UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Célia Márcia Fernandes Maia

Avaliação fenotípica e genotípica de famílias brasileiras com a síndrome de Jalili

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Célia Márcia Fernandes Maia

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Hospital Universitário Clemente Farias – HUCF
http://www.unimontes.br / ppgcs@unimontes.br
Telefone: (0xx38) 3224-8372 / Fax: (0xx38) 3224-8372
Av. Cula Mangabeira, 562, Santo Expedito, Montes Claros – MG, Brasil – Cep: 39401-001

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RESUMO

A síndrome de Jalili (SJ) é uma condição rara, hereditária, caracterizada pela combinação de distrofia das células fotorreceptoras da retina, os cones e bastonetes (DCB), e amelogênese imperfeita (AI), resultado de mutações em *Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 4* (*CNNM4*). O *CNNM4* é responsável pelo transporte de íons metálicos e uma variedade de mutações nesse gene tem sido reportadas em diferentes países. Este estudo teve como objetivo avaliar as características clínicas, imaginológicas, genéticas e o padrão de herança em duas novas famílias acometidas pela SJ. Foram estudados todos os membros de duas famílias identificadas com características da SJ, sendo a primeira família residente no município de Porteirinha - Minas Gerais, e a segunda em Americana - São Paulo, sem relação de parentesco entre as mesmas. Os membros das duas famílias foram submetidos a avaliações oftalmológica, odontológica e genética. Na primeira família, sendo os pais heterozigotos de casamento consanguineo, a SJ foi causada pela mutação homozigota p.L324P (c.971T> C) transição missensee a paciente afetada tinha DCB e AI. Na segunda família não havia casamento consanguineo, sendo a mãe normal, enquanto que pai e filha foram afetados. Encontrou-se nesses indivíduos uma combinação específica de uma mutação heterozigótica composta: a transição p.L324P (c.971T> C) missense e uma nova mutação p.Y581* (c.1743C> G), sendo que a filha mostrou características fenotipicas para DCB e AI, mas seu pai apenas desenvolveu alterações oculares. Juntos, esses achados sugeriram que a mutação p.L324P na homozigose induziu um fenótipo completo com DCB e AI, mas em heterozigose, e em composição com a nova mutação p.Y581 nonsense*no *CNNM4*, promoveu expressividade clínica variável, com a não manifestação do fenótipo dental. Esses diferentes fenótipos podem ser explicados por deleções que afetam o alelo homólogo, a epistasia ou as interações com fatores ambientais que levam à atividade residual da proteína. Estas duas famílias caracterizam os primeiros casos descritos da SJ no Brasil.

Palavras-chave: Síndrome de Jalil. Amelogênese imperfeita. Distrofia de cones e bastonetes. *CNNM4*. Mutação.

ABSTRACT

The Jalili syndrome (JS) is a rare, hereditary disorder characterized by the combination of retinal photoreceptor cell dystrophy, cones and rods (CRD), and imperfect amelogenesis (AI), resulting from mutations in Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 4 (CNNM4). The *CNNM4* gene is responsible for the transport of metal ions and a variety of mutations in this gene have been reported in different countries. This study aimed to evaluate the clinical, imaging, genetic and inheritance patterns in two new families affected by JS. All members of two families identified with characteristics suggestive of SJ were studied, being the first family resident in the city of Porteirinha - Minas Gerais, and the second resident in Americana - São Paulo, without relation of kinship of between both families. The members of the two families were submitted to ophthalmological, odontological and genetic evaluations. In the first family, being the heterozygous parents of consanguineous marriage, the JS was caused by the mutation homozygous p.L324P (c.971T> C) missense transition and the affected patient had CRD and AI. In the second family, there was no consanguineous marriage, being the normal mother, father and daughter were affected,a specific combination of a composite heterozygous mutation was found the p.L324P (c.971T> C) missense transition and a new mutation p.Y581* (c.1743C>G), and the daughter showed phenotypic characteristics for CRD and AI, but her father only developed ocular alterations. Together, these findings suggested that the p.L324P mutation in homozygousness induced a complete phenotype with CRD and AI, but in heterozygosis and in composition with the new mutation p.Y581 nonsense* in *CNNM4* promoted variable clinical expressiveness, with no manifestation of dental phenotype. These different phenotypes can be explained by deletions affecting the homologous allele, epistasis, or interactions with environmental factors that lead to the residual activity of the protein. These two families characterize the first described cases of JS in Brazil.

Keywords: Jalili Syndrome. Amelogenesis imperfecta. Cone-rod dystrophy. *CNNM4*. Mutation.

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LISTA DE ABREVIATURAS E SIGLAS

ABCA4	Gene - *ATP Binding Cassette Subfamily A Member 4*
ACDP4	*Ancient Conserved Domain-Containing Protein 4*
ACL	Amaurose congênita de Leber
AD	Autossômico dominante
AI	Amelogênese Imperfeita
AIPL1	Gene codificador da proteina *Aryl Hydrocarbon Receptor Interacting Protein Like 1*
AMBN	Gene codificador da proteína *Ameloblastin*
AMELX	Gene codificador da proteína *Amelogenin, X-Linked*
AR	Autossômico recessivo
Ca^{2+}	Ion Cálcio
CNGB3	Gene codificador da proteina *Cyclic Nucleotide Gated Channel Beta 3*
C40rf26	Gene codificador da proteina *Chromosome 40 Pen Reading Frame 26*
CFO	Conselho Federal de Odontologia
CNGA3	Gene codificador da proteina *Cyclic Nucleotide Gated Channel Alpha 3*
CNNM4	Gene *Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 4*
CRX	Gene codificador da proteina *Cone-Rod Homeobox*
DCB	Distrofia de cones e bastonetes
DLX3	Gene codificador da proteína *Distal-Less Homeobox 3*

DNA	Ácido desoxirribonucleico
EDTA	Ácido etilenodiamino tetra-acético
ENAM	Gene codificador da proteína *Enamelin*
ERG	Eletroretinograma
EUA	Estados Unidos da América
FAM20A	Gene - *FAM20A, Golgi Associated Secretory Pathway Pseudokinase*
FAM83H	Gene codificador - *Family with Sequence Similarity 83 Member H*
FOXC1	Gene codificador da proteína *Forkhead Box C1*
GUCA1A	Gene codificador da proteína *Guanylate Cyclase Activator 1 A (Retina)*
GUCY2D	Gene codificador da proteina *Guanylate Cyclase 2D, Retinal*
HCl	Cloreto de hidrogênio
ITGB6	Gene codificador da proteína *Integrin Subunit Beta 6*
KLK4	Gene codificador da proteína *Kallikrein Related Peptidase 4*
LAMB3	Gene codificador da proteína *Laminin Subunit Beta 3*
Mg^{2+}	Íon magnésio
MMP20	Gene codificador da proteína *Matrix Metallopeptidase 20*
MG	Minas Gerais
NaCl	Cloreto de sódio
OMIM	*Online Mendelian Inheritance in Man*
PCR	*Reação em cadeia da Polimerase*
PH	Potencial Hidrogeniônico
PITPNM3	Gene codificador da proteína *PITPNM Family Member 3*

