UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE GRADUAÇÃO EM BIOMEDICINA

Bruna Almeida Araújo

GENES DE REPARO DE DNA (*PMS1* E *PMS2*) COMO POSSÍVEIS MODIFICADORES DE FENÓTIPO DA DOENÇA DE MACHADO-JOSEPH/SCA3/MJD

Porto Alegre
2024

Bruna Almeida Araújo

GENES DE REPARO DE DNA (*PMS1* E *PMS2*) COMO POSSÍVEIS MODIFICADORES DE FENÓTIPO DA DOENÇA DE MACHADO-JOSEPH/SCA3/MJD

Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

Orientadora: Profa. Dra. Laura Bannach Jardim
Coorientadora: Ma. Ana Carolina Martins

Porto Alegre

2024

Dados Internacionais de Catalogação na Publicação

CIP - Catalogação na Publicação

Araújo, Bruna Almeida
Genes de reparo de DNA (PMS1 e PMS2) como possíveis modificadores de fenótipo da Doença de Machado-Joseph/SCA3/MJD / Bruna Almeida Araújo. -- 2024.
40 f.
Orientadora: Laura Bannach Jardim.

Coorientadora: Ana Carolina Martins.

Trabalho de conclusão de curso (Graduação) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Curso de Biomedicina, Porto Alegre, BR-RS, 2024.

1. Doença de Machado-Joseph. 2. Ataxia Espinocerebelar Tipo 3. 3. Reparo do DNA. 4. PMS1. 5. PMS2. I. Jardim, Laura Bannach, orient. II. Martins, Ana Carolina, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Bruna Almeida Araújo

GENES DE REPARO DE DNA (*PMS1* E *PMS2*) COMO POSSÍVEIS MODIFICADORES DE FENÓTIPO DA DOENÇA DE MACHADO-JOSEPH/SCA3/MJD

Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

Aprovado em: ___ de _____ de 2024.

BANCA EXAMINADORA

_____

Nome do professor - instituição

_____

Nome do professor - instituição

_____

Nome do professor - instituição (orientador)

AGRADECIMENTOS

Primeiramente agradeço a minha família (pai, mãe e irmã) por me acompanharem até Porto Alegre e apoiarem minhas decisões até aqui, possibilitando a realização do meu sonho de me formar em Biomedicina.

Quero agradecer também a todas as minhas amigas do curso de Biomedicina, principalmente Ellen, Grasiely e Taís, que me ajudaram em todos os momentos que precisei.

Por fim, sou grata pela oportunidade de fazer Iniciação Científica no Laboratório de Neurogenética Translacional, o que possibilitou também o desenvolvimento deste TCC. Agradeço a todos os integrantes do grupo de pesquisa pelo aprendizado compartilhado comigo, principalmente a minhas orientadoras Laura Jardim e Ana Carolina Martins, que me guiaram até aqui.

RESUMO

Introdução: A ataxia espinocerebelar ou doença de Machado-Joseph (SCA3/MJD) é causada por uma expansão dominante da sequência de trinucleotídeos CAG (CAGexp) no gene *ATXN3*. Sabe-se que a idade de início (AO) é 55% definida pelo comprimento de CAGexp, em que quanto maior a expansão, mais cedo aparece o primeiro sintoma, o qual pode variar para cada indivíduo. Alguns genes relacionados ao mecanismo de reparo do DNA, como o *PMS1* e o *PMS2* foram apontados como prováveis modificadores da AO, e que poderiam, portanto, ajudar a explicar os 45% de variabilidade restantes. A hipótese adotada consiste em averiguar se variantes associadas ao maior efeito da rota estão também associadas a adiamentos da AO ou vice-versa. **Métodos**: foram estudadas 160 pessoas sintomáticas portadoras de SCA3/MJD, diagnosticadas anteriormente pelo Serviço de Genética Médica (SGM) do Hospital de Clínicas de Porto Alegre (HCPA), cujos dados clínicos, tamanhos do CAGexp e amostras de DNA estavam guardados nos bancos de dados e biorrepositórios do grupo de neurogenética, que concordaram em participar do estudo, aprovado previamente pelo comitê de ética institucional (GPPG 2023-0302). A idade na qual as pessoas ou seus circunstantes notaram os primeiros sintomas foi considerada a idade de início da doença (AOfs). Nas suas amostras de DNA foram genotipados os seguintes polimorfismos de nucleotídeos únicos (SNPs), com prévia indicação de alteração de efeito no gene e com aceitáveis frequências dos alelos "menores" (MAF) definidas em bancos de dados públicos: o rs3791767 (alelos A e C, A sendo candidato a neuroprotetor) no *PMS1* e o rs1805323 (alelos G e T, G sendo candidato a neuroprotetor) no *PMS2*. Os três possíveis genótipos foram determinados em cada locus. A distribuição das AOfs foi comparada entre os genótipos usando-se a ANOVA ajustada para o CAGexp mediano do grupo, usando-se um p-valor de 0,05. **Resultados**: 151 pessoas atáxicas tiveram o rs3791767 (*PMS1*) genotipado: 81, 55 e 15 das mesmas apresentaram respectivamente os genótipos AA, AC e CC. Nestes grupos, as AOfs médias (DP) para um CAGexp de 75 repetições foram de 32,15 (9,66), 35,16 (11,28) e 31,07 (11,21) anos (p = 0,984). O rs1805323 (*PMS2*) foi genotipado nas 160 pessoas: 147, 12 e uma única delas apresentaram os genótipos GG, GT e TT, respectivamente. As AOfs destes genótipos, para uma CAGexp de 75 repetições, foram de 33,11 (10,35), 34,42 (12,54) e 20 anos (p= 0,337). **Discussão:** Nossos resultados descartam efeitos do rs3791767 (*PMS1*) sobre a AOfs da SCA3/MJD. O rs1805323 (*PMS2*) tampouco apresentou efeitos significativos sobre a AOfs, mas a MAF encontrada na nossa amostra para o alelo T (0,02) foi bem menor do que a esperada (0,11), o que pode ter prejudicado o poder do nosso estudo.

Palavras-chave: Doença de Machado-Joseph; ataxia espinocerebelar tipo 3; SCA3/MJD; *PMS1*; *PMS2*; reparo do DNA.

ABSTRACT

Introduction: Spinocerebellar ataxia or Machado-Joseph disease (SCA3/MJD) is caused by a dominant expansion of the CAG trinucleotide sequence (CAGexp) in the ATXN3 gene. It is known that the age of onset (AO) is 55% defined by the length of CAGexp, where the longer the expansion, the earlier the first symptom appears, which may vary for each individual. Some genes related to the DNA repair mechanism, such as *PMS1* and *PMS2*, have been identified as probable modifiers of OA, and could therefore help explain the remaining 45% of variability. The hypothesis adopted is to find out whether variants associated with a greater route effect are also associated with OA postponements or vice versa. **Methods:** 160 symptomatic people with SCA3/MJD, previously diagnosed by the Medical Genetics Service (SGM) of the Hospital de Clínicas de Porto Alegre (HCPA), whose clinical data, CAGexp sizes and DNA samples were stored in the neurogenetics group's databases and biorepositories, who agreed to participate in the study, previously approved by the institutional ethics committee (GPPG 2023-0302) were studied. The age at which people or their relatives noticed the first symptoms was considered the age of onset of the disease (AOfs). In their DNA samples, the following single nucleotide polymorphisms (SNPs) were genotyped, with prior indication of an effect change in the gene and with acceptable "minor" allele frequencies (MAF) defined in public databases: rs3791767 (alleles A and C, A being a candidate for neuroprotective allele) in *PMS1* and rs1805323 (alleles G and T, G being a candidate for neuroprotective allele) in *PMS2*. The three possible genotypes were determined at each locus. The distribution of AOfs was compared between the genotypes using ANOVA adjusted for the average CAGexp of the group, using a p-value of 0.05. **Results:** 151 ataxic people had rs3791767 (*PMS1*) genotyped: 81, 55 and 15 of them had the AA, AC and CC genotypes respectively. In these groups, the average AOfs (SD) for a CAGexp of 75 repeats were 32.15 (9.66), 35.16 (11.28) and 31.07 (11.21) years (p = 0.984). The rs1805323 (*PMS2*) was genotyped in 160 people: 147, 12 and only one of them had the GG, GT and TT genotypes, respectively. The AOfs of these genotypes, for a CAGexp of 75 repeats, were 33.11 (10.35), 34.42 (12.54) and 20 years (p= 0.337). **Discussion:** Our results rule out the effects of rs3791767 (*PMS1*) on the AOfs of SCA3/MJD. Neither did rs1805323 (*PMS2*) show significant effects on AOfs, but the MAF found in our sample for the T allele (0.02) was much lower than expected (0.11), which may have affected the power of our study.

