UNIVERSIDADE D
COIMBRA

Ana Filipa Silva Roque

Relatórios de Estágio e Monografia intitulada "The Influence of Gut Microbiota in the Development of Schizophrenia" referentes à Unidade Curricular "Estágio" sob orientação da Dra. Capitolina Pinho, do Dr. Vito Ladisa e da Professora Doutora Bárbara Rocha apresentados à Faculdade de Farmácia, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

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Eu, Ana Filipa Silva Roque, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2014201403, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "The Influence of Gut Microbiota in the Development of Schizophrenia" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

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Coimbra, 16 de julho de 2020.

Ana Filipa Silva Roque

(Ana Filipa Silva Roque)

Agradecimentos

Aos meu pais, por todo o esforço que fizeram para me proporcionar a possibilidade de ingressar no ensino superior. À minha irmã, quase uma segunda mãe, presente em todos os momentos, a voz da razão nos momentos de maior stress. A toda a família, pelo apoio incondicional.

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Índice

Part III – The Influence of Gut Microbiota in the Development of Schizophrenia

Resumo

O presente documento foi realizado no âmbito da Unidade Curricular "Estágio", inserido no plano de estudos do Mestrado Integrado em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade de Coimbra, obrigatório para aquisição do título de Farmacêutico.

O documento encontra-se dividido em três partes. A primeira parte, Relatório de Estágio em Farmácia Comunitária, diz respeito ao relatório do estágio em Farmácia Comunitária, realizado na Farmácia Figueiredo, no período de 17 de setembro de 2019 a 7 de janeiro de 2020, apresentando-se sob a forma de análise SWOT (*Strengths*, *Weaknesses*, *Opportunities*, *Threats*). Segue-se a segunda parte, Relatório de Estágio em Farmácia Hospitalar, referente ao relatório de estágio em Farmácia Hospitalar, realizado no *Istituto Nazionale dei Tumori*, em Milão, no período de 15 de janeiro a 15 de março de 2020, que resume o conhecimento e a experiência adquiridos nas três diferentes subunidades que constituem a Farmácia Hospitalar do *Istituto Nazionale dei Tumori*: fármacoeconomia e aquisição de medicamentos, ensaios clínicos e experimentais e centro de produção de medicamentos. Quanto à terceira e última parte, refere-se à Monografia intitulada "The Influence of Gut Microbiota in the Development of Schizophrenia".

Palavras-chave: Farmácia Comunitária; Farmácia Hospitalar; Microbiota intestinal; Microbioma; Eixo intestino-cérebro; Eixo microbiota-intestino-cérebro; Esquizofrenia.

Abstract

This document was executed within the scope of the Curricular Unit "Internship", included in the study programme of the Integrated Master's Degree in Pharmaceutical Sciences of the Faculty of Pharmacy of the University of Coimbra, mandatory for the acquisition of the title of Pharmacist.

The present document is divided into three parts. The first part, Internship Report in Community Pharmacy, regarding the report of the internship in Community Pharmacy, performed at Farmácia Figueiredo, from 17th of September 2019 to 7th of January 2020, in the form of SWOT analysis (Strengths, Weaknesses, Opportunities, Threats). Followed for the second part, Internship Report in Hospital Pharmacy, which concerns the internship report in Hospital Pharmacy, performed at the *Istituto Nazionale dei Tumori*, in Milan, from 15th of January to 15th of March 2020. This report aims to synthesize the knowledge and experience acquired in each of the three sub-units that constitute the Pharmacy Hospital of the *Istituto Nazionale dei Tumori*: pharmacoeconomics and acquisition of medicines, clinical and experimental trials and drugs production centre. The third and last part, refers to the Monograph entitled "The influence of the intestinal microbiota on the development of schizophrenia".

Keywords: Community Pharmacy; Hospital Pharmacy; Microbiota; Gut Microbiota; Microbiome; Gut-brain axis; Microbiota-gut-brain axis; Schizophrenia.

Parte I – Relatório de Estágio em Farmácia Comunitária

Lista de Abreviaturas

FF – Farmácia Figueiredo

IMC – Índice de Massa Corporal

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamento Não Sujeito a Receita Médica

MSRM – Medicamento Sujeito a Receita Médica

PIM – Preparação Individualizada de Medicação

PNV – Plano Nacional de Vacinação

PTS – Programa de Troca de Seringas

PVP – Preço de Venda ao Público

SWOT – *Strengths, Weaknesses, Opportunities and Threats*

VIH – Vírus da Imunodeficiência Humana

1. Introdução

O presente relatório de Estágio em Farmácia Comunitária foi realizado no âmbito da Unidade Curricular "Estágio", inserido no plano de estudos do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra, obrigatório para aquisição do título de Farmacêutico.

O estágio decorreu na Farmácia Figueiredo, em Coimbra, no período de 17 de setembro de 2019 a 7 de janeiro de 2020, sob orientação da Dra. Capitolina Pinho, farmacêutica proprietária e Diretora Técnica, completando as 648 horas mínimas obrigatórias em Farmácia Comunitária.

A Farmácia Figueiredo, inaugurada em 1928, conta atualmente com 91 anos de serviço prestado à comunidade. Situa-se no centro da cidade de Coimbra, na Rua da Sofia nº 107, apresentando um edifício renovado.

O relatório apresenta-se sob a forma de análise SWOT (*Strengths, Weaknesses, Opportunities, Threats*), começando pelos pontos fortes: *back-office*, aconselhamento farmacêutico, homeopatia e fitoterapia, Preparação Individualizada de Medicação (PIM), Programa de Troca de Seringas (PTS), VALORMED, fidelização de utentes, *marketing* farmacêutico e diversidade de serviços prestados; seguindo para os pontos fracos: atendimento ao público, preparação de manipulados, configuração da farmácia, erros de *stock* e administração de vacinas da gripe; passando às oportunidades: participação em diversas formações e participação na 'Campanha Nacional de Prevenção Cardiovascular'; chegando por fim às ameaças: medicamentos esgotados ou rateados, receitas manuais, locais de venda de Medicamentos Não Sujeitos a Receita Médica (MNSRM) e localização da farmácia.

2. Análise SWOT

	Fatores Positivos	Fatores Negativos
Fatores Internos	***Strenghts*** **(Pontos Fortes)** *Back-office* Aconselhamento farmacêutico Homeopatia e fitoterapia Preparação Individualizada de Medicação Programa de Troca de Seringas VALORMED Fidelização de utentes *Marketing* farmacêutico Diversidade de serviços prestados	***Weaknesses*** **(Pontos Fracos)** Atendimento ao público Preparação de manipulados Configuração da farmácia Erros de *stock* Administração de vacinas da gripe
Fatores Externos	***Opportunities*** **(Oportunidades)** Participação em diversas formações Participação na 'Campanha Nacional de Prevenção Cardiovascular'	***Threats*** **(Ameaças)** Medicamentos esgotados ou rateados Receitas manuais Locais de venda de MNSRM Localização da farmácia

2.1 Pontos Fortes

2.1.1. *Back-office*

O estágio começou no *back-office*, um ponto bastante desenvolvido ao longo do mesmo. Aqui é onde se procede à receção, reposição e arrumação de todos os medicamentos, produtos de cosmética, dietética e dispositivos médicos.

A receção começa com a chegada da encomenda entregue pelos armazenistas, seguindo para a verificação e receção informática, feita com auxílio do programa Sifarma 2000®. É necessário ter em conta as quantidades que foram encomendadas e as que efetivamente chegaram à farmácia, verificar o prazo de validade, o preço a que foi faturado, bem como o preço a que será vendido ao público. O preço a que será vendido ao público, para Medicamentos Sujeitos a Receita Médica (MSRM) não é possível alterar, seguindo o Preço de Venda ao Público (PVP) implementado, sendo que a margem de lucro vem essencialmente da escolha das marcas preferenciais, dando preferência às que são mais lucrativas para a farmácia. Quanto aos Medicamentos Não Sujeitos a Receita Médicas (MNSRM), produtos de cosmética, dietética e dispositivos médicos, existe uma margem de lucro superior, dando a possibilidade de alterar o preço consoante o que seja mais lucrativo para a farmácia, tendo sendo sempre em conta uma alteração responsável.

Após a receção da encomenda, segue a arrumação dos produtos nos locais estipulados. É necessário que a arrumação seja feita corretamente, de modo a evitar o aumento do tempo de espera dos utentes devido a erros de arrumação.

Quanto à reposição, esta é feita diariamente, duas vezes ao dia, de modo a evitar que os produtos que se encontram junto do local de atendimento estejam sempre disponíveis.

O estágio começar no *back-office* foi um ponto fulcral, pois possibilitou que tivesse um conhecimento de todo o funcionamento da farmácia, desde o funcionamento do Sifarma 2000®, aos vários produtos disponíveis e onde se encontram arrumados, de modo a facilitar o início do atendimento ao público.

2.1.2. Aconselhamento farmacêutico

O aconselhamento farmacêutico é o ponto principal para a Farmácia Comunitária. Não somos simples vendedores, que dispensam o que o utente pretende sem questionar, somos profissionais de saúde. O nosso principal foco é o utente e a sua saúde. Quando um utente chega à farmácia, devemos começar por avaliar o mesmo: elaborar perguntas de modo a perceber os sintomas e qual a frequência; possíveis causas. Segue-se a decisão: verificar se é necessária intervenção médica ou se o problema pode ser resolvido na farmácia; caso seja possível, seleção do tratamento, adaptado ao doente em questão. Chega então a parte do

aconselhamento, sendo dever do farmacêutico dar todas as informações necessárias para que o tratamento seja feito corretamente, de modo a melhorar a eficácia. Por fim vem o acompanhamento, mostrando ao utente a importância da sua saúde para nós.

Ao longo do estágio tive oportunidade de assistir a vários atendimentos, assimilando o conhecimento transmitido pelos vários profissionais que constituem a FF. Esse conhecimento irá ser bastante importante para exercer a minha profissão no futuro.

2.1.3. Homeopatia e Fitoterapia

O aumento da procura por medicamentos naturais, mais próximos das antigas mezinhas utilizadas pelos nossos antepassados, com menores efeitos secundários, levou a que a FF apostasse na homeopatia e fitoterapia como elementos diferenciadores.

A homeopatia utiliza as substâncias que provocam a doença para obter a sua cura, no entanto as doses são muito pequenas, obtidas através de diluições substanciais. Os sintomas são encarados como desequilíbrios no organismo, atuando a terapia de forma a estimular as suas defesas. Esta terapia começa a ganhar maior relevância, sendo que atualmente já existem médicos a reconhecer este método terapêutico e a prescreve-lo aos seus doentes.

A fitoterapia é um método terapêutico que tem por base substâncias de origem vegetal. Tendo a FF uma vasta linha de produtos nesta área, foram vários os aconselhamentos em que estes foram indicados e obtiveram boa aceitação. Os resultados benéficos comprovados, contribuíram para que vários utentes voltassem a adquirir estes produtos.

Apesar de ao longo do curso ter tido oportunidade de adquirir conhecimento na área da fitoterapia, na FF tive oportunidade de o aplicar em contexto real. Quanto à área da homeopatia, tão frequentemente desvalorizada, o conhecimento era escasso, pelo que considero que o conhecimento adquirido foi importante para a minha formação. Acredito que a oportunidade que tive de ter contacto com estas terapias complementares será positivo no futuro, pois é um elemento que assim como diferencia a FF, também me poderá diferenciar. Sendo o farmacêutico especialista do medicamento, é importante ter conhecimento dos vários tipos que existem disponíveis.

2.1.4. Preparação Individualizada de Medicação

A PIM é um dos serviços prestados pela FF, semanalmente, a várias instituições de Coimbra, na qual tive oportunidade de participar ativamente ao longo do estágio, com a supervisão da farmacêutica responsável. O principal objetivo é assegurar a utilização correta, segura e efetiva do medicamento, melhorando a adesão à terapêutica e evitando erros de medicação[1].

Este serviço consiste na organização da medicação de cada utente, de acordo com o mapa terapêutico prescrito pelo médico, enviado pela instituição semanalmente ou assim que ocorram alterações. A medicação é organizada num dispositivo com vários compartimentos, cada um corresponde a um determinado momento do dia (pequeno-almoço, almoço, jantar e deitar), para cada um dos dias da semana. O dispositivo é devidamente selado e descartado na farmácia, após a sua utilização. Estas preparações são efetuadas no laboratório da FF, onde se encontram armazenados os medicamentos de todos os utentes, devidamente identificados e separados, seguindo as condições de conservação, garantindo a sua qualidade, segurança e eficácia[1].

Outra vertente deste serviço, inclui a informação escrita e oral do mapa terapêutico do utente, juntamente com informações pertinentes para a compreensão do mesmo, de forma a auxiliar a correta administração dos medicamentos[1].

O público-alvo da PIM na FF são essencialmente utentes idosos, polimedicados, aos cuidados de diferentes instituições. Sendo os mapas terapêuticos complexos, estes utentes têm dificuldade em cumpri-los por si, pelo que as instituições recorrem à FF para garantir que a medicação é tomada de forma correta.

A participação na prestação deste serviço veio reforçar o contributo do farmacêutico em assegurar a utilização correta, segura e efetiva do medicamento, uma das suas funções prioritárias.

2.1.5. Programa de Troca de seringas

O objetivo do PTS, iniciado em 1993, é a prevenção de infeções pelo VIH e pelos vírus das Hepatites B e C, por via endovenosa, parentérica e sexual em pessoas que utilizam drogas injetáveis. O programa consiste na distribuição de material esterilizado, posterior recolha e destruição do material utilizado, evitando o abandono e reutilização de seringas. O kit disponibilizado contém duas seringas, um preservativo, dois toalhetes desinfetantes, duas ampolas de água bidestilada, dois filtros, dois recipientes, duas carteiras de ácido cítrico e um folheto informativo[2,3].

O impacto deste programa é notório na redução de casos diagnosticados por infeção VIH em toxicodependentes, bem com na sensibilização da população em geral[2].

Estando a FF localizada num dos principais focos de toxicodependentes em Coimbra, tive oportunidade de proceder a inúmeras trocas de kits. O contacto com esta realidade permitiu-me ter uma pequena noção da quantidade de utilizadores regulares na zona, bem como evitar o julgamento; as pessoas não são as suas escolhas.

2.1.6. VALORMED

A VALORMED é uma sociedade sem fins lucrativos, criada em 1999, responsável pela gestão de embalagens vazias e medicamentos fora de uso. Os resíduos de medicamentos são recolhidos em contentores na farmácia, assim que estão cheios são entregues aos distribuidores, seguindo por fim para Centros de Triagem, onde são separados e devidamente tratados. A acessibilidade dos pontos de recolha na farmácia permitiu consciencializar a população da importância ambiental de colocar os resíduos de medicamentos no devido local[4].

