK*
○ *AMPH*	○ *ASAH2*	○ *BLNK*
○ *AMY2B*	○ *ASAP1*	○ *BNIPL*
○ *ANAPC5*	○ *ASAP3*	○ *BRAF*
○ *ANGPT4*	○ *ASCL3*	○ *BRCA1*
○ *ANK1*	○ *ASH1L*	○ *BRCA2*
○ *ANK2*	○ *ASIC4*	○ *BRD4*
○ *ANK3*	○ *ASNA1*	○ *BRIP1*
○ *ANKAR*	○ *ASPHD1*	○ *BRPF1*
○ *ANKRD11*	○ *ASPM*	○ *BRSK1*
○ *ANKRD12*	○ *ASXL1*	○ *BSND*
○ *ANKRD13A*	○ *ASXL3*	○ *BTAF1*
○ *ANKRD13D*	○ *ATAD2*	○ *BTBD10*
○ *ANKRD16*	○ ATAD2B	○ BTN3A3

○ *BUB1*	○ *CAPRIN1*	○ *CEBPG*
○ *BZRAP1*	○ *CAPRIN2*	○ *CELA1*
○ *C10orf68*	○ *CAPZA1*	○ *CELA3B*
○ *C10orf71*	○ *CARD11*	○ *CELF4*
○ *C10orf90*	○ *CARKD*	○ *CELSR1*
○ *C11orf41*	○ *CASC5*	○ *CELSR2*
○ *C11orf9*	○ *CASK*	○ *CELSR3*
○ *C12orf56*	○ *CASKIN2*	○ *CEP135*
○ *C14orf28*	○ *CASR*	○ *CEP170B*
○ *C14orf80*	○ *CAT*	○ *CEP290*
○ *C15orf38-AP3S2*	○ *CATSPERG*	○ *CEP350*
○ *C15orf42*	○ *CBX5*	○ *CEP55*
○ *C15orf62*	○ *CBX8*	○ *CEP70*
○ *C16orf3*	○ *CCDC116*	○ *CEP85*
○ *C16orf48*	○ *CCDC125*	○ *CES4A*
○ *C16orf62*	○ *CCDC129*	○ *CGNL1*
○ *C17orf53*	○ *CCDC14*	○ *CHCHD10*
○ *C17orf97*	○ *CCDC15*	○ *CHD1*
○ *C18orf25*	○ *CCDC171*	○ *CHD2*
○ *C19orf44*	○ *CCDC173*	○ *CHD3*
○ *C1orf123*	○ *CCDC19*	○ *CHD4*
○ *C1orf173*	○ *CCDC22*	○ *CHD7*
○ *C1orf226*	○ *CCDC30*	○ *CHD8*
○ *C1orf94*	○ *CCDC47*	○ *CHEK2*
○ *C1QTNF6*	○ *CCDC57*	○ *CHIA*
○ *C20orf152*	○ *CCDC65*	○ *CHIC1*
○ *C20orf26*	○ *CCDC82*	○ *CHPF2*
○ *C2CD3*	○ *CCDC87*	○ *CHRM1*
○ *C2orf42*	○ *CCDC88A*	○ *CHRNB1*
○ *C3*	○ *CCDC88C*	○ *CHST2*
○ *C3orf20*	○ *CCKBR*	○ *CHSY1*
○ *C3orf38*	○ *CCL5*	○ *CHSY3*
○ *C5orf42*	○ *CCNB3*	○ *CHTF18*
○ *C7orf33*	○ *CCND2*	○ *CISH*
○ *C7orf60*	○ *CCNL1*	○ *CKAP2*
○ *C9orf11*	○ *CCNT1*	○ *CLASP1*
○ *C9orf114*	○ *CCPG1*	○ *CLCA2*
○ *C9orf64*	○ *CCT4*	○ *CLCN1*
○ *CA10*	○ *CCT7*	○ *CLCN4*
○ *CABIN1*	○ *CD14*	○ *CLCN6*
○ *CABP7*	○ *CD151*	○ *CLCN7*
○ *CACNA1A*	○ *CD163*	○ *CLCNKB*
○ *CACNA1B*	○ *CD163L1*	○ *CLEC16A*
○ *CACNA1C*	○ *CD3EAP*	○ *CLEC3A*
○ *CACNA1D*	○ *CD96*	○ *CLEC4A*
○ *CACNA1E*	○ *CDC42*	○ *CLIP3*
○ *CACNA1G*	○ *CDC42BPB*	○ *CLPB*
○ *CACNA1H*	○ *CDC42EP4*	○ *CLRN3*
○ *CACNA1I*	○ *CDCA7*	○ *CLSPN*
○ *CACNA1S*	○ *CDCA7L*	○ *CLSTN3*
○ *CACNA2D1*	○ *CDH10*	○ *CLTC*
○ *CACNB2*	○ *CDH13*	○ *CLTCL1*
○ *CAD*	○ *CDH23*	○ *CMAS*
○ *CALML6*	○ *CDH5*	○ *CNDP2*
○ *CAMK2A*	○ *CDHR5*	○ *CNGA3*
○ *CAMK2G*	○ *CDK13*	○ *CNGB1*
○ *CAMKK2*	○ *CDKL5*	○ *CNNM3*
○ *CAMSAP3*	○ *CDKN3*	○ *CNOT1*
○ *CAP1*	○ *CDO1*	○ *CNOT3*
○ *CAPG*	○ *CDON*	○ *CNST*
○ *CAPN10*	○ *CDR2*	○ *CNTF*
○ *CAPN15*	○ *CDS2*	○ *CNTN2*
○ *CAPN5*	○ *CDYL2*	○ *CNTN5*
○ *CAPN9*	○ *CEACAM16*	○ *CNTNAP4*

○ *CNTRL*	○ *CYP17A1*	○ *DLG1*
○ *COG4*	○ *CYP1A2*	○ *DLG4*
○ *COG7*	○ *CYP26C1*	○ *DLL1*
○ *COL12A1*	○ *CYP2J2*	○ *DLL4*
○ *COL16A1*	○ *CYP2U1*	○ *DLST*
○ *COL23A1*	○ *CYP4F12*	○ *DMBX1*
○ *COL27A1*	○ *DAAM2*	○ *DMC1*
○ *COL4A1*	○ *DACH1*	○ *DMD*
○ *COL4A3BP*	○ *DAO*	○ *DMPK*
○ *COL4A4*	○ *DAP3*	○ *DMXL2*
○ *COL6A1*	○ *DAPK1*	○ *DNAH1*