Keywords: Machado-Joseph Disease; spinocerebellar ataxia type 3; SCA3/MJD; *PMS1*; *PMS2*; DNA repair.

SUMÁRIO

1 INTRODUÇÃO COMPREENSIVA

1.1 A Doença de Machado-Joseph (SCA3/MJD)

A Doença de Machado-Joseph, também conhecida ataxia espinocerebelar tipo 3 (SCA3/MJD), é uma patologia neurodegenerativa dominante causada por uma expansão da sequência CAG (CAGexp) no gene *ATXN3* (Kawaguchi *et al.,* 1994). Esse gene está localizado no braço longo do cromossomo 14 (14q32.1) e a sequência CAG é encontrada no éxon 10 desse cromossomo (Takiyama *et al.*, 1993).

O tamanho dos alelos CAGn normais é polimórfico na população geral e varia entre 3 a 40 trinucleotídeos (Dürr *et al*., 1996; van de Warrenburg *et al*., 2005), que são transmitidos de forma estável para a prole (Sena *et al*. 2021). O intervalo entre 41 e 51 repetições é muito raramente encontrado nas populações normais. A menor sequência repetitiva associada à presença de sintomas atáxicos foi a de 52 repetições (Sena *et al*., 2021), mas esse CAGexp é muito raro. No Brasil, por exemplo, as menores CAGexp até hoje descritas tinham 65 repetições (de Castilhos *et al* 2014). CAGexp podem ter transmissões bastante instáveis e geralmente isso resulta em aumentos do CAGexp transmitido de uma geração para a outra (Sena *et al.*, 2021).

Como consequência do CAGexp, a proteína ataxina-3 adquire uma cadeia expandida de poliglutaminas (poliQ). Essa cadeia possui um efeito neurotóxico, caracterizado como um ganho de função nas células neuronais.

A proteína ataxina-3 é detectada em diversos tecidos embrionários e também adultos (Ichikawa *et al.,* 2001; Riess *et al.,* 2008), e possui funções diferentes no organismo, incluindo a ubiquitinização, por isso, expressão de ataxina-3 não é restrita às áreas do sistema nervoso central mais caracteristicamente afetadas pela doença (Riess *et al*., 2008). Algumas de suas isoformas, detectadas apenas em indivíduos SCA3/MJD, não têm sua função bem estabelecida, mas a expressão é

idêntica quando comparada à expressão em indivíduos saudáveis. Portanto, o nível de expressão não possui relação com a gravidade da doença ou com o tamanho de CAG (Paulson *et al.,* 1997a; Tait *et al.,* 1998).

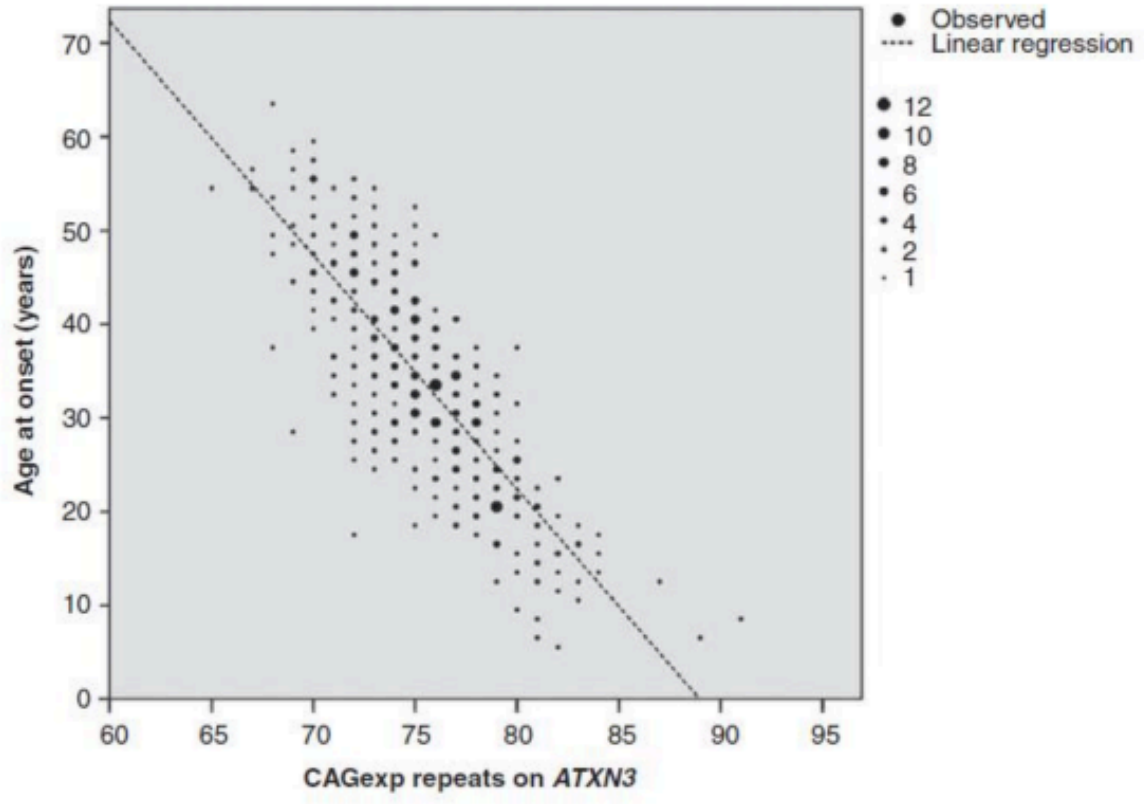
O tamanho da CAGexp é um importante modificador do genótipo da SCA3/MJD, ou seja, quanto maior a CAGexp, mais cedo a doença é desencadeada e mais graves são os sintomas. Essa correlação invertida entre CAGexp e a idade de início (AO, do inglês "*age at onset*") ocorre também em outras doenças de poliQs, como a doença de Huntington (HD) e outras SCAs. Porém, a CAGexp explica somente 55,2% da variabilidade da AO (de Mattos *et al*., 2018b). Na figura de correlação entre AO e CAGexp, como mostra a **Figura 1**, é possível perceber-se a grande variação na AO associada a cada tamanho de CAGexp (Saute & Jardim, 2015).

Os primeiros sintomas da SCA3/MJD são neurológicos e predominam os déficits motores, como a ataxia de marcha e de membros, a dificuldade de deglutição e o prejuízo na articulação da fala. Além disso, são comuns a síndrome piramidal, incluindo hiperreflexia e espasticidade, sinais extrapiramidais como distonias e parkinsonismo, distúrbios da motricidade ocular e também do sono, perda de peso e alterações cognitivas (Saute & Jardim, 2015), como mostra a **Figura 2**.

Geralmente, os primeiros sintomas da SCA3/MJD aparecem na vida adulta, sendo a idade de início do primeiro sintoma (AOfs, do inglês "*age at onset of first symptom*") média de aproximadamente 38 anos (Mattos *et al*., 2018). Porém, não há consenso na literatura científica sobre o que marcaria o início da doença. Alguns estudos utilizam o primeiro sintoma (AOfs), seja ele qual for, como o marcador do início da doença (por exemplo, Jardim *et al*., 2001 e França *et al*., 2009). Outros trabalhos (por exemplo, Tezenas du Montcel *et al*., 2014b; Tezenas du Montcel *et al*., 2014a; Raposo *et al*., 2015) utilizam a idade de início da ataxia de marcha (AOga, do inglês, "*age at onset of gait ataxia"*). Neste trabalho, foi adotada a AOfs, pois era o dado disponível para as amostras estudadas.