O conhecimento deste programa permitiu-me ter consciência, bem como perceber a importância de sensibilizar os utentes para o facto do medicamento, como resíduo específico, deve ser tratado de forma diferente dos restantes resíduos urbanos.

2.1.7. Fidelização de utentes

São vários os utentes fidelizados na FF, essencialmente idosos polimedicados. A fidelização de utentes traz vantagens económicas para a farmácia, porém é também fundamental para efetuar o acompanhamento do utente, pelo que é do interesse da farmácia aumentar a fidelização.

Estratégias que contribuem para o aumento da fidelização de utentes é o atendimento personalizado, desde pequenos pormenores, como decorar o nome do utente, a atender as suas necessidades de forma adequada. A simpatia e a capacidade de ouvir são duas características muito importantes. Especialmente no caso dos utentes idosos, estes sentem necessidade de maior atenção, pois muitos passam a maior parte do tempo sozinhos, procurando na farmácia algum conforto. É também importante que os produtos e serviços disponíveis sejam frequentemente revistos, de forma a atender as necessidades dos utentes.

2.1.8. *Marketing* farmacêutico

O *Marketing* farmacêutico atua não apenas a nível físico, na farmácia, como também virtual, através das redes sociais, tendo em vista o aumento do lucro da farmácia, potenciando as vendas e contribuindo para atrair e fidelizar utentes.

Podemos notar a sua presença assim que entramos na farmácia, desde a disposição dos produtos nos lineares, à disposição no espaço do balcão de atendimento. Os MNSRM, medicamentos homeopáticos, produtos de cosmética, dietética são privilegiados no campo visual do utente, de modo a potenciar o desejo de efetuar a compra.

A sua presença é também notória a nível virtual, pois a FF aposta na divulgação em diversas redes sociais, como o *Facebook* e o *Instagram*, nova rede social criada, através de publicações frequentes nas mesmas. Nestas redes sociais são partilhadas campanhas e

promoções a decorrer na farmácia, formações e consultas especializadas diversas a realizar, bem como partilha de informação útil aos utentes, contribuindo para a educação para a saúde. Sendo uma área que me cativa, pela parte criativa inerente, durante o estágio tive oportunidade de dar o meu contributo para algumas das publicações partilhadas.

A população que frequenta a FF com maior regularidade, é uma população essencialmente envelhecida. Para que a farmácia consiga atingir diferentes públicos-alvo, é necessário implementar diferentes estratégias, onde o marketing farmacêutico também atua. Uma das recentes estratégias implementadas foi a introdução de uma nova linha de cosmética e maquilhagem, tendo em vista um público mais jovem.

Outra estratégia relacionada com o *marketing* farmacêutico, fulcral na Farmácia Comunitária, é a venda cruzada, uma das capacidades que um farmacêutico a exercer nesta área precisa de desenvolver. A venda cruzada consiste em sugerir diferentes produtos, de modo a aumentar o valor da venda. Ao sugerir diferentes produtos, adequados às necessidades do utente, mostrando preocupação, ampliamos o nível de confiança depositado no farmacêutico. Importante referir que esta técnica deve ser utilizada de forma responsável, tendo sempre em vista o melhor para o doente. A ideia não é conseguir vender o máximo só por si, mas sim vender produtos que acrescentem valor para a resolução do problema do utente.

2.1.9. Diversidade de serviços prestados

São vários os serviços prestados na FF, pensados de forma a proporcionar saúde e bem-estar aos utentes, respondendo às suas necessidades.

Na área dos serviços farmacêuticos temos as medições de parâmetros bioquímicos (glicémia e colesterol total), pressão arterial e Índice de Massa Corporal (IMC). Ao longo do estágio realizei diversas medições de pressão arterial, pois existem utentes que frequentam habitualmente a farmácia para controlar esses valores. Durante a 'Campanha Nacional da Prevenção Cardiovascular' tive oportunidade não só de realizar medições da pressão arterial com deteção de fibrilação auricular, como também de colesterol total e IMC. Também é possível administrar vacinas que não constam no Plano Nacional de Vacinação (PNV), nomeadamente a vacina da gripe, sendo este outro dos serviços prestados.

Outro dos serviços prestados são consultas de diversas especialidades, nomeadamente podologia e nutrição. Recentemente foram introduzidos novos programas, o *beauty and shape system*, direcionado a regenerar e remodelar o rosto e o corpo e o programa de massagem "Menos *Stress* Mais Rendimento".

A FF aposta também na realização de diversos *workshops*, como por exemplo o *workshop* de aromaterapia dedicado às patologias de Inverno.

2.2. Pontos Fracos

2.2.1. Atendimento ao público

Comparando com as restantes atividades desenvolvidas na farmácia, o atendimento ao público acabou por ter um período de tempo substancialmente menor, sendo maioritariamente observacional. O facto de ter sido o meu primeiro contacto com Farmácia Comunitária, certamente influenciou negativamente o meu desenvolvimento nesta área, pois a confiança que desenvolvi não foi suficiente para ter a autonomia que gostaria de ter atingido.

Não obstante à necessidade de desenvolver competências de aconselhamento, tão fortemente abordado ao longo do curso, considero também relevante haver algum aprofundamento a nível do atendimento publico, nomeadamente, na forma de como lidar e abordar os mesmos.

2.2.2. Preparação de manipulados

Atualmente os manipulados preparados na FF são essencialmente à base de cremes, sendo que os pedidos são escassos, pois é bastante dispendioso. Apesar de que ao longo do curso tivemos contacto com várias preparações de manipulados, seria interessante ter tido oportunidade de aplicar o conhecimento adquirido numa situação real.

2.2.3. Configuração da farmácia

Um dos problemas da configuração da farmácia é o facto da sua dimensão total ser grande, porém nem todos os pisos têm as dimensões necessárias, sendo interligados por escadas.

Começando pelo piso -1, *back-office*, é notória a falta de espaço para todos os produtos que é necessário armazenar neste local. Seguindo para o piso 0, local de atendimento ao público, onde o espaço é demasiado limitado, desde falta de espaço para os lineares, os utentes da farmácia, até à área do balcão.

Quanto à ligação por escadas, esta dificulta as deslocações, essencialmente entre o piso -1 e 0, mais frequentes, contribuindo para um maior desgaste físico.

2.2.4. Erros de *stock*

Os erros de *stock* levam ao aumento do tempo de espera por parte dos utentes durante o atendimento, podem levar à impossibilidade de satisfazer as suas necessidades ou conduzir a um excesso de *stock*, ambos com impactos económicos negativos para a farmácia.

Estes erros podem surgir devido a erros na receção ou na saída do produto, bem como na incorreta arrumação do mesmo. É importante perceber quais os fatores que podem contribuir para a existência de erros de *stock*, de modo a minimizar ao máximo a sua ocorrência.

2.2.5. Administração de vacinas da Gripe

A administração de vacinas que não constam no PNV é um dos vários serviços prestados pela FF. O meu período de estágio coincidiu com o período em que a vacina da gripe deve ser administrada, de outubro a dezembro, pelo que tive oportunidade de observar a administração efetuada por uma das farmacêuticas da farmácia. Como não realizei o curso de administração de vacinas, não tive oportunidade de experimentar, porém será uma mais valia realizá-lo no futuro.

2.3. Oportunidades

2.3.1. Participação em diversas formações

Durante o período de estágio tive oportunidade de participar em várias formações nas mais diversas áreas, desde a homeopatia à cosmética. Assisti a formações das seguintes entidades: Boiron (homeopatia), Pranarom (fitoterapia), Skinerie e Darphin (cosmética), Pharma nord e Y Farma (suplementos alimentares).

Estas formações contínuas são de extrema importância para que se adquira confiança no aconselhamento dos produtos disponíveis na farmácia. Quanto mais informados estiverem os farmacêuticos, maior segurança apresentam no aconselhamento, maior a probabilidade de convencer o utente a adquirir o produto, levando assim ao aumento do lucro da farmácia.

2.3.2. Participação na 'Campanha Nacional de Prevenção Cardiovascular'

No período de 22 a 25 de outubro de 2019, a FF participou na 'Campanha Nacional de Prevenção Cardiovascular', promovida pelas Farmácias Apoteca Natura, *Network* internacional de Farmácias na qual está inserida, com o objetivo de promover a prevenção cardiovascular.

Esta campanha consistiu na realização de um questionário, de forma a aferir os dados da pessoa e registar as doenças que podem potenciar o risco cardiovascular tanto pessoal, como

familiar, acompanhado da realização de algumas medições pertinentes, como a pressão arterial, risco de fibrilação auricular, colesterol e perímetro abdominal. Estes testes foram realizados pelos estagiários, através de um sistema informático. A divulgação dos resultados aos utentes que participaram, foi feita assim que o teste terminou, acompanhada de um aconselhamento personalizado, de modo a sensibilizar as pessoas da importância do seu bem-estar cardiovascular, incentivando um estilo de vida mais saudável, adequado à diminuição do desenvolvimento de doenças cardiovasculares.

2.4. Ameaças

2.4.1. Medicamentos esgotados ou rateados

Os medicamentos esgotados ou rateados são uma das grandes ameaças com a qual me deparei ao longo do estágio.

Os medicamentos esgotados são aqueles que por diversos motivos se encontram temporariamente indisponíveis. Já os medicamentos rateados são aqueles cuja a sua comercialização se encontram consideravelmente reduzida. Ambas têm um impacto semelhante na farmácia, falta de medicamentos para suprir as necessidades dos utentes. A gestão destes medicamentos deve ser feita de forma racional. Diariamente é feita uma encomenda específica para este tipo de medicamentos, não só através do Sifarma 2000®, como pelo telefone, porém tendo em conta a dificuldade na sua aquisição, ficam em falta por longos períodos de tempo.

Verifiquei que para os utentes é difícil compreender que o problema dos medicamentos esgotados ou rateados não se deve a incompetência da farmácia, mas sim a indisponibilidade a nível nacional. O que vem reforçar a importância de explicar devidamente estas situações, de modo a evitar equívocos.

2.4.2. Receitas manuais

Apesar das receitas manuais serem cada vez menos utilizadas, ainda continuam a aparecer, sendo vários os cuidados a ter. É necessário conferir todos os detalhes, pelo que é exigido maior concentração para detetar possíveis erros. A maior dificuldade é a ilegibilidade da letra do prescritor, bem como as rasuras que impossibilitam a utilização da receita para efeitos de comparticipação. Os requisitos específicos que as receitas manuais têm obrigatoriamente de cumprir para serem aceites são nos ensinados ao longo do curso, porém a prática e a ajuda recebida ao longo do estágio foram crucias para que a dispensa e comparticipação de medicamentos através de receitas manuais seja bem-sucedida.

2.4.3. Locais de venda de MNSRM

Os locais de venda de MNSRM são uma das grandes ameaças às farmácias. Estes locais apresentam preços competitivos, muitas vezes inferiores aos da farmácia, pelo que se tornam bastante atraentes para o público em geral. Face a esta ameaça, um dos pontos que nos pode diferenciar destes locais é o aconselhamento. A aposta num bom aconselhamento irá contribuir para que os utentes fiquem mais satisfeitos com a aquisição do produto, potenciando a probabilidade de voltarem à farmácia.

2.4.4. Localização da Farmácia

A FF tem várias farmácias e locais de venda de MNSRM próximos, pelo que a necessidade de se destacar face a esta ameaça é elevada, de modo a atrair o maior número de utentes possível. São várias a estratégias adotadas, desde o aconselhamento aos produtos disponíveis, especialmente na área da homeopatia e fitoterapia, campanhas publicitárias, diversidade de serviços prestados.

3. Casos Práticos

3.1. Caso Prático 1

Uma senhora dirige-se à farmácia, alegando que o seu filho de 8 anos está constipado e necessita de algo para aliviar os sintomas. Refere que tem a garganta inflamada, nariz congestionado e tosse. Quando questionada quanto ao tipo de tosse, refere ser uma tosse seca. Até ao momento não apresenta febre. Não apresenta historial de doenças crónicas.

Começámos então por aconselhar Golamir 2Act® comprimidos para o alívio da dor e irritação de garganta. O produto é composto por ActiFilm DOL, complexo molecular vegetal que forma uma película com efeito barreira, que protege e normaliza a superfície da mucosa. Os óleos essenciais de eucalipto e hortelã proporcionam um efeito refrescante. Recomenda-se a toma de 1 comprimido, 3 a 4 vezes por dia, deixando dissolver lentamente na boca[5]. É importante que não ingira água logo após a toma do comprimido, pois irá reduzir a sua eficácia, quebrando o efeito barreira.

Para o alívio da tosse, aconselhámos a o xarope de inverno da Pranarom, à base de óleos essenciais de canela, anis e eucalipto, que promove a manutenção da saúde respiratória normal e das defesas naturais. A dose adequada é de 5 mL, 3 vezes por dia, durante 5 dias consecutivos.

Aconselhou-se ainda um *spray* nasal de água do mar hipertónica, para alívio da congestão. Referiu-se os cuidados para uma correta utilização, destacando a necessidade de limpar o aplicador. O *spray* deve ser aplicado até 4 vezes por dia, durante um máximo de 5 dias.

Como medidas não farmacológicas, referiu-se essencialmente a necessidade de ingerir água regularmente e bebidas quentes, como chá com mel e limão.

3.2. Caso Prático 2

Uma estudante universitária recorre ao aconselhamento farmacêutico, de modo a aliviar a ansiedade que sente, agravada em momentos de exposição pública, como apresentação de trabalhos, bem como em épocas de exames. Procura um produto que alivie os sintomas, pois sente um elevado nível de cansaço, mas que não provoque sonolência, de modo a não interferir com os estudos. Refere que não toma medicação regularmente, no entanto, por vezes, recorre à toma de paracetamol para alívio da dor de cabeça. Os sintomas persistem há um longo período de tempo, pretende algo com efeito rápido.

Aconselhou-se a toma de ANSIWELL® Fast, suplemento que ajuda no alívio da ansiedade, irritação, cansaço mental, palpitações, entre outros sintomas derivados. ANSIWELL® Fast é composto por L-Teanina, aminoácido que contraria os efeitos estimulantes do sistema nervoso, induzindo uma sensação de relaxamento e alerta mental, sem causar sonolência, aumento de peso, nem habituação. É composto também por vitamina C e magnésio, mineral fundamental nos processos de aprendizagem, formação de conexões neuronais e na memória.

Recomendou-se a toma de uma cápsula de ANSIWELL® Fast pela manhã após o pequeno almoço. Reforçar a dose à noite, se necessário, tomando uma cápsula 1 hora antes de ir dormir para promover um sono reparador. Quando se pretende uma ação rápida, antes dos momentos que sabe que irão gerar um elevado nível de ansiedade, tomar uma cápsula 30 minutos antes. A duração mínima recomendada para uma eficaz estabilização emocional é de 4 meses, não existindo período definido quanto à duração máxima[6]. Monitorizar os sintomas, caso não sinta alívio, sendo uma situação já prolongada, será necessário procurar ajuda especializada.

