UNIVERSIDADE D
COIMBRA

Sofia Grãos Rodrigues

Relatório de Estágio sob a orientação da Dra. Carina Cordeiro dos Santos e Monografia intitulada "Gene therapy in the treatment of Genetic Epilepsies" sob orientação do Professor Doutor Luís Pereira de Almeida", referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em ciências Farmacêuticas.

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Eu, Sofia Grãos Rodrigues, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o n.º 2018289217, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "Gene therapy in the treatment of Genetic Epilepsies" apresentado à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 14 de julho de 2023.

Sofia Grãos Rodrigues

(Sofia Grãos Rodrigues)

Agradecimentos

À Raquel, por ser sempre o meu porto seguro, só tenho a dizer " *And I know we'll be alright, child, just close your eyes and see, and I'll be by your side, any time you're needing me*". *Bikimana*, adoro-te incondicionalmente.

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"Prometeram que me sentiria em casa. E, de uma maneira estranha e confusa, sempre achei que teriam razão."

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Parte I

Relatório de Estágio Curricular em Farmácia Comunitária

Sob orientação da Dra. Carina Cordeiro dos Santos

ABREVIATURAS

DCI	Denominação Comum Internacional
MICF	Mestrado Integrado em Ciências Farmacêuticas
MNSRM	Medicamentos não sujeitos a receita médica
MSRM	Medicamentos sujeitos a receita médica
OTCs	*Over-the-counter products*
PIM	Preparação Individualizada da Medicação

INTRODUÇÃO

A profissão farmacêutica é uma parte integrante de toda a cadeia do medicamento, desde a sua génese ao seu aconselhamento e dispensa sendo, portanto, uma profissão crucial na área da saúde que, de forma integrada com os restantes profissionais desta área, procura e assegura a saúde e bem-estar da população. Os farmacêuticos destacam-se desde a farmácia comunitária, à área da Saúde Publica, bem como à área de investigação e desenvolvimento de medicamentos (Ordem dos Farmacêuticos, [s.d.]).

O farmacêutico comunitário é, muitas vezes, o primeiro contacto da comunidade com a área da saúde, sendo fulcral na prevenção, tratamento e monitorização de doenças tanto agudas, como crónicas. A profissão farmacêutica permite assim que o farmacêutico, em ambulatório, atue em várias áreas, das quais destaco a gestão da terapêutica, o aconselhamento, a administração de medicamentos, a determinação de parâmetros bioquímicos, a identificação precoce, a prevenção de certas doenças, assim como a promoção de estilos de vida saudáveis (Ordem dos Farmacêuticos, [s.d.]).

A distribuição geográfica das farmácias comunitárias em Portugal permite que as mesmas sejam, muitas vezes, o único contacto próximo com um espaço de saúde e a competência técnico-científica, a possibilidade de dispensa de medicamentos não sujeitos a receita médica (MNSRM) e de medicamentos de dispensa exclusiva em farmácia, assim como todos os serviços de prevenção, promoção e controlo de certas patologias, permite aos farmacêuticos oferecer à comunidade serviços que evitam o seu deslocamento a outros serviços de saúde (Ordem dos Farmacêuticos, [s.d.]).

Depois de vários semestres focados nas várias áreas teóricas que compõem a nossa formação académica, o estágio foi percecionado como o culminar de todas estas aprendizagens e o confrontar com aquilo que é ser farmacêutico em Portugal, com todas as suas vantagens, mas também com todas as dificuldades inerentes. Ao longo do curso, o papel do farmacêutico é recorrentemente um assunto de debate e discussão. Foram vários os trabalhos em que cheguei a abordar este tema; contudo, reconheço, agora, que não percebia a dimensão daquilo que escrevia até o experienciar na primeira pessoa. Ao dialogar e ao escutar os utentes que, diariamente, recorriam à farmácia, por questões do quotidiano ou por situações de maior urgência, vi que têm no farmacêutico um profissional de saúde próximo, a quem confidenciam e recorrem.

FARMÁCIA LIS

Entre os meses de janeiro e junho, realizei o meu estágio curricular em Farmácia Comunitária na Farmácia Lis, em Leiria, perfazendo um total de 816 horas de formação.

A farmácia localiza-se numa zona de Leiria que regista elevada movimentação diária, refletida na elevada movimentação dentro do estabelecimento, principalmente a horas chave como o início da manhã, e o período pós-laboral. A farmácia Lis encontra-se aberta de segunda-feira a domingo, das 9h às 00h. Pelo seu extenso horário de funcionamento, a equipa que integra esta farmácia é composta por dois turnos.

A Direção Técnica encontra-se ao encargo do Dr. António Antunes. Esta farmácia é parte integrante de um grupo de farmácias distribuídas por Leiria, Santarém e Figueira da Foz.

ANÁLISE SWOT

PONTOS FORTES

Equipa Técnica

O respeito, a confiança e a entreajuda são, na minha opinião, as melhores palavras para descrever os farmacêuticos e técnicos de farmácia que trabalham na Farmácia Lis e uma das principais, se não mesmo a principal razão do elevado número de clientes fidelizados à farmácia. São uma equipa com um conhecimento técnico-científico inquestionável, que prima pela dedicação, capacidade de resposta e adaptação a cada utente.

Durante o período de estágio, foi-me dada a oportunidade de, não só crescer a nível profissional, como a nível pessoal. Não posso deixar de enaltecer todos os profissionais com os quais trabalhei, convivi e tanto aprendi na minha passagem pela Farmácia Lis. Fui rapidamente acolhida e integrada nas equipas que compõem esta farmácia, observando e desempenhando as várias funções de cada membro que compõe a extensa equipa que ali trabalha, sentindo-me desde cedo uma parte integrante da mesma.

Estiveram sempre disponíveis para me ajudar e esclarecer, deram-me a oportunidade de aprender e melhorar e mostraram-se sempre recetivos e atentos às minhas opiniões e conhecimento.

Uma vez que a equipa técnica é composta por um elevado número de funcionários, achei interessante e educativa a possibilidade de ver as várias abordagens, quer no atendimento, quer na resolução de problemas, dando-me uma ideia geral e, por vezes, perspetivas diversificadas.

Horário, localização e espaço físico

A farmácia composta por três pisos e devidamente identificada, possui instalações adequadas para garantir a qualidade de todos os serviços por ela prestados, assim como espaço de estacionamento, acesso a pessoas com mobilidade condicionada, bem como o *Pharmadrive* (projetado principalmente para utentes com baixa mobilidade ou que estejam acompanhados por crianças pequenas), que garantem o fácil acesso à farmácia a todo o tipo de utentes.

No piso principal, encontramos seis balcões de atendimento prontos a ser utilizados pela equipa. Além disso, no interior da farmácia, encontramos expostos diversos produtos de Saúde, agrupados por áreas de atuação, entre as quais Saúde Oral, Dermofarmácia e Cosmética, Área do Bebé, Espaço Animal, Nutrição, entre outros. Todas estas áreas estão devidamente identificadas e planeadas para que o utente tenha a melhor experiência ao adquirir estes produtos. Grande parte dos MNSRM encontram-se expostos em lineares ou gavetas atrás dos balcões de atendimento, para que não estejam acessíveis ao utente da farmácia.

Para além da área destinada ao atendimento, a Farmácia Lis possui neste piso um gabinete destinado à prestação de variados serviços farmacêuticos, tais como a medição de parâmetros bioquímicos e a administração de injetáveis. É também neste piso que se encontra a área de receção e gestão de encomendas, as instalações sanitárias, lineares e armários deslizantes para armazenamento de medicamentos e dispositivos médicos, bem como um laboratório para a preparação de medicamentos manipulados.

O piso inferior é maioritariamente usado para armazenamento de OTCs (over-the-counter products) e para a receção de encomendas. No piso superior, encontramos o *robot* da farmácia, o *Robot ROWA®*, lineares para armazenamento de produtos e um gabinete onde se realizam alguns dos serviços desta farmácia, entre os quais consultas de Nutrição e consultas do Pé Diabético.

A localização privilegiada da farmácia, localizada perto de uma escola, variados estabelecimentos comerciais, bairros sociais, bem como uma estrada que liga o centro da cidade de Leiria à periferia, permite uma afluência grande e que os atendimentos sejam diversificados, abranjam todas as faixas etárias, classes sociais e áreas de atuação farmacêutica, sendo frequentada por utentes com necessidades muito díspares.

Adaptei o meu horário de estágio de forma a experienciar os vários horários da farmácia, incluindo o horário de fecho (à meia-noite) onde pude experimentar o atendimento ao postigo, realizar o fecho de caixa e proceder às seguranças da farmácia.

Retaguarda e receção de encomendas

Percecionada pela farmácia como uma atividade fulcral para o seu correto funcionamento, a farmácia Lis tem diariamente uma equipa de funcionários exclusivamente na retaguarda, para realizar tarefas como receção de encomendas, gestão de reservas pagas e não pagas, gestão de encomendas, reposição, gestão de *stocks,* aprovisionamento de medicamentos, entre outros. Este regime, ainda que não esteja interdito ao farmacêutico, possibilita aos farmacêuticos responsáveis pelo atendimento, focarem-se, quase que em exclusivo, no utente e no atendimento o que, na minha opinião, é altamente vantajoso. Além disso, permite diminuir os erros inerentes a estas atividades.

Tive a possibilidade de aprender a realizar estas tarefas com pessoas altamente qualificadas e que me explicaram de forma esclarecedora e concisa o importante a reter.

O Sistema Informático utilizado pela farmácia é o Sifarma, desenvolvido pela Associação Nacional de Farmácias e cuja gestão e manutenção está à responsabilidade da Glintt, empresa de Consultoria e Serviços Tecnológicos. Nesta zona, usa-se maioritariamente o sistema antigo, sendo o sistema novo utilizado no atendimento. Contudo, uma vez que nas aulas teórico-práticas da unidade curricular *Organização* e *Gestão Farmacêutica*, aprendemos a trabalhar com os dois sistemas informáticos, não considero que tenha sido complicado a adaptação a estes sistemas, ainda que o sistema antigo seja muito menos intuitivo que o recente.

Plano de Estágio

A estruturação do meu plano de estágio permitiu-me observar e fazer todas as atividades que estão ao encargo de um farmacêutico. Inicialmente, pude perceber como estavam dispostos os produtos na farmácia e como funcionava a retaguarda, onde não só fiz a receção de encomendas, como tratei da reposição, contactando com várias embalagens de produtos, nomes de marca e Denominação Comum Internacional (DCI), iniciando o longo processo de conhecer, reconhecer e associar as diferentes apresentações às aprendizagens teóricas prévias.

Rapidamente, passei a focar-me no atendimento farmacêutico, onde pude observar os meus colegas e comecei a fazer os meus próprios atendimentos. Os meus atendimentos começaram por ser no *Pharmadrive*, oferecendo-me a possibilidade de esclarecer as minhas dúvidas sobre o sistema ou sobre o próprio atendimento com os farmacêuticos, sem me sentir pressionada pelo utente, uma vez que este não se encontrava à minha frente. Foram nestes primeiros atendimentos que pude começar a perceber e a individualizar a maneira de abordar cada tipo de utente. Assim, quando atendi dentro da farmácia, já tinha desenvolvido uma forma mais sistematizada e personalizada de aconselhar e auxiliar aqueles que atendia, sentindo-me

mais confiante e proativa. Foi também aqui que percebi a elevada importância do contacto direto com o utente, preferindo estes atendimentos aos do *Pharmadrive* que acabam por ser, na minha opinião, mais acelerados e menos individualizados, ainda que nunca excluam etapas importantes da dispensa e aconselhamento.

PONTOS FRACOS

Promoção das atividades dinamizadas

A farmácia Lis, durante o período do meu estágio, dinamizou um elevado número de rastreios, atividades e promoções, de entre os quais destaco rastreios capilares, rastreios de insuficiência venosa crónica, rastreios de pele e aconselhamento dermofarmacêutico. Contudo, considero que estas atividades, muitas vezes não foram corretamente promovidas, acabando por não chegar ou chegar tardiamente aos potenciais interessados. No entanto, esta falta de promoção deve-se, muitas vezes, à elevada afluência que se regista na farmácia em momentos do dia considerados "chave" e à falta de meios e tempo que uma correta promoção exige.

Apesar de considerar que as redes sociais não possuem toda a relevância que, por vezes, lhes é atribuída, a nossa presença nas redes sociais não é elevada, especialmente quando comparada a outras farmácias ou espaços de saúde. Ainda assim, considero que a promoção nas redes sociais dos rastreios pela farmácia prestados, poderia aumentar a visibilidade dos mesmos, o que seria benéfico para a farmácia e, especialmente, para a população a que se destinam. Não obstante, informações como promoções nos produtos e algumas atividades promovidas pela farmácia são publicitadas e permitem aos utentes mais interessados um envolvimento que, de outra forma, estaria mais dificultado.

No final do meu estágio, esta dificuldade estava já a ser colmatada, uma vez que a farmácia começou a publicitar certas campanhas e rastreios aos utentes Saúda, via e-mail ou contacto telefónico, no entanto, não tive a oportunidade de assistir aos resultados adjacentes a esta crescente publicitação.

Reservas pagas e não pagas

Inerente à escassez de certos medicamentos que se tem registado em Portugal nos últimos meses, várias são as vezes que a farmácia não tem o produto pretendido aquando da vinda do utente à farmácia. Assim, a estratégia adotada pela equipa foi registar o contacto do utente interessado e fazer uma reserva não paga, avisando a pessoa quando esta reserva já se encontrar disponível para levantamento. Este método resulta muito bem e, no geral, os utentes

ficam satisfeitos com o sistema, ainda que apresentem alguma insatisfação com o tempo de espera de alguns produtos, problema que ultrapassa os limites de atuação da farmácia.

Ainda assim, muitas vezes, fazemos reservas pagas, quando o medicamento em falta na farmácia não se encontra esgotado nos fornecedores e temos a garantia de que este estará disponível para o utente, fazendo assim uma encomenda instantânea do mesmo. Contudo, considero um ponto negativo a quantidade de produto que temos ainda por levantar destas reservas pagas. Estes produtos, sendo propriedade do utente pagador, não podem ser repostos e vendidos, mas ficam na farmácia parados, sendo, na minha opinião, um desperdício de recursos. Assim, ainda que não ideal, penso que a farmácia beneficiaria se, de tempos a tempos, contactasse estes utentes com produtos pagos para que os venham levantar, libertando espaço na farmácia e evitando um certo constrangimento associado à devolução ou reciclagem destes produtos.