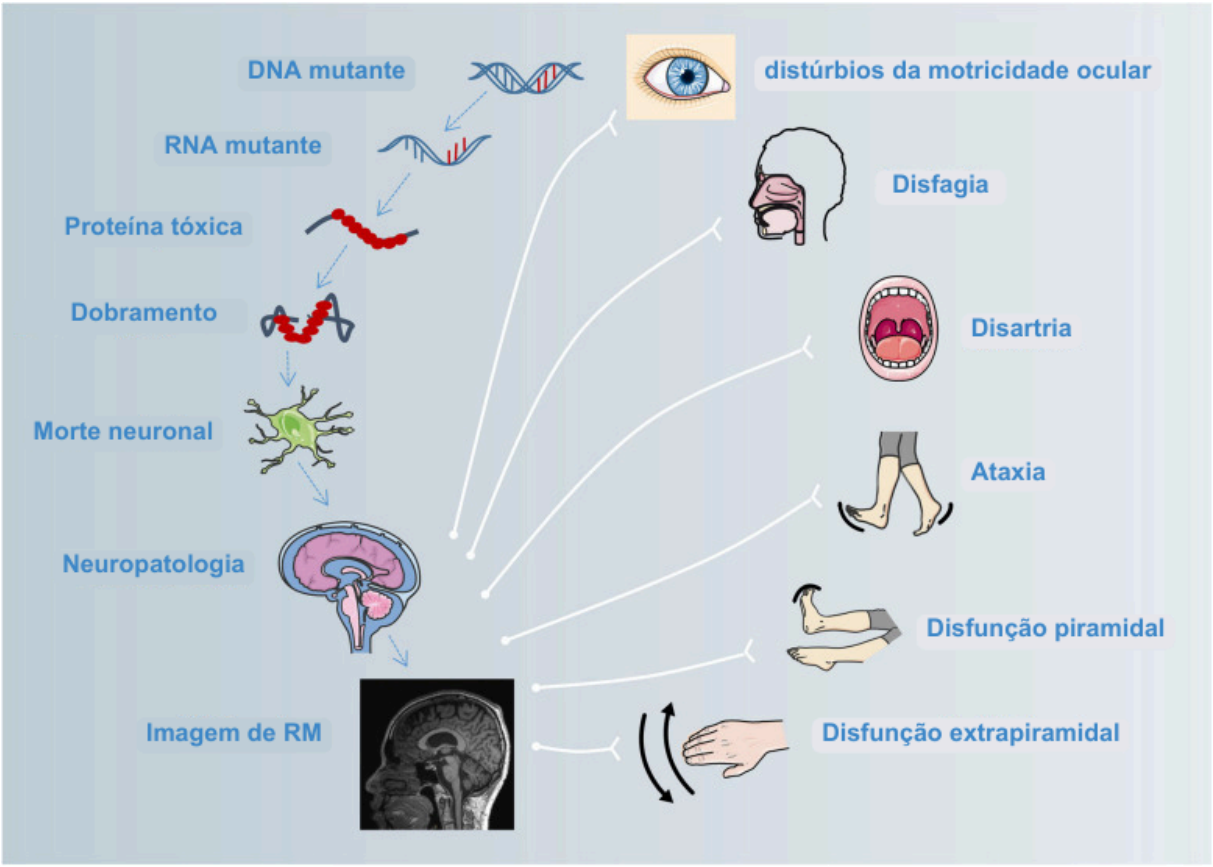
Os portadores de CAGexp em fases anteriores à sua percepção da doença, ou seja, não atáxicos, são caracterizados como portadores assintomáticos ou pré-atáxicos. Porém, já se percebem nesses indivíduos pré-atáxicos alterações subclínicas, como as do metabolismo da glicose no SNC (Soong and Liu 1998), no perfil de citocinas (da Silva Carvalho *et al*., 2016), na difusividade da água na substância branca (de Oliveira *et al*., 2023 PMID: 37193796), em escalas clínicas e nos movimentos oculares (de Oliveira *et al*., 2023 PMID: 33438269).

Figura 1. Associação entre o comprimento da sequência CAGexp e a idade de início dos sintomas em SCA3/MJD



Fonte: Saute e Jardim, 2015.

Figura 2. Um esquema da sequência de eventos patogenéticos e de suas consequências clínicas



Fonte: adaptado de Na Wan *et al*., 2020.

1.2 O reparo do DNA como modificador da AO e os genes *PMS1* e *PMS2*

Durante a replicação do material genético das células, podem ocorrer erros que resultam em danos ao DNA. O reparo do DNA acontece por meio de vários mecanismos como a reparação por excisão de bases, por excisão de nucleotídeos e reparação de "*missmatch*", além da recombinação homóloga e da união não-homóloga das extremidades.

A investigação dos genes envolvidos no reparo do DNA como possíveis modificadores da AO em poliglutaminopatias como a HD e as SCAs é recente. Entre os genes citados como possíveis modificadores de AO pela literatura científica recente estão *FAN1, PMS1, PMS2, LIG1, MSH3, TRIM29* e *RAG*.

Os genes do reparo do DNA não tinham sido cogitados como modificadores das doenças devidas a CAGexp, até que dois estudos de associação genômica ampla (GWAS) levantaram sua forte associação a variações nas AO dessas condições. O consórcio "*Genetic Modifiers of Huntington Disease*" (GeM-HD, de 2015 e sua atualização de 2019) identificou três genes de reparo de DNA (*PMS1, PMS2, LIG1*) como modificadores da idade de início de HD. O estudo de Bettencourt (2016) mostrou associação do gene *PMS2* e a AO da HD e também das ataxias espinocerebelares em conjunto, incluindo a SCA3/MJD. Nosso grupo de pesquisa demonstrou que o FAN1 em associação com o ATXN2 também modifica a AO, nos pacientes com SCA3/MJD da coorte do nosso estado (Mergener *et al*., 2020).

1.3 Justificativa

A SCA3/MJD é uma ataxia muito rara, mas de importante relevância no estado do Rio Grande do Sul (RS). Entre as SCAs, a SCA3/MJD é a mais frequente no RS (de Castilhos *et al*., 2014), a sua prevalência de indivíduos sintomáticos sendo de 7:100.000 (Rodriguez-Labrada *et al*., 2020). Além disso, é a SCA com maior prevalência tanto no Brasil quanto no mundo (Sequeiros *et al*. 2012). Por isso, o presente trabalho faz-se necessário para realizar novas investigações em relação à doença, contribuindo assim com o conhecimento sobre fatores que modulam a sua idade de início.

No presente trabalho, foram pesquisados os genes *PMS1* e *PMS2*, integrantes da rota MMR (do inglês, "*mismatch repair"*). Ambos os genes foram escolhidos para serem estudados em conjunto, pois são subunidades que, com o *MLH1*, formam complexos MutL. *PMS1* foi um dos modificadores identificados nos GWAS do GeM-HD, enquanto o gene *PMS2* demonstrou acelerar as expansões somáticas na célula (LAHUE, 2020), também em HD.

Para cada gene candidato, selecionamos então um polimorfismo de nucleotídeo único (SNP, do inglês "*single nucleotide polymorphism*") para ser estudado como marcador candidato. Para um SNP ser selecionado, sua frequência populacional deveria ser compatível com um estudo local em pacientes SCA3/MJD que tivesse poder - pois a SCA3/MJD é rara. A frequência do alelo mais raro (MAF, do inglês "*minor allele frequency*") deveria ser de pelo menos 10%. Além disso, os SNPs selecionados deveriam ter evidências ao menos iniciais de produzirem efeitos funcionais sobre os peptídios traduzidos.

Com base nesses critérios, selecionamos o rs3791767 no *PMS1* e o rs1805323 no *PMS2.* Os detalhes estão descritos na **Tabela 1**.

Tabela 1. Os SNPs rs3791767 e rs1805323

Gene	SNP	Alelo frequente	Alelo raro	MAF	Efeito do alelo raro		Alelo que parece ser neuroprotetor	Ref.
					Beta *	Outro		
PMS1	rs3791767	A	C	0,196	-0.8		A	GeM-HD, 2019
PMS2	rs1805323	G	T	0,112	-3.6		G	Bettencourt *et al*. 2016

* Beta = tamanho do efeito do alelo menor (Alelo 2), equivalente aos anos adicionados (+) ou subtraídos (-) da PAO, para cada cópia do alelo menor

MAF: minor allele frequency, ou frequência do alelo mais raro; SNP: single nucleotide polymorphism, ou polimorfismo de nucleotídeo único. Obtido de dbSNP NCBI (Sherry *et al*., 2001).