○ *COL6A5*	○ *DAPK3*	○ *DNAH10*
○ *COL6A6*	○ *DARC*	○ *DNAH11*
○ *COL7A1*	○ *DBN1*	○ *DNAH14*
○ *COLEC12*	○ *DBR1*	○ *DNAH17*
○ *COPB2*	○ *DCAF13*	○ *DNAH3*
○ *COPG1*	○ *DCAF4L2*	○ *DNAH5*
○ *COPZ1*	○ *DCAKD*	○ *DNAH6*
○ *CPA4*	○ *DCDC5*	○ *DNAH7*
○ *CPAMD8*	○ *DCHS1*	○ *DNAH9*
○ *CPD*	○ *DCHS2*	○ *DNAJA4*
○ *CPM*	○ *DCST1*	○ *DNAJB1*
○ *CPNE6*	○ *DCUN1D3*	○ *DNAJB13*
○ *CPSF1*	○ *DDB2*	○ *DNAJC13*
○ *CPSF3*	○ *DDHD1*	○ *DNAJC16*
○ *CPZ*	○ *DDI2*	○ *DNAJC5B*
○ *CR1*	○ *DDIT3*	○ *DNAJC6*
○ *CR1L*	○ *DDO*	○ *DNASE1L2*
○ *CR2*	○ *DDR2*	○ *DNASE2B*
○ *CRB2*	○ *DDX10*	○ *DNHD1*
○ *CREB5*	○ *DDX20*	○ *DNM1*
○ *CREBBP*	○ *DDX23*	○ *DNM3*
○ *CROCC*	○ *DDX3X*	○ *DNMT1*
○ *CRYBG3*	○ *DDX42*	○ *DNMT3A*
○ *CSAD*	○ *DDX50*	○ *DNMT3B*
○ *CSDE1*	○ *DEAF1*	○ *DOCK1*
○ *CSF1*	○ *DEF8*	○ *DOCK10*
○ *CSF2RB*	○ *DEFB128*	○ *DOCK11*
○ *CSF3R*	○ *DENND2C*	○ *DOCK2*
○ *CSMD1*	○ *DENND3*	○ *DOCK4*
○ *CSMD2*	○ *DENND4A*	○ *DOCK5*
○ *CSMD3*	○ *DENND4B*	○ *DOCK7*
○ *CSNK1G3*	○ *DENND6A*	○ *DOCK9*
○ *CSNK2A1*	○ *DEPDC7*	○ *DPP4*
○ *CSTF2T*	○ *DET1*	○ *DR1*
○ *CTCF*	○ *DFFB*	○ *DRAM1*
○ *CTNNA2*	○ *DGCR2*	○ *DRD5*
○ *CTNNB1*	○ *DGKH*	○ *DSC1*
○ *CTRC*	○ *DGKZ*	○ *DSCAM*
○ *CTTNBP2*	○ *DHDDS*	○ *DSEL*
○ *CUBN*	○ *DHTKD1*	○ *DSG2*
○ *CUL2*	○ *DHX29*	○ *DSG3*
○ *CUL3*	○ *DHX38*	○ *DST*
○ *CUL4A*	○ *DHX58*	○ *DTNA*
○ *CUL5*	○ *DIAPH3*	○ *DTX1*
○ *CUL7*	○ *DICER1*	○ *DTYMK*
○ *CUX1*	○ *DIDO1*	○ *DUOXA1*
○ *CUX2*	○ *DIEXF*	○ *DUOXA2*
○ *CUZD1*	○ *DIP2A*	○ *DUS1L*
○ *CXCR6*	○ *DIP2C*	○ *DUSP15*
○ *CYB561D1*	○ *DISC1*	○ *DVL1*
○ *CYBB*	○ *DISP1*	○ *DVL3*
○ *CYLC1*	○ *DLC1*	○ *DYNC1H1*
○ *CYP11A1*	○ *DLEC1*	○ *DYNC2H1*

○ *DYRK1A*	○ *EXD2*	○ *FLNA*
○ *DYRK1B*	○ *EXOC1*	○ *FLNC*
○ *DYSF*	○ *EXOC4*	○ *FLT4*
○ *DYTN*	○ *EXTL1*	○ *FN1*
○ *DZANK1*	○ *F5*	○ *FNBP1L*
○ *DZIP3*	○ *FA2H*	○ *FOCAD*
○ *EARS2*	○ *FABP2*	○ *FOXA1*
○ *EBF2*	○ *FABP4*	○ *FOXG1*
○ *ECSIT*	○ *FADS2*	○ *FOXK1*
○ *EED*	○ *FADS3*	○ *FOXK2*
○ *EEF1A2*	○ *FAF2*	○ *FOXM1*
○ *EEPD1*	○ *FAHD1*	○ *FOXN3*
○ *EFCAB12*	○ *FAM109A*	○ *FOXP1*
○ *EFHD2*	○ *FAM111B*	○ *FOXRED1*
○ *EFNA2*	○ *FAM129C*	○ *FPGT-TNNI3K*
○ *EFR3B*	○ *FAM134A*	○ *FPR2*
○ *EFTUD2*	○ *FAM135A*	○ *FRAS1*
○ *EHD1*	○ *FAM135B*	○ *FRAT2*
○ *EHMT1*	○ *FAM136A*	○ *FREM2*
○ *EID1*	○ *FAM160B2*	○ *FREM3*
○ *EIF3B*	○ *FAM161B*	○ *FRMD3*
○ *EIF3H*	○ *FAM168B*	○ *FRY*
○ *EIF4ENIF1*	○ *FAM171B*	○ *FRYL*
○ *EIF4G1*	○ *FAM178A*	○ *FSHB*
○ *ELAVL3*	○ *FAM184B*	○ *FUBP1*
○ *ELL*	○ *FAM200B*	○ *FUT9*
○ *ELMO2*	○ *FAM208A*	○ *FYCO1*
○ *EMC1*	○ *FAM208B*	○ *FYTTD1*
○ *EMC4*	○ *FAM20A*	○ *GAB3*
○ *EMILIN2*	○ *FAM21C*	○ *GABRA1*
○ *EMILIN3*	○ *FAM65C*	○ *GABRB1*
○ *EML6*	○ *FAM73A*	○ *GABRB2*
○ *EMR1*	○ *FAM76A*	○ *GABRB3*
○ *ENKD1*	○ *FAM83H*	○ *GABRG3*
○ *ENO3*	○ *FAM86C1*	○ *GABRP*
○ *EP300*	○ *FAN1*	○ *GALC*
○ *EPB41*	○ *FARSA*	○ *GALNT4*
○ *EPB41L1*	○ *FAS*	○ *GAN*
○ *EPB41L4A*	○ *FAT1*	○ *GANAB*