PROML1	Gene codificador da proteina *Prominin 1*
PITX2	Gene codificador da proteina *Paired Like Homeodomain 2*
RNA	Ácido Ribonucléico
RIMS1	Gene codificador - *Regulating Synaptic Membrane Exocytosis 1*
RPGR	Gene codificador da proteina *Retinitis Pigmentosa GTPase Regulator*
SDS	Dodecil Sulfato de Sódio
SP	São Paulo
SEMA4A	Gene codificador da proteina *Semaphorin 4 A*
SLC24A4	Gene codificador da proteina *Solute Carrier Family 24 Member 4*
Sp6	Gene codificador da proteina *SP6 Transcription Factor*
SJ	Síndrome de Jalili
TUFT1	Gene codificador da proteina *Tuftelin 1*
UNC 119	Gene codificador da proteina *Unc-119 Lipid Binding Chaperone*
UNIMONTES	Universidade Estadual de Montes Claros
WDR72	Gene codificador da proteina *WD Repeat Domain 72*

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1 INTRODUÇÃO

A síndrome de Jalili (MIM#217080) é uma condição hereditária, rara, autossômica recessiva, associada a mutações no gene *CNNM4*, localizado no cromossomo 2q11 (Luder *et al*., 2013; Purwar *et al*., 2015). É caracterizada por alterações oculares (distrofia de cones e bastonetes) e dental (amelogênese imperfeita) (Jalili & Smith, 1988; Michaelides *et al.*, 2004; Polok *et al.*, 2009; Parry *et al*., 2009). A primeira descrição da doença foi feita por Jalili & Smith, em 1988, após investigação em uma extensa família de origem Árabe, situada na Faixa de Gaza, com casamentos consanguíneos, na qual 29 membros apresentavam a associação entre distrofia de cones e bastonetes (DCB) e amelogênese imperfeita (AI). Uma análise da família foi realizada, indicando o modo de herança como autossômica recessiva (Jalili & Smith, 1988). Todos os membros afetados apresentavam fotofobia, nistágmo pendular, acromatopsia, alteração macular e redução da visão central, associado a alterações no esmalte dental, descritas como um esmalte áspero e cor amarelo-marrom. De acordo com as características dentais observadas, o diagnóstico das alterações dentais foi de AI (Jalili & Smith, 1988).

Ao realizar o estudo genético da família investigada na Faixa de Gaza, Downey *et al*. (2002) identificaram pela primeira vez o *loci* no intervalo 2cM/5Mb do cromossomo 2q11, entre os marcadores D2S2209 e D2S373, para as manifestações recessivas de DCB e AI.

Alterações na estrutura dental e ocular têm sido descritas em várias desordens como na displasia oculodentodigital, definida por anomalias craniofaciais, oculares, dentárias e neurológicas (Doshi *et al*., 2017). Como também a síndrome trico-dento-ósso, doença genética pertencente ao grupo das displasias ectodérmicas, que caracteriza-se pela presença de hipoplasia do esmalte dental, taurodontismo, alterações osseas e cabelos crespos (Al-Batayneh, 2012). Berezovsky *et al.* (2012) reportam a síndrome de Bardet-Biedl, um distúrbio complexo identificado por distrofia em retina, polidactilia, obesidade, retardo mental. Bateman *et al.* (1980) descrevem uma variedade de síndromes que apresentam alterações na retina associada a perda auditiva, entre elas a síndrome de Usher, que apresenta clinicamente retinite pigmentosa associada à alteração auditiva.

Em uma segunda família originária de Kosovo com características recessivas de DCB e AI, Michaelides *et al.* (2004) ao realizarem o sequenciamento genético dessa família, consideraram as mutações no gene *CNGA3* como responsáveis pelas alterações observadas. Esse gene localizado no

cromossomo 2q11 está associado a distrofia de cones e acromatopsia. No entanto, através da análise genética realizada por Michaelides *et al.* (2004) confirmaram a não expressão do gene *CNGA3* nos órgãos dental e ocular.

Novas famílias com a SJ, apresentando características de DCB e AI foram investigadas por Polok *et al*. (2009), originárias do Líbano, Kosovo e uma terceira de origem desconhecida. Foram realizados os sequenciamentos genéticos dessas famílias, confirmando mutações no gene *CNNM4*, no cromossomo 2q11 (Polok *et al*., 2009). Vários genes ligados a alterações oculares foram sequenciados na região já identificada por Downey *et al*. (2002). Para uma melhor confirmação da manifestação do gene *CNNM4*, estudos de imuno-histoquímica em olhos de camundongos foram realizados (Polok *et al*., 2009). Foram confirmadas manifestações do gene *CNNM4* em córnea e também em retina nas camadas de células ganglionares, nas camadas de plexiformes internas e externas e nos segmentos de fotorreceptor interno e externo (Polok *et al*., 2009). A análise imuno-histoquímica também foi realizada em dentes de ratos, sendo observada em ameloblasto (Polok *et al*., 2009).

Uma investigação mais abrangente foi realizada por Parry *et al.* (2009) em sete famílias apresentando a SJ, originárias da Faixa de Gaza A, Faixa de Gaza B, Kosovo, Irã, Turquia, Guatemala e Escócia. As mutações em *CNNM4*apresentadas, mostraram ser este o gene responsável pelas alterações genotípicas e fenotípicas observadas, corroborando com os achados de Polok *et al*. (2009). Diante das confirmações, o termo "síndrome de Jalili'' foi proposto por Parry *et al*. (2009), para a nova condição investigada, resultado de mutações no gene *CNNM4*, que afeta os órgãos dental e ocular. Novos casos foram apresentados em Kosovo, Canadá, Arábia Saudita, Argélia, China, USA, Turquia, Irã, India, Paquistão, Marrocos e Polônia e diferentes mutações no gene *CNNM4* foram identificadas.

As características fenotípicas para SJ, resultado de mutações no gene *CNNM4* (MIM#607805), localizado no cromossomo 2q11 (MIM#217080), são identificadas pela AI e DCB (Parry *et al*., 2009). AI constitui um grupo heterogêneo de condições hereditárias que afetam a formação do esmalte dental nas duas dentições decídua e permanente (Aldred *et al.* 2003). Enquanto as DCB são alterações em retina, herdadas e progressivas, pertencentes ao grupo de retinopatias pigmentares, indentificada por depósitos de pigmento, localizados principalmente em região macular (Hamel, 2007; Polok *et al*., 2009). As DCB são identificadas por exames de oftalmoscopia e fundoscopia e seu curso clínico leva a uma cegueira irreversível (Hamel, 2007; Polok *et al*., 2009).