1.4 Objetivos

1.4.1 Objetivo geral

Investigar se variantes nos genes *PMS1* e *PMS2* estão associadas a variações no fenótipo da SCA3/MJD.

1.4.2 Objetivos específicos

I. Genotipar os polimorfismos de nucleotídeos únicos (SNPs):
   a) rs3791767 no gene *PMS1*;
   b) rs1805323 no gene *PMS2*;

II. Avaliar se esses SNPs se associam com a AO da SCA3/MJD.

2 ARTIGO CIENTÍFICO

Preparado para ser submetido como um "*short report*" (1500 palavras e 15 referências) no periódico "*The Cerebellum*".

Title: Testing the DNA repair genes *PMS1* and *PMS2* as modifiers of Spinocerebellar Ataxia Type 3 Phenotype

Authors: Bruna Almeida Araújo, Ingrid Tuchtenhagen, Gabriel Vasata Furtado, Maria Luiza Saraiva-Pereira, Ana Carolina Martins, Laura Bannach Jardim

Affiliations/adresses:
Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
Bruna Almeida Araújo

Laboratório de Neurogenética Translacional, Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
Bruna Almeida Araújo, Ingrid Tuchtenhagen, Gabriel Vasata Furtado, Maria Luiza Saraiva-Pereira, Ana Carolina Martins, Laura Bannach Jardim

Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
Ingrid Tuchtenhagen, Maria Luiza Saraiva-Pereira, Ana Carolina Martins, Laura Bannach Jardim

Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porto Alegre, 90035-003, Brazil
Maria Luiza Saraiva-Pereira, Laura Bannach Jardim

Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
Maria Luiza Saraiva-Pereira

Departamento de Medicina Interna, FAMED, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
Laura Bannach Jardim

Correspondence author:
Prof. Laura Bannach Jardim
DMI FAMED UFRGS, and
Medical Genetics Service
Serviço de Genética Médica,
Hospital de Clinicas de Porto Alegre
Rua Ramiro Barcelos 2350
90035-003 Porto Alegre, Brazil
E-mail: ljardim@hcpa.edu.br

ABSTRACT

Introduction: Phenotype variations of spinocerebellar ataxia type 3/ Machado-Joseph disease (SCA3/MJD), a disease due to a CAG repeat expansion (CAGexp) at ATXN3, have been related to some DNA repair genes. Our aim was to investigate if polymorphisms at *PMS1* and *PMS2* might modify the age at onset of the first symptom (AOfs) in this disease.

Methods: 160 symptomatic carriers previously diagnosed whose clinical data, CAGexp lengths and DNA samples were stored in our biorepositories, were invited to participate. rs3791767 (A/C, A being the neuroprotective candidate) in *PMS1* and rs1805323 (G/T, G being the neuroprotective candidate) in *PMS2* were genotyped. AOfs was compared between genotypes using ANOVA adjusted for the group's median CAGexp, using a p-value of 0.05.

Results: 81, 55 and 15 persons had AA, AC and CC genotypes at rs3791767, respectively. Their mean (SD) AOfs for 75 repeats CAGexp were 32.15 (9.66), 35.16 (11.28) and 31.07 (11.21) years (p = 0.984). One hundred forty-seven, 12 and only

one of the subjects had GG, GT and TT genotypes at rs1805323; their AOfs, for 75 repeats CAGexp, were 33.11 (10.35), 34.42 (12.54) and 20 years (p= 0.337).

Discussion: Our results ruled out effects of rs3791767 (*PMS1*) over AOfs of SCA3/MJD. rs1805323 (*PMS2*) also did not show a significant association with AOfs. However, T allele frequency in our sample (0.02) was much lower than expected (0.11), and we cannot rule out potential effects of this SNP over SCA3/MJD onset of symptoms.

Keywords: Machado–Joseph disease; Spinocerebellar ataxia type 3; Age at onset; *PMS1*; *PMS2*.

To the Editor,

Spinocerebellar ataxia type 3 also known as Machado Joseph disease (SCA3/MJD) is an autosomal dominant, polyglutamine disease caused by a CAG repeat expansion (CAGexp) at *ATXN3* gene. Subjects carrying more than 51 CAG repeats present movement and other neurologic disorders, usually in adulthood. The average age at onset of the first symptoms (AOfs) is 38–40 years and is partly explained by the sequence's size. At least 45% of the variability of AOfs, however, should be related to other modifying genes, environmental factors and habits (de Mattos *et al.*, 2018).

SCA3/MJD patients have been reported in Rio Grande do Sul (RS), the southernmost state of Brazil, since 2001 (Jardim *et al.,* 2001). In this state, there is a large population of patients, called SCA3/MJD RS cohort (Souza *et al*., 2016): around 700 symptomatic individuals and over 1,500 individuals with a 50% risk were living there by 2020 (Rodriguez-Labrada *et al*., 2020).

Genes associated with DNA repair systems have been implicated as modifiers of the AO in polyQs diseases. The most studied disorders are Huntington's Disease (HD) and, to a lesser extent, SCA3/MJD. The genome-wide association study (GWAS)

performed by the "Genetic Modifiers of Huntington Disease" (GeM-HD) identified strong associations between AO and the DNA repair genes *FAN1, PMS1, PMS2, LIG1* and *MSH3* in a cohort of 9,000 HD patients, all genes directly associated with DNA maintenance/instability mechanisms (GeM-HD, 2019). Three publications described possible relationships between DNA repair genes and SCA3/MJD. An association between AO and *ANA1* and *PMS2* was found among 1,462 people with HD or a polyQ SCA (397 of them with SCA3/MJD) (Bettencourt *et al*., 2016). *FAN1 was related to the AOfs in a candidate gene study* (Mergener *et al*. 2020)*, whereas TRIM29* and *RAG* were raised as potential modifiers of SCA3/MJD in an unbiased approach that included less than 800 SCA3/MJD carriers (Akçimen *et al*., 2020).

PMS1 and *PMS2* encode proteins that are key components of the DNA mismatch repair system (MMR), which acts to correct DNA mismatches and small insertions and/or deletions that may occur (Pannafino & Alani 2021). *PMS1* was related to HD (GeM-HD, 2019) while *PMS2* might be related to SCA3/MJD (Bettencourt *et al*., 2016). None of them have been studied in the SCA3/MJD RS cohort. Single nucleotide polymorphisms (SNPs) with sufficient minor allele frequencies (MAF) and preliminary evidence of functional effects were identified, as were shown in **Table 1**.

Due to that, we tried to help with knowledge about the potential role of MMR components over SCA3/MJD phenotype. Our aim was to look for associations between the polymorphisms rs3791767 (*PMS1*) and rs1805323 (*PMS2*) and AOfs in the RS cohort.

Symptomatic carriers from the RS cohort and previously diagnosed in our institution were invited to participate, if their clinical data, CAGexp lengths and DNA samples were still available from our biorepositories.

DNA was isolated from peripheral blood leukocytes using standard methods. The CAG repeat length analysis was performed by the polymerase chain reaction (PCR) using fluorescent labeled primers flanking the CAG repeat tract at ATXN3, followed by capillary electrophoresis into the genetic analyzer ABI3130xl (Applied Biosystems, Foster City, CA, USA). Results were analyzed through GeneMapper® ID v 3.2 software (Applied Biosystems, Foster City, CA, USA).

rs3791767 (*PMS1*) and rs1805323 (*PMS2*) genotyping was performed using TaqMan SNP Genotyping Assay (C_483247_10) in a final volume of 8 µL containing 2 ng of DNA, according to assay protocol (Applied Biosystems, Foster City, CA, USA). Amplification was performed in the ABI 7500 Fast Real-Time PCR System® equipment (Applied Biosystems, Foster City, CA, vUSA) as follows: one cycle of 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Patients were divided in groups according to genotypes AA, AC and CC at rs3791767 (*PMS1*), and GG, GT and TT at rs1805323 (*PMS2*). The candidate alleles to be neuroprotective were A at rs3791767 (*PMS1*) and G at rs1805323 (*PMS2*). AOfs were compared across these groups by ANOVA, controlling for the CAGexp. Results were presented for the median CAGexp found, 75 CAG repeats. A p-value of 0.05 was adopted.

After all the data was collected, the patients' AO were compared by ANOVA with the CAG's size adjusted to 75 repetitions. P-value < 0.05 was adopted.