Relacionado com o não levantamento de produtos, destaco como ponto negativo, ainda, os medicamentos manipulados que ficam por levantar. Geralmente, medicamentos manipulados apresentam um custo superior aos restantes medicamentos e, por isso, apenas fazemos ou encomendamos o medicamento manipulado após fornecer um orçamento ao utente. Não obstante, muitos utentes acabam por nunca levantar os seus medicamentos manipulados, causando prejuízo à farmácia. Desta forma, a Farmácia Lis adotou uma forma que procura colmatar este prejuízo, exigindo, desde o passado mês de abril, que o utente pague uma caução quando faz a encomenda do manipulado.

OPORTUNIDADES

Rastreios Cardiovasculares

Durante o mês de maio, celebra-se o Mês do Coração, pelo que, com o apoio da marca Apoteca Natura, a Farmácia Lis realizou um Rastreio Cardiovascular, ao longo de todo o mês, que ficou a meu encargo. A Apoteca Natura realizou, com alguma antecedência, um Seminário Cardiovascular onde relembrou alguns aspetos teóricos relevantes e úteis para este rastreio.

Este tinha como principal objetivo a sensibilização dos utentes sobre o bem-estar cardiovascular, assim como a promoção da prevenção através da identificação de potenciais fatores de risco cardiovasculares.

Tive a oportunidade de dialogar e fazer o rastreio a 51 utentes da farmácia. O questionário, permitiu avaliar fatores como a pressão arterial, a deteção de fibrilação auricular, a hipercolesterolemia, o sedentarismo, entre outros. Assim, no final de cada questionário tive a oportunidade de ouvir, aconselhar e explicar aos utentes aquilo que podiam melhorar (principalmente a nível da prática regular de atividade física), os fatores a que deviam prestar

atenção e, principalmente relevante, pude detetar alguns casos de falta de adesão terapêutica ou incorreto uso da medicação.

Esta experiência desenvolveu as minhas *soft skills* a nível social, permitiu-me aplicar conhecimentos teóricos e aproximou-me da população da farmácia, pelo que demonstrou ser altamente benéfica para o meu estágio.

Preparação Individualizada da Medicação (PIM)

Um problema a destacar na área da saúde é a falta de adesão terapêutica. Esta não adesão pode ser categorizada em intencional e não intencional. O serviço de PIM permite minimizar a não adesão não intencional, permitindo promover e assegurar uma utilização correta e segura da medicação.

O serviço de PIM permite ao farmacêutico organizar as formas farmacêuticas sólidas, de uso oral, de acordo com a posologia prescrita num dispositivo próprio, selado de forma estanque na farmácia e descartado após a sua utilização. (Seara.com, [s.d.]) Alguns utentes da farmácia, devido à complexidade dos seus esquemas posológicos, à dificuldade de gestão da medicação por parte dos cuidadores ou às suas limitações físicas, entre outros, são elegíveis para este programa.

Assim, durante o meu estágio, tive a possibilidade de fazer a preparação manual da medicação e a revisão da medicação destes utentes. Esta oportunidade permitiu-me não só consolidar aspetos teóricos, como aumentar e promover o meu contacto com o medicamento, as suas aplicações e várias apresentações.

Preparação de Manipulados

A farmácia Lis possui um laboratório onde realizamos Fórmulas Magistrais (segundo receita médica que especifica o doente a quem o medicamento se destina) e Preparados Oficinais (medicamento preparado segundo indicações compendiais) (*Medicamentos manipulados*, [s.d.]). No laboratório da farmácia apenas realizamos a preparação das formas farmacêuticas "pomada", "xarope" e "solução". As restantes formas farmacêuticas são encomendadas à Farmácia Luciano & Matos, localizada em Coimbra.

A preparação e rotulagem destes medicamentos são da responsabilidade do farmacêutico, que deve assegurar o fornecimento de todas as informações relevantes, como posologia, modo de utilização, condições de conservação e prazo de validade.(*Medicamentos manipulados*, [s.d.]) Durante o estágio, foi-me dada a oportunidade de fazer a preparação de várias formas farmacêuticas, revendo as aprendizagens obtidas na unidade curricular de Farmácia Galénica, entre as quais técnicas de laboratório, preenchimento da ficha de

preparação e cálculo de preços. As fichas de preparação preenchidas são arquivadas durante 3 anos, e são passíveis de consulta.

Ação de Sensibilização sobre o Sol

Em conjunto com uma das farmacêuticas que integra a equipa técnica, realizei uma Ação de Sensibilização sobre o Sol, a duas turmas da pré-primária de um colégio, em Leiria. Dirigimo-nos a essas turmas para explicar aos meninos os benefícios e malefícios do Sol, como podem de forma segura e correta estar expostos ao sol e para lhes lançar um desafio que consistia em fazer um desenho que resumisse aquilo que explicámos. Uma vez que a ação foi feita para crianças entre os 3 e os 6 anos, optámos por recorrer à narração de uma história, com auxílio de uma apresentação PowerPoint com imagens ilustrativas. Este PowerPoint foi cedido pela *Uriage*.

Adorei a oportunidade de ter ajudado a planear e participado nesta atividade. Tive a possibilidade de interagir com uma faixa etária diferente daquela a que, usualmente, me dirijo durante os atendimentos e gostei do desafio de colocar a informação teórica numa linguagem simples e adequada.

Formações Pós-laborais

A transição entre a teoria e a aplicação prática da mesma, no estágio, pode ser um desafio. Somos confrontados com, por exemplo, várias marcas e nomes comerciais de produtos que dificultam esta transição e desaceleram os nossos atendimentos.

Assim, sempre que surgiu a oportunidade, participei e inscrevi-me em formações pós-laborais para tentar colmatar algumas das dificuldades sentidas. Destaco as formações das marcas *Isdin*®, *Apoteca Natura*®, *Aderma*®, *Telfast*® e *Nestlé*®, assim como webinares como "Gastroenterite Infantil: A Intervenção da Farmácia", da Humana.

Além disso, na própria farmácia, houve abordagem formativa por parte dos Delegados de Informação Médica de várias marcas, entre as quais *Fisiocrem*® e *Colgate*®. Estas formações permitiram-me conhecer as linhas dos produtos, esclarecer algumas dúvidas e dar-me maior segurança durante o aconselhamento destes produtos.

Projeto da Câmara Municipal

A farmácia Lis encontra-se localizada numa zona intermédia entre a zona urbana e rural, estando próxima de um bairro social, com uma população tendencialmente carenciada. Assim, a farmácia aderiu a um programa da Câmara Municipal de Leiria, que permite a atribuição de comparticipações em medicamentos a famílias carenciadas do concelho de Leiria.

As condições deste programa encontram-se devidamente regulamentadas e passíveis de consulta no *site* da câmara.

Este programa exige o conhecimento das condições e um responsável por farmácia. Tive a oportunidade de aprender mais sobre este programa, que valorizo e destaco pela proximidade, inclusão e solidariedade que apresenta. Programas como este, retiram o espaço Farmácia de uma visão "apenas comercial" e colocam-na no correto lugar, como um espaço de saúde que tem como prioridade o utente e a sua condição.

AMEAÇAS

Produtos Esgotados/ Rutura de *Stock*

No *site* do Infarmed, podemos ler "A garantia do acesso dos cidadãos aos medicamentos de que necessitam assume-se como uma das mais relevantes vertentes do direito fundamental à proteção da saúde." (*Gestão da disponibilidade do medicamento*, [s.d.]) Infelizmente, e por razões multifatoriais, durante o meu estágio curricular, vários foram os medicamentos que estavam indisponíveis nos fornecedores, causando constrangimentos de menor ou maior grau de preocupação.

Inúmeros foram os atendimentos onde os utentes demonstraram um nível de preocupação e insatisfação elevados, dificultando, por vezes, a comunicação.

Estagiários

A farmácia Lis acolheu, este ano, vários estagiários, vindos de várias faculdades e politécnicos do país. Apesar de rapidamente termos criado laços e tido a oportunidade de partilhar e comparar conhecimentos e experiência, considero que um número menor de estagiários poderia ser benéfico para a farmácia. Em nenhum momento, considerei a minha aprendizagem e estágio comprometidos, no entanto, reconheço que o número de estagiários alterou um pouco a dinâmica e funcionamento das equipas de farmacêuticos que trabalham na Farmácia Lis e que estes tinham, por vezes, dificuldade em gerir as suas tarefas para nos darem total disponibilidade e apoio.

Concorrência

A farmácia Lis encontra-se localizada nas proximidades de uma parafarmácia. Assim, comparação de preços, entre outros temas, são assuntos abordados em vários atendimentos.

Muitas vezes, os utentes deslocam-se à farmácia para pedir aconselhamentos nas áreas de Dermofarmácia e cosmética. No entanto, após ouvirem o aconselhamento e a apresentação das opções de produtos disponíveis, decidem deslocar-se a outros locais, como parafarmácias,

ou mesmo comprar os produtos *online*, devido aos preços mais baixos que estes locais normalmente conseguem praticar. Assim, o processo de aconselhamento, que é uma das mais valias que uma farmácia oferece, acaba por se tornar, por vezes, uma tarefa um pouco ingrata para o farmacêutico.

Foi com algum desagrado que constatei, ainda, que muitos utentes confundem estas parafarmácias e postos de venda de MNSRM com farmácias, desvalorizando inconscientemente, a meu ver, o papel do farmacêutico.

Homeopatia

Uma vez que a Homeopatia não é um tópico abordado nas unidades curriculares do plano curricular do Mestrado Curricular em Ciências Farmacêuticas (MICF), não me senti capacitada para aconselhar e vender produtos homeopáticos. Na farmácia Lis, uma pequena percentagem dos utentes recorre, quase que por exclusividade a este tipo de produtos e, por isso, várias foram as vezes em que tive que recorrer ao farmacêutico com pós-graduação nesta área ou à sua bibliografia, não me sentido de todo apta a fazer este tipo de aconselhamento.

MSRM sem receita médica

Os medicamentos sujeitos a receita médica (MSRM), estão presentes na generalidade dos atendimentos. Contudo, por variadas razões, entre as quais a falta de médicos nos centros de saúde, a demora na renovação de prescrições, dificuldades de acesso à consulta aberta, entre outros motivos que são de conhecimento do público geral e dos profissionais da área da saúde, muitos doentes não têm as receitas médicas necessárias para cumprir os esquemas posológicos habituais.

Além disso, muitos doentes não entendem a necessidade de receita médica para alguns medicamentos, especialmente aqueles que, apesar de serem sujeitos a receita, não são comparticipados pelo estado. Assim, muitos atendimentos ficam dificultados, levando a contestação por parte do utente.

CASOS CLÍNICOS

CASO CLÍNICO 1

LP, mulher de 40 anos, dirige-se à farmácia porque procura algo para alívio da tosse. Após algumas perguntas, concluo que a tosse é uma tosse produtiva, sem febre ou outros sintomas associados, que não piora à noite e que se encontra instalada à cerca de 3 dias. Após questionar a senhora, esta refere que é não fumadora, que tem asma controlada, não é diabética, nem apresenta nenhuma patologia adicional.

Para aliviar a sintomatologia, recomendei um mucolítico, neste caso, ambroxol 6mg/mL (xarope), com o esquema posológico "10mL, 2x/dia". O ambroxol possui propriedades mucocinéticas e expetorantes, atuando, portanto, nas células secretoras e favorecendo a produção de muco mais fácil de eliminar, bem como, aumentando a atividade ciliar. (*Resumo das Características do Medicamento - Benflux Forte 6mg/ml xarope*, [s.d.])

Sendo asmática, optei pelo ambroxol em detrimento da acetilcisteína, contudo esta administração deve ser, na mesma, cuidadosa.

Alertei a utente que inicialmente seria de esperar um ligeiro aumento da expetoração e tosse, devido ao modo de atuação do produto. Adicionalmente, informei que se não sentisse melhorias/notasse agravamento ou se apresentassem novos sintomas, nomeadamente, pressão nos pulmões ou falta de ar, deveria ser auscultada para descartar infeção pulmonar. Finalmente, relembrei a importância de uma hidratação adequada por ingestão ou até por inalação de vapor de água, como terapêutica não farmacológica.

CASO CLÍNICO 2

PA, um homem de 26 anos, pede auxílio na farmácia pois deixou cair água a ferver numa parte da coxa, provocando uma queimadura. A queimadura tem aspeto vermelho, inchado, com aparente início de acumulação de líquido de cor transparente. É dolorosa. Pode ser classificada como queimadura de segundo grau. (*Queimaduras - Lesões e envenenamentos*, [s.d.])

Assim, começo por recomendar um adjuvante de cicatrização como o óxido de zinco 100mg/g (pomada) ou a trolamina 6,7mg/g (emulsão cutânea). Uma vez que o utente refere ter *Biafine*® (ou seja, trolamina 6,7mg/g) em casa, informo que pode aplicá-lo em camada espessa, promovendo a penetração com massagem ligeira. Pode aplicar até 4x/dia, até regeneração total da pele. (*Indicações terapêuticas | www.biafine.pt*, [s.d.])

Recomendo ainda o uso do produto *Grassolind neutral*®, que são compressas esterilizadas, impregnadas com pomada vaselinada não medicamentosa, usadas na proteção e

tratamento de feridas com exsudado, que permitem manter intactos os bordos da ferida, evitam a adesão da compressa à ferida e promovem a granulação e epitelização da pele. (*Grassolind*®, [s.d.]) Assim, garanto a correta proteção da zona queimada ao mesmo tempo que promovo a correta regeneração dos tecidos.

A queimadura deve ser monitorizada nos próximos 3 a 6 dias, para avaliar o seu aspeto e a evolução da regeneração da pele.

Informo que a limpeza da queimadura é importante, mas que deve ser feita apenas com soro fisiológico ou água. Alerto, ainda, para que não rebente as bolhas que se estão a formar devido ao risco de infeção, e para a importância de proteger a área do sol até que a pele se encontre completamente regenerada.

CASO CLÍNICO 3

CF, uma mulher de 37 anos, desloca-se à farmácia para questionar se haveria algum produto que a ajudasse a dormir. Perante este problema, coloco algumas questões para avaliar melhor a situação, entre as quais "Tem dificuldade a adormecer ou em manter o sono?", "Qual a medicação que está a fazer?" ou "Quais os seus hábitos antes de se deitar?". Com as respostas obtidas, percebo que o problema passa pela dificuldade na indução mas também na manutenção do sono, acordando várias vezes ao longo da noite. Além disso, percebo que apenas toma medicação anti-hipertensora. A senhora refere que tem por hábito ver televisão antes de se deitar.