O gene *CNNM4* codificador da proteína *Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 4*, transportadora de metal, pertencente a uma família de quatro proteínas, localizadas na membrana basolateral de células de vários tecidos (Polok *et al*., 2009). A proteina codificada pelo gene *CNNM4* é responsável pelo transporte de íons metálico, em especial o ion magnésio (Mg^{2+}) (Polok *et al.*, 2009; Yamazaki *et al.*, 2013). O Mg^{2+} é o segundo elemento intra-celular mais abundante e importante para processos fisiológicos humano, sendo ele expresso em vários tecidos, como retina e esmalte (Parry *et al.*, 2009). O gene *CNNM4* atua como regulador da homeostase e transporte do Mg^{2+} em retina, participando da cascata de fototransdução, que é a conversão do estímulo luminoso em estímulo elétrico (Polok *et al.*, 2009). Mutações no *CNNM4* resulta em degeneração das células fotorreceptoras, observada já nos primeiros anos de vida do indivíduo (Polok *et al.*, 2009). Mutações no *CNNM4* têm como consequência alterações na regulação de ions de Mg^{2+}, causando danos ao esmalte dental, interferindo em sua biomineralização (Luder *et al*., 2013).

Nos diversos estudos genéticos realizados em famílias com características para SJ, as mutações em *CNNM4*, foram em sua maioria do tipo homozigotas, relacionadas a casamento consanguíneo mas, em 5 famílias com diagnóstico para a SJ, originárias da Guatemala, da Escócia, China, USA e Kosovo, foram identificadas mutações heterozigotas com ausência de casamentos consanguíneos (www.jalili.co.uk/cnnm4/cnnm4-mutations.xlsx').

A SJ tem sido relatada associada a outras anomalias. Zobor *et al.* (2012) descrevema SJ com a presença neurofibromatose tipo 1 e Purwar *et al.* (2015) mostraram a SJ em um paciente com *situs inversus totalis* (SIT) e ceratocone. Wawrocka *et al.* (2017) relatam a combinação da SJ e miopatia, o crescimento dos músculos das pernas, ampliando o espectro mutacional para esta desordem.

1.1 Alterações dentárias na síndrome de Jalili: amelogênese imperfeita

A amelogênese é o processo de formação do esmalte dental (Seymen*et al.*, 2015). Manifestações genéticas afetando estágios do processo de formação da amelogênese, resulta em uma mineralização do esmalte deficiente, caracterizando a amelogênese imperfeita (Seymen*et al*., 2015). A AI (OMIM#104530) é uma desordem genética, heterogênea, que afeta formação do esmalte dos dentes em sua qualidade e quantidade (Aldred *et al*., 2003; Crawford *et al*., 2007; Wright *et al.*, 2011;

Dure-Molla *et al.,* 2014; Seymen *et al.,* 2015). AI pode manifestar-se como traço autossômico dominante, autossômico recessivo e ligada ao cromossomo X, além de casos esporádicos, atingindo o esmalte de todos ou quase todos os dentes das dentições decídua e permanente (Aldred *et al.,* 2003; El-Sayed *et al.,* 2011; Jaouad *et al.,* 2015; Volodarsky *et al.,* 2015; Kim *et al.,* 2016). Sua incidência varia geograficamente 1:700 na Suécia e 1:14.000 nos EUA (Crawford *et al.,* 2007; Kim *et al.,* 2008; Dure-Molla *et al.,* 2014; Volodarsky *et al*., 2015). O tipo AI autossômica dominante é o mais comum nos Estados Unidos e Europa, sendo o tipo autossômico recessivo mais comum no Oriente Médio (Hart *et al.,* 2004; Kim *et al*., 2008). No Brasil, não existem estudos sobre a prevalência de AI.

A AI pode ocorrer isolada ou associada a algumas anormalidades. Martelli Júnior *et al*. (2012) reportam na síndrome de Bartter, a associação entre AI e nefrocalcinose. Diversas síndromes identificadas com outros sinais estão associadas a AI como a fibromatose gengival descrita por Martelli Júnior *et al*. (2008) e O'Sullivan *et al*. (2011).

O esmalte dental é o tecido mais mineralizado do corpo humano, com 95% do seu volume ocupado por cristais de hidroxiapatita, formados por células de origem *epitelial,* denominadas ameloblastos (Crawford *et al.,* 2007; Hu *et al.,* 2007; Kim *et al.,* 2017). São estruturas altamente organizadas e sua interação com moléculas da matriz orgânica resultará na modulação da deposição mineral e no crescimento do órgão de esmalte, controlados por vários genes (Crawford *et al.,* 2007). Esses genes têm função de realizar transcrição da matriz protéica e as proteinases, necessárias para o controle do processo de formação e mineralização do esmalte (Hart *et al*., 2004; Crawford *et al*., 2007; Hu *et al.,* 2007; O´Sullivan *et al*., 2011; Wright *et al*., 2011; Muto *et al.,* 2012; Jaouad *et al.,* 2015).

A formação da matriz de esmalte passa por três estágios: secretório (depósito de matriz orgânica pelos ameloblastos), transição ocorre degradação proteica e maturação (degradação da matriz orgânica e substituição por componentes minerais), e dependendo do estágio de formação do esmalte afetado, o aspecto clínico mudará, podendo o esmalte apresentar de uma forma ou mista, dos tipos hipoplásico, hipocalcificado ou hipomaturado (Hu *et al.,* 2007; Seymen *et al,* 2015; Volodarsky *et al.,* 2015). Assim, os defeitos no esmalte apresentados na AI são caracterizados por hipoplasia (defeito na formação, no estágio de secreção, resultando diminuição no volume da matriz proteica, causando alteração na espessura e forma do esmalte), hipocalcificado (defeito qualitativo, esmalte com espessura normal, mas facilmente perdido), hipomaturada (alterações na dureza e coloração) (Hu *et al*., 2007; El-Sayed *et al.,* 2011; Gadhia *et al.,* 2012).

O sistema de nomenclatura mais comumente utilizado para AI foi desenvolvido e mais tarde revisado por Witkop (1988), que categorizou os tipos de AI pelo maior fenótipo, ou seja, hipoplásico e hipomineralizado, que incorpora os subtipos hipocalcificada e hipomaturada (Witkop, 1989; Crawford *et al*., 2007). Após subdividir cada tipo de AI com base nas características fenotípicas adicionais e modo de herança, chegou-se a 14 subtipos diferentes reconhecidos de AI (Crawford *et al*., 2007).