One hundred and sixty subjects were included. Their demographic data was presented in **Table 2**.

For rs3791767 (*PMS1*), 151 subjects were included, as the genotyping assay did not work for all of them. Among the 302 chromosomes genotyped, 217 (71.85%) carried the A, and 85 (28.14%), the C allele, a MAF a little bit larger than the expected 19.6%, according to **Table 1**. Eighty-one subjects had the AA genotype, 55 had the AC genotype, and 15 had CC on the gene. The mean (SD) AOfs of these groups were 32.15 (9.66), 35.16 (11.28) and 31.07 (11.21), respectively, (p=0.984) (**Table 3)**.

The rs1805323 (*PMS2*) was genotyped, all 160 subjects. Among the 320 chromosomes, there were 306 (95.62%) with the G and 14 with the T allele (4.38%), a MAF quite smaller than the expected 11.2%. One hundred, forty-seven patients carried the GG, 12 carried the GT and only one subject carried the TT genotype. The mean (SD) AOfs of GG and GT subjects were 33.11 (10.35) and 34.42 (12.54),

while AOfs of the TT subject was 20 years of age. The calculated p-value is of 0.337 (**Table 3**).

As we have already mentioned, *PMS1* and *PMS2* encode key component proteins in the MMR DNA repair system (Panafino & Alani, 2021). Both proteins form heterodimers with the gene product of the *MLH1* gene. Bettencourt and colleagues (2016) demonstrated that the rs1805323 (*PMS2*) was related to the AO of a mixed group of polyQ patients that included HD, SCA1, SCA2, SCA3/MJD, SCA6 and SCA7. Evidence on *PMS1* effect over AO was so far obtained for HD, only (GeM-HD, 2019).

Our study excluded any effect of rs3791767 over SCA3/MJD AOfs. However, it is possible that we have missed *PMS1* modulating effects, unrelated to this polymorphism.

In spite of not achieving significance, the results related to rs1805323 (*PMS2*) were more difficult to interpret. Our cohort did not present the expected MAF of 11.2% of the T allele, the potentially non-protective variant. Twelve (7.5%) subjects were heterozygous, GT carriers, and only one patient (0.62% of the total sample) was a TT homozigote. This TT carrier would have an AOfs of 20 years, if the carrier of 75 repeats at CAGexp - while the usual PAOfs for 75 repeats, in our cohort, is 30 years of age (de Mattos *et al*., 2018b). If the T allele has a recessive effect, no consequence would be found on heterozygotes - as seen here - and more homozygotes would be needed to find a rs1805323 effect over SCA3/MJD AOfs. In any case, the minor allele at rs1805323 is so rare among the RS cohort that any potential effect could be negligible, for the point of view of the majority of patients and families.

In conclusion, rs3791767 (*PMS1*) and rs1805323 (*PMS2*) did not present any modulation effect over onset of symptoms of SCA3/MJD, at least in the RS cohort, either because there is no effect at all or because of the rarity of the minor variants in these loci.

Acknowledgements

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant 405963/2021-1, and by Financiamento e Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre (Fipe-HCPA), grant 2023-0302. IT was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). BAA, GVF, MLSP, ACM and LBJ were supported by CNPq.

References

AKÇIMEN, Fulya et al. Genome-wide association study identifies genetic factors that modify age at onset in Machado-Joseph disease. **Aging (Albany NY)**, v. 12, n. 6, p. 4742, 2020.

BETTENCOURT, Conceicao et al. DNA repair pathways underlie a common genetic mechanism modulating onset in polyglutamine diseases. **Annals of neurology**, v. 79, n. 6, p. 983-990, 2016.

DE MATTOS, Eduardo Preusser. Avaliação de modificadores do fenótipo na doença de Machado-Joseph. 2018.

GENETIC MODIFIERS OF HUNTINGTON'S DISEASE (GEM-HD) CONSORTIUM et al. Huntington's disease onset is determined by length of uninterrupted CAG, not encoded polyglutamine, and is modified by DNA maintenance mechanisms. **bioRxiv**, p. 529768, 2019.

JARDIM, Laura Bannach et al. A survey of spinocerebellar ataxia in South Brazil–66 new cases with Machado-Joseph disease, SCA7, SCA8, or unidentified disease–causing mutations. **Journal of neurology**, v. 248, p. 870-876, 2001.

MERGENER, Rafaella et al. Variation in DNA repair system gene as an additional modifier of age at onset in spinocerebellar ataxia type 3/Machado–Joseph disease. **Neuromolecular medicine**, v. 22, n. 1, p. 133-138, 2020.

PANNAFINO G, Alani E. Coordinated and Independent Roles for MLH Subunits in DNA Repair. **Cells**. 2021 Apr 20;10(4):948.

RODRÍGUEZ-LABRADA, Roberto et al. Founder effects of spinocerebellar ataxias in the American continents and the Caribbean. **The Cerebellum**, v. 19, p. 446-458, 2020.

SHERRY S. T, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. dbSNP: the NCBI database of genetic variation. **Nucleic Acids Res.** 2001.

SOUZA, G. N. et al. Spinocerebellar ataxia type 3/Machado-Joseph disease: segregation patterns and factors influencing instability of expanded CAG transmissions. **Clinical genetics**, *90*(2), 134–140, 2016.

Tables

Table 1. SNPs rs3791767 and rs1805323

Gene	SNP	Frequent allele	Rare allele	MAF	Rare allele effect		Allele that seems to be neuroprotective	References
					Beta *	Other		
PMS1	rs3791767	A	C	0.196	-0.8		A	GeM-HD, 2019
PMS2	rs1805323	G	T	0.112	-3.6		G	Bettencourt *et al.,* 2016

* Beta = effect of the minor allele (Allele 2), equivalent to the years added (+) or subtracted (-) from the PAO, for each copy of the minor allele.

MAF: minor allele frequency.

SNP: single nucleotide polymorphism; obtained from dbSNP NCBI (Sherry *et al.,* 2001).

Table 2. General characteristics of the present cohort

Characteristics	**SCA3/MJD**
Women/all subjects (%)	84/160 (52.5)
CAG (normal allele) mean (SD)	22.34 (4.96)
CAGexp mean (SD)	75.71 (3.35)
Age at onset of the first symptom in years mean (SD)	33.12 (10.51)

Table 3. Genotypic groups in rs3791767 (*PMS1*) and rs1805323 (*PMS2*) and their mean ages at onset of symptoms, adjusted for an expanded repeat with 75 CAGn

	Characteristics of the genotypic groups			**p***
rs3791767 (*PMS1)*	AA	AC	CC	
N	81	55	15	
Age at onset mean (SD)	32.15 (9.66)	35.16 (11.28)	31.07 (11.21)	0,984
rs1805323 (*PMS2*)	GG	GT	TT	
N	147	12	1	
Age at onset mean (SD)	33.11 (10.35)	34.42 (12.54)	20 (.)	0.337

* ANOVA

3 CONCLUSÕES E PERSPECTIVAS

Concluindo, os achados do estudo mostram que, para o SNP do *PMS1*, a maioria dos sujeitos é AA, pois o alelo C é mais raro. Já para o SNP do *PMS2*, a maioria é GG e apenas 1 é TT. Comparando os grupos de genótipo, não há diferença significativa entre a idade de início dos indivíduos. Ao calcular o p-value, os valores encontrados foram de 0,984 e 0,337, respectivamente.

Os resultados encontrados neste estudo, a partir dos 160 sujeitos genotipados, revelam que as variantes analisadas não modificam a AO em SCA3/MJD, visto que não foi encontrada correlação entre os SNPs e a idade de início, segundo os dados disponíveis.

Os resultados encontrados previamente na literatura, como nos estudos GeM-HD (2019) e Bettencourt (2016) podem ser devidos ao efeito nas outras doenças de poli-Q, como a Doença de Huntington e outras SCAs.

Além disso, o SNP do gene *PSM2* pode estar sujeito a estratificação populacional, devido a sua raridade da população do RS, como mostra o "*MAF*" apresentado na **Tabela 1.**

Assim sendo, faz-se necessário continuar investigando os diferentes fatores que contribuem na variação da idade de início em Machado-Joseph, incluindo os genes da rota MMR e outros, para entender o que pode acelerar ou atrasar o início da doença.