Então, sugiro que comece por experimentar produtos à base de ingredientes naturais. Recomendo, assim, o produto *Arkosono® Forte* 8H. Este produto é um suplemento à base de melatonina (1,9mg), Extratos de plantas (Passiflora, Valeriana e Papoila-da-califórnia) e vitamina B6. É um comprimido de dupla-camada que consegue atuar na indução e na manutenção do sono. Na camada de libertação rápida encontramos a dose ótima de melatonina em libertação rápida (1mg), assim como a Passiflora e a Valeriana, conhecidas pelas propriedades relaxantes e promotoras do sono. Na camada de libertação prolongada, fornece-se uma dose de 0,9mg de melatonina e encontra-se a Papoila-da-califórnia, contribuindo assim para a diminuição dos despertares noturnos. (*Arkosono® Forte 8H*, [s.d.])

Deve ser tomado 1 comprimido, cerca de 1 hora antes de ir dormir, com um grande copo de água. É importante alertar a utente que após a toma do comprimido deve colocar-se num ambiente escuro e propenso ao sono, sem uso de ecrãs, entre outros, para não contrariar os efeitos da melatonina e ajudar a regular o sono.

CASO CLÍNICO 4

FP, uma mulher de 60 anos, aproveitou a ida à farmácia para comentar que, depois de ter tomado um antibiótico, apresenta alguma comichão e ardor na zona genital, alguma vermelhidão e um corrimento branco anormal, sinais e sintomas característicos de uma candidíase. Explico-lhe que a toma oral de antibiótico pode provocar um desequilíbrio na flora vaginal, promovendo o crescimento fúngico.

Assim, para tratamento sugiro o uso do antifúngico clotrimazol 10mg/g (creme vaginal). Deve ser aplicado introduzindo o aplicador com creme vaginal, à noite, ao deitar, durante 6 dias consecutivos. (*Resumo das Características do Medicamento- Gino-Canesten 10mg/g creme vaginal*, [s.d.])

Adicionalmente, para restaurar e equilibrar a flora vaginal, sugiro a suplementação com um probiótico à base de estirpes de lactobacilos, como é o exemplo do *Floradela*®. Recomendei assim a toma de 2 cápsulas por dia, com um copo de água. (*Floradela*® | *Italfarmaco Portugal*, [s.d.])

Alertei, ainda, para a importância de uma correta higiene intima. Em seguimento deste alerta, e já a terminar o atendimento, a senhora refere que o desconforto vulvar já é persistente no tempo pelo que lhe recomendo uma solução de lavagem íntima adequado à sua situação, neste caso, da marca *Saforelle*®, que contém na sua composição a Bardana, uma planta com propriedades calmantes e suavizantes. (*Solução de lavagem - Saforelle*, [s.d.])

CASO CLÍNICO 5

JB, um rapaz de 25 anos, desportista, vai à farmácia com queixas de dor lombar (lombalgia) moderada derivada de uma lesão durante um treino, no dia anterior. Ainda não usou nenhum produto para alivio.

A minha sugestão inicial foi a aplicação de um anti-inflamatório não esteroide a nível tópico, sugerindo o uso do produto *Ib-u-ron*® gel mentol (50mg/g de ibuprofeno).

O utente refere que não queria um gel, perguntando se não existe algo diferente. Assim, sugiro o uso de pensos impregnados, como os pensos *TransAct*® *Lat*, que contêm 40mg de flurbiprofeno, podendo aplicar um só penso no local lesionado, a cada 12h. Em associação, poderá tomar um anti-inflamatório não esteroide via oral, como é o caso do *Voltaren*® 25mg cápsulas moles, de 8/8h, até um máximo de 5 dias. Este deve ser tomado durante ou após uma refeição (*Resumo das Características do Medicamento - Voltaren 25, cápsulas moles*, [s.d.]).

Alerto que, se a dor e inflamação não começarem a melhorar nas próximas 72h, poderá ser necessária avaliação médica. Adicionalmente, sugiro repouso e a aplicação, nos primeiros 2-3 dias, de gelo a nível local, com o intuito de reduzir a inflamação e, passado esse período,

poderá, se considerar necessário, aplicar calor húmido para relaxar o músculo. O retomar da atividade física deve ser gradual, após completa recuperação, evitando cargas excessivas no início.

CONCLUSÃO

O estágio realizado na Farmácia Lis superou as minhas expetativas e considero-o um desafio superado com sucesso e sem percalços. Desde cedo me adaptei à equipa e ao ritmo de trabalho exigido, podendo explorar as distintas tarefas da profissão farmacêutica, com colegas de trabalho que sempre estiveram disponíveis, atentos e me deram o espaço para aprender e crescer.

A oportunidade de estagiar numa farmácia movimentada, com um extenso horário de atendimento e com utentes com características muito díspares, permitiu-me vivenciar atendimentos complexos e diversificados, onde pude aplicar o conhecimento adquirido ao longo do curso de MICF. Destaco a capacidade de adaptação e de resolução de problemas, sendo que muitos dos pontos fracos e ameaças que abordo foram, ao longo do estágio, reconhecidos e estão já a ser solucionados.

Considero todo o estágio extremamente enriquecedor e sinto-me mais calma e preparada para os desafios futuros, com a certeza de que o curso e o estágio curricular me deram as bases para ser uma profissional capacitada, proativa e preparada para os entraves inerentes ao trabalho no atendimento ao público.

Para terminar, resta-me apenas agradecer a todos os profissionais que me acompanharam neste trajeto e me mostraram que, para ser um bom farmacêutico, temos que, não só saber a teoria e garantir a aprendizagem contínua, como procurar sempre ser pessoas preocupadas e humanas.

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Parte II

Monografia

"Gene therapy in the treatment of Genetic Epilepsies"

Sob orientação do Professor Doutor Luís Pereira de Almeida

ABSTRACT

Epilepsy is a prevalent disease of the nervous system that primarily results in recurrent unprovoked seizures. It is estimated that around 50 million people worldwide have epilepsy, with a especial prevalence in developing countries. Epilepsy has a great impact in quality of life, an increased mortality rate, as well as important economic implications. It is calculated that around 70-80% of epilepsies result from genetic factors. Traditional pharmacological approaches are currently seen as insufficient and the use of molecular diagnosis, genetic testing and genetic therapy tools are rapidly increasing and altering approaches surrounding epilepsy diagnosis and treatment. Here in, genetic epilepsies are discussed, particularly, Dravet Syndrome, Angelman Syndrome and *YWHAG* mutations, as well as promising therapeutic strategies for these diseases.

KEYWORDS: Epilepsy; Gene Therapy; Genetic Epilepsies; Dravet Syndrome; Angelman Syndrome; Epileptic Encephalopathy; *YWHAG* mutation.

RESUMO

A epilepsia é uma doença prevalente do sistema nervoso que resulta principalmente em convulsões recorrentes e não provocadas. Estima-se que cerca de 50 milhões de pessoas, mundialmente, tenham epilepsia, com uma prevalência especial em países em desenvolvimento. A epilepsia apresenta um grande impacto na qualidade de vida, um aumento da taxa de mortalidade, bem como importantes implicações a nível económico. Calcula-se que cerca de 70% a 80% das epilepsias sejam resultado de fatores genéticos. As abordagens farmacológicas tradicionais são, atualmente, consideradas insuficientes e a utilização de ferramentas de diagnóstico molecular, testes genéticos e terapia génica estão rapidamente a aumentar e a alterar as abordagens em torno do diagnóstico e tratamento da epilepsia. Aqui são discutidas epilepsias genéticas, particularmente, o Síndrome de *Dravet*, o Síndrome de *Angelman* e mutações YWHAG, bem como estratégias terapêuticas promissoras para estas doenças.

PALAVRAS-CHAVE: Epilepsia; Terapia génica; Epilepsias genéticas; Síndrome de *Dravet*; Síndrome de *Angelman*; Encefalopatia Epilética; mutação *YWHAG*.

ABBREVIATIONS

AAVs	Adeno-associated viruses
ACMG	American College of Medical Genetics and Genomics
AED	Antiepileptic Drug
Array-CGH	Array based comparative genomic hybridization
AS	Angelman Syndrome
ASO	Antisense oligonucleotides
BFNIE	Benign familial neonatal-infantile epilepsy
CAM	Complementary and alternative medicines
CRISPRa	CRISPR activation
CT	Computerized tomography
DALYs	Disability-adjusted life years
DEE	Developmental and Epileptic Encephalopathies
DS	Dravet Syndrome
EE	Epileptic Encephalopathy
EEG	Electroencephalogram
EIMFS	Epilepsy of infancy with migrating focal seizures
EMA	European Medicines Agency
FISH	Fluorescence in Situ Hybridization
GAT-1	GABA transporter
GoF	Gain-of-function
KD	Ketogenic diet
KO	Knock-out
LoF	Loss-of-function
MLPA	Multiplex Ligation-dependent Probe Amplification

MRI	Magnetic resonance imaging
NGS	Next Generation Sequencing
NPY	Neuropeptide Y
PCR-Sanger	Polymerase Chain Reaction-Sanger
PEI	Poliethyleneimine
rAAVs	Recombinant Adeno-associated viruses
RISC	RNA-induced silencing complex
RNAi	RNA interference
shRNAs	Short-hairpin RNAs
siRNAs	Small-interfering RNAs
ssDNA	Single-stranded DNA
SUDEP	Sudden Unexpected Death in Epilepsy
SV2A	synaptic vesicle protein 2A
TH	Tyrosine Hydroxylase
UBE3A-AS	UBE3A antisense transcript
WES	Whole Exome sequencing
WGS	Whole Genome Sequencing
WHO	World Health Organization

1. INTRODUCTION

Epilepsy is defined as a non-infectious chronic disease of the nervous system that targets the brain (Shaimardanova *et al.*, 2022), by occurrence of transient paroxysms of excessive or uncontrolled neuronal discharges, due to a variety of aetiologies, such as genetic, metabolic, structural, infectious, immune or even unknown, leading to epileptic seizures (Perucca, Bahlo e Berkovic, 2020; Regional office for South-East Asia, 2004). Established as a group of conditions without a homogenous manifestation or cause, it is considered as a difficult disease to develop unambiguous diagnostic criteria. It primarily results in recurrent unprovoked seizures, described as episodes of abnormal disturbance on the motor, sensory, autonomic or mental level (Shaimardanova *et al.*, 2022; Thijs *et al.*, 2019). Considered to be one of the most prevalent diseases of the nervous system, with over 50 million reported cases worldwide, epilepsy has been widely studied (Stafstrom e Carmant, 2015). In 2000, the World Health Organization (WHO) reported that epilepsy contributed to more than seven million DALYs (disability-adjusted life year) (0.5%) to the global burden of disease. It was also reported to have significant economic implications derived from health-care service needs, premature mortality and lost work productivity (World Health Organization, 2006).

It is stated that 70-80% of epilepsies are due to one or more genetic factors (Goodspeed *et al.*, 2022). Furthermore, seizures are known to be important manifestations of more than 150 single-gene disorders (Regional office for South-East Asia, 2004).

There are several pharmacological approaches for epilepsy. However the traditional pharmacological approach is sometimes seen as insufficient, in certain manifestations derived from genetic mutations, such as those in *SCN1A* and *YWHAG* genes, that usually cause drug-resistant seizures (Guella *et al.*, 2017; Isom e Knupp, 2021). Drug-resistance applies when the person does not respond to the combination of two appropriate and correctly administered anti-epileptic drugs (Riva *et al.*, 2021).

It is estimated that up to 70% of people diagnosed with epilepsy could have a seizure-free life if it was properly diagnosed and treated (*Epilepsy- World Health Organization*, [s.d.]). With that in mind, gene therapy becomes a main focus point of investigation, showing promising results in reduction of neuronal loss, inflammation, oxidative stress and the durations and frequency of epileptic seizures. By resorting to these new approaches, such as gene therapy, it is believed that besides creating new treatment options for those that can't use the traditional pharmacological approach, it might open a new range of options capable of increasing the neurons' survival, improving and providing neurogenesis and neuro protection, as well as preserving cognitive functions (Shaimardanova *et al.*, 2022). Gene therapy for

epilepsy aims mainly at reducing neuronal excitability by overexpression of certain neuro-modulatory peptides (such as galanin or neuropeptide Y), genetic modification of astrocytes, suppression of glutamate, as well as the overexpression of ion channels (Shaimardanova *et al.*, 2022).

2. EPILEPSY

Acknowledged as a noncommunicable chronic disease of the brain, with a prevalence of around 50 million people worldwide, it can be especially prevalent in developing countries. It is a disease characterized by recurrent seizures that may be partial (when they affect only a part of the body) or generalized (*Epilepsy- World Health Organization*, [s.d.]). But what is the definition of an epileptic seizure? Generally, we can define it as an occurrence in which an individual isn't completely or partially aware of their surroundings, with an abrupt beginning and that ceases on its own. During the seizure, the individual might experience various motor movements, sensory and behavioural experiences, accompanied by confusion and autonomic disturbances like loss of bowel or bladder function, as well as loss of consciousness (Regional office for South-East Asia, 2004). The seizures vary in several aspects from origin and symptomatology to duration and frequency. They can derive from different parts of the brain, resulting from excessive electrical discharges in a group of brain cells (*Epilepsy- World Health Organization*, [s.d.]).

Epilepsy is commonly defined as having two or more of these unprovoked seizures, >24 hours apart. (Fisher *et al.*, 2014) However, in some clinical circumstances this definition is considered inadequate, for example, when an epileptogenic abnormality is present but a seizure does not occur (Fisher *et al.*, 2014; World Health Organization, 2006). As mentioned before, the seizures provoke temporary symptoms such as loss of awareness, motor disturbances, changes in sensations, mood or even cognitive functions, that can lead to physical problems like fractures or other injuries related to falls, drowns or burns. The physical problems can cause premature death and a great portion of those are potentially preventable. People with epilepsy also tend to have higher rates of psychological conditions. The conjugation of the physical and psychological conditions derived from the seizures lead to a risk of premature death calculated as three times higher than for the general population (*Epilepsy- World Health Organization*, [s.d.]).