○ *EPB42*	○ *FAT2*	○ *GAPVD1*
○ *EPC1*	○ *FAT3*	○ *GATA6*
○ *EPDR1*	○ *FAT4*	○ *GATAD2B*
○ *EPHA10*	○ *FBLN2*	○ *GBA*
○ *EPHA2*	○ *FBN1*	○ *GBA1*
○ *EPHB1*	○ *FBN2*	○ *GBF1*
○ *EPHB2*	○ *FBXL19*	○ *GBP6*
○ *EPHX1*	○ *FBXO11*	○ *GCK*
○ *EPM1*	○ *FBXO18*	○ *GCLC*
○ *EPPK1*	○ *FBXO31*	○ *GCM2*
○ *EPRS*	○ *FBXO39*	○ *GCN1L1*
○ *EPT1*	○ *FBXW9*	○ *GCNT3*
○ *ERBB2*	○ *FCGR2B*	○ *GDAP2*
○ *ERBB2IP*	○ *FCGR3B*	○ *GDPD5*
○ *ERBB4*	○ *FER1L5*	○ *GEMIN5*
○ *ERCC6L*	○ *FETUB*	○ *GIGYF1*
○ *ERCC6-PGBD3*	○ *FGD3*	○ *GIGYF2*
○ *ERLIN2*	○ *FGFR1*	○ *GIMAP8*
○ *ERMP1*	○ *FGFR2*	○ *GIT1*
○ *ESAM*	○ *FGFR3*	○ *GLB1L3*
○ *ESPN*	○ *FGFR4*	○ *GLI3*
○ *ESRP1*	○ *FHDC1*	○ *GLIPR1L2*
○ *ESRRB*	○ *FIBIN*	○ *GLIS3*
○ *ETFB*	○ *FLAD1*	○ *GLMN*
○ *ETS1*	○ *FLG*	○ GLRA2

○ *GLRA3*	○ *HADHB*	○ *IFITM2*
○ *GLS*	○ *HAPLN2*	○ *IFNA10*
○ *GLT25D1*	○ *HAUS3*	○ *IFNAR2*
○ *GLYR1*	○ *HAUS6*	○ *IFT172*
○ *GMFG*	○ *HAUS8*	○ *IGDCC4*
○ *GNAI1*	○ *HBS1L*	○ *IGFN1*
○ *GNAO1*	○ *HCAR1*	○ *IGSF10*
○ *GNAS*	○ *HCAR2*	○ *IGSF21*
○ *GNB1*	○ *HCK*	○ *IGSF22*
○ *GNB5*	○ *HCN1*	○ *IGSF3*
○ *GNE*	○ *HCN2*	○ *IGSF9B*
○ *GNPDA1*	○ *HCN4*	○ *IKBIP*
○ *GNPTG*	○ *HDAC1*	○ *IKBKG*
○ *GOLGA3*	○ *HDAC10*	○ *IKZF2*
○ *GON4L*	○ *HDAC4*	○ *IL12RB1*
○ *GPCPD1*	○ *HDAC8*	○ *IL1R2*
○ *GPD1*	○ *HDLBP*	○ *IL20RA*
○ *GPHB5*	○ *HEATR5B*	○ *IL20RB*
○ *GPN3*	○ *HECTD1*	○ *IL2RB*
○ *GPR1*	○ *HECW2*	○ *ILVBL*
○ *GPR110*	○ *HELZ2*	○ *IMPDH2*
○ *GPR112*	○ *HEPACAM*	○ *INCENP*
○ *GPR114*	○ *HERC1*	○ *INF2*
○ *GPR132*	○ *HERC2*	○ *ING1*
○ *GPR139*	○ *HEXIM1*	○ *INHBB*
○ *GPR151*	○ *HFE*	○ *INIP*
○ *GPR153*	○ *HIF1A*	○ *INPP4B*
○ *GPR162*	○ *HIPK3*	○ *INPP5B*
○ *GPR84*	○ *HIRA*	○ *INPP5K*
○ *GPR98*	○ *HIST1H2AE*	○ *INSR*
○ *GPRASP1*	○ *HIST1H2BB*	○ *INSRR*
○ *GPRC5B*	○ *HIST1H3C*	○ *INTS1*
○ *GPS1*	○ *HIVEP1*	○ *INTS6*
○ *GRAMD1B*	○ *HIVEP2*	○ *IPMK*
○ *GRAMD2*	○ *HIVEP3*	○ *IPO9*
○ *GRAMD4*	○ *HJURP*	○ *IQCB1*
○ *GREB1L*	○ *HLTF*	○ *IQCC*
○ *GREM1*	○ *HMCN1*	○ *IQGAP3*
○ *GRHL3*	○ *HMHA1*	○ *IQSEC1*
○ *GRIA1*	○ *HMMR*	○ *IQSEC2*
○ *GRID2*	○ *HNRNPF*	○ *IQSEC3*
○ *GRIK5*	○ *HNRNPH1*	○ *IRF2BPL*
○ *GRIN1*	○ *HOXD1*	○ *ITGA10*
○ *GRIN2A*	○ *HPS1*	○ *ITGA11*
○ *GRIN2B*	○ *HRG*	○ *ITGA2B*
○ *GRIN3A*	○ *HRPT2*	○ *ITGA3*
○ *GRIP2*	○ *HS3ST2*	○ *ITGA4*
○ *GRM5*	○ *HSD17B2*	○ *ITGA6*
○ *GRM7*	○ *HSF2*	○ *ITGA7*
○ *GRM8*	○ *HSP90AA1*	○ *ITGA8*
○ *GSAP*	○ *HSP90B1*	○ *ITGAV*
○ *GSE1*	○ *HSPA6*	○ *ITGAX*
○ *GSG1*	○ *HSPA8*	○ *ITGB1*
○ *GSTM3*	○ *HSPG2*	○ *ITGB3*
○ *GTF2IRD1*	○ *HTATIP2*	○ *ITGB4*
○ *GTPBP1*	○ *HTR2A*	○ *ITIH3*
○ *GTPBP3*	○ *HTT*	○ *ITK*
○ *GTPBP4*	○ *HUWE1*	○ *ITM2B*
○ *GUCY1A3*	○ *HYDIN*	○ *ITPA*
○ *GXYLT1*	○ *HYKK*	○ *ITPR1*
○ *GXYLT2*	○ *HYPK*	○ *ITPR2*
○ *GYS2*	○ *IDUA*	○ *ITPR3*
○ *HACE1*	○ *IER2*	○ *JAG1*
○ *HADHA*	○ *IFI44L*	○ *JAK3*

○ *JARID2*	○ *KIF3C*	○ *LIG3*
○ *JMJD1C*	○ *KIFC3*	○ *LIMD1*
○ *JPH3*	○ *KLB*	○ *LIN52*
○ *KALRN*	○ *KLF12*	○ *LIN54*