REFERÊNCIAS

BETTENCOURT, C. et al. DNA repair pathways underlie a common genetic mechanism modulating onset in polyglutamine diseases. **Annals of Neurology**, v. 79, n. 6, p. 983–990, 1 jun. 2016.

DA SILVA CARVALHO, Gerson et al. Cytokines in Machado Joseph disease/spinocerebellar ataxia 3. **The Cerebellum**, v. 15, p. 518-525, 2016.

DE CASTILHOS, Raphael Machado et al. Spinocerebellar ataxias in Brazil—frequencies and modulating effects of related genes. **The Cerebellum**, v. 13, p. 17-28, 2014.

DE MATTOS, E. P. et al. Age at onset prediction in spinocerebellar ataxia type 3 changes according to population of origin. **European Journal of Neurology**, v. 26, n. 1, p. 113–120, 16 set. 2018.

DE MATTOS, Eduardo Preusser. Avaliação de modificadores do fenótipo na doença de Machado-Joseph. 2018.

DE OLIVEIRA C. M. et al. Pre-ataxic Changes of Clinical Scales and Eye Movement in Machado-Joseph Disease: BIGPRO Study. **Movement Disorder**. 2021.

DE OLIVEIRA C. M. et al. The longitudinal progression of MRI changes in pre-ataxic carriers of SCA3/MJD. **Journal of Neurology**. 2023.

DURR, Alexandra et al. Spinocerebellar ataxia 3 and Machado‐Joseph disease: clinical, molecular, and neuropathological features. **Annals of neurology**, v. 39, n. 4, p. 490-499, 1996.

FRANÇA, Marcondes C. et al. A combined voxel-based morphometry and 1 H-MRS study in patients with Friedreich's ataxia. **Journal of neurology**, v. 256, p. 1114-1120, 2009.

GENETIC MODIFIERS OF HUNTINGTON'S DISEASE (GEM-HD) CONSORTIUM et al. Huntington's disease onset is determined by length of uninterrupted CAG, not encoded polyglutamine, and is modified by DNA maintenance mechanisms. **bioRxiv**, p. 529768, 2019.

GENETIC MODIFIERS OF HUNTINGTON'S DISEASE (GEM-HD) CONSORTIUM. Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. **Cell**, 162(3), 516–526, 2015.

ISHIKAWA, K. et al. Cytoplasmic and nuclear polyglutamine aggregates in SCA6 Purkinje cells. **Neurology**, v. 56, n. 12, p. 1753-1756, 2001.

JARDIM, Laura Bannach et al. A survey of spinocerebellar ataxia in South Brazil–66 new cases with Machado-Joseph disease, SCA7, SCA8, or unidentified disease–causing mutations. **Journal of neurology**, v. 248, p. 870-876, 2001.

KAWAGUCHI, Yoshiya et al. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32. 1. **Nature genetics**, v. 8, n. 3, p. 221-228, 1994.

LAHUE, R. S. New developments in Huntington's disease and other triplet repeat diseases: DNA repair turns to the dark side. **Neuronal Signal**. 2020.

MERGENER, Rafaella et al. Variation in DNA repair system gene as an additional modifier of age at onset in spinocerebellar ataxia type 3/Machado–Joseph disease. **Neuromolecular medicine**, v. 22, n. 1, p. 133-138, 2020.

PAULSON, H. L. et al. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. **Neuron**, v. 19, n. 2, p. 333-344, 1997.

RAPOSO, Mafalda et al. Replicating studies of genetic modifiers in spinocerebellar ataxia type 3: can homogeneous cohorts aid?. **Brain**, v. 138, n. 12, p. e398-e398, 2015.

RIESS, Olaf et al. SCA3: neurological features, pathogenesis and animal models. **The Cerebellum**, v. 7, p. 125-137, 2008.

RODRÍGUEZ-LABRADA, Roberto et al. Founder effects of spinocerebellar ataxias in the American continents and the Caribbean. **The Cerebellum**, v. 19, p. 446-458, 2020.

SAUTE, J. A. M. et al. Planning future clinical trials in Machado Joseph disease: Lessons from a phase 2 trial. **Journal of the Neurological Sciences**, v. 358, n. 1-2, p. 72–76, nov. 2015.

SENA, Lucas Schenatto et al. Selective forces acting on spinocerebellar ataxia type 3/Machado–Joseph disease recurrency: a systematic review and meta‐analysis. **Clinical Genetics**, v. 99, n. 3, p. 347-358, 2021.

SEQUEIROS J. et al. Epidemiology and population genetics of degenerative ataxias. **Handb Clin Neurol.** 103:227–51. 2012.

SHERRY ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. dbSNP: the NCBI database of genetic variation. **Nucleic Acids Res.** 2001.

SOONG, Bing-wen; LIU, Ren-shyan. Positron emission tomography in asymptomatic gene carriers of Machado-Joseph disease. **Journal of Neurology, Neurosurgery & Psychiatry**, v. 64, n. 4, p. 499-504, 1998.

TAIT, Danilo et al. Ataxin-3 is transported into the nucleus and associates with the nuclear matrix. **Human molecular genetics**, v. 7, n. 6, p. 991-997, 1998.

TAKIYAMA, Y. et al. The gene for Machado–Joseph disease maps to human chromosome 14q. **Nature Genetics**, v. 4, n. 3, p. 300–304, jul. 1993.

TEZENAS DU MONTCEL, Sophie et al. Modulation of the age at onset in spinocerebellar ataxia by CAG tracts in various genes. **Brain**, v. 137, n. 9, p. 2444-2455, 2014.

VAN DE WARRENBURG, Bart PC et al. Age at onset variance analysis in spinocerebellar ataxias: a study in a Dutch–French cohort. **Annals of neurology**, v. 57, n. 4, p. 505-512, 2005.

WAN, Na et al. MR Imaging of SCA3/MJD. **Frontiers in neuroscience**, vol. 14 749, 2020.

ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA THE CEREBELLUM

Submission guidelines

Contents

Instructions for Authors

Editorial policy

The Cerebellum is a high-quality quarterly journal which will publish material relevant to the understanding of the functions of the cerebellum and cerebellar ataxias. The Cerebellum aims to bring the most significant advances in research devoted to the cerebellum.

Areas of particular interest include:

Fundamental neurosciences, including molecular and cellular biology, behavioural neurosciences and neurochemistry, Genetics, Fundamental and clinical neurophysiology, Neurology and neuropathology, Cognition, Neuroimaging.

The Cerebellum will consider:

In-depth reviews; mini-reviews focusing on important new developments; original research; new ideas, opinion and controversies; clinicopathologic case presentations; single case studies that significantly advance the understanding of mechanisms underlying cerebellar disturbances; letters to the editor; meeting reports; book reviews; commentary; editorials.

The journal is committed to high standards of presentation. Original reports and reviews can be up to 10,000 words in length. Please contact the Editor to discuss the suitability of topics. Short reports should be approximately 1500 words long, with no more than 15 references. Correspondence items should be no more than 1000 words in length, with 1 figure or table.

Transparent standards of reporting of research help to improve the reliability and reproducibility of the research literature. We advocate for complete and transparent reporting in line with the standards of the research community. Springer and BMC both previously had policies that encouraged the use of relevant reporting checklists, but now the five biological/biomedical checklists below will be mandatory. Randomized controlled trials: CONSORT Randomized controlled trial protocols: SPIRIT Systematic reviews and meta-analyses: PRISMA Case reports: CARE Preclinical animal studies: ARRIVE The above checklists must be completed before peer review, and made available to the editors and reviewers for evaluation during peer-review.

Back to top

Online Manuscript Submission

Springer now offers authors, editors and reviewers of The Cerebellum the use of our fully web-enabled online manuscript submission and review system. To keep the review time as short as possible, we request authors to submit manuscripts online to the journal's editorial office. Our online manuscript submission and review system offers authors the option to track the progress of the review process of manuscripts in real time. Manuscripts should be submitted to: http://www.editorialmanager.com/cere

The online manuscript submission and review system for The Cerebellum offers easy and straightforward log-in and submission procedures.