Epilepsy is a disease that can have several causes, divided into six main categories: genetic, structural, infectious, metabolic, immune and unknow. It can be divided into active epilepsy, when in the last five years there are two or more epileptic seizures that are unprovoked by a direct discernible cause, or inactive epilepsy (Regional office for South-East

Asia, 2004). The underlying cause needs to be investigated. For that, imaging tests like Computerized Tomography (CT) scans, brain magnetic resonance imaging (MRI), as well as Electroencephalogram (EEG) are important tools (*Epilepsies*, 2021).

The recurrence of a seizure depends on multiple factors such as the type of epilepsy, age, aetiology, syndrome, treatment, alongside many others. With currently available drugs, combined with good compliance from the patient, it is possible to achieve a seizure-free life. Even more, in about 70% of the cases, epilepsy is short-lived and relapses are uncommon (Regional office for South-East Asia, 2004). Epilepsy is, generally, considered to be "resolved" when an individual with an age-dependent epilepsy syndrome is past the applicable age or, for those individuals who remain seizure free for 10 years, without taking any anti-seizure medicine for the last 5 years (Fisher *et al.*, 2014).

Epilepsy can commonly be divided into two categories, the first being "idiopathic" when the cause is unknown and the second being "secondary seizures" when there is a known cause. The seizures, in turn, can be categorized as generalized or partial (also known as focal). When generalized, the abnormal electrical charges responsible for the seizure affect both halves of the brain, whereas in the focal ones the electrical abnormalities start from a focal point in one side of the brain. It is important to point out that this focus can then spread to the other side, known as secondary generalization. We can, subsequently, subcategorise generalized seizures in tonic-clonic seizures, clonic seizures, tonic seizures, absence seizures, myoclonic seizures, and infantile spasms (Table I). It is important to highlight the fact that a person can have a combination of types of seizure and that they may change over time, depending on age and maturation of the brain (Regional office for South-East Asia, 2004).

Table I- Classification of Seizure types (Regional office for South-East Asia, 2004).

Seizure types		
Generalized	Tonic	Characterized by sudden muscle contraction, that fixes the limbs in strained positions. There is immediate loss of consciousness. Often it is registered a deviation of the eyes and head towards one side or even rotation of the whole body.
	Clonic	Characterized by repetitive rhythmic flexing and stretching of the limbs.
	Tonic-clonic	Characterized by a tonic phase followed by a clonic phase. Either the tonic phase or the clonic phase can predominate in the seizure.
	Absence	Short periods of loss of consciousness (no more than 30 seconds). Usually, there are not motor manifestations. Described often as a blank stare, brief upward rotation of the eyes and an interruption of ongoing activities, with an absence of response when spoken to. When they are over, the individual returns to activity, without memory of these seizures. They are typically seen in school-aged children and can occur multiple times a day.

	Myoclonic	Shock-like muscle contractions that are described as sudden, brief and that can be felt in one limb or bilaterally. In terms of periodicity, they can be single jerks or repeated over long periods of time. Often seen combined with other seizure types occurring in distinct epileptic syndromes.
	Infantile spasms	Occurring in the first year of life, are perceived as difficult to treat. Characterized as flexor spasms of the head, bending of the knees and flexion with abduction of the arms.
Focal	Simple partial	Consciousness is maintained during these seizures. They can be perceived as a motor or a sensory phenomenon, depending on the specific area of the brain involved. These are only commonly recognized as epileptic seizures when they develop into generalized seizures.
	Complex partial	There is an impairment of consciousness, even though there is no complete loss of it. Usually, the patient is aware of the seizure but cannot respond to external stimuli nor change the behaviour during the episode. The seizures can occur with hallucinations or psychomotor symptoms, known as automatisms.

Certain children under six years old suffer convulsions when they have high fevers, known as febrile seizures. However, even though they have these seizures, they are not labelled as "epileptic". Usually, febrile seizures do not require anti-epileptic drugs and tend to spontaneously disappear after that age, not causing long-term problems (*Febrile Seizures*, [s.d.]). Febrile seizure symptoms can include loss of consciousness, eye rolling, stiff limbs and uncontrollable shaking. They tend to last from one to two minutes, however they might last for over fifteen minutes (*Febrile Seizures*, [s.d.]).

The diagnosis of epilepsy relies a lot on reports from patients and witnesses. Alongside, EEG is sometimes used to support a diagnosis. The EEG evaluates brain function, by looking for the presence or absence of specific brain activity, in specific areas of the brain, so it can detect abnormal brain activity when a seizure happens during the evaluation. However, it is important to note that the EEG alone cannot lead to a diagnosis (*A closer look at EEG | Epilepsy Society*, 2020). For a correct patient management, beyond diagnosis it is of extreme importance to document the type, the cause and the patient's social and personal background (*Epilepsies*, 2021).

There are seizure precipitating factors. These can be subdivided into seizure-inducing factors, with an environmental or endogenous origin and that induce a lowering in the seizure threshold, and seizure-triggering factors, when they involve a chemical or physiological stimulation capable of inducing a seizure (Nakken *et al.*, 2005). Knowing these seizure precipitants can be an important tool to avoid seizures, achieve a good control of epilepsy and increase quality of life. There are over 40 precipitating factors described in the literature such as sleep deprivation, flickering lights, alcohol abuse, stress or physical exercise, among others (Nakken *et al.*, 2005). Despite their importance, these factor tend to be neglected and underestimated, with the clinicians paying them little to no attention. In the study entitled

"Which seizure-precipitating factors do patients with epilepsy most frequently report?" (Nakken *et al.*, 2005) conducted simultaneously in Denmark, Norway and the USA, out of the 1677 patients with epilepsy, 53% reported at least one precipitating factor, with 30% reporting two or more. The most frequently reported factors were emotional stress, sleep deprivation and tiredness. It is important to note that sleep deprivation and flickering lights (about 5% of those with epilepsy are photosensitive) were more prevalent in patients with generalized seizures when compared to the ones with partial seizures. Contrariwise, menstruation was a factor more relevant in women with partial seizures than in women with generalized ones (Nakken *et al.*, 2005). These are examples that leads us to assume that there may be differences in the distribution of these factors between epilepsy types. It is important to emphasize that in previous studies, the percentage of people reporting at least one precipitating factor was higher (62-91%). The author justifies this discrepancy due to the sample used in the study, which included patients who had seizures in the past and that may have forgotten about the precipitating factors, since 73% of the patients with an active form of epilepsy stated the presence of these factors vs only 42% of those with a non active form of epilepsy (Nakken *et al.*, 2005).

2.1 Pharmacological Therapy

As referred before, epilepsy is a polymorphic disease that results in recurrent seizures that can be classified by a physician. Its complex and proper classification is of extreme importance for the correct therapeutic choice of the Antiepileptic Drug (AED) used.

Several new AED have been introduced and there are about 30 different drugs available worldwide (Johannessen Landmark *et al.*, 2023).

Physio pathological mechanisms of the seizures haven't been quite clarified, yet it is established that it correlates to electric instability of neurons' cell membranes. This instability seems to be linked to three of the following main reasons: alterations in the potassium conductance, deficiency in membranal ATPases or in the voltage-gate calcium channels. Since these are the main reasons for the seizures, the pharmacological approach can classically be divided into three main categories: the ones that enhance the inhibition of the sodium channels, the ones which potentiate GABA mediated inhibitory transition and the ones that interfere with voltage-gate calcium channels (*Prontuário Terapêutico online*, [s.d.]). There is also another mechanism of action that invokes glutamate antagonists. These mechanisms of action can have several molecular targets, an extensive pharmacokinetic variability and show a great tendency to have drug interactions (Johannessen Landmark *et al.*, 2023). It is of extreme

importance to recall that these approaches are targeted to the symptoms and not the cause of epilepsy. Figure 1 summarizes the principal mechanism of action of some of these AEDs.

2.1.1 Inhibition of Sodium Channels (voltage-gated)

These cause blockage of the pre-synaptic and post-synaptic voltage-gated sodium channels, leading to the stabilization of the neuronal membrane, preventing the repetitive neuronal firing that results in seizures. Therefore, these drugs narrow the maximum development of the epileptic crisis and reduce their progression (Johannessen Landmark *et al.*, 2023; Kwan e Brodie, 2006).

Some examples of drugs that have this mechanism of action are Oxcarbazepine, Carbamazepine, Valproate, Phenytoin, Lamotrigine, Zonisamide, Felbamate and Topiramate (Kwan e Brodie, 2006).

2.1.2 Inhibition of Calcium Channels

There are two main types of voltage-gate calcium channels: T-type and L-type.

The blockage of L-type channels inhibits pre-synaptic neurotransmitters' release. Some examples of drugs that have this mechanism of action are Pregabalin and Gabapentin (Kwan e Brodie, 2006).

The inhibitors of T-type channels function as pacemakers of the normal cerebral rhythmic activity by inhibition of thalamic rhythm. These drugs, like Ethosuximide, Zonisamide and, presumably, Valproic acid are only effective in absence seizure crisis (Johannessen Landmark *et al.*, 2023; Kwan e Brodie, 2006).

2.1.3 Potentiation of GABA mediated inhibitory transition

There are four main ways to potentiate GABA's actions: promoting its synthesis, reducing its metabolism, reducing its capture and using agonists.

The synthesis can be promoted by increasing the levels of Glutamic acid decarboxylase. An example of a drug with these properties is Gabapentin (Kwan e Brodie, 2006).

By reducing GABA transaminase and Succinic semialdehyde dehydrogenase, GABA is not metabolised, therefore increasing its time in the synaptic cleft. Vigabatrin is an example of a drug that decreases GABA transaminase and Valproate of a drug that decreases both enzymes (Kwan e Brodie, 2006).

One alternative is reducing the capture of GABA capture, by reducing GAT-1 (GABA transporter), for example, by using Tiagabine.

Finally, GABA-A agonists, like Phenobarbital and Benzodiazepines, can provoke influx of chlorine, that result in hyperpolarization of the membrane, therefore holding off the membrane potential from the neuronal excitability threshold. These AEDs have a high risk of overdose, so they have limited use (Johannessen Landmark *et al.*, 2023; Kwan e Brodie, 2006).

2.1.4 Inhibition of glutamate exocytosis

Levetiracetam is a drug that acts in the pre-synaptic neuron, by stimulating and binding to synaptic vesicle protein 2A (SV2A) inhibiting glutamate release. However, it does not affect normal neurotransmission, only modulating SV2A under pathophysiological conditions (*Levetiracetam*, [s.d.]).

2.1.5 Glutamate Antagonists

Glutamate is an excitatory neurotransmitter of the central nervous system. Therefore, its antagonists seem to be a resourceful source to help preventing seizures. Felbamate, by blocking NMDA receptors and Topiramate and Perampanel, by blocking AMPA receptors, are drugs that have a potential antiepileptic effect. Nonetheless, the therapeutic action of the blockage of these receptors is still unclear (Kwan e Brodie, 2006).

2.1.6 Other mechanisms

Fenfluramine, used in Dravet Syndrome and Lennox Gastaut Syndrome, indirectly stimulates serotonergic 5-HT_{2C} and 5-HT_{1D} receptors and interacts as well with sigma-1 receptors (Johannessen Landmark *et al.*, 2023).

Cannabidiol's mechanisms have been reported to involve: antagonism of G protein-coupled receptor-55, decrease of Ca-mediated excitation, enhancement of adenosine-mediated signalling and desensitization of transient receptor potential vanilloid (Johannessen Landmark *et al.*, 2023).

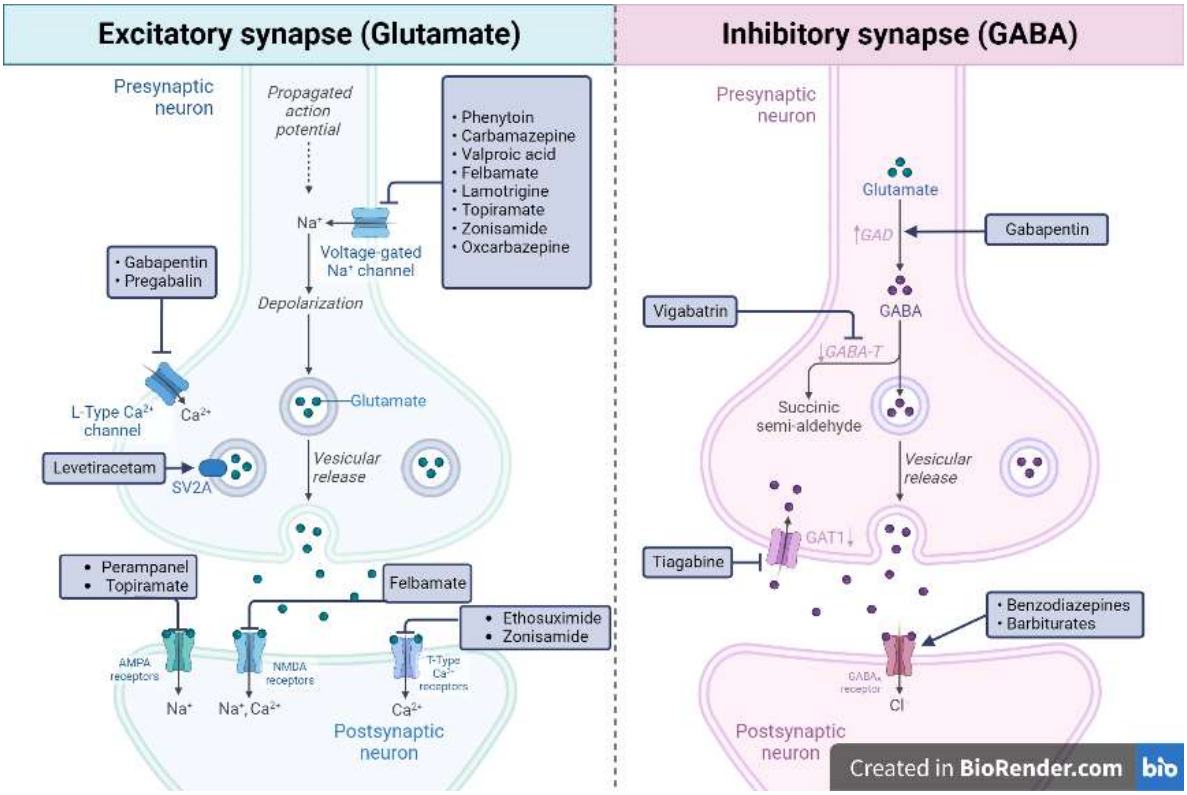

Figure 1- Antiepileptic drugs' mechanism of action, based on Johannessen Landmark *et al.*, 2023

2.2 Drug interactions with AEDs

About 25% of patients with epilepsy and over 75% of patients with drug-resistant epilepsy are treated with two or more antiepileptic drugs, that can interact between them. Moreover, the co-prescription of AEDs with other drugs and over-the-counter products is common and increases the risk of adverse reactions related to drug-drug interactions, increasing morbidity and mortality. For example, it can alter pharmacokinetic aspects, by inducing or inhibiting drug metabolism, since many AEDs are substrates, inducers or inhibitors of drug metabolizing enzymes, such as CYP450 (Johannessen Landmark *et al.*, 2023).