3.3. Caso Prático 3

Um senhor, com cerca de 50 anos, recorre à farmácia no sentido de adquirir um laxante para melhorar a obstipação. Quando questionado em relação aos sintomas e à sua duração, referiu sentir dificuldade a defecar, evacuando menos de 3 vezes por semana, há cerca de 2 semanas. Até ao momento não se encontrava a tomar medicação regularmente. Referiu já ter recorrido a laxantes anteriormente, porém não obteve o efeito pretendido.

Inicialmente começou-se por aconselhar medidas não farmacológicas: aumentar o conteúdo de fibras faseadamente na alimentação, através da ingestão de fruta, vegetais, leguminosas e cereais integrais; mastigar bem os alimentos; beber muita água, entre 1 a 2 L por dia; reeducar o intestino através do estabelecimento de horários regulares, adequados a responder à vontade de evacuar; não ignorar o reflexo de defecação; prática regular de exercício físico, idealmente 30 minutos por dia, sendo de melhor utilidade, neste caso, o que atue sobre a musculatura abdominal; não utilizar roupas apertadas; limitar o consumo de álcool e cafeína; evitar alimentos que induzam ou agravam a obstipação, como os alimentos ricos em cálcio, arroz e chocolate. É impreterível que o utente siga estas medidas, pois é frequente que apenas alterações no estilo de vida sejam suficientes para prevenir o a ocorrência de novos episódios.

Seguiu-se o aconselhamento de um laxante para o alívio imediato dos sintomas, o MeliLax® adulto, um microclister à base de Promelaxin, um complexo de méis, enriquecido com polissacáridos de Aloé e Malva. O MeliLax é dotado de uma ação evacuante, induz um estímulo não agressivo de ativação da defecação, bem como de uma ação protetora na mucosa retal, reduzindo a sensação de incómodo, a irritação e a inflamação associadas à obstipação. Explicou-se o modo correto de utilização, essencial para que o produto tenha o efeito desejado. Primeiro deve-se abrir o microclister, removendo o cobre-cânula, de modo a separá-lo completamente do anel. De seguida aplica-se algumas gotas de produto na zona perianal e introduz-se delicadamente a cânula no reto. Pressionar a fundo o microclister até à extração completa da cânula. O MeliLax® deve ser aplicado quando se pretende evacuar, levando cerca de 15 a 30 minutos a exercer a sua ação[7]. Não deve ser utilizado por longos períodos de tempo.

Aconselhou-se o doente a seguir as indicações referidas, monitorizar os sintomas. Se após uma semana os sintomas melhorarem, deve reforçar as medidas não farmacológicas, diminuindo progressivamente a toma do MeliLax®. Se não se verificar alívio dos sintomas, deve procurar ajuda médica.

3.4. Caso Prático 4

Uma senhora, com cerca de 30 anos, dirige-se à farmácia, com o intuito de adquirir um produto para o alívio da infeção urinária.

Inicialmente tentou-se perceber quais os sintomas que sentia. Referiu sentir bastante prurido, vermelhidão na zona vulvar e dor ao urinar. Quando questionada quanto à frequência urinária, referiu que a frequência era normal, contrariando um dos sintomas mais característicos da infeção urinária, a vontade constante de urinar. Perguntou-se ainda se tinha

algum tipo de corrimento vaginal anormal. Afirmou ter corrimento branco, espesso e sem cheiro.

Pelas respostas dadas, levou-nos a suspeitar que o autodiagnóstico da utente era incorreto e que estávamos efetivamente perante um caso de candidíase vaginal, infeção causada por um fungo oportunista do género *Candida*, sendo a espécie mais comum a *Candida Albicans*.

Aconselhámos então a toma de LIBIFEME® *Optimal* óvulos vaginais. Estes óvulos proporcionam uma ação protetora, rápida e localizada, reduzem o ardor, prurido e irritações. Ajudam a manter as defesas naturais da vagina, a equilibrar o pH e a flora vaginal, proporcionam regeneração celular, bem como hidratação da mucosa vaginal. Deve colocar 1 óvulo/dia, durante 5 dias consecutivos. Se necessário, deve aplicar 2 óvulos/semana durante mais 2-3 semanas[8].

Para um tratamento eficaz, antes da aplicação do óvulo deve-se efetuar uma correta lavagem da zona íntima, recorrendo a produtos adequados, de modo a não alterarem o pH vaginal. Sugeriu-se a utilização de WOMAN ISDIN® Higiene Íntima, um gel íntimo de uso diário que neutraliza os odores, alivia o prurido e evita proliferações bacterianas[9].

Referiu-se também medidas não farmacológicas que ajudam a prevenir a ocorrência de candidíase: higienizar corretamente a área genital, medida referida anteriormente; evitar o uso de roupas apertadas; usar roupa interior de algodão; evitar o uso de pensos diários; evitar comer alimento ricos em hidratos de carbono, pois desregulam o pH, promovendo o desenvolvimento de microrganismos oportunistas, como é o caso da *Candida*. Caso os sintomas persistam após o tratamento, aconselha-se consultar o médico.

4. Conclusão

O Estágio em Farmácia Comunitária, obrigatório, incluído no plano de estudos, é uma oportunidade que nos é concedida de ter um primeiro contacto com a área em questão, em contexto real. Permite-nos passar do conhecimento teórico adquirido ao longo do MICF, para a prática do exercício da profissão farmacêutica, fundamental para nos tornarmos farmacêuticos de excelência num futuro próximo.

A Farmácia Comunitária é a área com maior visibilidade da profissão farmacêutica, sendo um dos primeiros locais ao qual os utentes se dirigem para resolver questões de saúde, daí a sua elevada importância na atividade farmacêutica e a necessidade de adquirir experiência nesta área.

O processo de aquisição de conhecimento é contínuo ao longo da vida, é importante procurar sempre a melhoria das nossas capacidades, tanto como pessoa, como profissional. Este contacto com a realidade possibilitou o desenvolvimento de várias características, como a simpatia, a escuta ativa, a comunicação, essenciais para proporcionar um bom atendimento ao utente. Apesar de sentir que a confiança que desenvolvi não foi suficiente para ter a autonomia que gostaria de ter atingido, certamente no futuro irei ter mais oportunidades para melhorar.

Considero que a minha experiência de Estágio em Farmácia Comunitária foi bastante enriquecedora, essencial para ter uma visão do caminho que pretendo seguir como farmacêutica.

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Parte II – Relatório de Estágio em Farmácia Hospitalar

Nota: O presente relatório de estágio segue as normas exigidas pelo orientador de estágio da instituição de acolhimento, Dr. Vito Ladisa, responsável pela unidade de Farmácia da *Fondazione IRCSS Istituto Nazionale dei Tumori*, localizado em Milão, Itália.

FONDAZIONE IRCSS ISTITUTO NAZIONALE DEI TUMORI

FINAL REPORT

Internship in Hospital Pharmacy

HOSPITAL PHARMACY

- Pharmacoeconomics and acquisition
- Clinical and experimental trials
- Drugs production center

INTRODUCTION

Trainee: Ana Filipa Silva Roque
Tutor: Dr. Vito Ladisa

This report aims to synthesize the knowledge and experience acquired in each of the three sub-units that constitute the pharmacy of this hospital, during the 2-month internship (January 15th to March 15th of 2020).

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FACULDADE DE FARMÁCIA
UNIVERSIDADE D
COIMBRA

FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

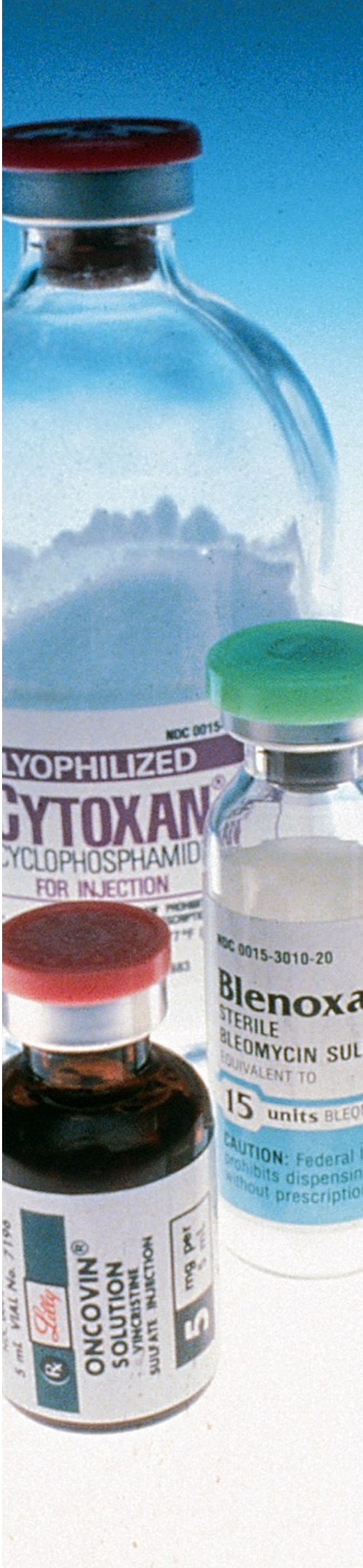
Pharmacoeconomics and acquisition

Functions:

- Checking all drugs in the hospital.
- Connecting entity between the pharmacy and patients. Availability of drugs to be taken at home. It is the duty of the pharmacist to inform the patient how to administer the medication.
- Connecting entity between the pharmacy and the other departments. Availability of the necessary medication for each hospital department.
- Monitoring of certain drugs through AIFA, in order to obtain future reimbursement from the state.
- Prepare some simple galenical preparations.
- Lead and follow some experimental studies.

Tasks preformed:

- Cognizance of operation of the various programs used in the sub-unit.
- Observing the pharmacists´ interaction to the public.
- Supervising order of drugs sourced by storage to departments.
- Weekly assistance for keeping track of specific drugs.
- Supervising the preparation of various galenical preparations.
- Evaluation of protocols for its usefulness and viability further leading to possible implementation in the hospital.



Clinical and experimental trials

Functions:

- Checking all drugs used in clinical and experimental trials developed at the hospital.
- Receive the drugs used in the various studies and provide them to patients and to all hospital departments.
- Elaboration of protocols to be followed by health professionals responsible for the administration of the new tested drugs introduced.
- Validation and preparation of drugs for blind and double blind studies.
- Validation of experimental drugs intended to be prepared in the drugs production center.
- Daily check of the temperature of the storage of all experimental drugs.
- Evaluation of the usefulness and viability of possible studies to be developed in the hospital.

Tasks preformed:

- Cognizance of GMP and guidelines followed in clinical trials.
- Awareness of recently introduced clinical and experimental study protocols.
- Proficiency in the operation of the various programs used in this sub-unit.
- Assisting in receiving experimental drugs.
- Passively supervising the process of providing experimental drugs.
- Observer during the drug accounting process by the person responsible for the study.
- Attend kick-off meetings of new clinical trials.

Drugs production center

Functions:

- Validation, production and control of chemotherapeutic drugs administered intravenously.
- Validation, production and control of hydration solutions used before and after chemotherapy.
- Production and control of experimental drugs by centralized process.
- Sending drugs and hydration solutions to all departments of the hospital.
- Verification of new chemotherapy regimens, suggested by doctors. After verification, insert in the data base (cartella terapia).
- Evaluation of the possibility of centralizing experimental drugs.

Tasks preformed:

- Basic knowledge of the operation of the various programs used in this sub-unit.
- Verification of the expiration date of unused drugs that have not yet been reused, in order to follow the disposal process for those that are expired.
- Sending drugs and hydration solutions to the various departments of the hospital.
- Knowledge of the characteristics of the various drugs used.
- Attend meetings to evaluate the centralization of experimental drugs.

Conclusion

During the duration internship I had the opportunity to perceive the role of a pharmacist, as a health professional in an hospital, and which different areas can he be envolved. As an oncological hospital, I was able to have a closer contact with the drugs used in this area, understand better their characteristics, functions and what procedures to follow to maintain their safety, quality and effectiveness. It helped me to have a perception of the functioning of the pharmacy of an hospital in another country, Italy, making it possible to compare with Portugal in the future. This internship allowed me not only to practice what I learned theoretically, but also to acquire more knowledge in practice. The experience has added more value to my learning, and i see it contributing in becoming a better professional in the future.

Part III – The Influence of Gut Microbiota in the Development of Schizophrenia

Resumo

O microbiota intestinal humano é constituído por um vasto número de microrganismos, maioritariamente bactérias, que se co-desenvolveram com o seu hospedeiro humano. Recentemente, neurocientistas começaram a apreciar a influência da interação dinâmica entre os micróbios intestinais e os sistemas gastrointestinal e nervoso do seu hospedeiro, atualmente conhecido por eixo microbiota-intestino-cérebro. Esta comunicação bidirecional envolve vias imunológicas, neuronais, endócrinas e metabólicas. Evidências de estudos realizados nos últimos anos sugerem que o microbiota pode estar associado a doenças neuropsiquiátricas, em particular a esquizofrenia.

Esta monografia resume e discute as informações atualmente disponíveis sobre a influência do ambiente gastrointestinal no sistema nervoso central, com foco no eixo microbiota-intestino-cérebro, os mecanismos subjacentes à comunicação bidirecional entre o microbiota intestinal e o cérebro, o impacto que o eixo microbiota-intestino-cérebro pode ter na esquizofrenia e novas estratégias terapêuticas, de modo a estabelecer perspetivas para o futuro. Com efeito, estudos recentes relataram que distúrbios neuropsiquiátricos, como a esquizofrenia estão associados a alterações do microbiota intestinal, um fenómeno conhecido por disbiose. Alterações no microbiota intestinal induzem a ativação anormal das principais vias de comunicação do eixo microbiota-intestino-cérebro através de mecanismos imunológicos, neurais, endócrinos, metabólicos e epigenéticos, levando a uma inflamação exacerbada da mucosa intestinal e a alterações nas respostas ao *stress*.

Em jeito de conclusão, o eixo microbiota-intestino-cérebro emerge agora como uma nova estratégia terapêutica para prevenção e tratamento de distúrbios neuropsiquiátricos, incluindo a esquizofrenia. No entanto, mais estudos são necessários para que a visão tradicional da etiologia das doenças neuropsiquiátricas seja alterada, revelando o papel real do eixo microbiota-intestino-cérebro e o seu potencial como alvo de novos tratamentos.

Palavras-chave: Microbiota intestinal; Microbioma; Eixo intestino-cérebro; Eixo microbiota-intestino-cérebro; Esquizofrenia.