○ *KAT6A*	○ *KLF2*	○ *LINGO2*
○ *KAT6B*	○ *KLF4*	○ *LIPE*
○ *KBTBD12*	○ *KLHDC1*	○ *LIPJ*
○ *KCNA3*	○ *KLHDC4*	○ *LKB1*
○ *KCNB1*	○ *KLHL11*	○ *LMBRD2*
○ *KCNC1*	○ *KLHL17*	○ *LMTK2*
○ *KCNC3*	○ *KLHL28*	○ *LMTK3*
○ *KCND3*	○ *KLHL4*	○ *LMX1A*
○ *KCNH1*	○ *KLKB1*	○ *LOC100288142*
○ *KCNH5*	○ *KMT2A*	○ *LOC101060181*
○ *KCNH6*	○ *KMT2B*	○ *LONP1*
○ *KCNJ11*	○ *KMT2C*	○ *LOXL2*
○ *KCNJ15*	○ *KMT2D*	○ *LPA*
○ *KCNK12*	○ *KMT2E*	○ *LPAR3*
○ *KCNMA1*	○ *KNDC1*	○ *LPHN1*
○ *KCNQ2*	○ *KPNA1*	○ *LPHN2*
○ *KCNQ5*	○ *KPRP*	○ *LPHN3*
○ *KCNT1*	○ *KRBA1*	○ *LRFN2*
○ *KCNU1*	○ *KRT1*	○ *LRFN3*
○ *KCNV2*	○ *KRT15*	○ *LRP1*
○ *KCTD19*	○ *KRT16*	○ *LRP11*
○ *KCTD6*	○ *KRT20*	○ *LRP1B*
○ *KDM1A*	○ *KRT25*	○ *LRP2*
○ *KDM5A*	○ *KRT27*	○ *LRP4*
○ *KDM5B*	○ *KRT80*	○ *LRP5*
○ *KDM6A*	○ *KRT82*	○ *LRPPRC*
○ *KIAA0090*	○ *KRTAP10-3*	○ *LRPS*
○ *KIAA0100*	○ *KRTAP13-3*	○ *LRRC31*
○ *KIAA0195*	○ *KRTAP9-3*	○ *LRRC40*
○ *KIAA0196*	○ *L1TD1*	○ *LRRC48*
○ *KIAA0319*	○ *L3MBTL1*	○ *LRRC8B*
○ *KIAA0319L*	○ *LAMA1*	○ *LRRC8D*
○ *KIAA0556*	○ *LAMA2*	○ *LRRCC1*
○ *KIAA0586*	○ *LAMA3*	○ *LRRFIP1*
○ *KIAA0664*	○ *LAMA4*	○ *LRRIQ1*
○ *KIAA1009*	○ *LAMA5*	○ *LRRK1*
○ *KIAA1109*	○ *LAMB1*	○ *LSM3*
○ *KIAA1161*	○ *LAMB2*	○ *LSS*
○ *KIAA1217*	○ *LAMB3*	○ *LTBP1*
○ *KIAA1244*	○ *LAMC1*	○ *LTK*
○ *KIAA1324*	○ *LAMC3*	○ *LTN1*
○ *KIAA1324L*	○ *LAMTOR5*	○ *LUZP1*
○ *KIAA1432*	○ *LAPTM5*	○ *LYST*
○ *KIAA1456*	○ *LARGE*	○ *LZTR1*
○ *KIAA1462*	○ *LARP1*	○ *MACC1*
○ *KIAA1468*	○ *LARP4*	○ *MACF1*
○ *KIAA1731*	○ *LARS2*	○ *MAGT1*
○ *KIAA1737*	○ *LAX1*	○ *MAK16*
○ *KIAA2018*	○ *LCE1A*	○ *MAMDC4*
○ *KIAA2022*	○ *LCE1B*	○ *MAML2*
○ *KIF11*	○ *LCMT2*	○ *MAMLD1*
○ *KIF13A*	○ *LCT*	○ *MAN2A2*
○ *KIF14*	○ *LDLRAD1*	○ *MANSC1*
○ *KIF18A*	○ *LEMD3*	○ *MAP2*
○ *KIF1A*	○ *LENG1*	○ *MAP2K1*
○ *KIF1B*	○ *LENG8*	○ *MAP2K7*
○ *KIF20B*	○ *LGR5*	○ *MAP3K1*
○ *KIF21A*	○ *LHFPL3*	○ *MAP3K19*
○ *KIF26B*	○ *LIFR*	○ *MAP4*
○ *KIF2A*	○ *LIG1*	○ *MAP4K1*

○ *MAPK8*	○ *MPG*	○ *MYT1L*
○ *MAPK8IP1*	○ *MPI*	○ *N4BP1*
○ *MAPK8IP3*	○ *MPO*	○ *N4BP2L2*
○ *MAPKBP1*	○ *MPZL3*	○ *N6AMT1*
○ *MAPT*	○ *MR1*	○ *NAA10*
○ *MARCH2*	○ *MRC2*	○ *NAA15*
○ *MARCH5*	○ *MREG*	○ *NAA16*
○ *MARK2*	○ *MROH5*	○ *NAA40*
○ *MASP1*	○ *MRS2*	○ *NACA*
○ *MASP2*	○ *MSANTD1*	○ *NAGLU*
○ *MAST1*	○ *MSH2*	○ *NALCN*
○ *MAST4*	○ *MSH6*	○ *NAP1L2*
○ *MASTL*	○ *MST1R*	○ *NARF*
○ *MAT1A*	○ *MTF1*	○ *NARG2*
○ *MBD5*	○ *MTF2*	○ *NAT8L*
○ *MC4R*	○ *MTG2*	○ *NAV1*
○ *MCCC2*	○ *MTHFS*	○ *NAV2*
○ *MCM6*	○ *MTMR11*	○ *NAV3*
○ *MCM9*	○ *MTMR2*	○ *NBEA*
○ *MCT8*	○ *MTOR*	○ *NBEAL1*
○ *MDM1*	○ *MTR*	○ *NCAN*
○ *MDM2*	○ *MTRF1*	○ *NCAPD2*
○ *MDN1*	○ *MTSS1*	○ *NCAPD3*
○ *MECP2*	○ *MTTP*	○ *NCDN*
○ *MED13L*	○ *MTUS1*	○ *NCKAP1*
○ *MED24*	○ *MUC15*	○ *NCKAP5*
○ *MED26*	○ *MUC16*	○ *NCKIPSD*
○ *MED29*	○ *MUC17*	○ *NCOA1*
○ *MEF2C*	○ *MUC2*	○ *NCOR2*
○ *MEGF11*	○ *MUC5B*	○ *NDST4*