When prescribed with enzyme-inducing AEDs (for example, Carbamazepine or Phenytoin), some drugs like anticoagulants, oral contraceptives, statins among others require adjustments. Using contraception as an example, oral contraception can decrease serum levels of certain AEDs like Lamotrigine or, less pronounced, valproic acid, resulting in increased prevalence of seizures in some women (Johannessen Landmark *et al.*, 2023; Zaccara e Perucca, 2014). Furthermore, these AEDs can make certain forms of birth control — for instance the combined oral contraceptive pill, among other types of pills, patches and vaginal rings, as well as, postcoital contraceptives — less effective. The enzyme-inducing AEDs will induce CYP450, leading to an increasing metabolism rate of ethinylestradiol and progestogen, lowering their blood concentration (O'Brien e Guillebaud, 2010). Therefore, contraceptive methods such as intrauterine devices are preferred, since they are not affected by enzyme-inducing AEDs

(*Contraception*, [s.d.]; O'Brien e Guillebaud, 2010; Zaccara e Perucca, 2014) It is important to clarify that most hormonal contraceptives and other medications can be safely and effectively managed if physicians follow the correct protocols.

3. AN OVERVIEW OF GENETIC EPILEPSIES

As mentioned before, epilepsy can have a genetic cause and several epileptic syndromes have been identified as having a prevalent genetic alteration. The evolution of genetic testing techniques allowed the investigation of such alterations and the association to certain types of epilepsies, such as the one that occurs in Dravet Syndrome (DS), Angelman Syndrome (AS), *YWHAG* mutations, among several others, exemplified in Table 2.

Genetics seem to play a large role in epilepsies in infants with 0 to 36 months of age (Treadwell, Wu e Tsou, 2022). These early-life epilepsies seem to be a consequence of neurodevelopmental disorders with a genetic origin. Such complex syndromes have some particularities worth mentioning and are some of the main targets when it comes to the potential use of genetic therapy in the treatment of epilepsy (Berg *et al.*, 2017).

Table 2 - Examples of Gene Mutations associated with the development of epilepsy and potential therapy

Gene mutation	Mutation Type	Protein Coded	Type of epilepsy	Gene therapy proposed	References
KCNT1	Gain of function	Kca4.1	Infantile epilepsy with migrating focal seizures (EIMFS), nocturnal frontal lobe epilepsy Ohtahara and West syndrome	Gene silencing (ASO)	(Turner *et al.*, 2021; Zimmern, Minassian e Korff, 2022)
SCN8A	Gain of function	Nav1.6	DEE	ASO	(Turner *et al.*, 2021; Zimmern, Minassian e Korff, 2022)
CDKL5	Loss of function	CDKL5	DEE	AAV-mediated (Gene replacement)	(Zimmern, Minassian e Korff, 2022)
TSC1/2	Loss of function	Hamartin/Tuberin	Infantile spasms, focal seizures	AAV-mediated (Gene replacement)	(Rahman e Fatema, 2020; Zimmern,

					Minassian e Korff, 2022)
DNM1	Dominant negative/ Loss of function	Dynamin 1	DEE	RNAi	(Zimmern, Minassian e Korff, 2022)
KCNA1	Loss of function	Kv1.1	EE	CRISPRa	(Paulhus, Ammerman e Glasscock, 2020; Zimmern, Minassian e Korff, 2022)
SCN1A	Loss of function/ Haploinsufficiency	Nav 1.1	DS	ASO, CRISPRa	(Goodspeed *et al.*, 2022; Turner *et al.*, 2021; Zimmern, Minassian e Korff, 2022)
SCN2A/8A	Gain of function	Nav1.2/ Nav1.6	Early infantile onset EE, myoclonus	ASO	(Turner *et al.*, 2021; Zimmern, Minassian e Korff, 2022)
SCN2A	Loss of function	Nav1.2	Later-on set mild epilepsy	CRISPR	(Turner *et al.*, 2021; Zimmern, Minassian e Korff, 2022)r
EPMA2A/2B	Loss of function	Glycogen phosphatase/Ubiquitin E3 ligase	Lafora Disease	ASO CRISPR-Cas9	(Zimmern, Minassian e Korff, 2022) (Goodspeed *et al.*, 2022)
SLC13A5	Loss of function	NaCT	Early infantile epileptic encephalopathy	AAV-mediated (Gene replacement)	(Goodspeed *et al.*, 2022)
SLC6A1	Loss of function/ Haploinsufficiency	GAT-1	Absence seizures	Gene replacement	(Goodspeed *et al.*, 2022)
KCNQ2/Q3	Loss of function	Kv7.2/Kv7.3	Benign familial neonatal-infantile epilepsy (BFNIE)	Lentivirus-mediated (Gene replacement)	(Turner *et al.*, 2021; Zhang e Wang, 2021)
YWHAG	Haploinsufficiency/ Loss of function	14-3-3γ	DEE	Non-reported yet	(Guella *et al.*, 2017; Kanani *et al.*, 2020; Ye *et al.*, 2021)
UBE3A	Loss of function	Ubiquitin E3A ligase	AS	ASO	(Samanta, 2021; Turner *et al.*, 2021)

KCNC1	Loss of function/ Dominant negative	Kv3.1	Progressive myoclonus, DEE	Non-reported yet	(Muona *et al.*, 2015; Turner *et al.*, 2021)
STXBP1	Loss of function/ Haploinsufficiency	Stxbp/Munc18-1	DS, Lennox-Gastaut, Ohtahara, West	Non-reported yet	(Turner *et al.*, 2021)
PRRT2	Loss of function	PRRT2	BFNIE, DS	Non-reported yet	(Turner *et al.*, 2021)
GRIN2A	Loss of function/Gain of function	GluN2A	DEE	Non-reported yet	(Turner *et al.*, 2021)
GRIN2B	Loss of function	GluN2B	DEE, Lennox-Gastaut syndrome	Non-reported yet	(Turner *et al.*, 2021)
GRIN2B	Gain of function	GluN2B	West syndrome	Non-reported yet	(Turner *et al.*, 2021)

3.1 Dravet Syndrome

Dravet Syndrome (DS), a severe developmental and epileptic encephalopathy, usually develops during the first year of life. It is believed to have a prevalence of about 1/15,700 live births, and known to be a drug-resistant epilepsy, with typically myoclonic seizures that evolve over the lifespan alongside non-seizure symptoms connected to the underlying pathophysiology of this syndrome (Isom e Knupp, 2021; Turner *et al.*, 2021). Even with current pharmacological and non-pharmacological treatments, this pathology still reduces significantly patients' quality of life, since seizures usually are not under control and non-seizure symptoms, such as feeding issues, sleep disruption, behavioural issues, parkinsonism, among others, remain present, impacting the daily life of patients equally or even more than seizures (Isom e Knupp, 2021).

Genetic testing revealed that about 70-80% of children that are suspected to have DS have pathogenic variants in the gene *SCN1A.* Knowing this, DS is largely considered to be a monogenic disease with a characteristic clinical presentation. The gene *SCN1A* encodes the voltage-gated sodium channel Nav1.1 α subunit and over 3000 monoallelic pathogenic variants have been reported in this gene. The mutations are mostly occurring *de novo* and, in most cases, haploinsufficiency of Nav1.1 is sufficient to produce clinical disease. Usually, these pathogenic variants result in loss of function, with some reporting cases of gain of function (Isom e Knupp, 2021).

The Nav1.1 channel, a channel expressed widely in the inhibitory neurons of the brain, regulates the sodium transportation, therefore, essential in the creation of action potential that controls the excitability of the inhibitory interneurons of the hypothalamus, cerebellum,

thalamus and cortex. Loss of function will subsequently affect inhibitory neurons but not the excitatory ones, resulting in an imbalance that justifies the convulsions (Perucca e Perucca, 2019).

3.2 Angelman Syndrome

Angelman Syndrome (AS) is a neurodevelopmental disorder, considered to be severe, caused by the dysfunctional (usually by loss of function or deletion) maternally inherited *UBE3A* gene, since only the maternal expression of the gene is seen in the brain (*GTX-102 for Angelman Syndrome (AS)*, [s.d.]; Samanta, 2021). This gene provides information for making the ubiquitin protein ligase E3A, that is responsible for targeting other proteins to be degraded within proteosomes (Samanta, 2021; *UBE3A ubiquitin protein ligase E3A [Homo sapiens (human)] - Gene - NCBI*, [s.d.]). It is also important for synaptic plasticity and maintenance of suitable levels of GABA (Samanta, 2021).

AS is characterized by severe development delays such as speech impairment, movement abnormalities like ataxia or tremors and behaviours like frequent laughter. About 80-90% of AS patients develop epilepsy. Seizures are present since early childhood and are associated with characteristic abnormalities in both the EEG and MRI neuroimaging of the brain. These seizures are usually drug-resistant and can be of multiple types (Samanta, 2021).

The alteration in GABA levels was initially the main focus point. Originally, for AS research, Knock-out (KO) animal models using disrupted GABA receptor genes were used, such as GAbrb3 (15q11-13 deletion) KO mice. Those have shown similarities with deletion subtype AS patients. However, 30% of AS patients do not have the 15q11-13 deletion and the animal models do not express all characteristics features of AS. Therefore, GABA impairment was abandoned as the focal point that has since shifted to the ubiquitin pathway. However, it is important to clarify that the alteration in the ubiquitin pathway associated with the GABA gene deletion seen in some AS patients might be responsible for the higher severity of this phenotype when compared to the other AS phenotypes (Samanta, 2021).

Multiple seizure types have been described in this syndrome, with generalized ones being more prevalent, especially myoclonic seizures during infancy, as well as infantile spams. Status epilepticus is remarkably common in this syndrome, especially in the 15q11-13 deletion subtype. Status epilepticus is considered a medical emergency, since it is a seizure that presents a long duration, with a great risk of causing permanent brain damage (*Status Epilepticus*, 2019). In AS, these episodes of status are the non-convulsive type, but myoclonic status with altered mental status and drooling, as well as other manifestations of non-convulsive status epilepticus

(Samanta, 2021). Therefore, regression in milestones can be noticeable. Typically, AS patients tend to have less frequently these episodes of Status epilepticus after puberty (Samanta, 2021).

Currently, there is no approved treatment for AS, with available treatment options focused on symptomatology. Some treatment options are Topiramate, Lamotrigine, Ethosuximide, Levetiracetam and Clobazam. Carbamazepine and oxcarbazepine can cause worsening of seizures in AS patients, alongside, Vigabatrin (Samanta, 2021). However, gene therapy is undoubtedly capable to revolutionise this field by presenting a long-term solution capable of applying a gene-specific regulation with a maximum and durable phenotypic alteration (Samanta, 2021).

3.3 Developmental and Epileptic Encephalopathies (DEE) associated to YWHAG mutations

DEE are a series of severe heterogenous genetically epilepsy syndromes that are typically characterised by having an early-onset, being drug-resistant (causing refractory seizures) and causing developmental delay or regression (Guella *et al.*, 2017; Kanani *et al.*, 2020).

There are over 50 gene-associated DEE reported, mostly associated with *de novo* mutations. Recently, with increased access to Next-Generation Sequencing (NGS) techniques several new candidates are emerging (Guella *et al.*, 2017; Kanani *et al.*, 2020). An example of mutations found *de novo* and resulting in early-onset epilepsy, including epileptic encephalopathy and intellectual disability, are the mutations associated with the *YWHAG* gene (Guella *et al.*, 2017; Kanani *et al.*, 2020). There are currently at least eight mutations demonstrated to be associated with DEE (Ye *et al.*, 2021). Haploinsufficiency in the *YWHAG* gene is one of the candidates for causing, for example, infantile spasms and myoclonic seizures (Kanani *et al.*, 2020). The spectrum of epilepsies potentially caused by *YWHAG* mutations seem to range from mild myoclonic epilepsies and febrile seizures to severe DEE, depending on the mutation type associated (Ye *et al.*, 2021).

The *YWHAG* gene is widely expressed in the brain and in the skeletal and heart muscle (Kanani *et al.*, 2020). It encodes an adapter 14-3-3γ protein, a member of the 14-3-3 protein family (Ye *et al.*, 2021). This family is involved in intracellular signalling pathways, by modulation of cell survival and apoptosis, therefore having an influential role in signal transduction leading to mitosis and cellular proliferation. Consequently, the numerous mutations found in the gene will result in loss-of-function (LoF), which will lead to, for example, a delayed neuronal migration in the developing cerebral cortex, with development delay being widely reported,

leading to DEE, among other effects (Kanani *et al.*, 2020). However, the diminished presence of tyrosine hydroxylase (TH), a result of the deficient 14-3-3γ protein, cannot alone explain the pathogenicity associated with *YWHAG* mutations (Ye *et al.*, 2021).

All mutations presently found are located within or close to the 14-3-3γ binding groove or at residues critical for dimerization. Furthermore, all of the mutations associated with epileptic encephalopathy were found to be located at the primary binding site (Ye *et al.*, 2021).

Unlike the typical drug resistance reported in most genetic epilepsies and DEE, *YWHAG* mutations don't tend to result in refractory seizures. Most patients were not resistant to treatment and seizure control was achieved, even if most needed multiple AEDs. Sodium Valproate, alone or in combination with other AED seems to be efficient (Kanani *et al.*, 2020).

4. MOLECULAR DIAGNOSIS IN EPILEPSY

Molecular diagnostic methods and genetic testing

The genetic variants are classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Scala *et al.*, 2020).

An increased knowledge of the genetics behind epilepsy is being displayed in recent years (Orsini, Zara e Striano, 2018). There are several genetic analysis techniques currently available, with emphasis for the ones on Table 4. It is important to keep in mind that none is able to detect all types of variants with pathogenic potential (Lesca *et al.*, 2022).