Abstract

The human gut microbiota (GM) comprise a large number of microorganisms, mostly bacteria, which co-evolved together with their human host. Recently, neuroscientist began to appreciate the influence of the dynamic interaction between gut microbes and host gastrointestinal and central nervous system, the now known microbiota-gut-brain (MGB) axis. This bidirectional communication involves immune, neural, endocrine and metabolic pathways. Recent evidences suggest that microbiota may be associated with the pathogeny of neuropsychiatric diseases, in particular schizophrenia (SCZ).

This document summarizes and discusses currently available information on the interaction between the gastrointestinal and central nervous system, focusing on the MGB axis, the mechanisms underlying the bidirectional communication between the GM and the brain, the impact that MGB axis may have in SCZ and novel therapeutic strategies, to establish future perspectives. Indeed, recent studies reported that neuropsychiatric disorders, such as SCZ, are associated with changes in the GM, a phenomenon known as dysbiosis. Alterations in the GM induce aberrant activation of key pathways of MGB axis communication, including immune, neural, endocrine, metabolic and epigenetic mechanisms, leading to exacerbated intestinal mucosal inflammation and altered responses to stress.

The MGB axis may provide a novel therapeutic strategy for the prevention and treatment of neuropsychiatric disorders, including SCZ. However, further research is required to change the traditional view of neuropsychiatric diseases, revealing the feasibility and potential of the of MGB axis as a target for novel treatment.

Keywords: Microbiota; Gut Microbiota; Microbiome; Gut-brain axis; Microbiota-gut-brain axis; Schizophrenia.

List of Abbreviations

5-HT – 5-Hydroxytryptamine

αMSH – Alpha-melanocyte stimulating hormone

ACTH – Adrenocorticotrophic hormone

AhR – Aryl hydrocarbon receptor

BBB – Blood-brain barrier

BDNF – Brain-derived neurotrophic factor

BMI – Body mass index

CCK – Cholecystokinin

CRF – Corticotrophin-releasing factor

CNS – Central nervous system

DNMT – DNA (cytosine-5)-methyltransferase

FFAR – Free Fatty Acid Receptor

FGA – First-generation antipsychotics

FMT – Fecal microbiota transplantation

FOS – Fructo-oligosaccharide

GABA – Gamma-aminobutyric acid

GALT – Gut associated lymphoid tissue

GI – Gastrointestinal

GLP-1 – Glucagon-like peptide 1

GM – Gut microbiota

GOS – Galacto-oligosaccharide

HDAC – Activity of histone deacetylases

HPA – Hypothalamic-pituitary–adrenal

IDH – Isocitrate dehydrogenase

IL – Interleukin

LC-PUFA – Long-chain polyunsaturated fatty acids

LPS – Lipopolysaccharide

LTL – Shorter leucocyte telomere length

MGB – Microbiota-gut-brain

miRNA – MicroRNA

mRNAs – Messenger RNA

MTT – Microbiota transfer therapy

NMDA – N-methyl-D-aspartate

PAMP – Pathogen-associated molecular patterns

pri-miRNA – Precursor molecules of micro RNA

PRR – Pattern recognition receptor

PYY – Peptide-YY

RISC – RNA-induced silencing complexes

SAM – S-adenosylmethionine

SCFA – Short-chain fatty acids

SCZ – Schizophrenia

SGA – Second-generation antipsychotics

SHGM – Standardised human gut microbiota

TET – Ten-eleven translocation

TGF-β – Transforming growth factor-β

Th – T helper

TLR – Toll-like receptor

TS – Telomere shortening

TNF – Tumour necrosis factor

1. Introduction

The abstract idea of the gut-brain axis has raised interest since a thousand years. In Greece, 2000 years ago, Hippocrates, the father of modern medicine, proclaimed that "All disease begins in the gut". William Beaumont, in the 1800s, in notes of "pain and uneasiness" at corporeal sites far from the wound, linked digestion with disease and emotion. He realised that the emotional state affected digestion, suggesting a link between the gut and the brain[23]. Even into our everyday language, the implicit concept of the gut-brain axis is commonly used in expressions as gut feelings, gut instinct, it takes guts, butterflies in one's tummy[2].

Neuroscientists began to appreciate the influence of gut-brain axis, that later evolved to MGB axis, the now known dynamic interaction between gut microbes and host gastrointestinal and central nervous system. This axis influences modulating brain function and maintaining homeostasis, especially during stressful situations. Through the years, the effort to understand the critical role that the MGB axis plays in homeostatic processes in health and disease is constantly increasing[2]. Recent studies have demystified the role of MGB axis in neuropsychiatric diseases, in particular SCZ which is a severe neuropsychiatric disease, affecting relatively young people, with a still elusive pathophysiology[3,13].

The present review is divided into five chapters, starting with the definition of GM, explaining its structure, the development through lifespan, its functions and how it affects homeostasis. Then, the concept of MGB axis and the mechanisms underlying the bidirectional communication between the GM and the brain are exploited. The third chapter discusses the mechanisms through which MGB axis may impact on SCZ pathophysiology, including the discussion of novel epigenetic data. The final chapters discuss new therapeutic strategies targeting the MGB axis in SCZ patients and, finally, an overview of the limitations of the MGB axis approach in mental illness and future perspectives.

2. Gut microbiota

2.1. Definition and structure

The microbiota is defined as an ecosystem made by a complex group of microorganisms residing on or within human tissues and fluids[4,17,19]. The human microbiota is unequally distributed in the body, with the vast majority (c.a. 70%) inhabiting the gut[19]. Microorganisms in the human body approach the ratio of nearly 1:1 the number of human cells, according to recent estimates, with over one hundred times the number of genes than the human genome[4,9,13,19]. Considering its unique functions and the significant part occupied by the microbiota, it is now recognised as an organ of the human body[4,17].

Every niche of the human body has a distinct microbiota[18]. In order to understand the role of GM, it is first necessary to identify its composition[7]. The human gut hosts a diverse population of microorganisms, which include bacteria, archaea, protozoa, fungi and viruses[4,17,18,19]. Bacterial populations, mainly strict anaerobes, is predominant, hence the best characterized[3,4,23]. At a phylum level, the *Bacteroidetes* and *Firmicutes* are the dominant bacterial phylotypes[3,16,21]. *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* phyla were also identified, although in low abudance[3,23]. The vast majority of bacterial populations reside in the distal small and large intestines since those are the regions where they can extract energy from the non-digestible food components with higher efficiency[19,23,46]. Deeper knowledge on the number, composition and diversity of human microbiota is mandatory to understand its function and thereby how it can be modulated towards a healthy phenotype[23].

2.2. Development: from birth to adulthood

The gut microbiota is formed in three stages, varying through the lifespan (Figure 1)[4]. Infancy is a critical period for microbiota and neurodevelopment[3,18].

The colonisation of the gut starts at birth. Due to uterine asepsis, the fetus is sterile[3,4]. At the moment of delivery, with the exposure to the mother's microbiota, the newborn receives an initial maternal microbial signature that persists into adulthood[3,4,5,7]. The mode of delivery has an impact on the infant's microbiota[3,4,7]. In caesarean section, the microbial composition resembles the one residing the mother's skin, with *Staphylococcus* and *Corynebacterium* as the main bacterial population. On the other hand, in vaginal delivery, the microbiota is more similar to the vaginal flora, with *Bifidobacterium*, *Lactobacillus* and *Prevotella* being predominant[3,4]. The gestational period also affects microbiota composition, where preterm infants lack two of the main bacterial genera detected in normal-term infants, *Bifidobacterium* and *Lactobacillus*, compensating with a dominance of the *Proteobacteria*[3,7]. The feeding mode also modulates microbiota[3,4]. Although exclusively breastfed newborns have a microbiota with lower diversity comparing to those fed with formula, the composition is more stable, with increased *Bifidobacterium* species, which evolved to utilise human milk oligosaccharides[3,4]. When infants initiate solid foods, the diversity of the microbiota increases and bacteria from the *Firmicutes* phylum starts to increase. At the age of three years old, the microbiota is already as complex as the one of an adult[4].

In addition to infancy, also adolescence and ageing are sensitive periods to microbiota (Figure 1). During these critical periods, vulnerability to external insults is likely to increase, resulting in enhanced susceptibility to diseases, including brain disorders. In fact, disturbances of the GM in early life may significantly impact neurodevelopment, leading to adverse mental

health outcomes later in life. Similarly, the microbiota is also linked with the ageing process and the possibility of development of neurodegenerative disorders[3,18].

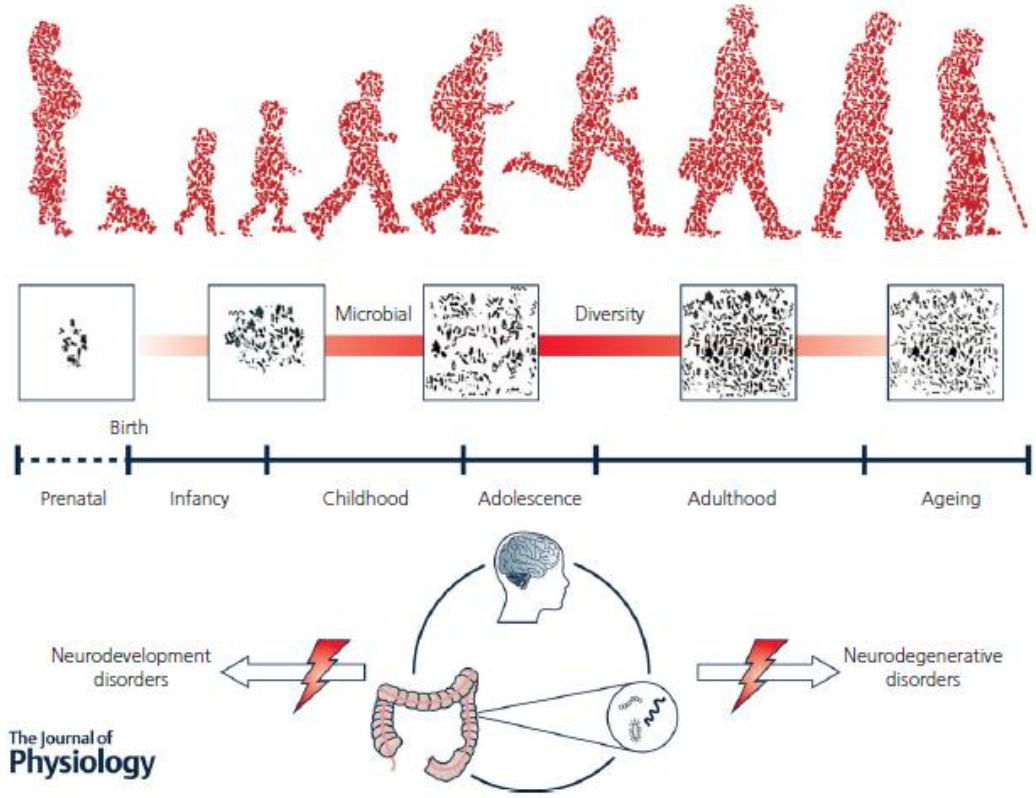


Figure 1. Microbial changes through lifespan and their impact on adverse mental disorders outcomes[3].

2.3. Physiological functions

The microorganisms and their human host co-evolved together, establishing a symbiotic relationship, where they are both fundamentally dependent on each other for survival[3,4,23]. While the microbiota guarantees an environment to live and a source of nutrients, the host receives physiological capacities, such as harvesting energy, strengthening gut integrity or shaping the intestinal epithelium, protecting against pathogens and regulating host immunity[19]. The gut barrier, a multi-layer system, is responsible for providing these physiological capacities to the host. The first layer, intestinal mucosa, is a superficial physical barrier that comprises the interactions between internal and external components, such as harmful microorganisms and antigens, but also acts as a lubricating agent for intestinal motility[7,61]. This layer consists of a single layer of epithelial cells that are sealed by tight junction proteins, such as occludin, claudin, and zonulin-1, preventing paracellular passage, intraepithelial lymphocytes, M cells, mucus-producing Goblet cells and bacteriocin-producing Paneth cells[62]. The second layer, a deep functional barrier, has the ability to distinguish pathogens from commensals, inducing an immune reaction against pathogens. This layer is composed by a complex network of immune

cells, known as gut-associated lymphoid tissue (GALT), that consists of both isolated and aggregated lymphoid follicles, and contains up to 70% of the immune cells of the whole human body. Specific immune cells, as the dendritic cells and the M-cells within the Peyer's patches, have the ability to acquire microorganisms and macromolecules and to present antigens to T lymphocytes, which produce cytokines that activate the immune response. Other mechanisms, such as gastric juice and pancreatic enzymes, both with antibacterial properties, participate in the luminal integrity of the gut barrier. A healthy gut barrier relies on the composition of its microbiota, immune cells function, intestinal epithelial cells and their intercellular junctions integrity (adherents and tight junctions), as well as on the secretion of anti-microbial peptides by Paneth cells. A dysfunctional barrier alters the intestinal permeability, allowing the pathogens and harmful substances, such as bacteria, viruses, antigens and food allergens to penetrate and enter the bloodstream[7,61,62].

Gut microbiota, in healthy individuals, serves a physiological function of fermenting not digestible food ingredients, thereby providing not only micronutrients (amino acids and short fatty acids), but also aiding absorption and degradation. Thus, GM act as a source of nutrients and energy to the host and ensures the homeostasis of the immune system, which means, the steady-state of the immune system where internal, physical, and chemical optimal conditions are maintained[4,15,18]. Recently a complex interaction between microorganisms and its host was found to be responsible for releasing cytokines, chemokines, neurotransmitters, neuropeptides, endocrine messengers, and microbial by-products. These substances can infiltrate the blood and lymphatic systems, or influence neural messages carried by the vagal and spinal afferent neurons to continually communicate with the brain and update health status, which possibly may have a significant role in modulating brain function and behaviour[3,4,11,23].

The microbiota shows a critical impact on human health and disease, being a key regulator of host physiology[19,21,23]. The state of balance between essential and pathogenic microbes, fundamental to maintain host homeostasis, it is known as "eubiosis". The disruption of this homeostasis, named "dysbiosis", is associated with the development of several pathological conditions, such as stress, anxiety, depression and autism spectrum disorders[15,23]. In order to maintain the resistance and metabolic homeostasis, the microbiota works synergistically with the immune system, informing the brain of potential threats using cytokines as a supplement to the direct interactions through the vagus nerve, to avoid colonisation from pathogenic microbes[15,19].

2.4. Factors that affect the gut microbiota

Although the human genome is mostly stable through the lifespan, the microbiome, the collection of genes of the microbiota, is extremally variable, responding to external factors[23]. Multiple factors influence the composition and abundance of the human microbiota, such as delivery mode (vaginal or caesarean section), feeding mode during infancy, infections, antibiotic exposure, stress, diseases, diet, age and host genetics[3,4,7,25,40]. Disturbances in microbiota may lead to long-term effects on physiology and behaviour, that may negatively impact health by increasing vulnerability to diseases, ranging from asthma and allergic pathologies to severe and life-threatening forms of colitis[3].