○ *MEIS2*	○ *MUC6*	○ *NEB*
○ *MEN1*	○ *MUM1*	○ *NECAB3*
○ *MEOX2*	○ *MYADML2*	○ *NEDD4L*
○ *MEP1B*	○ *MYBBP1A*	○ *NEK1*
○ *MEST*	○ *MYBL2*	○ *NEK10*
○ *METRNL*	○ *MYBPC2*	○ *NEK5*
○ *METTL16*	○ *MYBPC3*	○ *NEO1*
○ *METTL20*	○ *MYBPHL*	○ *NEURL2*
○ *MFAP1*	○ *MYEF2*	○ *NF1*
○ *MFN1*	○ *MYH10*	○ *NFASC*
○ *MFSD8*	○ *MYH11*	○ *NFATC2*
○ *MGA*	○ *MYH14*	○ *NFE2L3*
○ *MGAT4C*	○ *MYH2*	○ *NFIA*
○ *MGAT5B*	○ *MYH3*	○ *NFIL3*
○ *MGME1*	○ *MYH6*	○ *NFIX*
○ *MIB1*	○ *MYH9*	○ *NFKBIB*
○ *MICALCL*	○ *MYO10*	○ *NFKBIL1*
○ *MIF*	○ *MYO15A*	○ *NFXL1*
○ *MINK1*	○ *MYO16*	○ *NGEF*
○ *MIOS*	○ *MYO18A*	○ *NHSL1*
○ *MKI67*	○ *MYO18B*	○ *NID1*
○ *MKL2*	○ *MYO1D*	○ *NID2*
○ *MKLN1*	○ *MYO1E*	○ *NINL*
○ *MKRN2*	○ *MYO3A*	○ *NIPA1*
○ *MLH1*	○ *MYO5A*	○ *NIPAL3*
○ *MLL2*	○ *MYO5B*	○ *NIPBL*
○ *MLLT1*	○ *MYO5C*	○ *NISCH*
○ *MMP15*	○ *MYO6*	○ *NLGN1*
○ *MMP3*	○ *MYO7A*	○ *NLRC4*
○ *MMS22L*	○ *MYO7B*	○ *NLRC5*
○ *MN1*	○ *MYOF*	○ *NLRP1*
○ *MOGAT1*	○ *MYOG*	○ *NLRP12*
○ *MOV10*	○ *MYOM3*	○ *NLRP3*
○ *MOV10L1*	○ *MYOZ2*	○ *NLRP5*

○ *NLRP8*	○ *OR10J3*	○ *PDE3B*
○ *NLRX1*	○ *OR10S1*	○ *PDE4DIP*
○ *NOL9*	○ *OR10Z1*	○ *PDGFB*
○ *NOLC1*	○ *OR11A1*	○ *PDGFRB*
○ *NOMO1*	○ *OR11L1*	○ *PDHA1*
○ *NOP2*	○ *OR1D2*	○ *PDIA2*
○ *NOP58*	○ *OR1S2*	○ *PDIK1L*
○ *NOS2*	○ *OR2D2*	○ *PDPN*
○ *NOS3*	○ *OR2G3*	○ *PDS5A*
○ *NOTCH1*	○ *OR2K2*	○ *PDSS2*
○ *NOTCH3*	○ *OR2T10*	○ *PDZD2*
○ *NPDC1*	○ *OR4E2*	○ *PDZD3*
○ *NPFFR2*	○ *OR5AR1*	○ *PDZRN3*
○ *NR*N1*	○ *OR6C76*	○ *PDZRN4*
○ *NR0B1*	○ *OR6N2*	○ *PEAK1*
○ *NR0B2*	○ *OR8B4*	○ *PEAR1*
○ *NR1D1*	○ *ORC1*	○ *PEG3*
○ *NR1H2*	○ *OS9*	○ *PEPD*
○ *NR2E3*	○ *OSBP*	○ *PES1*
○ *NR3C2*	○ *OSBPL5*	○ *PEX1*
○ *NR4A2*	○ *OSBPL9*	○ *PEX16*
○ *NR6A1*	○ *OSMR*	○ *PFKL*
○ *NRG3*	○ *OXTR*	○ *PFKM*
○ *NRN1L*	○ *P4HA2*	○ *PGAP1*
○ *NRROS*	○ *p63*	○ *PGBD3*
○ *NRSN2*	○ *PABPC4L*	○ *PGD*
○ *NRXN1*	○ *PACS2*	○ *PGM3*
○ *NRXN2*	○ *PAFAH1B2*	○ *PGRMC1*
○ *NSD1*	○ *PAK6*	○ *PHC2*
○ *NSUN6*	○ *PALLD*	○ *PHEX*
○ *NSUN7*	○ *PAPD4*	○ *PHF14*
○ *NTM*	○ *PAPSS1*	○ *PHF19*
○ *NTN1*	○ *PAPSS2*	○ *PHF2*
○ *NTN3*	○ *PAQR4*	○ *PHF3*
○ *NTN5*	○ *PARD3B*	○ *PHIP*
○ *NTNG1*	○ *Parkin gene*	○ *PHRF1*
○ *NTRK1*	○ *PARM1*	○ *PI4KA*
○ *NTRK2*	○ *PARP10*	○ *PIGM*
○ *NTRK3*	○ *PASK*	○ *PIGR*
○ *NUAK1*	○ *PATE2*	○ *PIK3AP1*
○ *NUB1*	○ *PAX3*	○ *PIK3C2B*
○ *NUCB1*	○ *PAX5*	○ *PIK3CD*
○ *NUDCD1*	○ *PBRM1*	○ *PIK3R3*
○ *NUDT17*	○ *PCBP3*	○ *PIK3R4*
○ *NUMA1*	○ *PCDH1*	○ *PIKFYVE*
○ *NUP133*	○ *PCDH10*	○ *PINK1*
○ *NUP188*	○ *PCDH12*	○ *PIP4K2C*
○ *NUP210*	○ *PCDH15*	○ *PISD*
○ *NUP214*	○ *PCDH7*	○ *PITPNM1*
○ *NUP62*	○ *PCDHA13*	○ *PITPNM3*
○ *NUP98*	○ *PCDHA2*	○ *PITX2*
○ *NUPL2*	○ *PCDHA4*	○ *PKD1*
○ *NVL*	○ *PCDHB13*	○ *PKD1L3*
○ *NXF3*	○ *PCDHB4*	○ *PKD2*
○ *NYAP2*	○ *PCDHGA10*	○ *PKHD1*
○ *NYNRIN*	○ *PCDHGA2*	○ *PKM*
○ *OAZ3*	○ *PCLO*	○ *PKN1*
○ *OBSCN*	○ *PCNT*	○ *PKN3*
○ *OCRL*	○ *PCNX*	○ *PKNOX2*
○ *ODF2L*	○ *PCNXL2*	○ *PKP3*