Table 4 - Use of Genetic Analysis Techniques

Genetic Analysis Technique	Characteristics/ Benefits	Limitations
Array-CGH	Suitable for DNA copy number variants like deletions or duplications; (Scala *et al.*, 2020) Defines the exact genomic region altered and its genes. (Orsini, Zara e Striano, 2018)	Lower diagnostic impact when epilepsy is not associated with intellectual disability or dysmorphic features and malformations.(Scala *et al.*, 2020)
FISH	Used when there are suspicions of microdeletion syndromes, duplication or when there is a need to characterize abnormalities in the chromosomes that were identified by other techniques. (Orsini, Zara e Striano, 2018)	
MLPA	Detects deletions and duplications of several exonic sequences; (Scala *et al.*, 2020) Screening of entire genes in experimental sessions; (Scala *et al.*, 2020) Identification of intragenic deletions and duplications (Orsini, Zara e Striano, 2018)	
Gene Panel	Low cost;	Limited number of genes sequenced for each panel;(Scala *et al.*, 2020)

	Used to validate WES findings and potentially identify additional variants in candidate genes; (Scala *et al.*, 2020) Particularly useful in the diagnosis of infantile epilepsies; (Scala *et al.*, 2020) Increasingly used in the clinical diagnosis of epileptic disorders with a presumed genetic epilepsy (Scala *et al.*, 2020)	Needs continuous updating of the included genes;(Scala *et al.*, 2020)
WES	Sequencing of the exomes (Orsini, Zara e Striano, 2018) Sequences multiple genes at the same time (Scala *et al.*, 2020) Able to detect *De novo* mutations (Scala *et al.*, 2020) It creates a profile of the identified gene variants and compares it with the polymorphic variants distributed in the general population so that it identifies possible pathogenic variants (Orsini, Zara e Striano, 2018) Show advantages in clinical settings (for example, no periodically updates needed) and scientific research (potentiates the discovery of new candidates for a specific disorder) (Scala *et al.*, 2020) and allows reanalysis of prior data (Lesca *et al.*, 2022)	Interpretation can get difficult since there is a large output that requires complex filtering;(Scala *et al.*, 2020) Focus on protein coding regions only(Dunn *et al.*, 2018) High costs(Scala *et al.*, 2020)
WGS	Sequencing also includes non-coding regions; (Scala *et al.*, 2020) Increasingly used in the clinical diagnosis of epileptic disorders with a presumed genetic epilepsy (Scala *et al.*, 2020) Able to detect *De novo* mutations. (Scala *et al.*, 2020)	High costs(Scala *et al.*, 2020)
PCR-Sanger	Fast technique that determines DNA sequences.(Orsini, Zara e Striano, 2018)	Being replaced by next-generation sequencing(Orsini, Zara e Striano, 2018)

Abbreviations: Array-CGH: Array based comparative genomic hybridization; FISH: Fluorescence in Situ Hybridization; MLPA: Multiplex Ligation-dependent Probe Amplification; WES: Whole Exome Sequencing; WGS: Whole Genome Sequencing; PCR-Sanger: Polymerase Chain Reaction-Sanger

Whole exome sequencing, also known as WES, is one of the techniques of NGS, alongside gene panels and Whole Genome Sequencing (WGS) that is improving new knowledge of the genetic causes behind epilepsy, as well as enhancing the understanding of its pathophysiology (Orsini, Zara e Striano, 2018). NGS techniques are quick, cost effective and allow the sequencing of several genes with a large number of DNA segments that can either be the exons of selected gene panels or the entire exome. Therefore, they have become the first-line for the diagnosis of genetic epilepsies patients with a suspicion of a monogenetic epilepsy, (Lesca *et al.*, 2022) seeming remarkably useful in the diagnosis of infantile epilepsies (Scala *et al.*, 2020). These techniques allow a comparison between the gene variants found with polymorphic variants distributed in the general population. With these comparisons, the

techniques allow to identify possible pathogenic variants, as well as classify the findings as "de novo mutation" when it is not present in the parents, and according to the state of homozygosity (if it is homozygous or heterozygous, depending if it affects or not both alleles) and compound heterozygous (when there are two different mutations in the two alleles of the same gene) (Orsini, Zara e Striano, 2018). They can also be classified based on the nature (we can classify them as missense, nonsense and frameshift) and based on the effect on the structure and function of the protein that encodes (Scala *et al.*, 2020).

Therefore, with NGS techniques it is of extreme importance to be able to do the correct interpretation of the detected variants, as well as the correct prediction of their pathogenicity (Orsini, Zara e Striano, 2018).

5. GENE THERAPY

5.1 Therapeutic Strategies

Gene therapy medicines are, according to European Medicines Agency (EMA), one of the Advanced Therapy Medicinal Products, alongside Somatic-cell therapy medicines and Tissue-engineered medicines (EMA, 2018).

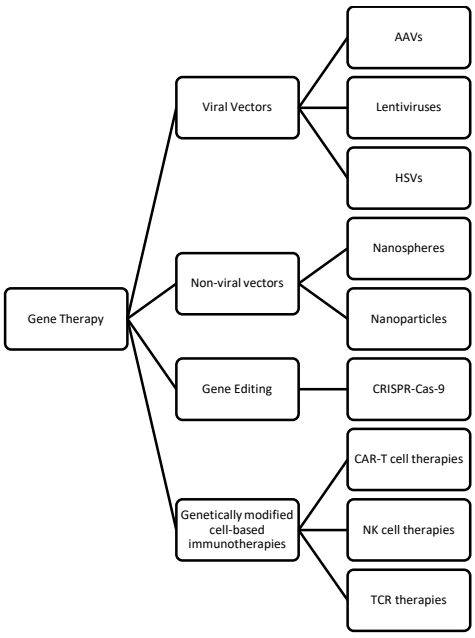

Figure 2 - Examples of Gene Therapy Strategies.

These gene therapy medicines are based on gene therapy technologies that mostly work by inserting recombinant genes into the body, leading to a therapeutic, prophylactic or diagnostic effect. But what is a recombinant gene? It is a short string of DNA, selected and created in the laboratory, which may be used to correct the effects of a mutated gene (EMA, 2018).

All therapeutic strategies, for example the ones mentioned in Figure 2, use one of the following approaches: supplementation of genes that help treat the disease, replacing a missing gene or a gene that is causing a problem, or turning off or editing genes that are causing problems (Commissioner, 2022; Turner *et al.*, 2021).

A. Modalities of gene therapy:

A.1 Gene addiction

One of the most straightforward approaches is delivering a supplemental transgene to cells that are lacking the corresponding gene product, allowing them to restore their healthy functions (Turner *et al.*, 2021). Vector-mediated gene transfer provides this possibility, as well as providing the possibility to modify the therapeutic targets, especially by resorting to non-viral and, mainly, viral vectors to deliver these transgenes (Zhang e Wang, 2021).

The transgene is the gene or genetic material that has been artificially altered and introduced into the genome of the organism (*Transgenic*, 2022). For the correct construction of this transgene it is necessary to carefully choose the promoter sequence, the sequence of the gene that codes for amino acids of the desired recombinant protein and the poly (A) signal and termination sequences. This will ensure that the expression of the transgene occurs in the appropriate cells and that the mRNA produced is properly processed and stable for subsequent translation (Nishu *et al.*, 2020).

A.1.1 Promoter

The promoter is a major DNA regulatory element. Alongside the vector, it is a key factor that determines the expression dynamics of the transgene. The promoter influences the strength of expression and the type of cells that will express the transgene (Nieuwenhuis *et al.*, 2021). When choosing a promoter, it is important to consider its size, since most times the packaging capacity of the vector is limited, especially in adeno-associated viruses (AAVs). Some examples of promoters are sCAG, hCMV, mPGK and hSYN. In a comparative study between this four promoters, it was reported that the mPGK and hSYn promoters were able to activate transgenes in more neurons when compared to the other two. Moreover, the hSYN promoter was able to mediate neuron-specific transgene expression (Nieuwenhuis *et al.*, 2021).

Another example of a neuron-specific promoter is the CamKII promoter (Mesraoua *et al.*, 2019). This promoter can be used in therapies that aim to manipulate excitatory pathways. Promoters that are suitable for targeting inhibitory interneurons have been recently showing promising results in mice, however, until now, they were poorly defined (Mesraoua *et al.*, 2019).

A.1.2 Poly (A) signal and termination sequence

The poly(A) signal, also known as polyadenylation sequence, is a sequence that signals the addition, to the mRNA molecule, of a long chain of adenine nucleotides, the poly(A) tail, making RNA more stable, allowing exportation and subsequent translation and preventing its degradation (Kantor *et al.*, 2014). Usually, this tail marks the end of the coding region and is important for transcription termination, since this termination typically occurs directly after the poly(A) signal (Rehfeld *et al.*, 2013).

A.2 Gene silencing by RNA interference (RNAi) based Therapies

RNA interference therapy allows the silencing of gene expression. Therefore, RNAi allows to selectively reduce the expression of target genes, decreasing the encoded protein (Bumcrot *et al.*, 2006).

The RNAi based therapeutic approaches are in general the non-viral delivery of chemically synthetised double stranded small-interfering RNAs (siRNAs) and the viral delivery of short-hairpin RNAs (shRNAs) (Bumcrot *et al.*, 2006; Rao *et al.*, 2009). These two approaches can achieve similar outcomes, however, since they are different molecules, the mechanism of action, the RNAi pathway or applications may be different (Rao *et al.*, 2009).

It is important to consider the potential off-target effects of both of these approaches as well as the potential immune stimulation that they might trigger (Bumcrot *et al.*, 2006).

In past years, several studies were published demonstrating the successful silencing of both endogenous and exogenous genes in animal models of human disease. Particularly in the nervous system, RNAi has also been used to validate disease targets *in vivo*, potentiating the knowledge about these diseases. The delivery is usually by local administration. In the particular case of the nervous system, intracerebroventricular, intrathecal or intraparenchymal administrations resulted in the silencing of specific neuronal molecular mRNA targets (Bumcrot *et al.*, 2006).

Evidence shows that RNAi therapies could be used to treat, for example, DEEs caused by dominant-negative or gain-of-function (GoF) mutations (Aimiuwu *et al.*, 2020).

A.3 Antisense Oligonucleotides (ASO) Therapies

Despite not being formally considered gene therapy products as they are generated by synthesis and not by recombinant DNA technology, ASOs can silence expression of genes. Oligonucleotides are single-stranded DNA (ssDNA) sequences that can go up to 25 nucleotides. This ssDNA sequences may or not be chemically modified. They hybridize to

specific complementary mRNAs, and promote RNA degradation or prevent the translational machinery by steric blockage, therefore inhibiting the formation of proteins. (Riva *et al.*, 2021) By inhibition of specific sequences, ASOs can downregulate or upregulate certain proteins, being useful in both loss-of-function and gain-of-function mutations (McGinn *et al.*, 2022).

ASO and RNAi both have similar principles, as mentioned before. However, ASO have to survive and function as single-strands, but in the RNAi technique a small double strand RNA, composed of a passenger and a guide strand, associates with the RISC (RNA-induced silencing complex), where the passenger strand is discarded, while the guide strand collaborates with RISC to bind to complementary RNA molecules. In RNAi, the guide strand is always associated to a complementary strand or part of a protein complex, whereas in ASO the oligonucleotides are found as a single strand (Watts e Corey, 2012).

A.4 Gene editing by CRISPR-Cas9

CRISPR-Cas9 is a versatile technology that allows the treatment of some disorders, for instance neurological disorders by repairing or knocking-out mutant genes and by editing other related genes. Therefore, it is a promising tool that potentially allows the treatment of genetic diseases, as well as creating animal models used for research (Guan *et al.*, 2022).

Thus, CRISPR base editors are an example of gene editing tools based on impaired Cas proteins fused to deaminases that are able to convert C-G to T-A and A-T to G-C, allowing the possible correction of certain pathologies, including certain epilepsy syndromes (Turner *et al.*, 2021).

To succeed, there has to be an effective delivery strategy, with a specific targeting ability, low immunogenicity and high editing efficiency (Guan *et al.*, 2022). Besides that, it is a priority to profoundly study the off-target effects of this therapy, as well as creating appropriate CRISPR inhibitors (the so called anti-CRISPRs) to avoid the potential deleterious effects of the off-target editing (Turner *et al.*, 2021).

B. Gene Delivery vehicles

Vectors are vehicles genetically engineered that were created to deliver the genes needed to treat the disease directly into the cells, supressing some of the limitations related to therapies based on insertion of new genes (Commissioner, 2022).

In general, they are divided into viral vectors and non-viral vectors. Viral vectors seem to be better candidates for gene therapy for focal seizures, while non-viral vectors seem to be better candidates for gene therapy for generalized ones (Zhang e Wang, 2021). According to Shaimardanova, in gene therapy investigations for epilepsy found promising results upon use

of an AAV encoding a potassium channel or Neuropeptide Y (NPY) genes (Shaimardanova *et al.*, 2022).

The main obstacle is the blood-brain barrier, since it prevents genetic vectors from entering the brain from the bloodstream, demanding more invasive approaches than originally intended (Riva *et al.*, 2021). The correct selection of the delivery vehicle is of extreme importance to achieve the appropriate therapeutic effect.

B.1 Viral vectors

Taking advantage of their natural ability to deliver genetic material into cells, viruses are a strong and well-studied class of vectors (Commissioner, 2022).

The most common viral vectors used in clinical trials concerning the brain are adeno-associated viruses, lentiviruses and Herpes simplex viruses. A brief characterisation can be found in Table 3. These vectors can efficiently carry exogenous genes into the host nucleus (Zhang e Wang, 2021). An important aspect to consider is that these viruses are modified so that they loose their ability to cause infectious diseases (Commissioner, 2022).

In multiple reviews, they have demonstrated capacity to achieve high levels of transgene delivery in *in-vivo* models of disease and in clinical trials. They, however, present risks of triggering an immunogenic response or of transgene mis-insertion and have large-scale production limitations (Riva *et al.*, 2021).

Table 3- Characteristics of viral vectors

Adeno-associated viruses	Lentiviruses	Herpes simplex viruses
Loading capacity of about 5.2 Kb of DNA (Zhang e Wang, 2021) Well-established for long-term gene transduction in neurons (Zhang e Wang, 2021)	Loading capacity of about 9 Kb of DNA (larger than AAVs) (Zhang e Wang, 2021) Only effective in restricted areas, since its dimensions difficultes diffusion (Zhang e Wang, 2021) Theoretical risk of disrupting oncogenes (Mesraoua *et al.*, 2019)	Loading capacity of about 150 Kb of DNA (Zhang e Wang, 2021) Can generate neurotoxicity or immunogenic reactions (Zhang e Wang, 2021)

According to Mesraoura *et al.*, herpes simplex is not suitable as a viral vector in epilepsy, but lentiviruses as well as AAVs are suited since they both have the ability to infect neurons and lead to stable expression (Mesraoua *et al.*, 2019).