A forma hipoplásica da AI caracteriza-se por esmalte fino associado com defeitos na síntese da matriz, com mineralização aparentemente normal, podendo apresentar desgastes, ranhuras ou amplos defeitos na superfície, o dente apresenta cor amarelo/marrom (Hu *et al.,* 2007; Dure-Molla *et al.,* 2014). A forma AI hipocalcificada é caracterizada por um esmalte com espessura normal, mas insuficiente mineralização, resultando em um esmalte macio e áspero, que é facilmente perdido logo após a erupção do dente (Hu *et al.,* 2007; Dure-Molla *et al.,* 2014). Já a AI hipomaturada representa um defeito associado com a falha na remoção de proteínas do esmalte para subsequente substituição pelos cristais, o que resulta em um esmalte de espessura normal, macio e manchado, o qual apresenta propensão a soltar-se em lascas, ao invés de se desgastar (Crawford *et al*., 2007; Kim *et al*., 2008, Muto *et al.,* 2012; Dure-Molla *et al.,* 2014).

Mutações em mais de 10 genes foram identificadas (Tabela1), envolvidas na patogênese da AI (Seymen *et al.,* 2015). Esses genes codificam as proteinas da matriz do esmalte como a amelogenin (*AMELX*,Xp22.3) que representa de 80-90% do total de proteínas do esmalte (Hu *et al.,* 2007). A enamelin (*ENAM,* 4q21) maior estrutura proteica no desenvolvimento do esmalte, passa por uma série de clivagens proteolíticas que irá gerar polipeptídeos, que participarão da nucleação e extensão dos cristais de esmalte e também em sua regulação (Hart *et al*., 2004; Hu *et al.,* 2007). Mutações em *AMELX* e *ENAM* causam AI ligada ao cromossomo X e autossômica dominante (Hart *et al*., 2004).

O gene de ameloblastin (*AMBN,* 4q21) atua na adesão de amelobastos na formação do esmalte (Hart *et al.,* 2004). Duas proteinases que regulam o processamento proteico da matriz de esmalte, definindo a estrutura e composição do esmalte a enamelysin (*MMP20*, 11q22.3) expressado no estágio secretório e kallikrein 4 (*KLK4, 19q13*), descrita por Hart *et al*. (2004) confirmando sua expressão em uma família com AI autossômica recessiva do tipo hipomaturada (Hart *et al.,* 2004; Hu *et al.,* 2007).

Outros genes importantes na formação do esmalte e candidatos para AI têm sido estudados. Kim *et al*. (2008) analisaram duas famílias com AI autossômica dominante hipocalcificada, identificaram mutação no gene *FAM83H*, localizado no cromossomo 8q24.3. Muto *et al*. (2012) descrevem uma nova mutação para AI autossômica recessiva, identificada no gene *Sp6* através de análise genética em ratos. El-Sayed *et al.* (2011) reportaram mutação no gene *WDR72* para AI recessiva hipomaturada após análise da estrutura de dentes decíduos, revelando redução da densidade mineral do esmalte, resultado da mutação apresentada, que afetou o estágio de maturação da formação do esmalte. Estudo realizado por O´Sullivan*et al.* (2011), em cinco membros de uma consanguínea família afetada com anomalias dental e hiperplasia gengival, foi identificada mutação no gene *FAM20A*, como sendo a causa para AI e sua associação com hiperplasia gengival, também descrito em trabalho realizado por Jaouad *et al*. (2015). Outros casos de mutações no gene *FAM20A* na forma recessiva associando AI com nefrocalcinose foram descritos, denominado síndrome de esmalte renal (Dure-Molla *et al.*, 2014).

Mutações no cromossomo 4 (*C4orf26*), no gene da família (*SLC24A4*) integrin beta 6 (*ITGB6*) e *LAMB3* foram identificados como fatores etiológico para AI (Hart *et al*., 2004; Seymen *et al*., 2015; Volodarsky *et al*., 2015).

Descrições realizadas por Jalili & Smith (1988), em que os dentes dos indivíduos investigados na Faixa de Gaza com características fenotípicas dental para SJ, apresentavam uma morfologia anormal, com superfícies rugosas e cor amarelo-marron, mostrando características da AI hipoplásica/hipomineralizada. Estudo em uma família de Kosovo foi identificado mutações no gene *CNNM4*, as quais interferiram na formação do esmalte dental, resultando em uma mineralização anormal, com uma densidade reduzida em relação aos dentes de estrutura normal, estando essas alterações associadas a concentração alterada de Mg^{2+} (Luder *et al*., 2013).

Tabela 1: Genes associados à amelogênese imperfeita.

Padrão de herança	Gene	Cromossomo humano
Ligado ao X	*AMELX X*	X
Autossômico dominante	*AMBN*	4
	ENAM	4
	DLX3	17
	FAM83H	8
Autossômico recessivo	*ENAM*	4
	MMP20	11
	KLK4	19
	WDR72	15
	FAM20A	17

Muto *et al.*, 2012

1.2 Alterações oculares na síndrome de Jalili: distrofia de cones e bastonetes

A DCB (MIM#120970) representa um amplo espectro de doenças progressivas nas células fotorreceptoras da retina, caracterizada pela degeneração das células fotorreceptoras, os cones seguida pelos bastonetes (Jalili, 2010). As manifestações clínicas encontradas para DCB como fotofobia, perda da visão central, acromatopsia, nistagmos, lesão macular, são observadas já nos primeiros anos de vida (Hamel, 2007).

Mutações nos genes *ABCA4* (MIM#601691), *AIPL1* (MIM#604392), *CRX* (MIM#602225), *GUCA1A* (MIM#600364), *GUCY2D* (MIM#600179), *PITPNM3* (MIM#608921), *RIMS1* (MIM#606629), *SEMA4A* (MIM#6072920), *RPGR* (MIM#312610), *PROML1* (MIM#604365) e *UNC119* (MIM#604011) levam a alterações na retina, a DCB (Parry *et al.*, 2009). O modo de herança para a DCB é variado, podendo apresentar-se na forma autossômico dominante, autossômico recessivo ou ligado ao cromossomo X, de maneira isolada ou relacionadas a outras

desordens como síndrome Bardet-Biedl e ataxia espinocerebelar tipo 7 (Hamel, 2007). O diagnóstico para as distrofias de retina baseia-se na história clínica, oftalmoscopia e eletrorretinografia, mostrando como uma alteração progressiva não existindo tratamento que possa interromper a evolução da doença (Hamel, 2007).

Jalili (2010) mostra que as células fotorreceptoras presentes na retina são gravemente afetadas, devido a mutação no gene *CNNM4*, levando a progressiva DCB, na qual os cones fotorreceptores são os mais comprometidos. As anormalidades oculares são observadas já nos primeiros anos de vida em indivíduos afetados pela SJ, que apresentavam fotofobia, nistagmo, acromatopsia, atrofia da área do epitélio pigmentar da retina, formando máculas (Jalili & Smith, 1988; Jalili, 2010). As características das lesões maculares podem apresentar-se em diferentes aspectos, desde aparência de olhos de búfalo com depósitos pigmentares a alterações mais grave, escavação e estafiloma (coloboma) (Jalili, 2010). O nervo óptico mostrou-se sem alterações no início da doença, mas durante seu curso, atrofia de sua estrutura foi observada. Além das alterações descritas, a cegueira noturna não foi confirmada nos indivíduos com SJ (Jalili, 2010).