○ *ODZ4*	○ *PCNXL3*	○ *PLA1A*
○ *OIP5*	○ *PCSK9*	○ *PLA2G4C*
○ *OLFM2*	○ *PDCD11*	○ *PLBD2*
○ *OR10H2*	○ *PDCD1LG2*	○ *PLCB4*

○ *PLCD1*	○ *PRKG2*	○ *RANBP17*
○ *PLCD3*	○ *PRMT8*	○ *RANBP6*
○ *PLCG1*	○ *PROC*	○ *RANGAP1*
○ *PLCG2*	○ *PROM1*	○ *RAPGEF1*
○ *PLCL1*	○ *PROX2*	○ *RARA*
○ *PLCZ1*	○ *PRPF18*	○ *RARS*
○ *PLD5*	○ *PRPF4B*	○ *RASGRF1*
○ *PLEKHA6*	○ *PRR14*	○ *RASIP1*
○ *PLEKHH1*	○ *PRR19*	○ *RASSF10*
○ *PLEKHM1*	○ *PRR22*	○ *RASSF5*
○ *PLEKHO1*	○ *PRR25*	○ *RAVER1*
○ *PLEKHO2*	○ *PRRC2A*	○ *RB1*
○ *PLVAP*	○ *PRRC2B*	○ *RBBP5*
○ *PLXDC1*	○ *PRRC2C*	○ *RBFOX2*
○ *PLXNA1*	○ *PRSS38*	○ *RBL2*
○ *PLXNA3*	○ *PRUNE2*	○ *RBM20*
○ *PLXNB1*	○ *PRX*	○ *RBM27*
○ *PLXNB2*	○ *PSAPL1*	○ *RBM3*
○ *PLXNB3*	○ *PSD4*	○ *RBM8A*
○ *PLXND1*	○ *PSEN1*	○ *RBMS3*
○ *PM20D1*	○ *PSG11*	○ *RBP7*
○ *PMEL*	○ *PSMD2*	○ *RCC1*
○ *PMFBP1*	○ *PSME4*	○ *RCOR2*
○ *PML*	○ *PSPC1*	○ *RD3*
○ *PMP22*	○ *PTAFR*	○ *RDH5*
○ *PMS1*	○ *PTAR1*	○ *RELA*
○ *PNLIPRP2*	○ *PTBP1*	○ *RELN*
○ *PNMAL1*	○ *PTCH1*	○ *RERE*
○ *PNPLA7*	○ *PTDSS1*	○ *REV3L*
○ *POGZ*	○ *PTEN*	○ *RFC5*
○ *POLD2*	○ *PTGFRN*	○ *RFWD2*
○ *POLL*	○ *PTGIS*	○ *RFX3*
○ *POLQ*	○ *PTH*	○ *RFX8*
○ *POLR2M*	○ *PTK2B*	○ *RGL1*
○ *POLR3B*	○ *PTK7*	○ *RGPD4*
○ *POM121C*	○ *PTMS*	○ *RGS13*
○ *POU3F1*	○ *PTPN12*	○ *RGS14*
○ *POU6F1*	○ *PTPN3*	○ *RGS22*
○ *PPAP2A*	○ *PTPN7*	○ *RHOG*
○ *PPAPDC2*	○ *PTPRF*	○ *RHPN2*
○ *PPCS*	○ *PTPRG*	○ *RIF1*
○ *PPFIA1*	○ *PTPRJ*	○ *RIMS1*
○ *PPFIA2*	○ *PTPRK*	○ *RIN2*
○ *PPL*	○ *PTPRS*	○ *RINL*
○ *PPM1B*	○ *PTPRU*	○ *RIOK3*
○ *PPP1R13B*	○ *PUF60*	○ *RLIM*
○ *PPP1R3B*	○ *PURA*	○ *RNF112*
○ *PPP2R1B*	○ *PXDNL*	○ *RNF123*
○ *PPP2R5D*	○ *PXMP4*	○ *RNF20*
○ *PPWD1*	○ *PYCARD*	○ *RNF213*
○ *PRAMEF1*	○ *PYHIN1*	○ *RNF38*
○ *PRCP*	○ *QPCT*	○ *RNF44*
○ *PRDM1*	○ *QRSL1*	○ *RNPEPL1*
○ *PRDM4*	○ *QTRT1*	○ *ROCK1*
○ *PRDM6*	○ *RAB10*	○ *RP1*
○ *PRDX3*	○ *RAB11FIP4*	○ *RP1L1*
○ *PREPL*	○ *RAB25*	○ *RP2*
○ *PRIM1*	○ *RAB43*	○ *RPF1*
○ *PRKAA1*	○ *RABGAP1L*	○ *RPGRIP1*
○ *PRKACB*	○ *RAD18*	○ *RPGRIP1L*
○ *PRKAR2B*	○ *RAD51*	○ *RPL5*
○ *PRKCA*	○ *RAD54L2*	○ *RPP40*
○ *PRKCE*	○ *RALGAPB*	○ *RPRD1A*
○ *PRKD2*	○ *RALGDS*	○ *RPS15*

○ *RPS3A*	○ *SETBP1*	○ *SLC5A7*
○ *RPS6KA1*	○ *SETD2*	○ *SLC5A8*
○ *RPS6KA3*	○ *SETD5*	○ *SLC5A9*
○ *RPUSD4*	○ *SETDB2*	○ *SLC6A1*
○ *RRAGC*	○ *SEZ6*	○ *SLC6A13*
○ *RREB1*	○ *SEZ6L2*	○ *SLC6A17*
○ *RRP1B*	○ *SF1*	○ *SLC6A8*
○ *RRP8*	○ *SFPQ*	○ *SLC7A5*
○ *RS1*	○ *SFTPC*	○ *SLC7A6*
○ *RSBN1*	○ *SFXN5*	○ *SLC7A7*
○ *RTKN2*	○ *SGK223*	○ *SLC8A3*
○ *RTN4*	○ *SGSM2*	○ *SLC9A1*
○ *RTN4R*	○ *SGSM3*	○ *SLC9A3*
○ *RUFY2*	○ *SH2B1*	○ *SLC9A3R1*
○ *RUNDC3A*	○ *SH2D3C*	○ *SLC9A3R2*
○ *RUNDC3B*	○ *SHANK1*	○ *SLC9A8*
○ *RUNX1*	○ *SHANK3*	○ *SLC9C2*
○ *RUNX3*	○ *SHH*	○ *SLCO1B3*
○ *RUSC1*	○ *SHKBP1*	○ *SLCO1B7*
○ *RUVBL1*	○ *SHOX2*	○ *SLCO1C1*
○ *RXFP1*	○ *SHPRH*	○ *SLCO4A1*
○ *RXFP3*	○ *SHQ1*	○ *SLFN5*
○ *RYR1*	○ *SHROOM1*	○ *SLIT2*