As previously mentioned, the perinatal stage and childhood are critical to shape GM[3]. Disturbances during these periods may print a signature in the infant's microbiota that persists through adulthood[3,4,7].

The increase in the relative composition of strict anaerobes due to food preferences and environment occurs early in life. Specific types of diet may induce different microbiota patterns, endowing the organism with the capability to digest that particular type of food[3,23]. For instance, the Mediterranean diet, that consists mostly of cereals, nuts, vegetables and fruits results in distinctive GM characteristics, such as an increased abundance of *Bacteroides* and *Clostridium* phyla, and a reduction in *Proteobacteria* and *Firmicutes* phyla[23]. Ingested food, with plenty of external microorganisms, transfer the microorganisms through the gastrointestinal (GI) tract, threatening intestinal integrity by unbalancing the equilibrium between commensals, opportunistic and pathogenic bacteria[19].

Infections, antibiotic exposure, stress and diseases can temporarily alter the stability of the GM composition, resulting in harmful effects on the host, lead to long-term dysbiosis, inducing accelerated maturation and exacerbation of diseases[3,4]. Once the insult is resolved or suspended, the composition may return to its previous level of diversity[3].

As we age, microbiota composition suffers a dynamic shift, narrowing in number and diversity (Figure 1). *Bifidobacteria* strains considerably decreases with age[3,7]. Yet, healthy ageing is associated with a diverse microbiota, contributing to a healthy brain across lifespan[3].

Part of the variability of the microbiome is inter-individual, which means each microbiota is unique to individuals. It can be identified as microbial genomic structural variants that demonstrate a strong association with host metabolic health[3,23].

3. The microbiota-gut-brain axis

The gut-brain axis is a bidirectional communication between the gut and the central nervous system (CNS), modulating not only brain function and behaviour but also multiorganic physiological mechanisms. Although the hypothalamic-pituitary-adrenal (HPA) axis has been already described to link gut, brain and other systemic functions, the GM is now emerging as a new player in this intricate interaction[4,13,15,23]. Thus, the novel MGB axis concept, deals with the influence that GM can exert over host cognition, learning and memory, behaviour and mental health[3,23]. The MGB axis is a complex physiological network including the immune system, autonomic nervous system (efferent and afferent neurons), endocrine system and enteric nervous system (Figure 2)[4].

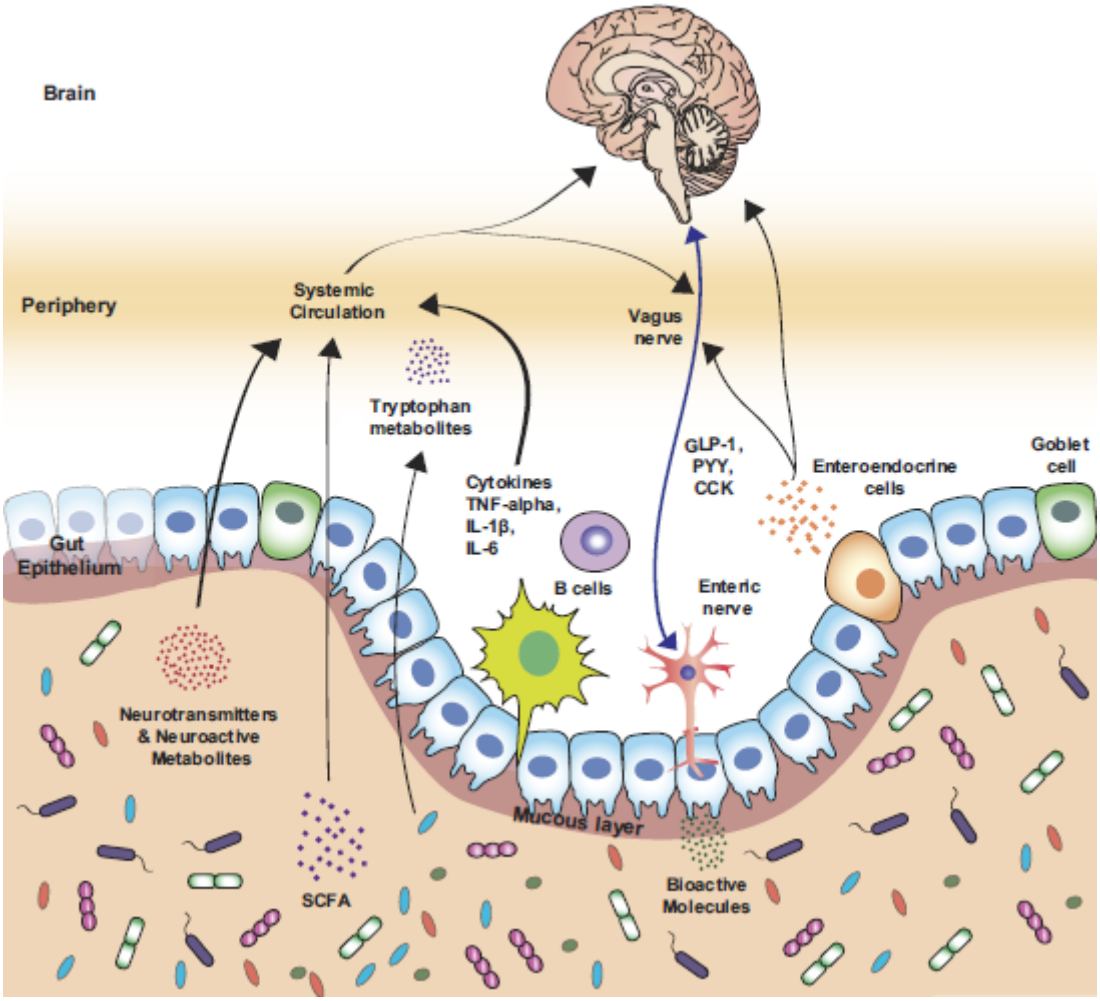


Figure 2. Main bidirectional pathways of communication between the GM and the brain. Cholecystokinin (CCK); Glucagon-like peptide-1 (GLP-1); Interleukin (IL); Peptide YY (PYY); Tumour necrosis factor (TNF); Short-chain fatty acid (SCFA)[23].

3.1. Mechanisms of communication

When considering the MGB axis, the main questions are not only how gut microbes may modulate brain function and vice versa but also how both systems are able to communicate. In fact, there are multiples mechanisms of communication, enabling the link between the GM and the brain, both direct and indirect, relying broadly upon immune, neural, endocrine and metabolic pathways[3,4,16,17].

3.1.1. Immune pathways

One critical function of the multi-layer system of the gut lumen and mucosa is to limit the contact of GM with the visceral tissue by secreting a protective viscous mucus layer from goblet cells of the epithelium. This is where the majority of host-microbe interaction occurs, and the exchange of molecules through the mucous layer and epithelium serves as a physical barrier, facilitating communication between the gut microbes and the host immune cells through the recognition of self and non-self-antigens, and thus to help the immune system to identify potentially harmful pathogens[3,23].

In addition, peptidoglycans, polysaccharides and other antigens that confer beneficial roles for the bacteria, such as protection against degradation, also allow the host immune cells to identify GM composition and changes in the homeostatic balance of the gut. Epithelial pattern recognition receptor (PRR), of which the Toll-like receptor (TLR) family are the most studied, recognise molecular patterns unique to bacteria and other microorganisms, such as pathogen-associated molecular patterns (PAMP). Once TLR are activated, inflammatory cells are recruited with consequent cytokine production and chemokine mediated recruitment of more acute inflammatory cells[23]. These inflammatory responses promote the release of cytokines or other chemicals-related substances into the blood, further affecting the immune system[4,7,19].

Chronic inflammation can lead to large dysfunctions in brain networks[14]. Lipopolysaccharide (LPS) and peptidoglycan are some of the typical inflammatory mediators produced by the GM that may trigger systemic inflammation. LPS is recognised by the TLR-4, widely distributed in CNS monocytes, macrophages and microglia. The indirect effects of GM on the innate immune system may lead to alterations in the circulatory levels of pro-inflammatory and anti-inflammatory cytokines, which in turn have direct impacts on the brain functions due to their capacity to permeate the blood-brain barrier (BBB)[4,7]. Immune mediators produced or induced by the microbiota include cytokines such as interleukin (IL)-1α, IL-1β, tumour necrosis factor (TNF)-α and IL-6, which act as signalling mediators. The

mediators cross the BBB, act on specific receptors expressed by neurons and glial cells, changing their physiological function[7].

3.1.2. Neural

The bidirectional communication between the GM, enteric nervous system and the brain is ensured by the vagus nerve, a mainly sensory nerve that has also some motor fibres[7]. Lipopolysaccharide is a significant component of the outer membrane of gram-negative bacteria, capable of inducing the release of cytokines such as IL-1β, which in turn may trigger pathogenic mechanisms through the vagus nerve. The c-Fos expression, an indicator of neural activity of vagal nerve, proved the increased activity of vagal nerve during dysbiosis, indicating that bacteria can directly affect behaviour through the vagus nerve[4]. Activation of the vagus nerve plays an essential role in behaviour, cognition and emotions through mechanisms associated with immune activation, increased intestinal permeability, endocrine signalling and enteric reflexes. The efferent fibres modulate gut functions such as secretion of acid and bicarbonates, motility and mucus stability by maintaining the normality of the GM[7].

The GM may also modulate the production of host neurotransmitters by altering the levels of its precursors, but bacteria themselves may also synthesise and release neurotransmitters[3]. Bacteria can produce neurotransmitters such as gamma-aminobutyric acid (GABA), catecholamines (noradrenaline and dopamine), serotonin, histamine and potential bioactive neuropeptides[7]. Thus, dysbiosis, induced by different insults, as described above, may perturb the production of CNS neurotransmitters which may ultimately lead to cognitive alterations[15].

GABA is the main inhibitory neurotransmitter in the CNS, produced efficiently by both host and GM, in particular by *Lactobacillus* and *Bifidobacterium*. The production of GABA derives from the conversion of glutamate or glutamic acid into GABA. It is likely that host GABA production can influence GM, leading to increased organisms' virulence through variation of the production of a quorum-sensing-regulated factor and modulation of enzymes involved in the oxidative stress response[63]. The way that GABA derived from GM affects GABAergic neurotransmission is still unclear. Gut microbiota-derived GABA may be capable of crossing the BBB through diffusion or active transport. There is also evidence of expression of a GABA transporter at the BBB, implying the possibility that GM-derived GABA may enter the CNS via this transporter, suggesting that GM is capable of regulating central GABAergic transmission, increasing central levels of this neurotransmitter. Gut microbiota may also regulate central GABAergic transmission directly through the vagus nerve. Indeed, disruption of GABA production is associated with mood disorders, such as depression and anxiety[7,23,36,48].

Catecholamines, a group of neurotransmitters that include noradrenaline and dopamine, are involved in the regulation of endocrine axes, motor control and cognitive activities[7]. Bacterial production of these neurotransmitters is associated with increased levels within the brain. Curiously, decreased levels of catecholamines are associated with psychiatric diseases, including SCZ[7,37]. Finally, serotonin and histamine, produced by GM, are associated with circadian cycles, cognition, food ingestion and pain perception[7].

Interestingly, recent data suggest that neurogenesis is also regulated by microbiota[3]. At the early stages of life, synaptogenesis exceeds elimination, an essential phenomenon to establish neural networks. From infancy to adolescence, synaptogenesis is outpaced by synaptic pruning, a critical process to the maturation of synapses and neural circuits which eliminates ineffective synapses and strengths vital neuronal connections. An equilibrium between synaptic formation and pruning is fundamental to normal brain function. In parallel, the GM evolves from infancy to adult age, characterised by less diversity between species. The GM reaches its maturity at adulthood, becoming more diverse and stable over time and more resistant to perturbations, such as antibiotics, dietary changes and stress. Neural function and GM co-evolve, suggesting the intriguing possibility of a bidirectional influence between brain and GM on each other's maturation[9,50].

Microglia, the innate immune cells of the CNS, have a key role in synaptic pruning by sparing the brain of infrequently used synapses. Microglial activation and function during critical periods of development are modulated by GM. Microbiota dysbiosis displayed microglia alterations in morphological characteristics and gene expression profiles, accompanied by inhibition in their maturation state. This suggests that GM may directly contribute to the maturation progress of microglia. Microglial alterations were enriched in pathways related to synapse organization and synapse assembly, suggesting that dysfunctions of the GM may alter microglia-mediated synaptic pruning and disrupt dendritic spine remodelling, causing behavioural impairments. Mutations in several genes that encode the required proteins for synapse formation (such as postsynaptic density protein 95, synaptophysin, and spinophilin), development, plasticity, and pruning were linked to the psychopathology of multiple complex neuropsychiatric disorders, including SCZ, supporting the hypothesis that neuropsychiatric disorders are, in part, a consequence of a developmental synaptopathy[9,38].

3.1.3. Endocrine

The GM works as an endocrine organ, controlling the HPA axis, the central responses to stress and the activity of enteroendocrine cells by releasing a number of different hormones[1,4,7,17].

Disturbances in the HPA axis are associated with stress responses and the secretion of cortisol. Stress induces secretion of corticotrophin-releasing factor (CRF) by the hypothalamus. Consequently, the pituitary gland secretes adrenocorticotrophic hormone (ACTH), leading to the release of cortisol by the adrenal cortex. A dysfunctional stress response can result in hypersecretion of cortisol, which regulates many physiological processes, as immunity, metabolism and neurodevelopment[7,39].

Bacterial populations of GM can regulate the activity of enteroendocrine cells, specialised intestinal cells that produce signalling molecules that may establish the link between CNS and other organs[7,17]. The abundancy of enteroendocrine cells in the gut, where the majority of bacterial populations reside, enables the direct contact with the luminal constituents via the apical surface, including bacterial metabolites. These cells have a long lifespan, potentially allowing them to integrate into the local signalling network of the ENS, glia and immune cells of the GI submucosa[23]. Several different hormones are secreted by these specific cells, such as glucagon-like peptide 1 (GLP-1), peptide-YY (PYY), cholecystokinin (CCK) and serotonin (5-hydroxytryptamine, 5-HT). These hormones modulate nutrient absorption, metabolism, appetite and anxiety-like behaviors[17].

3.1.4. Metabolic

In the metabolic pathway, it is included not only the tryptophan metabolism but also the molecular signalling of short-chain fatty acids (SCFA), important metabolic products of GM activity, along with long-chain polyunsaturated fatty acids (LC-PUFA)[3,7].