1.3 Alterações genéticas na síndrome de Jalili

A SJ é causada pelas mutações heterozigótica/homozigótica no gene *CNNM4* (MIM#607805) mapeado no intervalo 2 cM/5.4 Mb, entre os marcadores D2S2209 e D2S373, no cromossomo 2q11 por Downey *et al*, (2002) em indivíduos afetados com amelogênese imperfeita e distrofia de cones e bastonetes.

O gene *CNNM4* é o codificador da proteína transportadora de metal ACDP4 (Cyclin And ABS *Domain Divalent Metal Cation Transport Mediator4*, sendo uma família de quatro proteínas, expresso em células de vários tecidos como esmalte e retina, localizadas na membrana plasmática (Polok *et al.*, 2009; Yamazaki *et al.*, 2013). *O CNNM4* está envolvido no transporte de íons metálico, em especial o Mg^{2+}atuando em seu transporte e homeostase (Yamazaki *et al*., 2013). Na retina o Mg^{2+} participa da cascata de fototransdução, que é a conversão do estímulo luminoso em estímulo elétrico e em esmalte atua em sua biomineralização (Polok *et al*., 2009). Mutação no gene *CNNM4* resultará em alteração na regulação do Mg^{2+}, reduzindo seus níveis intracelulares, como

consequência haverá uma limitação na função da retina caracterizando sua degeneração e uma desorganização na biomineralização do esmalte (Polok *et al*., 2009; Yamazaki *et al*., 2013).

Através de análise de imuno-histoquímica em ratos confirmou-se sinais da expressão do gene *CNNM4* em membrana basolateral do tecido formador do esmalte dos dentes, o ameloblasto, interferindo em sua mineralização (Yamazaki *et al*., 2013). O papel do Mg^{2+} na mineralização do esmalte dental ainda é desconhecida, mas sugere-se que o Mg^{2+} precisa ser removido do tecido do esmalte para promover sua mineralização (Yamazaki *et al*., 2013).

Luder *et al*. (2013) ao investigarem pacientes afetados pela SJ, descreveram mutações no gene *CNNM4*, homozigota de um par de base c.1312dupC, resultando em uma mineralização deficiente em esmalte e dentina, de acordo com análise da densidade e composição de elementos mineralizados, resultado de uma concentração anormal de Mg^{2+} nos tecidos dentários.

O Mg^{2+} é o segundo cátion intracelular mais abundante no corpo humano após o potássio, sendo sua homeostase regulada entre a absorção intestinal e a excreção renal (Meyer *et al*., 2010; Garcia *et al*., 2011; Yamazaki *et al*., 2013). O Mg^{2+} está presente em ossos, músculo esquelético, tecidos moles e sangue, estando seus níveis séricos relacionados a doenças crônicas como hipertensão, diabetes, osteoporose (Meyer *et al*., 2010). Várias condições têm como característica alterações na homeostase do Mg^{2+}, incluindo síndrome de hipomagnesemia, síndrome de Bartter (Meyer *et al*., 2010).

Diferentes mutações têm sido identificadas no gene *CNNM4* em várias famílias de diferentes países (Tabela 2). As primeiras mutações no *CNNM4*, foram confirmadas por Parry *et al*. (2009) em sete famílias (Faixa de Gaza A, Faixa de Gaza B, Kosovo, Guatemala, Escócia, Turquia e Irã) e por Polok *et al*. (2009) em três famílias originárias do (Líbano, Kosovo e uma de origem desconhecida).

Tabela 2: Mutações identificadas no gene *CNNM4*.

Origem (Referência)	**Histórico familiar de consanguinidade**	**Mutação1/Mutação2**
Gaza A (Parry *et al.*, 2009)	Sim	c.599C>A; Ser200Tyr/c.599C>A; Ser 200Tyr

Tabela 2: Mutações identificadas no gene *CNNM4*.

Kosovo (Parry *et al.*, 2009)	Sim	c.1312C dupC; Leu438ProfsX9/c.1312 dupC; Leu438ProfsX9
Gaza B (Parry *et al.*, 2009)	Sim	c.1813 C>T; Arg605X/c.1813C>T; Arg605X
Guatemala (Parry *et al.*, 2009)	Não	c.2149C>T; Gln717X/c.62_145 del; del;L21HisfsX185
Turquia (Parry *et al.*, 2009)	Sim	c.586T>C; Ser196Pro/c.586T>C; Ser196Pro
Irã (Parry *et al.*, 2009)	Sim	c.1-?1403+? del/c.1-?1403+? del
Escócia (Parry *et al.*, 2009)	Não	c.971T>C; Leu324Pro/c.1690C>T; Gln564X
Kosovo (Polok *et al*, 2009)	Sim	c.1312dupC; p.L438ProfX9
Líbano (Polok *et al.*, 2009)	Sim	c.707G>A; PR236Q/c.707G>A
Desconhecida (Polok *et al*., 2009)	Sim	c.971T>C/p.1324p
Canadá (Doucette, *et al.*, 2013)	Sim	c.1555C>T/p.R519*
Kosovo (Zobor *et al.*, 2012)	Sim	c.1312 dupc; Leu438 Profs*9
Arábia Saudita (Abu-Safieh *et al.*, 2013)	Sim	c.1484C>T/p.T495lle
Arábia Saudita (Lopez Tores *et al.*, 2015)	Sim	c.1474G>T/p.C492
Kosovo (Luder *et al.*, 2013)	Sim	c.1312dupC/p.L438Pfs*9x
Kosovo (Gerth-Kahlert *et al.*, 2013)	Sim	c.1312dupC/p.L438Pfs*9
Argélia (Coppleters *et al.*, 2014)	Sim	c.189del/Asp63Glufs*12
Argélia (Prasad *et al*., 2015)	Sim	c.1495G>A/p.[V499M]
China (Wang H *et al*., 2015)	Não	c.896_89T/p.A300CfsX22
EUA Portland – Oregon (Pennesi *et al*., 2015)	Não	c.1307delC/p.T436fs/c.C1690T/p.Q564X
Kosovo (Kiessling *et al*., 2016)	Não	c.1312dupC/p.L438PfsX9/c.694_722del/ p.lle232ProfsX80
Turquia (Topçu *et al*., 2016)	Sim	c.1781A>G(p.N594S)
Irã (Rahimi-Aliabadi *et al*., 2016)	Sim	c.1091delG

Tabela 2: Mutações identificadas no gene *CNNM4*.

India (Purwar *et al.*, 2015)	Sim	-
Paquistão (Malik, *et al*., 2016)	Sim	-
Marrocos (Jaouad *et al*., 2017	Sim	c.1682-1G>C;p.Glu561Glyfs*5
Polônia (Wawrocka *et al*., 2017)	Sim	c.1076T>C/p.Leu359Pro

Os estudos realizados em famílias com características para SJ em diferentes países totalizaram 87 casos examinados, em 32 famílias, com 48 pacientes masculinos e 39 femininos acometidos (Tabela3).