○ *RYR2*	○ *SHROOM3*	○ *SLIT3*
○ *RYR3*	○ *SI*	○ *SLITRK5*
○ *SACS*	○ *SIGLEC5*	○ *SLK*
○ *SAFB*	○ *SIK3*	○ *SLMO2*
○ *SAFB2*	○ *SIN3A*	○ *SLX4*
○ *SATB2*	○ *SIN3B*	○ *SMAD2*
○ *SBF1*	○ *SIX3*	○ *SMAD4*
○ *SBNO1*	○ *SKIL*	○ *SMAP2*
○ *SBNO2*	○ *SLAIN2*	○ *SMARCA2*
○ *SCAF11*	○ *SLAMF1*	○ *SMARCC2*
○ *SCAF4*	○ *SLAMF6*	○ *SMC1A*
○ *SCARB2*	○ *SLC10A6*	○ *SMC3*
○ *SCHIP1*	○ *SLC12A6*	○ *SMC6*
○ *SCN10A*	○ *SLC12A9*	○ *SMCHD1*
○ *SCN11A*	○ *SLC16A13*	○ *SMG1*
○ *SCN1A*	○ *SLC17A6*	○ *SMG6*
○ *SCN2A*	○ *SLC18A2*	○ *SMG7*
○ *SCN4A*	○ *SLC1A2*	○ *SMS*
○ *SCN4B*	○ *SLC1A5*	○ *SMURF2*
○ *SCN5A*	○ *SLC22A18*	○ *SMYD1*
○ *SCN8A*	○ *SLC22A25*	○ *SMYD4*
○ *SCNN1D*	○ *SLC22A6*	○ *SNAP25*
○ *SCRN1*	○ *SLC22A9*	○ *SNAP91*
○ *SCYL1*	○ *SLC25A27*	○ *SNAPC5*
○ *SDC3*	○ *SLC25A4*	○ *SNX13*
○ *SDCBP2*	○ *SLC26A11*	○ *SNX19*
○ *SDK1*	○ *SLC26A4*	○ *SNX30*
○ *SEC16B*	○ *SLC26A5*	○ *SNX31*
○ *SEC22C*	○ *SLC26A8*	○ *SOAT1*
○ *SEC31A*	○ *SLC26A9*	○ *SOGA3*
○ *SEMA3E*	○ *SLC2A1*	○ *SOHLH2*
○ *SEMA4G*	○ *SLC30A5*	○ *SON*
○ *SERHL2*	○ *SLC35A2*	○ *SORBS2*
○ *SERINC4*	○ *SLC38A1*	○ *SORCS1*
○ *SERPINA3*	○ *SLC38A3*	○ *SOS1*
○ *SERPINB7*	○ *SLC39A12*	○ *SOS2*
○ *SERPINB8*	○ *SLC40A1*	○ *SP2*
○ *SERPINH1*	○ *SLC4A8*	○ *SP7*
○ *SESN2*	○ *SLC52A1*	○ *SPAG17*
○ *SESN3*	○ *SLC5A10*	○ *SPAG9*
○ *SESTD1*	○ *SLC5A2*	○ *SPAST*

○ *SPATA2*	○ *SYNM*	○ *THSD7A*
○ *SPATA2L*	○ *SYNRG*	○ *TIAL1*
○ *SPATA4*	○ *SYT14*	○ *TIFA*
○ *SPATA5*	○ *SYT15*	○ *TINF2*
○ *SPATA9*	○ *SYT7*	○ *TIPIN*
○ *SPDYC*	○ *SYT9*	○ *TJP3*
○ *SPEG*	○ *SYVN1*	○ *TLK1*
○ *SPEN*	○ *TAAR2*	○ *TLK2*
○ *SPG11*	○ *TACC1*	○ *TLN1*
○ *SPRED2*	○ *TAF13*	○ *TLR10*
○ *SPRR1A*	○ *TAF1C*	○ *TLR5*
○ *SPTAN1*	○ *TAF6*	○ *TLR8*
○ *SPTBN1*	○ *TANC1*	○ *TM2D2*
○ *SPTBN5*	○ *TANC2*	○ *TM9SF2*
○ *SQLE*	○ *TAOK3*	○ *TM9SF3*
○ *SRCAP*	○ *TAPBPL*	○ *TMC4*
○ *SRCIN1*	○ *TARBP1*	○ *TMCO5A*
○ *SREBF2*	○ *TARS2*	○ *TMEM120A*
○ *SRGAP3*	○ *TAS2R10*	○ *TMEM125*
○ *SRPR*	○ *TATDN2*	○ *TMEM173*
○ *SRRM2*	○ *TBC1D30*	○ *TMEM201*
○ *SS18L1*	○ *TBC1D31*	○ *TMEM206*
○ *SSC5D*	○ *TBC1D4*	○ *TMEM41A*
○ *SSH2*	○ *TBC1D8B*	○ *TMEM54*
○ *SSPO*	○ *TBCK*	○ *TMEM62*
○ *SSRP1*	○ *TBL1*R1*	○ *TMEM63A*
○ *ST*1B*	○ *TBL1XR1*	○ *TMEM87B*
○ *ST*BP1*	○ *TBR1*	○ *TMEM8A*
○ *ST20-MTHFS*	○ *TBX22*	○ *TMPRSS11D*
○ *ST3GAL3*	○ *TBX5*	○ *TNFAIP3*
○ *ST3GAL6*	○ *TCERG1L*	○ *TNFRSF10B*
○ *STAB1*	○ *TCF20*	○ *TNKS*
○ *STAB2*	○ *TCF3*	○ *TNKS2*
○ *STAC2*	○ *TCF4*	○ *TNNI3K*
○ *STAG1*	○ *TCF7L1*	○ *TNPO2*
○ *STAG2*	○ *TCP10L2*	○ *TNRC18*
○ *STAG3*	○ *TCP11L2*	○ *TNRC6B*
○ *STARD10*	○ *TDRD1*	○ *TNRC6C*
○ *STARD13*	○ *TDRD10*	○ *TOM1*
○ *STARD9*	○ *TDRD12*	○ *TOMM40L*
○ *STAT3*	○ *TDRD6*	○ *TOR1AIP1*
○ *STIL*	○ *TDRD9*	○ *TP53*
○ *STK10*	○ *TDRKH*	○ *TPR*
○ *STK31*	○ *TECTA*	○ *TPTE2*
○ *STK32A*	○ *TEKT1*	○ *TRAPPC8*
○ *STK36*	○ *TENM1*	○ *TRAPPC9*
○ *STK38L*	○ *TEPP*	○ *TREH*
○ *STT3B*	○ *TERF2*	○ *TRIB2*