NOTE: In case you encounter any difficulties while submitting your manuscript online, please get in touch with the responsible Editorial Assistant by clicking on "CONTACT US" from the tool bar.

http://www.editorialmanager.com/cere

Back to top

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Back to top

Preparation of manuscripts

Manuscripts should be typed in Word. Any special points should be emphasized in the covering letter. The number of heading levels used should be kept to a maximum of three. Papers describing original research should be arranged as follows:

1. Title page (including correspondence address)

2. Structured Abstract (maximum 250 words)

3. Keywords (maximum 6) taken from Index Medicus

4. Introduction

5. Materials (or patients) and methods

6. Results

7. Discussion

8. Conclusion or summary

9. Acknowledgements

10. References (see below)

11. Tables (with captions)

12. Figures (with captions)

The Title page should include:

• The name(s) of the author(s)

• A concise and informative title

• The affiliation(s) and address(es) of the author(s)

• The e-mail address of the corresponding author

The Abstract should not exceed 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Please provide 4 to 6 Keywords which can be used for indexing purposes.

The Introduction should assume that the reader is knowledgeable in the field and should, therefore, be as brief as possible.

In the Materials and methods section, methods that have been published in detail elsewhere should not be described in detail.

Abbreviations and symbols used should be defined at first mention and used consistently thereafter. Abbreviations must be standard and SI units used throughout. Acronyms should be used sparingly and must be fully explained when first used. Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention. Statistical analyses must explain the methods used.

Text Formatting

For submission in Word:

• Use a normal, plain font (e.g., 10-point Times Roman) for text.

• Use italics for emphasis.

• Use the automatic page numbering function to number the pages.

• Do not use field functions.

• Use tab stops or other commands for indents, not the space bar.

• Use the table function, not spreadsheets, to make tables.

• Use the equation editor or MathType for equations. Note: If you use Word 2007, do not create the equations with the default equation editor but use MathType instead.

Back to top ↑

Ethical Responsibilities of Authors

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.

The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').

A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').

Concurrent or secondary publication is sometimes justifiable, provided certain conditions are met. Examples include: translations or a manuscript that is intended for a different group of readers.

Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are used for verbatim copying of material, and permissions secured for material that is copyrighted.

Important note: the journal may use software to screen for plagiarism.

Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).

Research articles and non-research articles (e.g. Opinion, Review, and Commentary articles) must cite appropriate and relevant literature in support of the claims made. Excessive and inappropriate self-citation or

coordinated efforts among several authors to collectively self-cite is strongly discouraged.

Authors should avoid untrue statements about an entity (who can be an individual person or a company) or descriptions of their behavior or actions that could potentially be seen as personal attacks or allegations about that person.

Research that may be misapplied to pose a threat to public health or national security should be clearly identified in the manuscript (e.g. dual use of research). Examples include creation of harmful consequences of biological agents or toxins, disruption of immunity of vaccines, unusual hazards in the use of chemicals, weaponization of research/technology (amongst others).

Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or deleting authors during the revision stages is generally not permitted, but in some cases may be warranted. Reasons for changes in authorship should be explained in detail. Please note that changes to authorship cannot be made after acceptance of a manuscript.

*All of the above are guidelines and authors need to make sure to respect third parties rights such as copyright and/or moral rights.

Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

If there is suspicion of misbehavior or alleged fraud the Journal and/or Publisher will carry out an investigation following COPE guidelines. If, after investigation, there are valid concerns, the author(s) concerned will be contacted under their given e-mail address and given an opportunity to address the issue. Depending on the situation, this may result in the Journal's and/or Publisher's implementation of the following measures, including, but not limited to:

If the manuscript is still under consideration, it may be rejected and returned to the author.

If the article has already been published online, depending on the nature and severity of the infraction:

- an erratum/correction may be placed with the article

- an expression of concern may be placed with the article

- or in severe cases retraction of the article may occur.

The reason will be given in the published erratum/correction, expression of concern or retraction note. Please note that retraction means that the article is **maintained on the platform**, watermarked "retracted" and the explanation for the retraction is provided in a note linked to the watermarked article.

The author's institution may be informed

A notice of suspected transgression of ethical standards in the peer review system may be included as part of the author's and article's bibliographic record.

Fundamental errors

Authors have an obligation to correct mistakes once they discover a significant error or inaccuracy in their published article. The author(s) is/are requested to contact the journal and explain in what sense the error is impacting the article. A decision on how to correct the literature will depend on the nature of the error. This may be a correction or retraction. The retraction note should provide transparency which parts of the article are impacted by the error.

Suggesting / excluding reviewers

Authors are welcome to suggest suitable reviewers and/or request the exclusion of certain individuals when they submit their manuscripts. When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. It is strongly recommended to suggest a mix of reviewers from different countries and different institutions. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer, or, if this is not possible to include other means of verifying the identity such as a link to a personal homepage, a link to the publication record or a researcher or author ID in the submission letter. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Back to top ↑

Authorship principles

These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, **before** the work is submitted.

The Publisher does not prescribe the kinds of contributions that warrant authorship. It is recommended that authors adhere to the guidelines for authorship that are applicable in their specific research field. In absence of specific guidelines it is recommended to adhere to the following guidelines*:

All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

Disclosures and declarations

All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

The decision whether such information should be included is not only dependent on the scope of the journal, but also the scope of the article. Work submitted for publication may have implications for public health or general welfare and in those cases it is the responsibility of all authors to include the appropriate disclosures and declarations.

Data transparency
All authors are requested to make sure that all data and materials as well as software application or custom code support their published claims and comply with field standards. Please note that journals may have individual policies on (sharing) research data in concordance with disciplinary norms and expectations.

Role of the Corresponding Author
One author is assigned as Corresponding Author and acts on behalf of all co-authors and ensures that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

The Corresponding Author is responsible for the following requirements:

ensuring that all listed authors have approved the manuscript before submission, including the names and order of authors;

managing all communication between the Journal and all co-authors, before and after publication;*

providing transparency on re-use of material and mention any unpublished material (for example manuscripts in press) included in the manuscript in a cover letter to the Editor;

making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate (see above).

* The requirement of managing all communication between the journal and all co-authors during submission and proofing may be delegated to a Contact or Submitting Author. In this case please make sure the Corresponding Author is clearly indicated in the manuscript.

Author contributions
In absence of specific instructions and in research fields where it is possible to describe discrete efforts, the Publisher recommends authors to include contribution statements in the work that specifies the contribution of every author in order to promote transparency. These contributions should be listed at the separate title page.

Examples of such statement(s) are shown below:

- Free text:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [full name], [full name] and [full name]. The first draft of the manuscript was written by [full name] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Example: CRediT taxonomy:

- Conceptualization: [full name], ...; Methodology: [full name], ...; Formal analysis and investigation: [full name], ...; Writing - original draft preparation: [full name, ...]; Writing - review and editing: [full name], ...; Funding acquisition: [full name], ...; Resources: [full name], ...; Supervision: [full name],....

For **review articles** where discrete statements are less applicable a statement should be included who had the idea for the article, who performed the literature search and data analysis, and who drafted and/or critically revised the work.

For articles that are based primarily on the **student's dissertation or thesis**, it is recommended that the student is usually listed as principal author:

A Graduate Student's Guide to Determining Authorship Credit and Authorship Order, APA Science Student Council 2006

Affiliation
The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may additionally be stated. Addresses will not be updated or changed after publication of the article.

Changes to authorship
Authors are strongly advised to ensure the correct author group, the Corresponding Author, and the order of authors at submission. Changes of authorship by adding or deleting authors, and/or changes in Corresponding Author, and/or changes in the sequence of authors are **not** accepted **after acceptance** of a manuscript.

Please note that author names will be published exactly as they appear on the accepted submission!

Please make sure that the names of all authors are present and correctly spelled, and that addresses and affiliations are current.

Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that journals may have individual policies on adding and/or deleting authors during revision stage.

Author identification
Authors are recommended to use their ORCID ID when submitting an article for consideration or acquire an ORCID ID via the submission process.

Deceased or incapacitated authors
For cases in which a co-author dies or is incapacitated during the writing, submission, or peer-review process, and the co-authors feel it is appropriate to include the author, co-authors should obtain approval from a (legal) representative which could be a direct relative.

Authorship issues or disputes
In the case of an authorship dispute during peer review or after acceptance and publication, the Journal will not be in a position to investigate or adjudicate. Authors will be asked to resolve the dispute themselves. If they are unable the Journal reserves the right to withdraw a manuscript from the editorial process or in case of a published paper raise the issue with the authors' institution(s) and abide by its guidelines.