B.2 Non-viral vectors

When compared with viral vectors, these are less explored but provide advantages regarding, for example, safety profile and production costs (Riva *et al.*, 2021). These vectors have large packaging capacity but lower delivery efficiency and rapid clearance. Since non-viral vectors might have special ligands that interact with specific receptors on the blood-brain

barrier, they can be administered peripherally (Zhang e Wang, 2021). They are complex systems with potential to carry large therapeutic genes and that have in their composition the required nucleic acids and other materials. These materials can be, for instance, cationic lipids, peptides or polysaccharides (Riva *et al.*, 2021).
Usually, they do not induce a detectable immune response, even after repeated administrations.

Non-viral vectors can be divided into two main categories: Polymer-based and Lipid-based. Note that, in research related to gene therapy in epilepsy, the use of polymers is more developed than the use of lipid-based vectors (Zhang e Wang, 2021).

Polymer-based vectors

Polymer-based vectors are very attractive considering their chemical diversity and potential for functionalization (Riva *et al.*, 2021).

The most used polymer is Polyethyleneimine (PEI), since it forms a stable and homogeneous nanoparticle with DNA and other small molecules (Zhang e Wang, 2021).
Besides PEI, there's Dendrimers and Encapsulated cell bio delivery. Dendrimers are spherical and highly branched structures that contain amine groups on the surface that facilitate DNA condensation and cellular uptake (Zhang e Wang, 2021). Encapsulated cell biodelivery is a technique that encapsulates, in the polymer membranes, molecule-secreting cells that are genetically modified. These polymer membranes have large pores and allow the diffusion of therapeutic agents to the surrounding tissues. However, they are small enough to protect the exogenous cells from the host immune reactions. This technique's main advantages are its safety and retrievability since it is subject to removal (Zhang e Wang, 2021).

In epilepsy treatment, these kind of vectors are more used to carry small molecules other than genes.

Lipid-based vectors

Cationic liposomes are currently the most used non-viral vehicle (Riva *et al.*, 2021). They show better biocompatibility and lower cytotoxicity, as well as the capability to transfect larger nucleic acids with reasonable efficiency (Zhang e Wang, 2021). Some limitations worth noting are their low efficacy due to poor stability, rapid clearance and the potential of generating an inflammatory as well as an anti-inflammatory response (Riva *et al.*, 2021).

The structure of cationic liposomes usually is the following: a cationic head group, a hydrophobic tail and a linkage, also known as helper lipids, between these two domains. These helper lipids can be potentially modified to improve structural stability and transfection (Zhang e Wang, 2021).

5.2 Examples of Promising Therapeutic Strategies for Epilepsy Treatment

In epilepsy, the premise for the use of genetic therapy is the fact that usually there's an imbalance between excitation and inhibition pathways. Therefore, gene therapy can, for example, act by modulating the expression and regulation of neuropeptides, by overexpressing regulating channels (such as potassium or sodium) or by inhibition of translational machinery. Another interesting application is chemogenetics.

5.2.1 Neuropeptide-Y based therapy

A great amount of data show the involvement of NPY in epilepsy, since it acts as neuromodulators, controlling excitability and homeostasis, and since epilepsy modifies the expression pattern of NPY-encoding genes and receptors (Cattaneo *et al.*, 2021).

NPY is widely expressed in the central and peripheral nervous systems. Typically, it is co-released with other neurotransmitters. Being expressed in different areas of the brain NPY can, for example, exhibit an inhibitory effect on excitatory synapses in the hippocampus, primarily by reducing glutamate release (Cattaneo *et al.*, 2021).

Four out of the five different NPY receptors found in mammals are functional. Particularly, NPY shows a significant high affinity for the Y1, Y2 and Y5 receptors. Evidence shows that Y2, and to a minor degree, Y5 receptors have an anti-epileptic role. However, it is believed that Y1 receptors have a pro-epileptic effect. As a result, a simple increase of NPY levels might have an unpredictable effect. So, strategies ideally have to not only increase NPY levels but also re-shape the NPY ligand-receptor system, by the delivery of genes encoding for specific NPY receptors (Cattaneo *et al.*, 2021).

Thus, gene therapy-mediated overexpression of NPY and its receptors have shown promising results (Cattaneo *et al.*, 2021; Mesraoua *et al.*, 2019). Furthermore, direct infusions of recombinant AAVs (rAAVs) designed to modulate NPY levels are greatly emphasized. Most studies in the area use rAAV serotype 2 vectors or chimeric AAV1/2 serotype vectors. In comparison, AAV1/2 have a more widespread expression in diverse subtypes of neurons, not being only effective in a subtype of interneurons (Cattaneo *et al.*, 2021). When tested in epileptic rats, the effect of hippocampal injections of primarily an AAV1/2 vector expressing NPY and next of a rAAV1 vector expressing NPY, it was concluded with great success that there was a decrease in spontaneous epileptic seizures (Noè *et al.*, 2008; Noe' *et al.*, 2010). It should be highlighted that there was no detectable evidence of immune response or cognitive impairment. Even with the preclinical proven success of NPY in modulating excitatory imbalance, therapies like the ones mentioned before still need to undergo extended long-time studies to exclude side effects and neuropathological changes (Cattaneo *et al.*, 2021).

5.2.2 STK-001 for Dravet Syndrome: ASO Therapy

Presented by Stoke Therapeutics, STK-001 is an example of an antisense oligonucleotide therapy developed using Targeted Augmentation of Nuclear Gene Output technology. It intends to increase the levels of productive SCN1A mRNA and, therefore, upregulate the expression of the Nav1.1 protein by RNA modulation (Isom e Knupp, 2021).

Briefly, the mechanism of action is, as shown in Figure 3, binding the ASO to the non-productive exon of SCN1A wild-type and mutant pre-mRNA and promoting the skipping of this exon, leading to a decrease of non-productive mRNA (that would be eliminated) and incrementing levels of productive mRNA, therefore, increasing the levels of wild-type Nav1.1 protein to near normal levels, compensating the loss-of-function mutant alleles (Isom e Knupp, 2021).

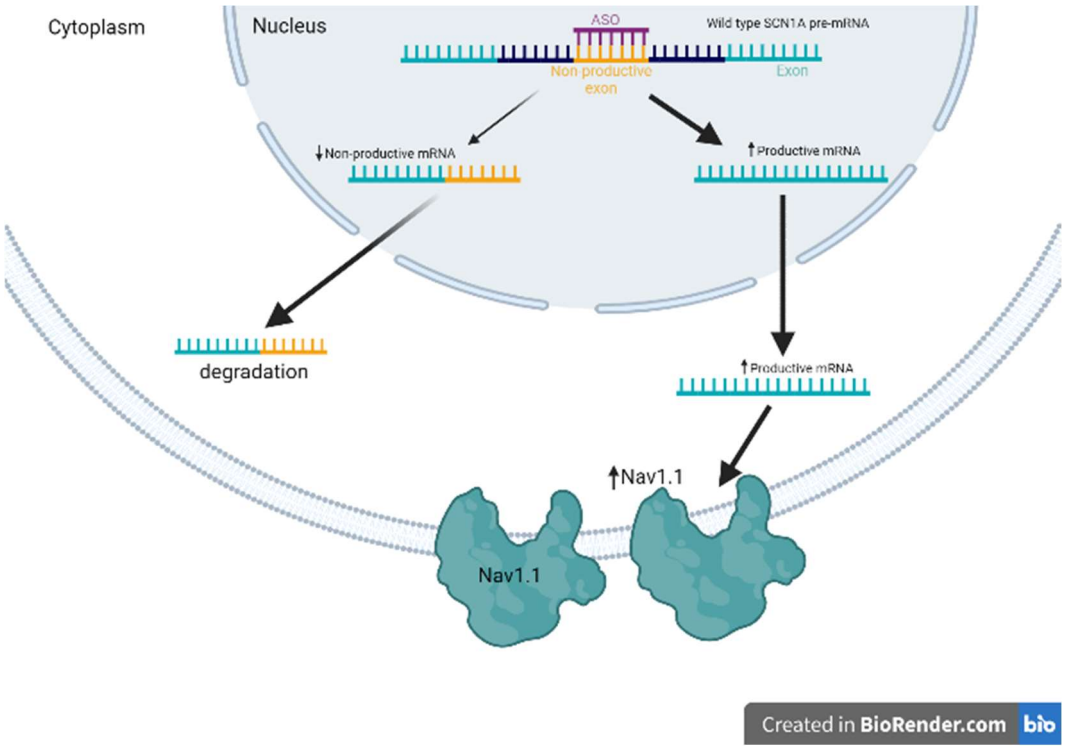

Figure 3- Mechanism of Action of STK-001, based on Isom e Knupp, 2021.

Currently undergoing a phase 2 open-label extension, this study intends to evaluate the long-term safety and tolerability of repeated doses of STK-001, as well as look for changes in seizure frequency, quality of life and overall clinical status (*An Open-Label Extension Study of STK-001 for Patients With Dravet Syndrome - Tabular View - ClinicalTrials.gov*, [s.d.]).

5.2.3 ETX101 for Dravet Syndrome: AAV-mediated therapy

ETX101 is another gene therapy that is being developed to treat DS. It is being developed by Encoded Therapeutics, and is a cell-selective gene therapy that aims to treat the underlying cause of DS. The therapeutic uses an AAV9 capsid as the vector and is delivered

via an intracerebroventricular infusion. The mechanism of action is the delivery of a transgene coding for a SCN1A-specific transcription factor that allows the increasing expression of the endogenous *SCN1A* gene in the GABAergic interneurons, therefore increasing the non-mutated coded protein and restoring normal function (*ETX101 for Dravet Syndrome*, [s.d.]). This therapeutic is undergoing clinical trials to evaluate safety and efficacy in infants and children with SCN1A+ DS (Encoded Therapeutics, 2022).

5.2.4 GTX-102 for Angelman syndrome: ASO mediated therapy

GTX-102 is an ASO therapy under investigation for the treatment of Angelman Syndrome. It is being developed by Ultragenyx. AS is caused by LoF or deletion of the motherly inherited allele of the *UBE3A* gene. The paternally inherited gene is silenced by the UBE3A antisense transcript (UBE3A-AS) in the nervous system. GTX-102 aims to inhibit UBE3A-AS so that the paternally inherited gene can be expressed in the nervous system. That expression would allow the expression of the protein missing and treating AS. It is currently under clinical trials (phase1/2) to evaluate tolerability and safety and the effectiveness in AS paediatric patients (*GTX-102 for Angelman Syndrome (AS)*, [s.d.]).

While allowing the expression of *UBE3A* gene it is important to consider and avoid the overexpression of the gene, since it is strongly associated with autism spectrum disorders (Turner *et al.*, 2021). Studies suggest that this and, in the future, other AS gene therapies have to be used during early development, since epileptical phenotypes cannot be corrected during adulthood (Turner *et al.*, 2021).

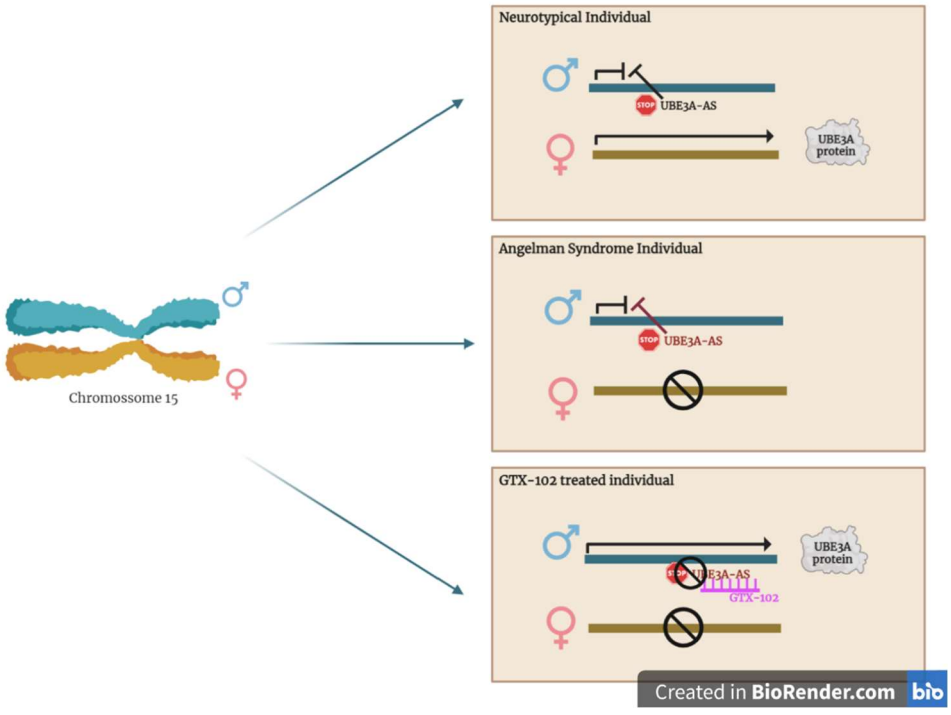

Figure 4 - Mechanism of action of GTX-102, based on GTX-102 for Angelman Syndrome (AS), [s.d.]

5.2.5 CRISPR mediated therapy

A study conducted in mice by Colasante *et al.* (2020) targeted a transcriptionally active promoter of the *SCN1A* gene that aimed to activate the expression of SCN1A in interneurons, by intermediate of an AAV-mediated CRISPR activation (CRISPRa) system, that intended to compensate the typical haploinsufficiency seen in DS, therefore attenuating the disease (Colasante *et al.*, 2020; Guan *et al.*, 2022). The success of this study in mice proved that DS caused by haploinsufficiency can be rescued by activating SCN1A expression through the CRISPRa system (Guan *et al.*, 2022). However, further studies are required to assess certain details. It also opens a pathway to explore this strategy in other diseases caused by haploinsufficiency. The observations made in the study also imply that interneurons can potentially recover their normal activity if the correct amount of Nav1.1 channels are available, consequently indicating that some DS pathological effects can be reversible (Colasante *et al.*, 2020).