Tabela 3: Totalidade de casos síndrome de Jalili examinados, número de famílias acometidas e sexo.

Origem	**Casos examinados**	**Famílias**	**Masculino**	**Feminino**
Argélia	4	2	3	1
Arábia Saudita	3	2	3	-
América do Norte	2	2	1	1
China	1	1	-	1
Escócia	1	1	1	-
Faixa de Gaza	33	3	18	15
Guatemala	5	1	5	-
India	4	4	2	2
Irã	5	2	3	2
Kosovo	11	6	6	5
Líbano	3	1	2	1
Marrocos	6	2	-	6

Tabela 3: Totalidade de casos síndrome de Jalili examinados, número de famílias acometidas e sexo.

Paquistão	1	1	1	-
Polônia	3	1	3	-
Turquia	4	2	-	4
Não descrito	1	1	-	1
Total	**87**	**32**	**48**	**39**

1.4 Famílias estudadas com características fenotípicas-genotípicas da sindrome de Jalili

O primeiro relato sobre a síndrome de Jalili, em uma extensa família da Faixa de Gaza, com 29 membros afetados, apresentando alterações oculares (distrofia de cones e bastonetes) e dental (amelogênese imperfeita), foi realizado por Jalili & Smith (1988). Após uma análise da linhagem dessa família, foi confirmado o modo de herança como autossômica recessiva, resultado de casamentos consanguíneos. As alterações oculares encontradas foram fotofobia, nistagmo pendular, acromatopsia, atrofia do epitélio pigmentar da retina e distrofia macular. AI apresentada foi do tipo hipomineralizada/hipocalcificada, e os dentes tinham cor amarelo/marrom.

Um sequenciamento genético dos membros afetados da Faixa de Gaza A foi realizado por Downey *et al*. (2002) identificando o locus no cromossomo 2q11 para AI e DCB, homozigota e de caráter recessivo. Os estudos de Michaelides *et al.* (2004) em uma nova família afetada em Kosovo, onde dois meninos também apresentavam alterações oculares e dentais já descritas na família da Faixa de Gaza A, além da AI, os primeiros molares permanentes apresentavam taurodontismo, e presença de cárie nos dentes decíduos. O tipo de AI foi do tipo Hipoplásica/hipomineralizada. Neste trabalho o gene *CNGA3* também foi analisado, descartado como causador das alterações oculares e dentais encontradas.

Polok *et al*. (2009) analisaram três famílias afetadas com DCB e AI originárias de Kosovo, Líbano e terceira de origem desconhecida. Vários genes ligados a alterações oculares foram sequenciados, sendo observado mutações no gene *CNNM4* em dois irmãos de Kosovo, uma duplicação homozigótica de um par de bases c.1312 dupC. Na família do Líbano com duas irmãs e um primo, o

sequenciamento mostrou uma mutação homozigótica (c.707G>A) no gene *CNNM4* nos três indivíduos afetados. Na família de origem desconhecida, com um único indivíduo afetado, também uma mutação homozigótica c.971T>C foi encontrada no gene *CNNM4.* Assim, através deste trabalho, mutações em *CNNM4* foram confirmadas como responsáveis pelas alterações DCB e AI encontradas nestas famílias. Trabalho realizado por Parry *et al*. (2009) em sete famílias sendo da Faixa de Gaza A, Kosovo, Faixa de Gaza B, Guatemala, Turquia, Irã e Escócia investigadas com DCB e AI, através do sequenciamento genético de todos os membros afetados, foi confirmado o gene *CNNM4* como responsável pelas mutações apresentadas. Diante dos achados, Parry *et al*. propuseram o termo ″ síndrome de Jalili ‶ para a desordem investigada.

Os relatos apresentados por Zobor *et al*. (2012) e Purwar *et al*. (2015) mostram que a SJ pode manifestar-se associada a outras anormalidades. Na paciente de Kosovo foi identificada uma mutação no gene *CNNM4* igual à encontrada em outra família já estudada de Kosovo. Os achados no paciente da Índia, oculares e dentais, foram semelhantes aos observados em outras famílias estudadas. O estudo genético dessa família não foi realizado.

A necessidade de melhores estudos dos fenótipos dentais foi observada por Luder *et al*. (2013), porque todos os trabalhos publicados até aquele período sobre a SJ foram direcionados para área oftalmológica. Então, realizou-se análise genética de uma família de Kosovo, 2 meninos com distrofia de cones e bastonetes e amelogênese imperfeita, que revelaram mutação no gene *CNNM4* homozigota c.1312dupC; p.L438Pfs9X, pais heterozigotos. Seis dentes decíduos desses pacientes foram analisados, através de microscopia eletrônica, com espectropia de raios X. Observaram que o esmalte dental apresentava deficiência em sua mineralização, com decréscimo de cálcio (Ca^{2+}), mas elevado nível de Mg^{2+}. Em dentina também apresentou mineralização deficiente e redução no nível de Mg^{2+} e nível normal de Ca^{2+}. As alterações oculares foram: fotofobia, redução da visão central, nistagmo, estrabismo, distrofia macular. A alteração dental foi AI do tipo hipoplásica/hipomineralizada, com dentes cor amarelo/marrom.

Jalili (2010) ao reportar três membros afetados de uma segunda família da Faixa de Gaza B, sendo 1 menino (10 anos de idade), e 2 meninas (5 e 6 anos de idade), apresentando DCB e AI. Os três irmãos não apresentavam outras condições médicas associadas. As características fenotípicas encontradas na família Faixa de Gaza B são diferentes das encontradas na primeira família. Foi encontrado AI do tipo hipomaturada/hipoplásica na dentição decídua e permanente, sendo observado também mordida aberta anterior (MAA), premaxila pequena em todos os três irmãos, o

que os difere dos membros da família Faixa de Gaza A, e também presença de cálculo nos dentes no irmão mais velho. As alterações oculares presentes já na primeira infância foram: acuidade visual diminuída, fotofobia, acromatopsia, nistagmo pendular fino, lesão macular e todos os irmãos negaram cegueira noturna. Os pacientes de 5 e 6 anos apresentaram acuidade visual diminuída, com hipermetropia. Foi observado elevada consanguinidade e mutações presentes na primeira e segunda geração: mutação c.1813C>T; Arg605X. Através de análises realizadas, mutações diferentes no gene *CNNM4* confirmaram o não parentesco entre as duas famílias da Faixa de Gaza (A e B). Foi sugerido que essas alterações possam estar associadas ao excesso de flúor encontrado nas águas subterrâneas da região, devido à ação mutagênica relacionada ao flúor.