○ *STXBP1*	○ *TET1*	○ *TRIM10*
○ *STXBP5L*	○ *TET2*	○ *TRIM17*
○ *SUFU*	○ *TEX15*	○ *TRIM29*
○ *SULT1B1*	○ *TFAP2D*	○ *TRIM33*
○ *SULT4A1*	○ *TFEB*	○ *TRIM41*
○ *SUPT16H*	○ *TFG*	○ *TRIM46*
○ *SUPT5H*	○ *TFIP11*	○ *TRIM6*
○ *SUV420H1*	○ *TG*	○ *TRIM8*
○ *SV2A*	○ *TGFB1*	○ *TRIO*
○ *SV2B*	○ *TGM2*	○ *TRIP11*
○ *SVEP1*	○ *TGM3*	○ *TRIP12*
○ *SVIL*	○ *TGM6*	○ *TROAP*
○ *SVOPL*	○ *THADA*	○ *TRPM1*
○ *SYNCRIP*	○ *THAP1*	○ *TRPM5*
○ *SYNE1*	○ *THOC2*	○ *TRPM6*
○ *SYNGAP1*	○ *THRAP3*	○ *TRPV3*

○ *TRPV4*	○ *UTRN*	○ *ZBTB18*
○ *TRPV5*	○ *UVRAG*	○ *ZBTB20*
○ *TRRAP*	○ *VAMP4*	○ *ZBTB40*
○ *TSC2*	○ *VCAN*	○ *ZBTB41*
○ *TSEN2*	○ *VCL*	○ *ZBTB45*
○ *TSHZ1*	○ *VCP*	○ *ZBTB49*
○ *TSHZ2*	○ *VHL*	○ *ZBTB7B*
○ *TSNAX*	○ *VPS11*	○ *ZC3H11A*
○ *TSNAXIP1*	○ *VPS13A*	○ *ZC3H18*
○ *TSPAN17*	○ *VPS13B*	○ *ZC3H6*
○ *TSPEAR*	○ *VPS13C*	○ *ZC3HAV1*
○ *TSR2*	○ *VPS33B*	○ *ZDHHC13*
○ *TTBK1*	○ *VPS35*	○ *ZDHHC16*
○ *TTC21A*	○ *VPS53*	○ *ZDHHC18*
○ *TTC28*	○ *VWA5B1*	○ *ZDHHC5*
○ *TTC39A*	○ *VWA9*	○ *ZEB1*
○ *TTC4*	○ *VWCE*	○ *ZFC3H1*
○ *TTK*	○ *VWF*	○ *ZFHX3*
○ *TTLL5*	○ *WAC*	○ *ZFP62*
○ *TTN*	○ *WARS*	○ *ZFP69*
○ *TTPA*	○ *WDFY2*	○ *ZFPL1*
○ *TTYH1*	○ *WDFY3*	○ *ZFPM2*
○ *TUBA1A*	○ *WDFY4*	○ *ZFR*
○ *TUBG1*	○ *WDHD1*	○ *ZFYVE1*
○ *TULP4*	○ *WDR1*	○ *ZFYVE26*
○ *TUSC3*	○ *WDR19*	○ *ZFYVE9*
○ *TWF2*	○ *WDR20*	○ *ZIC2*
○ *TYK2*	○ *WDR26*	○ *ZMYM1*
○ *TYW5*	○ *WDR33*	○ *ZMYM6*
○ *UACA*	○ *WDR4*	○ *ZMYND11*
○ *UBE2B*	○ *WDR45*	○ *ZMYND12*
○ *UBE2O*	○ *WDR5*	○ *ZMYND8*
○ *UBE3A*	○ *WDR64*	○ *ZNF182*
○ *UBE3C*	○ *WDR87*	○ *ZNF213*
○ *UBR3*	○ *WDR96*	○ *ZNF219*
○ *UBR4*	○ *WFS1*	○ *ZNF221*
○ *UBR5*	○ *WHSC1L1*	○ *ZNF25*
○ *UCHL3*	○ *WIBG*	○ *ZNF263*
○ *UFL1*	○ *WIPI2*	○ *ZNF266*
○ *UGGT1*	○ *WIZ*	○ *ZNF268*
○ *UIMC1*	○ *WNK1*	○ *ZNF292*
○ *UMODL1*	○ *WNK2*	○ *ZNF318*
○ *UNC13A*	○ *WNK4*	○ *ZNF326*
○ *UNC13B*	○ *WNT5A*	○ *ZNF335*
○ *UNC13C*	○ *WRN*	○ *ZNF34*
○ *UNC45B*	○ *WTX*	○ *ZNF397*
○ *UNC80*	○ *WWC2*	○ *ZNF407*
○ *UPF2*	○ *WWC3*	○ *ZNF410*
○ *UQCRC2*	○ *WWP2*	○ *ZNF419*
○ *URB1*	○ *XIRP1*	○ *ZNF420*
○ *USF2*	○ *XIRP2*	○ *ZNF423*
○ *USH1C*	○ *XKR6*	○ *ZNF425*
○ *USH2A*	○ *XPA*	○ *ZNF44*
○ *USP34*	○ *XPO1*	○ *ZNF460*
○ *USP38*	○ *XPO4*	○ *ZNF462*
○ *USP4*	○ *XPOT*	○ *ZNF490*
○ *USP40*	○ *XRCC5*	○ *ZNF521*
○ *USP44*	○ *YARS*	○ *ZNF532*
○ *USP45*	○ *YIPF5*	○ *ZNF536*
○ *USP54*	○ *YME1L1*	○ *ZNF541*
○ *USP8*	○ *YTHDC1*	○ *ZNF544*
○ *USP9X*	○ *YWHAG*	○ *ZNF555*
○ *UTP14C*	○ *YWHAZ*	○ *ZNF559*
○ *UTP6*	○ *ZBTB17*	○ *ZNF576*

○ *ZNF598*	○	○
○ *ZNF607*	○	○
○ *ZNF623*	○	○
○ *ZNF644*	○	○
○ *ZNF665*	○	○
○ *ZNF675*	○	○
○ *ZNF700*	○	○
○ *ZNF761*	○	○
○ *ZNF77*	○	○
○ *ZNF778*	○	○
○ *ZNF79*	○	○
○ *ZNF800*	○	○
○ *ZNF804B*	○	○
○ *ZNF831*	○	○
○ *ZNF839*	○	○
○ *ZNF880*	○	○
○ *ZNF99*	○	○
○ *ZNFX1*	○	○
○ *ZP4*	○	○
○ *ZRANB3*	○	○
○ *ZSCAN12*	○	○
○ *ZSCAN2*	○	○
○ *ZSWIM8*	○	○
○ *ZWILCH*	○	○
○ *ZZEF1*	○	○
○ *ZZZ3*	○	○