One of the mechanisms through which GM and the CNS are believed to communicate is related to food. Tryptophan is an essential amino acid that the host obtains from the diet. However, some bacterial strains can get tryptophan from tryptophanase[7]. Once absorbed in the gut, tryptophan crosses the BBB, allowing the production of serotonin in the CNS. Serotonin is a neurotransmitter involved in the development of the brain, visceral target organs and potentially the gastrointestinal tract. Higher levels of serotonin are observed mainly in the critical development ages, through childhood to adolescence, influencing behaviour, appetite, emotions, motor function and cognition. Kynurenine, a tryptophan metabolite, is metabolised by enzymes as tryptophan-2,3-dioxygenase (TDO) and ubiquitous indoleamine-2,3-dioxygenase (IDO) in the liver into two sub-products, neuroprotective kynurenic acid and neurotoxic quinolinic acid. The tryptophan catabolites generated by IDO are indole products, aryl hydrocarbon receptor (AhR) ligands, whose activity may be affected, decreasing levels of tryptophan. The majority of tryptophan is metabolised through the kynurenine pathway.

Disturbances in the tryptophan metabolism have been reported in some diseases, including SCZ[7,42,49].

The GM metabolizes dietary, non-digestible, carbohydrates into SCFA, a group of small molecules, with several physiologic functions, ranging from energy balance and metabolism to modulation of neurotransmission, regulation of the immune system and suppression of inflammation. Acetic, propionic and butyric acids, are the main SCFA produced by gut microbes. Short-chain fatty acids may exert central effects indirectly or directly either through G-protein coupled receptors or in the case of butyrate as an epigenetic modulator acting through histone deacetylases. Therefore, SCFAs act as mediators between the GM and the brain, contributing to mechanisms by which GM affects brain physiology and, consequently, behaviour. Butyric and propionic acids increase the expression of the gene encoding tyrosine hydroxylase, an enzyme that limits the rate of synthesis of dopamine and noradrenaline, and dopamine-β-hydroxylase, an enzyme that converts dopamine to noradrenaline. Propionic acid also lowers levels of GABA, serotonin, and dopamine. Propionate may activate intestinal gene expression associated with gluconeogenesis through a gut-brain circuit involving the fatty acid receptor FFAR3. Short-chain fatty acids produced by GM can affect the CNS by acting on glial cells, including microglia and astrocytes, but the specific effect depends on the type of SCFA and the target cell. Butyric acid demonstrates an anti-inflammatory effect in LPS-induced microglial cells. SCFA are also capable of restoring microglial malformation and immaturity due to activation of FFAR2. Accordingly, propionic acid has been shown to affect cytoskeletal integration and increased glial fibrillary acidic protein in cultured astrocytes, inducing cognitive and sensorimotor impairment[3,4,7,23].

Long-chain polyunsaturated fatty acids, especially omega-3 and omega-6, may protect neuronal cells from oxidative damage, control inflammation, regulate neurogenesis and preserve neuronal function and therefore are key molecules in neurodevelopment and brain function. The great interest in LC-PUFA resides in their biophysical properties in cell membranes. Due to their long carbon chains and a high degree of unsaturation, LC-PUFA confer specific properties on the lipid bilayer that make it dynamic and flexible, implying that these fatty acids affect the brain by altering biophysical properties of cell membranes. Decreased levels of LC-PUFA are involved in several mental health conditions[7,35,51].

4. The role of the microbiota-gut-brain axis in schizophrenia

4.1. Schizophrenia

Schizophrenia is a chronic, neurodevelopmental disease, with a significative genetic component, characterised not only by psychiatric and emotional symptoms but also permanent cognitive impairment[7,13,15,18,20]. The symptoms usually appear in adolescence or early adulthood, between the ages of 15 and 25, in male individuals[9,13,45]. In females, the onset of SCZ has a bimodal age distribution, with a first peak between the ages of 25 and 30, and later above 40 years old[45]. Schizophrenia affects approximately 1% of the global population, with a significantly higher incidence in male individuals[3,24,26,45]. The impact on the quality of life is significant, being one of the most debilitating illnesses affecting young people while carrying a considerable economic and social burden[3,20,22,24,26].

As a major psychiatric disease, SCZ reduces life expectancy in 10 to 25 years, mostly due to suicide attempts and the significant impact on cardiovascular system[7,15,18]. The hight rate of mortality of SCZ is also related to the higher prevalence of comorbid chronic organic disorders and age-related illnesses, such as cardiovascular and metabolic disorders, which are associated with a chronic inflammatory component[6,15]. Also, the adverse effects associated with the antipsychotic treatments, the lack of physical activity, a diet rich in sugars and saturated fats as well as substances abuse, such as alcohol and smoking, contribute to the development of the comorbidities[45].

Early diagnosis and efficient treatment, allied with family and social support, is crucial to improve quality of life in SCZ patients[45].

4.1.1. Pathophysiology

The pathogenesis of SCZ is multifactorial, that is to say, results from a complex gene-environment interaction[7,13,20,25,45]. Hereditary factors seem to have an important contribution to the development of SCZ since there is a high number of genes involved in the pathophysiology of the disease. The most probable way of transmission implies a polygenetic model, capable of interfering in the neural migration and plasticity, synaptogenesis, and in the signalling pathways of dopamine and glutamate and acetylcholine. Increased levels of dopamine and decreased levels of glutamate and acetylcholine are observed. The ambiental factors include obstetric and perinatal complications, infections, maternal malnutrition and substances abuse. Psychological theories also implicate pathological familiar relationships, rejection and hostility in the onset of SCZ[45].

Some neuroanatomical alterations are associated with SCZ patients. It was discovered two distinct and highly reproducible subtypes. The subtype 1 displayed widespread grey matter

volume reduction, related to illness duration and worse premorbid functioning. The subtype 2 had normal and stable brain anatomy, except for larger basal ganglia and internal capsule, not explained by antipsychotic dose. These findings challenge the notion that brain volume loss is a general feature of SCZ, suggesting the existence of differential aetiologies, which may facilitate strategies for clinical trial enrichment and stratification and also precision diagnostics. Both grey and white matters can be used as SCZ biomarkers[64].

4.1.2. Symptoms

The symptoms of SCZ are highly heterogeneous, complex and not always clearly perceived. The symptoms fluctuate through the development of the disease, most of them also characteristic of other neuropsychiatric diseases[45]. Symptoms are classified as positive, the productive symptoms, including delusions and hallucinations, and negative, related to loss or decrease of normal functions, including avolition of speech, affective flattening and social distancing[15,17,26,45]. The severity of negative symptoms has an influence on global functions of the patient, autonomy, prognostic and evolution of SCZ. Other symptoms, such as cognitive and emotional impairments are also present (Table 1)[45].

Table 1 – Key symptoms in the diagnosis of SCZ[45].

Positive	**Negative**	**Other**
Delusions	Affective flattening	Cognitive and social impairments
Hallucinations	Alogia	
Changes in thinking	Avolition	
Behavioural changes	Anhedonia	
	Apathy	
	Social distancing	

4.1.3. Diagnosis

The diagnosis of SCZ is often hard to be precise since it is mostly based on the clinical history and psychopathological observation. Currently, there are no laboratory exams that can be done to diagnose SCZ, although the existence of some neuroanatomic alterations and biomarkers can aid in the diagnosis. However, none are specific of SCZ and their validity is controversial[45]. The most used system of diagnosis requires the coexistence of two or more symtoms[17,45].

4.1.4. Evolution and prognostic

The symptoms usually appear in adolescence, recognised retrospectively. External environment changes, such as changing cities, start college, substance abuse, may trigger the symptoms. They may persist through several years until the onset of the psychotic traits[45].

The classical evolution of SCZ is driven by multiple exacerbations of the disease, with remissions and relapses with several hospitalisations. Most patients respond to the antipsychotic treatment, although the majority relapse in the first 5 years after the first episode, partially due to therapeutic discontinuation. Each relapse represents a deterioration of the previous patient's functional level, a differential factor that separates SCZ from humour perturbations, such as bipolar disorder. Positive symptoms tend to decrease, with a predominance of the negative and cognitive symptoms[45].

Schizophrenia is a deteriorating and debilitating disease, usually with a poor prognostic. The majority of patients suffers multiple hospitalisations, symptom's exacerbations and suicidal attempts[45].

4.1.5. Treatment

The treatment of SCZ is based on a multidisciplinary approach, considering the different stages of the disease. Biological, psychological and social domains must be considered, in order to improve functioning and prevent cognitive decline[45].

Currently, the pharmacological treatment for SCZ is the antipsychotic medication, the first-line approach to the disease. It is important to critically analyse the adverse effects of these drugs, in order to prevent therapeutic discontinuation[45]. In the first episode, it should be prescribed an oral antipsychotic drug at the lowest therapeutic dose which must be progressively increased until it becomes effective. A strict monitorisation of the effects of the medication is essential to a successful treatment[44].

The antipsychotic drugs are divided into two distinct groups, first-generation (typical) and second-generation (atypical), as demonstrated in Table 2. The main goal of the antipsychotic therapy is to treat psychosis, through the antagonism or partial agonism of the receptors of the dopamine, especially D2. These drugs may also affect other receptors, such as serotonergic, M1 cholinergic, α1 adrenergic, H1 histamine, contributing to a singular mechanism of action that must be taken in considerations while prescribing[45].

First-generation antipsychotics (FGA) block the D2 receptors, resulting not only in their antipsychotic effects, but also in extrapyramidal effects, such as akathisia, acute dystonia, parkinsonism, and hyperprolactinemia, clinically expressed as amenorrhea, galactorrhoea and sexual dysfunction[45].

Second-generation antipsychotics (SGA) block the D2 receptors and also the 5-HT2A serotonergic receptors. Inhibition of the serotonergic receptors leads to an increase of the dopamine levels, alleviating the negative symptoms, which is an advantage of their use instead of FGA. According to the antipsychotic drug used, other receptors may be blocked, causing different adverse effects. Blocking of the α1 adrenergic receptors may be associated with hypotension, dizziness and tachycardia, on the other hand, blocking of M1 muscarinic receptors are associated with blurred vision, xerostomia and cognitive deficits. H1 receptors, when blocked lead to sedation and increased appetite. Other adverse effects should be considered, such as electrocardiographic changes (prolongation of the QT interval) and metabolic changes, as weight gain, dyslipidemia and development of insulin resistance, increasing the risk of metabolic syndrome[45].

Table 2 – Classification of the antipsychotic drugs used in the treatment of SCZ, divided as first- and second-generation[45].

First-generation	Second-generation
Chloropromazine	Amilsuprida
Fluphenazine	Aripiprazole
Flupentixol	Asenapine
Haloperidol	Clozapine
Levomepromazine	Lusaridone
Pimozida	Olanzapine
Sulpiride	Paliperidone
Zuclopentixol	Quetiapine
	Risperidone
	Ziprasidone
	Zotepina

Besides the pharmacological treatment, non-pharmacological treatments shall also be considered, such as psychological therapy and social reintegration[45].

4.2. Evidence of MGB axis affecting the pathophysiology of schizophrenia

As aforementioned, GM may have a critical impact on brain function mainly by controlling local inflammatory pathways, modulating neurotransmitters levels, secretion of

hormones and metabolites[7,13,19,23]. Dysbiosis is associated with neuropsychiatric disorders, such as autism spectrum disorders, major depression, anxiety, bipolar disease and also SCZ, suggesting that perturbations of the GI system may have central consequences[13,15,23]. Dysbiosis induces aberrant activation of key pathways of MGB axis communication, through immune, neural, endocrine, metabolic and epigenetic mechanisms, leading to exacerbated intestinal mucosal inflammation and altered responses to stress. As consequence behaviour may be affected with a direct impact on mood, sociability and anxiety[23]. The role of GM in SCZ emerged has an attempt to explain the mechanisms underlying the pathophysiology of this complex disease[13,15].

Most evidence regarding the influence of MGB axis in SCZ were performed in animal models. The most common animal model used mice that grow up devoid of a microbiome, referred to as germ-free mice[2].

4.2.1. Immune mechanisms

Schizophrenia is associated with a disruption of the gut epithelial barrier, leading to neurological dysfunction and atypical behaviour. In short, infections increase GI permeability allowing the translocation of bacteria or their metabolites, which may explain part of the pathophysiology of SCZ[7].

Dysfunction of immune and inflammatory pathways is associated with the development of SCZ[7,24]. Increased levels of IgA reported in SCZ patients may imply innate immune imbalances in the pathophysiology of SCZ. In addition, the serum markers of bacterial translocation, including soluble CD14 and LPS-binding protein, were also significantly elevated in patients with SCZ and were significantly associated with elevated C-reactive protein in the serum[24]. Dysbiosis of GM increases systemic inflammation, and the resulting neuroinflammation may be linked with SCZ. The GM has the capacity to modulate inflammation and immune function, acting directly or indirectly through microbial metabolites. For instance, GM may produce bioactive immunomodulins and regulatory cytokines, such as IL-10 and TNF-β, that attenuate systemic inflammation. Furthermore, microbial biosynthesis and modulation of neurotransmitters that cross the BBB, such as serotonin and GABA can influence brain function[26]. Hence, the immune-inflammatory response, depending (or not) on GM, is a key mechanism in SCZ pathophisiology[7].

Infections and activated immune response may compromise the BBB, allowing inflammatory markers to enter the brain, which may contribute to an increased risk of mental disorders[18]. Also, telomere shortening (TS) is observed under inflammatory conditions that may lead to neuropsychiatric disorders[15]. In SCZ is observed a shorter leucocyte telomere

length (LTL) and increased levels of pro-inflammatory cytokines, suggesting that both signalling pathways may play a role in the neurobiology of SCZ[7,15]. Cytokines modify signals sent from nerve afferents into the CNS and increased levels of IL-1 β, IL-6 and transforming growth factor-β (TGF-β) are observed during exacerbations of SCZ and normalised during pharmacological treatment[7,18].

Alterations of microbiota eubiosis can profoundly disturb CNS neurotransmission, and that microbiota alterations with induced acute bacterial infection may lead to cognitive alterations. Microbiota imbalances verified in SCZ are associated with immunomodulatory actions, including the activation of inflammatory pathways[15].

Gastrointestinal inflammation can be assessed based on microbial biomarkers. In SCZ, antibodies against *S. cerevisiae* are increased, particularly at the onset of the disease. *Candida albicans* antibodies are also increased, especially in patients with GI symptoms, such as constipation. Curiously, these patients experience worse psychiatric symptomes[6]. Also, SCZ patients present increased bacterial translocation markers[6,22].

Translocation of microbial components into circulation, via a leaky gut, can include other substances, such as digested foods[6]. Antigens present in food induce intestinal inflammation, leading to further damage of the GI tract epithelial barrier. This damage causes excessive permeability of the gut barrier, allowing the passage of harmful pathogens, such as *Toxoplasma gondii*. Once in the systemic circulation, these microbes may cross the BBB, resulting in brain alterations, associated with the development of psychiatric diseases. For instance, it has been shown a connection between celiac disease, gluten sensitivity and SCZ[6,7,52]. The factors responsible for the development of celiac disease are exposure to gluten in genetically predisposed individuals. The abnormal immune response against gluten induces a pro-inflammatory activity, increasing gut permeability[7]. The immune response to gluten includes the production of antibodies, such as anti-gliadin IgA antibody, which is also increased in schizophrenic patients. This observation positively correlates with kynurenine levels and the kynurenine/tryptophan ratio[5,7,52]. In addition, the levels of anti-milk casein, associated with lactose intolerance, are also increased in schizophrenic patients. The inflammatory response generated by lactose sensitivity is similar to the one generated by gluten[6].