Doucette *et al*. (2013), estudando acromatopsia no Canadá diagnosticou também a SJ em uma família de quatro membros afetados após sequenciamento dos genes *CNGA3, CNGB3, CNAT2, CNNM4.* Os achados oftalmológicos foram: acuidade visual diminuída, nistagmo e acromatopsia. Alterações dentais encontradas: displasia severa do esmalte e dentes extraídos. Em algumas famílias foram encontradas mutações nos genes *CNGA3, CNGB3, CNAT2,* o que justificou a presença da acromatopsia em alguns membros. No gene *CNNM4* foram identificadas as seguintes mutações*:* c.1555C>T: p.R519X constituindo assim o primeiro caso de SJ na America do Norte.

Gerth-Kahlert *et al*. (2013) realizaram análises genéticas em duas irmãs (15 e 16 anos de idade) de Kosovo. Em uma das irmãs foi observado, através de exame de fundoscopia, atrofia macular severa em aspecto de olho de boi e na outra uma distrofia retiniana menor. Também apresentava nistagmo pendular. As duas pacientes apresentavam AI. Duas mutações foram identificadas c.1312dupC, p.L438Pfs9.

Topçu *et al*. (2016) descreveram 3 irmãs com 14, 12 e 8 anos de idade, originárias da Turquia, apresentando AI e DCB. Após análise genética dos pais de casamento consanguíneo, foi confirmada mutação no gene *CNNM4*, homozigoto c.21781A>G; p.N594S no exon 4. As alterações oculares encontradas nas três irmãs foram: acuidade visual diminuída com hipermetropia. Não apresentavam dificuldade visual à noite, fotofobia e nistagmo. Fenótipo dental observado nas 3 irmãs: a de14 anos apresentava dentição permanente com diastemas generalizados e tamanho pulpar normal. Paciente 12 anos apresentava dentição mista e diastemas generalizados, tamanho pulpar normal, lesão apical, higiene bucal deficiente, cálculos, periodontite, restaurações nos dentes 26/46, extração do dente 36, tratamento endodôntico dos dentes 31/41, dentes 38/48 impactados na mandíbula. Paciente 8 anos

apresentava dentição mista, dentes permanentes em erupção 11/ 21/ 16/ 26/ 31/ 36/ 41/ 46 e o tipo de AI encontrado foi hipoplásica/hipomineralizadas, dentes cor amarelo/marrom.

Kiessling *et al*. (2016) analisaram 2 pacientes: uma mulher com 20 anos e um homem com 16 anos de idade, ambos com DCB e AI, pais de casamento não consanguíneo, que não apresentavam alterações visuais e dentais, sendo naturais de Kosovo. Ambos pacientes apresentavam acuidade visual diminuída desde quatro anos de idade, também os dentes decíduos e permanentes apresentavam cor amarelada com defeito no esmalte, sinais de AI e cariados. As alterações oculares encontradas aos seis anos foram DCB com presença de degeneração macular, nistagmo e dificuldade de enxergar no escuro. Aos dez anos os exames oftalmológicos confirmaram também acromatopsia, aos 17 anos constatada uma progressão do quadro clínico. O sequenciamento genético revelou a presença de mutação heterozigota c.1312dupC e segunda mutação c.694_722del; pII no exon 1 localizado no CBS domínio do gene *CNNM4*.

Malik *et al.* (2016) confirmaram em estudo de um homem, origem Paquistão, 35 anos de idade, sem alterações física e mental, apresentava alteração de acuidade visual diminuída e miopia. Ao exame de fundo de olho, foi observado pigmentação em aspecto de espículas ósseas e alteração macular. Os dentes com anormalidades em sua formação, cor amarelada. Não foi realizado estudo genético.

Rahimi-Aliabadi *et al*. (2016) relataram um trabalho em uma extensa família com alto grau de consanguinidade, do Iran, com 24 membros afetados. O estudo genético foi realizado em apenas 4 membros, de acordo com o heredograma. Dois pacientes, um com 32 anos e outro com 39 anos, ambos do sexo feminino, apresentavam retino-distrofia desde período neonatal, com grave deficiência visual, fotofobia, nistagmo fino, progressiva deficiência visual, catarata, coloboma e aglomerados de pigmentos e pontos esbranquiçados na retina. No paciente de 25 anos do sexo masculino foram encontrados alterações apresentadas desde primeira infância, fenótipo menos grave com suave atrofia macular nos dois olhos, pigmentação na região periférica da retina, nistagmo pendular latente, fotofobia, deficiência visual média e estrabismo. No paciente de 27 anos do sexo masculino apresentou alterações desde o nascimento, como nistagmo, fotofobia, deficiência visual, coloboma macular, pigmento região mácula e periférica da retina e exames dentais foram diagnosticados com AI. Análise do sequenciamento do DNA revelou mutação homozigota e mutação heterozigota para os pais. A mutação foi deleção nucleotídeo G (c.1091delG), no exon 1 do gene *CNNM4*.

Jaouad *et al*. (2017) analisaram estudo genético de uma extensa família consanguínea do Marrocos, com três irmãos afetados pela síndrome de Jalili., identificado uma nova mutação homozigoto (c.1682-1G> C) no gene *CNNM4.*

Wawrocka *et al*. (2017) identificaram em 3 irmãos poloneses, com SJ associada ao crescimento excessivo dos músculos das pernas, e uma nova mutação homozigota (c.1076T>C, p. Leu 359Pro) para o gene *CNNM4* foi observada. Os resultados encontrados ampliam o espectro mutacional associado à SJ, sugerindo que a hiperplasia dos músculos da perna, pode ser uma manifestação identificada da desordem, sendo este o primeiro relato desta manifestação. Para os pacientes com SJ foi recomendado exames de creatina quinase e EMG.

Outras famílias com características para SJ foram identificadas, com novas mutações no gene *CNNM4:* Coppieters *et al.* (2014) c.189del; Asp63Glufs*12, Prasad *et al* (2015) c.1495G>A; p.[V499M], Lopez Torres *et al.* (2015), c.[1474G>T]; p. C492, Wang *et al.* (2015), c.896_897insT; p.A300CfsX22, Pennesi *et al.* (2015) c.1307delC, p.T436fs/c.C1690T, p.Q564X, Huang *et al* .(2012) c.47G>A; p.Arg16His.

O presente estudo se justifica pelo fato de serem as primeiras famílias brasileiras descritas com a SJ, sendo este diagnóstico importante para avaliar outros quadros clínicos semelhantes como hipótese diagnóstica. Enfatiza-se a importância do tratamento multiprofissional adequado aos indivíduos afetados, bem como orientação genética às famílias, contribuindo assim com uma melhora na qualidade de vida desses pacientes. Além disso, a importância da amelogênese imperfeita como manifestação de condições sistêmicas. Este trabalho faz parte da continuidade das investigações científicas de doenças genéticas com envolvimento orofacial, que têm sido desenvolvidas no Programa de Pós-graduação em Ciências da Saúde da Universidade Estadual de Montes Claros.

2 OBJETIVOS

2.1 Objetivo Geral

- Avaliar as características clínicas, imaginológicas e genéticas em duas famílias acometidas pela síndrome de Jalili.