Another study conducted by Colasante *et al.* (2020), used CRISPRa-mediated Kv1.1 upregulation to reduce neuronal excitability in epileptic mice. The positive results of the study also granted a promising pathway to treat neurological disorders where there are abnormal ion channels by using CRISPRa-based treatments (Colasante *et al.*, 2020; Guan *et al.*, 2022).

5.2.6 Chemogenetics

It is possible to express receptors extremely sensitive for exogenous drugs but insensitive to endogenous neurotransmitters, allowing the achievement of optimal dosage without interfering with normal brain function, increasing the suppression of seizures (Riva *et al.*, 2021). On-demand suppression of seizures using the human M4 muscarinic receptor has been demonstrated (Mesraoua *et al.*, 2019).

This technique doesn't require the irreversible and permanent gene transfer of channels and neuropeptides, which is seen as an advantage (Mesraoua *et al.*, 2019). Another advantage is the potentiality of being administered on demand in events like status epilepticus or clusters of seizures. However promising, this method is still a work-in-progress and important aspects like immunogenicity still need to be evaluated (Riva *et al.*, 2021).

A further improvement of this technique consists of using receptors able to detect pathological elevations of the endogenous neurotransmitter glutamate, thus, inhibiting neurons. This dispenses the use of exogenous drugs (Mesraoua *et al.*, 2019).

6. GENETIC THERAPY VS CLASSICAL PHARMACOLOGICAL TREATMENT

Regarding classical pharmacological treatments, parents state that the main reasons for initiating complementary and alternative medicines (CAM) for their children were the side effects of the AEDs, such as behavioural changes and systemic and metabolic disturbances, or their lack of efficacy (Zhu *et al.*, 2023). Moreover, around 90% of people with epilepsy in developing countries do not have access to pharmacological treatment, with a great treatment gap registered (World Health Organization, 2006; Zhu *et al.*, 2023). Some people with epilepsy also refuse AEDs because of social, cultural and personal motivations, mostly resorting to CAM as treatment (Zhu *et al.*, 2023).

Gene therapy can potentially revolutionize the treatment of epilepsies with a genetic underlying cause. Especially in drug-resistant epilepsies, genetic therapy is seen as a way to significantly improve the condition of epilepsy's patients (Shaimardanova *et al.*, 2022). Presently, with the improved notions around the pathophysiology of epilepsy and genetics, the treatment line is and should develop into a more "precision" approach (Riva *et al.*, 2021).

6.1 Parental Concerns around infantile epilepsies

Several epilepsy syndromes derived from genetic mutations are developed and diagnosed in early-life. Therefore, an important topic is "how does the diagnosis impact family life and what are the main concerns to parents and caregivers?". Uncontrolled seizures have serious short-term health risks and, in children between 1 and 36 months is associated with impairments of developmental, behavioural and psychological order (Treadwell, Wu e Tsou, 2022).

Epilepsy as mentioned before can have life-long impact, having the potential to agitate all aspects of family life. However, most fears and concerns go most times unaddressed by health care professionals that mostly look into seizure management (Carter *et al.*, 2022).

In a review conducted in March 2021 and entitled "Parents"/caregivers' fears and concerns about their child's epilepsy: A scoping review", it is stated that a diagnosis of epilepsy in childhood can affect widely certain aspect of the family life and that it has huge and adverse influences over the child and people around. Increased levels of depression, stress and anxiety were reported, as well as a decrease in quality of life accompanied by higher stigma around the disease. The results of this review refer to three main themes: fears and concerns regarding their child's epilepsy; impact of epilepsy-related fears and concerns on the daily lives of children

and parents/caregivers and impact of epilepsy-related fears and concerns on their social and emotional well-being (Carter *et al.*, 2022).

It is important to highlight particular findings such as most parents/caregivers are highly concerned about overnight seizures and the unexpectedness of them. They also emphasised the fear felt around Sudden Unexpected Death in Epilepsy (SUDEP), that sometimes led to sleep deprivation and other changes around daily-life. SUDEP is the death in people with epilepsy that are not caused by injury, drowning or other known causes. These events usually occur during or immediately after a seizure, and possible triggering factors are heart rhythm changes/cardiac arrest and breathing issues like prolonged apnoea or obstruction of the airways (*Sudden Unexpected Death in Epilepsy (SUDEP) | CDC*, 2020).

Other concerns were around school, mostly around bullying or learning difficulties. Moreover, it is emphasized the concern around various aspects of the child's future including seizures, health, development and medication use. In this review it was stated that four studies specifically reported on parents' concerns around medication, such as adverse side effects, specially the impact of medication over learning, behaviour and mental illnesses (Carter *et al.*, 2022).

With this in mind, it is important to raise awareness around the healthcare community about the impact of epilepsy on the psychosocial health of the patient as well as parents/caregivers, so that those families can have the help and support needed throughout their daily lives. It is also important to improve awareness and information about epilepsy in schools and overall community to reduce stigma (Carter *et al.*, 2022).

In a comparative effectiveness systematic review, it was stated that even though most infants were given pharmacological interventions before other interventions, such as dietary or surgical, there was limited evidence for pharmacological interventions (there were included studies assessing 10 drugs, however oxcarbazepine was not included as it is commonly prescribed for age 0-36 months) (Treadwell, Wu e Tsou, 2022). The studies revealed that, although many combinations of drugs are used in this population, there was only evidence for five of the ten drugs. Moreover, only levetiracetam had sufficient evidence to conclude effectiveness (Treadwell, Wu e Tsou, 2022).

For some infants, it was found that dietary interventions, for example, the ketogenic diet (KD) is effective in reducing seizure frequency and achieving seizure freedom, as well as the modified Atkins diet, that also reduced seizure frequency, even though this was less likely then KD.

The studies used in the review mentioned before had some gaps worth mentioning, such as: few studies that assess treatments for children with less than 36 months and studies

that reported on seizure frequency and seizure freedom, but failed to describe other outcomes such as hospitalization, neurodevelopment, quality of life and sleep outcomes, that are very important outcomes to caregivers and to assess the overall control of epilepsies (Treadwell, Wu e Tsou, 2022). In the study, it was also remarked that the long-term potential harms of pharmacological and dietary treatments is still unclear, because few studies followed patients for over a year (Treadwell, Wu e Tsou, 2022). Therefore, in the future, it is of extreme importance to assess these evidence gaps.

7. CASE STUDY- YWHAG GENE MUTATION

An interview was conducted to a mother of a 5 year old girl (born in 2017), from Portugal, that has been diagnosed with EED resultant from an *YWHAG* mutation.

In this particular case, the mutation was in *YWHAG*: c.169C>T (p.Arg57Cys), which means that a substitution of arginine by cysteine occurred at residue 57. This Arg57Cys substitution is located at a primary interaction site, and is part of a conserved triad responsible for fixing the orientation of phosphopeptides at the binding site. (Ye *et al.*, 2021) The mutation is, therefore, responsible for the destruction of the original hydrogen bond with the ligand. This missense mutation is in the binding site of ser19-phosphorilated peptide of tyrosine hydroxylase (Ye *et al.*, 2021).

At first, when the girl was 9 months old, she started suffering from febrile seizures. Initially, the convulsions were after the fever had settled, but rapidly, the febrile seizures were the first symptom of disease, previous to fever or any other symptom. She had some admission to the hospital and, while there, the EEG and MRIs were both normal. She started having some myoclonic seizures.

In 2019, the neuropediatric doctor recommended WES genetic testing, to search for an eventual mutation, since morphological and structural problems had already been discarded. The neuropediatric doctor was inclined to DS, however the girl's development, even if borderline, was not as delayed as it is characteristic of DS. Therefore, she did a WES panel that screened for 137 genes associated with Epileptic Encephalopathies, and the mutation mentioned above was discovered (*YWHAG*: c.169C>T (p.Arg57Cys). Afterwards, the parents were also submitted to genetic testing but no mutations were detected. Moreover, gonadal mosaicism was also discarded. This mutation was *de novo* and considered to be "probably pathogenic".

Currently with 5 years old, she has a social and behavioural development within normal parameters, however the gross and fine motor skills are delayed and in her particular case the most affected area from EE. To increase development she does speech therapy, occupational

therapy and psychomotricity exercises. Seizures are under control with an association of sodium valproate (20mg/kg/day) and levetiracetam (6mg/day). However, it is worth mentioning that when levetiracetam is removed the seizures are not controlled and that the myoclonia is only controlled with sodium valproate. Recent EEG show that the epileptic activity is decreasing.

Since mutations in the *YWHAG* gene associated with DEE are relatively new, there are still only a few reported cases, however the reports are increasing with the help of NGS diagnostic techniques.

A foundation was created to raise awareness, promote investigation in the topic and to connect parents with children with *YWHAG* mutations, to compare, help and discuss treatments, concerns, the development of children, and discuss the most up to date investigation, among other topics. They have raised money to promote investigation in drug repurposing, since the investigators and parents behind the foundation believe that, in the future, it can be the way to control seizures and diminish the side effects associated with, for example, sodium valproate, which is one of the main concerns of parents and caregivers. During the interview the mother stated that her child suffers from these side-effects and that her development has been more delayed since taking sodium valproate. She also mentioned this concern from other parents of the foundation.

7.1 Drug Repurposing

Drug repurposing is the term that defines the research and use of new therapeutic indications for previously approved or investigational drugs, since it is understood that one drug can have several targets and mechanisms of action, beyond the one originally intended (Krishnamurthy *et al.*, 2022).

The use of repurposing drugs is a cheaper, faster and less risky way of finding effective therapies when compared to the traditional drug development, since drug safety and early-phase clinical trials have already been established (Krishnamurthy *et al.*, 2022). There are several examples of successfully repurposed drugs, such as, Azidothymidine, Daptomycin, Sildenafil and Thalidomide (Krishnamurthy *et al.*, 2022).

There are several approaches for drug repurposing discovery, for instance data mining, molecular docking, machine learning, network analysis, among others (Ahmad, Qazi e Raza, 2021). The foundation mentioned before is researching promising drugs that could be repurposed for the treatment of symptoms of the *YWHAG* mutation, especially drugs whose mechanism of action could alter the regulation of the 13-4-4γ protein. There are no reported studies of application of gene therapy techniques to treat *YWHAG* mutations, however it is a

future promising pathway considering the on-going research of gene therapy techniques in other genetic epileptic syndromes.

8. FUTURE CHALLENGES

Unfortunately, even with the recent advances concerning epilepsy pathophysiology and potential new treatments, there is still a lot of uncertainty surrounding certain topics.

In the future, it is of extreme importance to develop long-term studies assessing themes like the potential risks of pharmacological approaches, treatment for children with less than 36 months and studies that measure important outcomes such as hospitalization, neurodevelopment, quality of life and sleep outcomes alongside the traditional seizure frequency and seizure freedom (Treadwell, Wu e Tsou, 2022).

Since epilepsy is prevalent in developing countries that, unfortunately, have some limited access to health care and cannot afford the high cost of some treatments, complementary and integrative medicine should also be a focus study point (Zhu *et al.*, 2023).

It is primordial to study the long-term effects of genetic therapies in the treatment of epilepsy, as well as reduce the stigma and fear around them. It is, of course, important to fund clinical trials and investigations like the ones mentioned above, so that the treatment of syndromes and drug-resistant epilepsies, so deleterious to the health of patients, are in sight.

Clinicians should not underestimate and undertreat important non-epileptic problems such as depression or sleep deprivation and even stigma and discrimination, since these are questions commonly found in people with epilepsy (Schachter, 2010). They should also bet on a "Medicine Based on Evidence" approach and use precision medicine concepts, so that the treatment is individualised and the best medical decision for each patient is obtained (Riva *et al.*, 2021; Schachter, 2010). They also ought to adapt and listen more to parents fears and concerns, not just focusing on seizure management, as well as focus on seizure precipitants since they seem to be an important tool to avoid seizures, achieve a good control of epilepsy and increase quality of life (Carter *et al.*, 2022; Nakken *et al.*, 2005).

Since studies indicate that about 70% of people diagnosed with epilepsy could have a seizure-free life if it was properly diagnosed and treated, it is of extreme importance to improve diagnostic and treatment approaches (*Epilepsy - World Health Organization*, [s.d.]). Diagnosis relies extensively on self reports, which have been shown to be inaccurate, therefore, compromising the effectiveness and optimization of treatments (Schachter, 2010). So the development of new diagnostic criteria, relying on not only self-reports alongside EEG and MRI, but also on genetic testing, precision medicine, and criteria that would help predict

treatment response, seizure type and frequency and other comorbidities could be potentially life-changing.

Moreover, the growing technological development in the last decades can and is being used to help people with epilepsy. Firstly, the NGS techniques and its potential to identify and characterize epilepsy predisposition genes cannot be overlooked (Rees, 2010). They have to be seen as valuable tools to identify genetic alterations that have potential applications to certain forms of epilepsy (Steinlein, 2008). On the other hand, systems able to predict and detect seizures are already being developed and can increase quality of life, especially in people with drug-resistant seizures (Schachter, 2010).

Concerning the *YWHAG* genetic mutations, further studies must be developed to explore the mechanisms involved in epilepsy, since the TH decrease alone cannot explain pathogenicity. Moreover, repurposing should be an investigational focal point since there are repurposed drugs that seem to potentially be able to treat the symptoms of *YWHAG* genetic mutations, diminishing the DEE and controlling the seizures without the characteristic side-effects of other AEDs currently used. Likewise, a promising pathway is the investigation and application of gene therapy techniques in this and other known mutations that result in genetic epilepsies.

9. FINAL CONSIDERATIONS

In conclusion, the technological advancements in recent years, alongside the implementation of a personalized medicine model, have led to the development of new diagnostic, profiling and therapeutic techniques for several diseases, including epilepsy. Because genetic factors are believed to cause up to 80% of epilepsy cases, genetic therapy emerges as an important tool for accurate diagnosis and the development of new therapeutic techniques.

The application of NGS techniques has allowed clinicians and investigators to understand the genetic basis of epilepsy, enhancing our knowledge of pathophysiology and improving diagnosis and treatment options. While current therapeutic techniques may not yet include genetic therapy, it is undoubtedly a promising avenue, particularly for the treatment of refractory epileptic syndromes such as DS and AS.

Despite recent advances in these areas, there is still much uncertainty surrounding not only the management of epilepsy, but also the use of genetic therapy. Therefore, further research is needed to improve our understanding of the disease and to provide better treatment options for patients. However, it is important for clinicians to remain focused not only on seizure control, but also on other quality of life indicators, as well as in addressing patients' and caregivers' concerns.

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