Immune cells, such as T cells, are essential for the adaptative immune response, in particular, T helper (Th) 17 cells. T helper 17 produce IL-17, which is increased in SCZ and is associated with the severe forms of the disease. Interestingly, dysbiosis activates Th17 cells, thereby inducing GI inflammation, playing a critical role in neuropsychiatric diseases, including in SCZ[7].

4.2.2. Neural mechanisms

Abnormalities of the neural system are typical in schizophrenic patients. These include altered synaptogenesis, synaptic pruning, dysregulated neurotransmitters levels and dysfunctional vagal activity[20].

Brain function, at the most fundamental level, is based mainly on synapses[9]. Disruption of synaptic structure and function, as well as defective synaptic formation, elimination and plasticity results in altered neuronal function in complex neuropsychiatric disorders, such as SCZ[9,20].

In schizophrenic patients, synaptic pruning ends after SCZ onset, which further links the dysregulation of synaptic pruning to the SCZ pathophysiology. The extent of synaptic pruning is measured by the grey matter volume, which is reduced in SCZ and is associated with reduced cognitive performance. Since GM can impact on synaptic pruning via microglia activation, as previously mentioned, microbiota alterations may be associated with SCZ[9].

Dysbiosis of GM may contribute to the onset of SCZ by modulating the hippocampal glutamate-glutamine-GABA cycle. Increased levels of glutamine and GABA, and decreased glutamate after acquiring SCZ are observed[13,20,24]. Disruption of GABA production is associated with symptoms such as depression and anxiety[7]. Decrease of catecholamines production is also related to SCZ, such as reduced 5-HT synthesis[7,17].

Vagal efferent activity is lost in SCZ, probably due to disturbed cortical-subcortical circuits modulating the autonomic nervous system in acute psychosis. The definite mechanisms responsible by suppression of the vagal activity in SCZ are still unknown[7,47].

4.2.3. Endocrine mechanisms

Gut microbiota may disturb the HPA axis and immune system, due to the release of behaviour-altering chemicals including glucocorticoids, mineralocorticoids and catecholamines that result in disruption the brain neurochemistry. Schizophrenic patients may have hyper- and hypo-activity of the HPA axis, confirmed by the cortisol levels. Although psychological stressors induce the increase of cortisol levels, some schizophrenic patients may experience hypocortisolemia. Different cortisol variations in patients might be related to the fluctuation of symptoms, antipsychotic medication (reduce stress and consequently cortisol levels) and environmental exposure to antibiotics or stress[7,53].

The MGB axis is related to emotional and cognitive centres, such as central, autonomic and enteric nervous systems. Disturbances of these systems may be associated with the etiology of psychiatric disorders, including SCZ[7].

4.2.4. Metabolic mechanisms

Functional alterations associated with SCZ include differences in SCFA and LC-PUFA synthesis as well as tryptophan metabolism[10].

Acetate, one of the SCFA produced by the GM, cause physiological changes in the hypothalamus, modifying the levels of neurotransmitters, such as decreasing levels of glutamate and increasing levels of glutamine and GABA and also increasing the expression of the anorectic neuropeptide and alpha-melanocyte-stimulating hormone (αMSH). Curiously, acetate may also potentiate the feeling of satiety. SCFA can cause anxiety and behavioural changes, typical of neuropsychiatric disorders and SCZ. SCFA can also reduce the activity of histone deacetylases (HDAC), facilitating access of DNA repair enzymes and decreasing the excessive activity of HDAC in SCZ[7].

Levels of LC-PUFA are involved in many health conditions, including SCZ, that shows low levels of omega-3 in their cell membranes[7]. Antipsychotic treatments, currently used in SCZ treatment, may also decrease the levels of LC-PUFA[7,24]. In cerebellum and striatum, LC-PUFA levels are decreased, although in the cortex are increased in SCZ[20].

Disturbances induced by the GM in the tryptophan metabolism may be associated with the pathogenesis of SCZ. Kynurenic acid is a tryptophan metabolite and a N-methyl-D-aspartate (NMDA) receptor antagonist. Increased levels of kynurenic acid are observed in SCZ patients. However, a deficit of peripheral kynurenic acid is associated with relapse[5,7]. Moreover, increased levels of anthranilic acid, a downstream metabolite of kynurenic acid, has also been found in SCZ patients. The GM has the ability to substantially influence plasmatic tryptophan levels, and thereby affect tryptophan metabolism. Although the exact role of the kynurenic pathway of tryptophan metabolism in the pathogenesis of SCZ has not been elucidated, there is evidence pointing to the possibility that increased kynurenine level in the CNS is associated with SCZ, leading to downregulation of NMDA activity or promotion of an immune response[5].

In addition, bacteria-derived metabolites could affect the CNS expression of brain-derived neurotrophic factor (BDNF) and other important cognition-related proteins, affecting host behaviour. Decreased levels of BDNF were found in SCZ patients, leading to hypoactivity of NMDA receptor[3,5,26].

4.2.5 Epigenetic mechanisms

Epigenetics is the term used to define the stable, heritable traits, phenotypes, that cannot be explained by changes in the DNA sequence. Epigenetics tries to unveil the dynamic interaction between genetic and environmental factors, such as lifestyle[7,25]. Hence, the

phenotype is a result of complex interactions between epigenome, genotype and environment[7,25,27].

Some epigenetic mechanisms, such as DNA methylation and hydroxymethylation, histone acetylation, and microRNA (miRNA) expression are essential for the regulation of several physiological processes, including learning and memory, but also to understand pathological processes (Figure 3)[7,33].

Schizophrenia is a multifactorial disease likely resulting, as previously mentioned, from an environmental insult in a genetic susceptible person[7,13]. Transgenerational inheritance might have a role in divergent trajectories in psychopathologies[7,28]. In psychiatric diseases, the heritability of phenotypes generally implies the direct exposure to stressors by the previous generation[7,29]. Epigenetic programming, mediated by recognition of the group of molecular mechanisms underlying gene-environment interactions, controls gene expression and is responsible for human embryonic development[7,28]. Although the difficulty in finding a single causal gene, several studies addressed the link between major histocompatibility complex (MHC) genes on chromosome 6 and SCZ. One longitudinal study also demonstrated a direct and linear relationship between blood C-reactive protein (CRP) levels in adolescents and the risk of SCZ in adulthood[60]. A locus on chromosome 1p21.3 was identified as highly associated with the miRNA-137, one of the strongest factors associated with SCZ. QKI was identified as a potential SCZ gene because it is the only gene located in the chromosome susceptibility locus, 6q25-6q27[59].

The main epigenetic mechanisms associated with SCZ are DNA methylation, hydroxymethylation, histone modifications and miRNA expression. DNA methylation leads to complete gene silencing cells[7,30]. DNA methylation is a life-time inductive molecular mechanism that consists of methylation of cytosine residues followed by guanine or adenine, suppressing gene expression. However, hydroxymethylation of the same residues can stimulate gene expression. These changes are mediated via a group of enzymes that promote DNA methylation, such as DNA (cytosine-5)-methyltransferase (DNMT) 1, DNMT3A, and DNMT3B or hydroxymethylation, such as ten-eleven translocation (TET) 1-3 and isocitrate dehydrogenase (IDH) 1-3. A study demonstrated DNA methylation in key neurodevelopmental genes, suggesting that epigenetic mechanisms mediate some of the effects observed in SCZ[7,30,54,60].

Histone modification is a reversible and complex process. Different amino acids of histone tails can be acetylated or deacetylated, leading to an increase or decrease of gene expression, depending on the type of change and its position. For example, acetylation of histone residues leads to increased accessibility of nucleosomal DNA to transcription factors,

consequently increasing the expression of corresponding genes. Histone acetylation is catalysed by lysine acetyltransferases. Conversely, HDACs remove the acetyl group from lysine residue[7,27,60].

These complex histone modifications interact with small non-coding RNA molecules, miRNA. In turn, each miRNA can target the transcript of several genes in a tissue-specific manner, thus increasing the complexity of the transcriptional response of an organism without increasing the number of genes. Therefore, miRNA emerged as potential neuroregulators, crucial for normal neurodevelopment[7,33,60]. Hence, a new term emerged, neuroepigenetics, to define the external conditions that activate the regulation of the tridimensional DNA structure in the CNS, the endpoint in the regulation of epigenetic mechanisms. Neuroepigenetics have multiple consequences at the level of the CNS, including changes in the modulation of neuronal plasticity, neurotoxicity, substance addition and ultimately in behaviour, such as regulation of anxiety and propensity of commit suicide, associated with psychiatric disorders, including SCZ[7,31,55].

Gut microbiota produces molecules capable of interfering in genetic, epigenetic and metabolic processes. For instance, GM-derived metabolites can directly interact with the epigenome. The GM generates a variety of SCFA for ATP production. Bacteria from *Clostridium*, *Eubacterium*, and *Butyrivibrio* genera are able to synthetize butyrate from non-digestive fibres in the GI lumen, inducing inhibitory effects on HDAC. The GM also contributes to the absorption and secretion of minerals such as zinc, selenium, cobalt and other cofactors that participate in epigenetic processes. Additionally, some other key metabolites of GM, including S-adenosylmethionine (SAM), acetyl-CoA, NAD, alpha-KG, and ATP serve as essential cofactors for epigenetic enzymes that regulate DNA methylation and histone modifications. Besides the impacts of GM that indirectly affects the epigenetic landscape, infection with some bacteria, such as *Helicobacter pylori* are specifically linked to DNA methylation and may decrease expression of O6-methylguanine DNA methyltransferase, resulting in changes of local epigenetic signatures[7,60]. Factors misbalancing GM composition may also cause epigenetic abnormalities and, consequently, the progression of metabolic diseases[7,43].

Schizophrenia is a polygenic and heritable disease and therefore it has been challenging to identify individual causative genes. Phenotypic expression of SCZ includes complex interactions between risk alleles and environmental risk factors, including prenatal and postnatal stressors, stressors of the parental generation, as in hypomethylation of the paternal copy of the schizophrenia risk gene LRRTM1. DNA methylation and histone modification with subsequent modulation of chromatin structure as well as miRNA regulation of signalling

pathways, including those involved in DNA methylation and histone activity, play important roles in neurodevelopment and are capable of regulate large numbers of genes at once. Therefore, it is possible that epigenetic mechanisms mediate the interaction between genetic risk alleles and environmental factors by dynamic modification of the genome in response to positive or negative environmental stimuli in SCZ[55,56].

Environmental factors and epigenetic factors interact with the GM and modify gene expression. Therefore, GM could be the converging point where environmental risk factors trigger pathological changes in epigenetic modulation, eventually leading to the onset of SCZ[7,27,32,58].

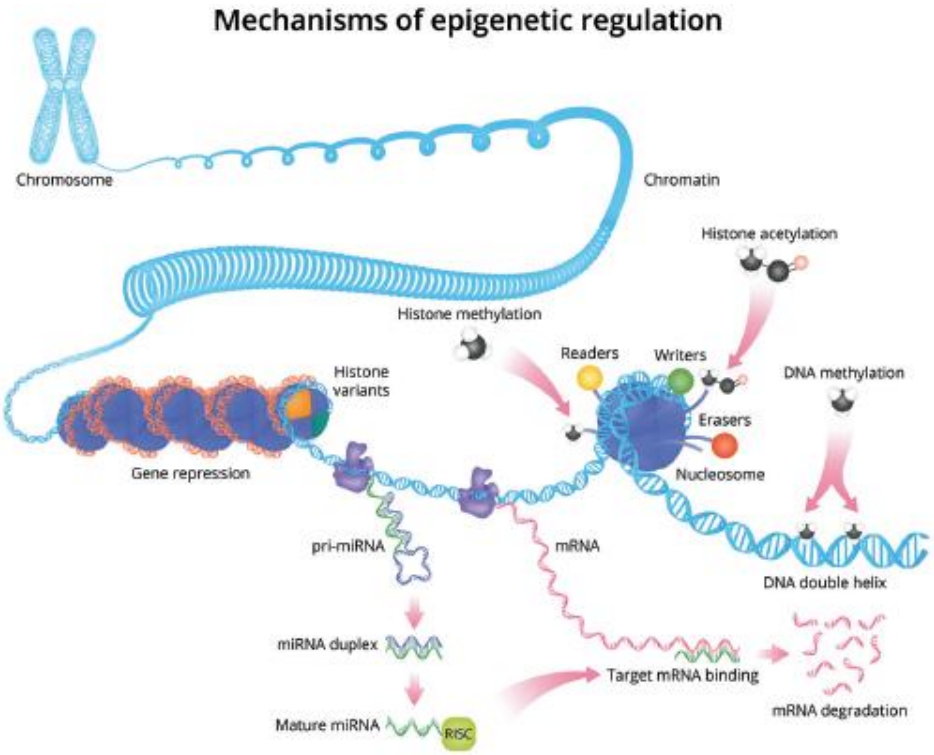


Figure 3. Overview of epigenetic regulatory mechanisms. Methylation involves the addition of methyl groups to DNA sequences. Histone modifications, usually through methylation or acetylation, exert effects through the relaxation or compaction of nucleosomes, thus activating or repressing transcription, respectively. The miRNA affect gene expression as a post-transcriptional mechanism, through the inhibition of protein translation or the destabilisation of target transcripts. miRNA are transcribed as primary precursor molecules (pri-miRNA) that undergo nuclear cleavage. The miRNA duplex binds to RNA-induced silencing complexes (RISC), while the mature miRNA assembles into RISC, which catalyse the degradation of messenger RNAs (mRNAs)[57].

4.3. Analyses of microbiota composition in SCZ patients

Correlations between compositional changes in the microbiota and SCZ are difficult to define due to the intra-individual structure of healthy microbiota[21,23]. However, some clear differences in microbiota composition may be observed between healthy volunteers and SCZ patients (Figure 4)[22,24].