Confidentiality
Authors should treat all communication with the Journal as confidential which includes correspondence with direct representatives from the Journal such as Editors-in-Chief and/or Handling Editors and reviewers' reports unless explicit consent has been received to share information.

Back to top ↑

Compliance with Ethical Standards

To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors

should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

Disclosure of potential conflicts of interest

Research involving Human Participants and/or Animals

Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

The Editors reserve the right to reject manuscripts that do not comply with the above-mentioned guidelines. The author will be held responsible for false statements or failure to fulfill the above-mentioned guidelines.

Back to top ↑

Competing Interests

Authors are requested to disclose interests that are directly or indirectly related to the work submitted for publication. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

Editorial Board Members and Editors are required to declare any competing interests and may be excluded from the peer review process if a competing interest exists. In addition, they should exclude themselves from handling manuscripts in cases where there is a competing interest. This may include – but is not limited to – having previously published with one or more of the authors, and sharing the same institution as one or more of the authors. Where an Editor or Editorial Board Member is on the author list they must declare this in the competing interests section on the submitted manuscript. If they are an author or have any other competing interest regarding a specific manuscript, another Editor or member of the Editorial Board will be assigned to assume responsibility for overseeing peer review. These submissions are subject to the exact same review process as any other manuscript. Editorial Board Members are welcome to submit papers to the journal. These submissions are not given any priority over other manuscripts, and Editorial Board Member status has no bearing on editorial consideration.

Interests that should be considered and disclosed but are not limited to the following:

Funding: Research grants from funding agencies (please give the research funder and the grant number) and/or research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through publication of this manuscript.

Employment: Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through publication of this manuscript. This includes multiple affiliations (if applicable).

Financial interests: Stocks or shares in companies (including holdings of spouse and/or children) that may gain or lose financially through publication of this manuscript; consultation fees or other forms of remuneration from organizations that may gain or lose financially; patents or patent applications whose value may be affected by publication of this manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

Non-financial interests: In addition, authors are requested to disclose interests that go beyond financial interests that could impart bias on the work submitted for publication such as professional interests, personal relationships or personal beliefs (amongst others). Examples include, but are not limited to: position on editorial board, advisory board or board of directors or other type of management relationships; writing and/or consulting for educational purposes; expert witness; mentoring relations; and so forth.

Primary research articles require a disclosure statement. Review articles present an expert synthesis of evidence and may be treated as an authoritative work on a subject. Review articles therefore require a disclosure statement.Other article types such as editorials, book reviews, comments (amongst others) may, dependent on their content, require a disclosure statement. If you are unclear whether your article type requires a disclosure statement, please contact the Editor-in-Chief.

Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under 'summary of requirements' (see below) funding information should be included in the '**Declarations**' section.

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

Partial financial support was received from [...]

The research leading to these results received funding from [...] under Grant Agreement No[...].

This study was funded by [...]

This work was supported by [...] (Grant numbers [...] and [...]

Examples of statements to be used when there is no funding:

The authors did not receive support from any organization for the submitted work.

No funding was received to assist with the preparation of this manuscript.

No funding was received for conducting this study.

No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

Financial interests: Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.

Non-financial interests: Author C is an unpaid member of committee Z.

Financial interests: The authors declare they have no financial interests.

Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

Financial interests: Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

Non-financial interests: none.

Financial interests: Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

The authors have no relevant financial or non-financial interests to disclose.

The authors have no competing interests to declare that are relevant to the content of this article.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

The authors have no financial or proprietary interests in any material discussed in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Back to top ↑

Research involving human participants, their data or biological material

Ethics approval

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

Retrospective ethics approval

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

Ethics approval for retrospective studies

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

Ethics approval for case studies

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: *Filip1*$^{tm1a(KOMP)Wtsi}$ **RRID:MMRRC_055641-UCD**

Cell Line: RST307 cell line **RRID:CVCL_C321**

Antibody: Luciferase antibody DSHB Cat# LUC-3, **RRID:AB_2722109**

Plasmid: mRuby3 plasmid **RRID:Addgene_104005**

Software: ImageJ Version 1.2.4 **RRID:SCR_003070**

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as "A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions" and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

- All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

- This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

- Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

- The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

- Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

- This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

- This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

- This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

- The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Back to top ↑

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

Back to top ↑

Tables

• All tables are to be numbered using Arabic numerals.

• Tables should always be cited in text in consecutive numerical order.

• For each table, please supply a table heading. The table title should explain clearly and concisely the components of the table.

• Identify any previously published material by giving the original source in the form of a reference at the end of the table heading.

• Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Back to top ↑

Artwork

For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

Supply all figures electronically.

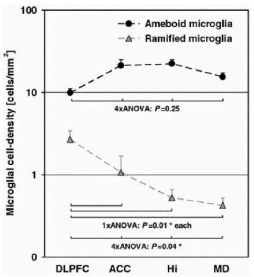
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



Definition: Black and white graphic with no shading.

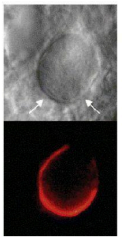
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

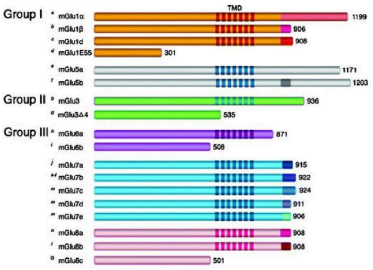


Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for print and online publication.

Color illustrations should be submitted as RGB.

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

When preparing your figures, size figures to fit in the column width.

For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

Permissions

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s) for both the print and online format. Please be aware that some publishers do not grant electronic rights for free and that Springer will not be able to refund any costs that may have occurred to receive these permissions. In such cases, material from other sources should be used.

Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (color-blind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

Back to top

Electronic Supplementary Material

If Electronic supplementary material (ESM) is submitted, it will be published as received from the author in the online version only. ESM may consist of:

· information that cannot be printed: animations, video clips, sound recordings

· information that is more convenient in electronic form: sequences, spectral data, etc.

· large original data, e.g. additional tables, illustrations, etc.

· If supplying any ESM, the text must make specific mention of the material as a citation, similar to that of figures and tables (e.g., "... as shown in Animation 3.").

Back to top

Proofreading

The purpose of the proof is to check for typesetting errors and the completeness and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor. After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

Back to top

Guidelines for Electronically Produced Illustrations for Print

General

Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.

Vector (line) Graphics

Vector graphics exported from a drawing program should be stored in EPS format. Suitable drawing program: Adobe Illustrator. For simple line art the following drawing programs are also acceptable: Corel Draw, Freehand, Canvas.

· No rules narrower than .25 pt.

· No gray screens paler than 15% or darker than 60%.

· Screens meant to be differentiated from one another must differ by at least 15%.

Spreadsheet/Presentation Graphics

· Most presentation programs (Excel, PowerPoint, Freelance) produce data that cannot be stored in an EPS format. Therefore graphics produced by these programs cannot be used for print.

Halftone Illustrations

· Black and white and color illustrations should be saved in TIFF format. Illustrations should be created using Adobe Photoshop whenever possible.

Scans*

· Scanned reproductions of black and white photographs should be provided as 300 ppi TIFF files.

· Scanned color illustrations should be provided as TIFF files scanned at a minimum of 300 ppi with a 24-bit color depth. Line art should be provided as TIFF files at 600 ppi.

· *We do prefer having the original art as our printers have drum scanners which allow for better reproduction of critical medical halftones.

Graphics from Videos

· Separate files should be prepared for frames from a video that are to be printed in the journal. When preparing these files you should follow the same rules as listed under Halftone Illustrations.

Back to top

Guidelines for Electronically Produced Illustrations for ONLINE

Video

· MPEG (.mpg) is the preferred format, but .rm, .avi, .mov, etc. are acceptable.

· No video file should be larger than 2MB. To decrease the size of your file, consider changing one or more of the following variables: frame speed, number

of colors/greys, viewing size (in pixels), or compression. Video is subject to Editorial review and approval.

Back to top ↑

Copyright Transfer

Authors will be asked to transfer copyright of the article to the Publisher (or grant the Publisher exclusive publication and dissemination rights). This will ensure the widest possible protection and dissemination of information under copyright laws. Open Choice articles do not require transfer of copyright as the copyright remains with the author. In opting for open access, they agree to the Springer Open Choice Licence.

Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.