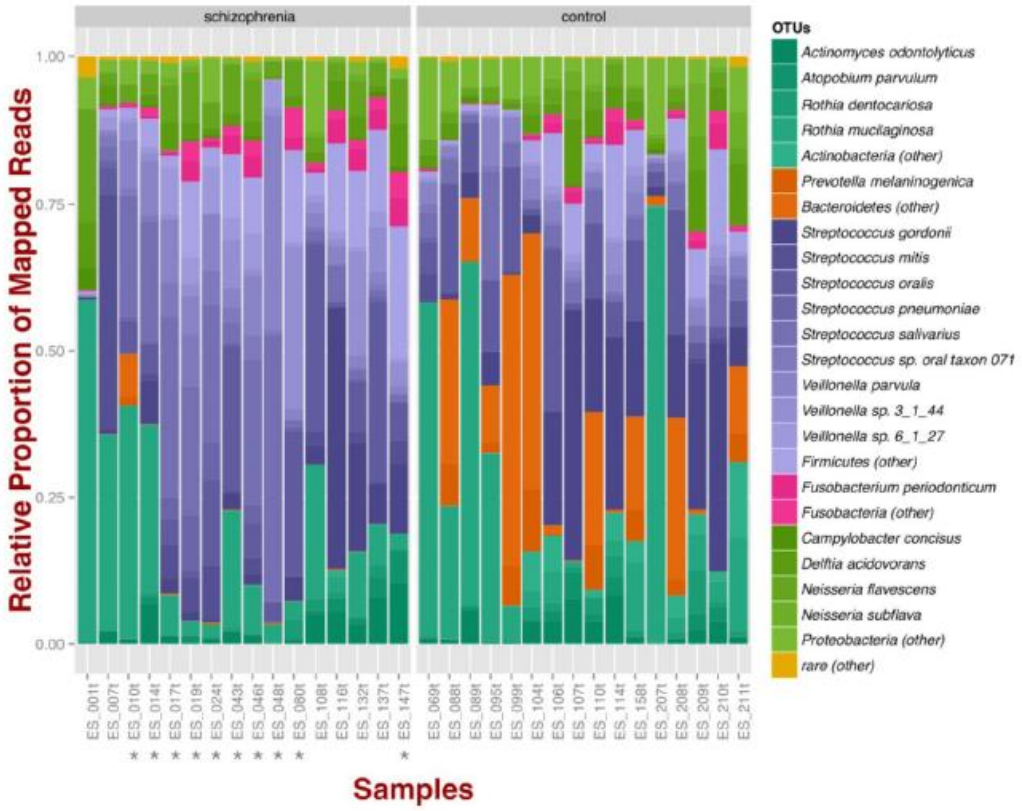


Figure 4. Differences between microbial composition patterns for SCZ and control samples. The stacked bar chart shows the most prevalent species present in SCZ and controls. Green, Actinobacteria; Orange, Bacteroidetes; Blue, Firmicutes; Green, Proteobacteria. The symbol (*) indicates samples from smokers[6].

In patients with a first-episode of psychosis, the abundance of *Lactobacillaceae*, *Halothiobacillaceae*, *Brucellaceae*, and *Micrococcineae* are increased, whereas *Veillonellaceae* is decreased. This is particularly evident in patients with more negative symptoms and minor global functioning[26]. Note that the virus *Lactobacillus phage phiadh* is the main responsible for the *Lactobacillaceae* abundance, which might be associated with co-morbid immune-related disorders in SCZ patients. The most likely mechanism that might underlie this association is that *Lactobacillus phage phiadh* modulates the level of its host bacteria *Lactobacillus gasseri* with subsequent effects on the host immune systems[65]. Also, a lower abundance of *Firmicutes* was observed. Communities of *Faecalibacterium* and *Lachnospiraceae*, SCFA-producing genera, were

also decreased, altering intestinal permeability and, thereby, the gut resident opportunistic pathogens translocate to the mesenteric lymph nodes or systemic circulation, triggering a peripheral immune-inflammatory response. The opportunistic pathogens, *Bacteroidetes* and *Proteobacteria*, are increased in SCZ patients. Levels of *Actinobacteria* were also increased, furthermore positively associated with sparing of and well-organised brain microstructure, in contradiction to the previous findings[6,16,22]. The abundance of *Megasphaera*, associated with poor cognition and inflammation, and *Clostridium*, an ubiquitous pathogen producer of toxins, are significantly higher in patients with SCZ[24]. *Lactobacillus* and *Bifidobacterium*, regulators of chronic stress-induced inflammation and behavioural changes, were enriched considerably, implying gut inflammation. This observation is associated with the severity of the symptoms and gut inflammation since these bacterial strains attempt to re-establish homeostasis[5,6,24].

Trichoderma, the main cellulose-producing genus of fungi in the gut, is markedly decreased in schizophrenic patients. Although the role of Trichoderma in human health is unknown, some strains can work as bacterial substrates, enhancing the growth of health-promoting strains, like *Lactobacillus* and *Bifidobacterium*, beneficial for psychiatric disorders[22,41].

5. New therapeutic strategies targeting the MGB axis

The success of the classical pharmacological treatment of SCZ is often low[4]. Psychopharmacological treatment, namely antipsychotic drugs targeting the dopaminergic system, demonstrate incomplete or ineffective response in resistant patients[3,7,21,24]. Many patients stop treatment due to the associated side effects of antipsychotics. These may be extrapyramidal, such as acute dystonia and parkinsonism or metabolic, such as weight gain, dyslipidemia and insulin resistance[22,45]. Negative symptoms associated with SCZ are particularly difficult to treat. Since the current pharmacological options are limited, the symptoms persist, resulting in loss of cognitive functions and productivity, contributing to the massive personal and socioeconomic burden of SCZ[26]. Novel therapeutic approaches and the search for more effective therapeutic targets is essential[3,7,24].

5.1. MGB targeting treatments

The MGB axis has recently emerged as a potential novel target for the treatment of SCZ[4,21]. New therapeutic strategies aim to reshape GM structure by using probiotics, prebiotics, antibiotics, fecal microbiota transplantation (FMT), microbiota transfer therapy (MTT) and LC-PUFA[7,9,21]. The tolerability and safety of these therapies are particularly encouraging[21].

5.1.1. Probiotics

Probiotics are live microorganisms that provide benefits to the host when administrated in adequate amounts by restoring gut homeostasis[4,7,9,21]. Probiotics contribute to reconstruct and modulate GI barrier, increase mucin production and reduce intestinal permeability, blocking bacterial translocation[7]. The bacteria most frequently used include *Lactobacillus* and *Bifidobacterium*, both being lactic acid-producing bacteria[7,9,21].

Neurotransmitters, such as serotonin and GABA and acetylcholine and hormones, such as PYY and GLP-1, are some of the several molecules influenced by probiotics, revealing their important role in different pathways, in particular in inflammation and immunomodulation[21,26,66]. Inflammatory parameters occupy a significant role in psychiatric diseases, and probiotics seem to beneficially impact them[21]. Studies on probiotics have shown positive results, such as reducing low-grade inflammation, increasing BDNF levels, restoring gut permeability and changing composition of the GM towards a healthy phenotype[4,9].

Although no study has yet proven successful in altering any positive or negative behavioural symptoms while examining the potential for probiotic intervention in SCZ, some alleviation of bowel problems commonly associated with SCZ may be possible[4,9,21,23]. Probiotics can also be used to reduce the adverse metabolic side effects of current treatments[19]. *Candida albicans* is associated with gut discomfort; probiotic formulations have been shown to improve positive psychiatric symptoms in males who were seronegative for *Candida albicans*[23].

Novel probiotics and probiotics combinations seem to be promising therapies for the treatment of psychiatric illnesses, including comfort in therapeutic analyses[7,23].

5.1.2. Prebiotics

Prebiotics are a substrate that is selectively utilised by host microorganisms conferring a health benefit. One of the main classes of prebiotics is a specific type of dietary ingredients, non-digestible fibres[7,9,21,23]. These fibres resist to gastric acidity, enzymatic hydrolysis and absorption in the upper GI tract, reaching lower regions of the gut, where they are selectively metabolised, promoting growth and improving the activity of the probiotic strains, beneficiating host health[4,7,21].

The major prebiotics includes galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), inulins and oligofructose[4]. Prebiotics have a bifidogenic effect, acting as a specific substrate to the probiotic strains, resulting in increment level of *Bifidobacterium* and normalise the composition of *Lactobacillus*, *Bacteroides*, and *Bifidobacterium*[4,26]. GOS and FOS also increase the production of SCFA[4]. However, prebiotics do not always change the composition and activity of the GM in a selective and predictable manner[23].

Prebiotic supplementation promotes the achievement of a healthy GM in the elderly, reducing the risk of age-related illnesses associated with neurogastrointestinal diseases, such as SCZ[7]. Prebiotics also demonstrated to reduce stress responsiveness, anxiety, and depressive-like behaviour, as well as to facilitate changes in hippocampal synaptic efficacy, including general hypothalamic neuronal activity, and enhanced cognition and learning [4,9,23].

5.1.3. Antibiotics

Antibiotics are a class of drugs used in the treatment of bacterial infections. Although antibiotics target pathogenic bacteria, GM can also suffer secondary adverse effects. Acute antibiotic treatment is sufficient to decrease bacterial diversity and may affect the GM for a long period after therapy discontinuation[7,33].

Treatment with antibiotics is associated with abnormal adult behaviours, such as anxiety and cognitive disorders. Also, exposure to antibiotics influence not only the GM but may also contribute to bacterial resistance and is a potential risk factor for metabolic and neoplastic diseases[3,7]. To avoid potential abuse and the repercussion of their harmful effects associated with microbiota dysbiosis, their use should be monitorized[7].

Despite the referred negative impact that antibiotics exert in GM composition, intriguingly the alterations might be beneficial in SCZ, enhancing the action of antipsychotic drugs while preventing their metabolic side effects[7,12,19]. Antibiotics may reduce the increased ratio of *Bacteroidetes,* leading to less weight gain, a known adverse effect of antipsychotics and has also be shown useful to control obesity due to sedentary life induced by negative symptoms of SCZ[12]. By reducing bacterial load, antibiotics may also promote treatments where the goal is to colonise the gut with a new microbiota, such as FMT and MTT, showing that GM composition could be reshaped to an extent not anticipated in the previous studies[34].

5.1.4. Fecal microbiota transplantation

Fecal microbiota transplantation is a procedure to modify the GM, by transplantation of healthy human feces to the gut of the patient suffering microbiota dysbiosis, to re-establish eubiosis[4,7,9].

The microorganisms can be introduced orally or through enemas or colonoscopy[9]. Recently, the oral capsules administration was introduced as an improvement in FMT safety[8,9]. The capsules have equal effectiveness, preventing adverse event due to the conventional colonoscopy-delivered routes of FMT[8].

Fecal microbiota transplantation therapy proved to be effective, well-tolerated, with minimal associated adverse events. This alternative improves significantly the GI symptoms

and shows particular efficacy in the treatment of neurological diseases[7,9]. Further studies are needed to confirm the therapeutic benefit and long-term safety of FMT for neuropsychiatric disorders, in particular, SCZ[9].

5.1.5. Microbiota transfer therapy

Microbiota transfer therapy is the process of transplantation of healthy microbiota into a patient suffering microbiota disturbances. It is considered a modified protocol of FMT.

A dose of Standardized Human Gut Microbiota (SHGM) is administrated, either orally or rectally, followed by a lower maintenance oral doses with a stomach acid suppressant. The stomach-acid suppressant aids to increase the survival of SHGM in the gastric environment.

This novel therapy is a promising treatment for neurobiological disorders where microbiota plays an essential role, due to its safety, tolerability and its effects on GI symptoms. As in the case of FMT, further studies need to confirm the benefits provided by the MTT in neuropsychiatric illnesses, such as SCZ[7,34].

5.1.6. LC-PUFA

Intake of LC-PUFA increases the abundance of healthy microbes and also prevents alterations of the GM[23]. LC-PUFA omega-3 can reduce the severity of symptoms related to psychiatric disorders, leading to functional improvements and preventing exacerbations. Exogenous supplementation increases LC-PUFA blood levels, benefiting cardiovascular diseases associated with psychiatric disords[7].

Concomitant use of supplementation with LC-PUFA and antipsychotic drugs results in improvements in psychiatric symptoms without notable side effects. These findings support the administration of LC-PUFA in routine psychiatric clinical practice, refining the current pharmacological therapy[7,35].

6. Limitations and future perspectives

6.1. Limitations:

- Most evidence regarding the influence of MGB axis in SCZ were performed in germ-free mice[2]. Although germ-free mice have been instrumental in advancing GM research, there are some limitations in their use, especially regarding the marked alterations in the immune system and GI tract, and lacking any true clinical translation. The clinical use of this animal model not directly mimic the human condition but provides a platform to explore the role of bacteria on host

development and function, answering the question whether the microbiome is involved or not[3].

- The existence of a core healthy microbiota profile is still controversial. Due to the individuality of the microbial population in the gut, it is difficult to find a consistent pattern[21].
- A limited number of experimental studies tested the effect of the new therapeutic strategies targeting the MGB axis. The results are controversial[21].
- Difficulty in obtaining samples from SCZ patients[6].
- It remains unclear whether a causal relationship exists between GM dysbiosis and SCZ or if anomalies in the GM composition represent an epiphenomenon of the underlying pathological processes[3,4,15]. The behaviour of the microbiome during psychotic exacerbations in SCZ is unknown[6].

6.2. Future perspectives:

- To investigate if the data emerged from animal studies can be translated to humans[3].
- To improve the understanding of the mechanisms underlying the MGB axis in SCZ patients; larger studies are needed[5,16,23].
- Large-scale longitudinal studies with measurements of immune markers from multiple biological samples, such as material close to the brain, cerebrospinal fluid, brain-scans targeting neuroinflammation, analysis of blood and intestinal microbiota[18].
- Technological advances concerning the MGB axis, more sensitive methods to monitor molecular markers[7,21].
- Gather information about factors that influence the result of a microbiota analysis, such as body mass index (BMI), smoking, alcohol consumption, diet habits, antibiotics, sample handling, wet laboratory methods and statistics and control them[6,16].
- Define the effects of psychiatric medication on the GM composition in terms of psychiatric activity and side effects[6].
- Larger cohorts studies characterising the GM in SCZ patients[22].
- Future of neuropsychiatry should be based on the study of the MGB axis as a multifaceted system, essential to understanding the complexity of the CNS[7].

- Cross-disciplinary collaborative work englobing immunologic, genetic, microbiological and psychiatric expertise is needed to clarify the pathophysiology of SCZ, verifying if GM changes are core to the pathophysiology or merely epiphenomenal[3,15,18].
- Increase research substantiating the clinical use of novel and inexpensive treatments targeting the MGB axis[3,4,17,21]. Further implementation of innovative treatment guidelines in SCZ[17].

7. Conclusion

Although the concept of MGB axis has been implicit since ancient history, scientific proofs, especially regarding its influence in neuropsychiatric disorders, such as SCZ, are a relatively recent subject of study. It is crucial that we fully understand this concept, associated mechanisms, microbiota interactions, modulators, and neuro-gastrointestinal response[7].

Research still has a long route to follow until finding the scientific evidence necessary to completely change the traditional overview of neuropsychiatric diseases, revealing the real role of MGB axis and its potential as a target for novel treatments[3,4,17,21]. In the future, innovative treatment guidelines might be implementated[17].

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