2.2 Objetivos Específicos

- Avaliar e descrever as características clínicas e imaginológicas dentárias dos indivíduos afetados com a síndrome de Jalili.
- Avaliar as características oculares dos indivíduos afetados com síndrome de Jalili.
- Realizar o sequenciamento genético nos membros das famílias com a síndrome de Jalili e descrever as expressões gênicas manifestadas.

3 METODOLOGIA

3.1 Tipo de pesquisa

Trata-se de um estudo transversal, clínico e laboratorial.

3.2 Populações do estudo

Foram avaliados membros de duas famílias, que apresentam características da síndrome de Jalili, sendo uma residente no município de Porteirinha – Minas Gerais e a segunda residente no município de Americana – São Paulo.

3.3 Avaliações clínica e radiográfica

Foram avaliados e fotografados todos os membros afetados e não afetados pela SJ, identificados: dados referentes exame intrabucal foram coletados através de uma ficha (Anexo B). As avaliações clínicas foram realizadas por dentistas especialistas em odontopediatria, estomatologia e patologia. Radiografias intra e extrabucais foram realizadas em clínica especializada. E exames oftalmológicos foram realizados por oftalmologistas e geneticistas, apresentando exames: Retinografia, eletroretinografia (ERG), Tomografia de coerência óptica (TCO).

3.4 Avaliação genética

Após coletados os dados clínicos, foi confeccionado um heredograma de cada família, para melhor entendimento da transmissão da condição entre os membros das famílias, através das gerações

afetadas. Foi realizado sequenciamento genético a partir do DNA dos indivíduos afetados e não afetados de cada família.

3.5 Amostras

Membros de uma família residente no município de Porteirinha - MG, sendo o pai, a mãe e um filho normais e uma filha com características fenotípicas da SJ. Também membros da família residente no município de Americana - SP, sendo mãe sem alterações, pai também apresentava sem características fenotípicas e uma filha com características fenotípicas da SJ.

3.6 Coletas das Amostras de células bucais

As amostras de células bucais foram coletadas por meio de um bochecho, por 60 segundos com 5 mL de uma solução aquosa de sacarose a 3%. O conteúdo resultante do bochecho foi transferido para um tubo de 15 mL, o qual continha o volume de 3 mL de uma solução 66% alcoólica contendo 17 mM Tris-HCl pH 8, 5 mM NaCl e 7 mM EDTA.

3.7 Isolamento do DNA

A cada tubo foi adicionado água destilada e deionizada autoclavada q.s.p. 15 mL (FANEM, mod.415). Após centrifugação e descarte do sobrenadante, o precipitado foi lavado e centrifugado (Eppendorf, centrifuge 5430R) em solução aquosa, contendo 17 mM Tris-HCl pH 8, 50 mM NaCl e 7 mM EDTA. Novamente o sobrenadante foi descartado e o precipitado ressuspendido em 1 mL de solução de lise contendo 10 mM Tris-HCl pH 8, 0,5% dodecil sulfato de sódio (SDS), 5 mM EDTA, incubado a 50ºC por 1 hora, após este tempo foi adicionado 10 µl de proteinase K (Invitrogen, USA) e encubado em movimento contínuo semicircular. Após 16 horas de incubação, adicionamos 470 µl de uma solução aquosa de 8M de acetato de amônio. A mistura foi centrifugada por 10 min a 14.000 rpm a 4ºC. O precipitado de DNA foi adquirido após adição de 540 µl

isopropanol ao sobrenadante e centrifugado (14.000 rpm por 5 min a 4°C). Para o término da purificação foi adicionado 1mL de etanol 70% gelado e centrifugado (14.000 rpm por 5 min a 4°C), removemos o sobrenadante, seco e foi ressuspendido em tampão Tris-EDTA (TE Buffer). A determinação da concentração e pureza das amostras foram determinadas por espectrofotometria utilizando a razão 260/280 nm em que valores inferiores a 1,7 indicam contaminação com proteína e valor superior a 2 indica contaminação com RNA.

3.8 Sequenciamento genético

Inicialmente, foram selecionados os primers para avaliação de todos os éxons do gene *CNNM4* através da realização de reação em cadeia de polimerase (PCR) e separação dos fragmentos amplificados através da eletroforese em gel.

Todos os fragmentos amplificados foram purificados com Quick Gel Extraction Kit da Invitrogen seguindo as recomendações do fabricante. Em seguida, foram quantificados e analisados a sua integridade através do espectrofotometro Nanodrop 2000 (Nanodrop 2000c Thermo Scientific) conforme o fabricante.

Para a reação de sequenciamento dos fragmentos gel purificados utilizou-se o Big Dye Cycle Sequencing Kit. Na reação de sequenciamento, um primer se liga especificamente a sequência complementar e os nucleotídeos são incorporados de acordo com a fita molde. Nesta etapa ocorre a incorporação da fluorescência aos nucleotídeos da fita de DNA. As amostras receberam formamida e foram encaminhadas para o termociclador (Applied Biosystems) para a reação de desnaturação. Após esta etapa, as amostras foram encaminhadas para o sequenciador, modelo ABI Prism 3500 Genetic
Analyzer (Applied Biosystems, Foster City, CA, USA).

A análise do sequenciamento foi realizada a través do programa *Sequence Scanner Software* 2.

4 PRODUTOS

4.1 Produto 1: *Report of two unrelated families with Jalili syndrome and a novel nonsense heterozygous mutation in CNNM4 gene*, formatado segundo as normas para publicação (ANEXO C) do periódico *European Journal of Medical Genetics*.

Running title: Mutations in *CNNM4* gene and Jalili syndrome.

4.1 PRODUTO 1

Report of two unrelated families with Jalili syndrome and a novel nonsenseheterozygous mutation in *CNNM4* gene

Célia Márcia Fernandes Maia[a], Renato Assis Machado[*,b], Vera Lúcia Gil-da-Silva-Lopes[c], Elaine Lustosa-Mendes[c], Priscila Hae Hyun Rim[c], Verônica Oliveira Dias[a], Daniella Reis Barbosa Martelli[a], Luciano Sólia Nasser[a], Ricardo D. Coletta[b] and Hercílio Martelli-Júnior[a,b]

[a]Health Science Program, State University of Montes Claros, Montes Claros, Minas Gerais, Brazil;

[b]Department of Oral Diagnosis, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil;

[c]Department of Medical Genetics, School of Medical Sciences, State University of Campinas, Campinas, São Paulo, Brazil.

*Corresponding author.Department of Oral Diagnosis, School of Dentistry, State University of Campinas, CEP 13414-018, Piracicaba, São Paulo, Brazil.

E-mail: renatoassismachado@yahoo.com.br (R.A. Machado).

Abstract

Jalili syndrome (JS) is an autosomal recessive disease characterized by a combination of cone-rode retinal dytrophy (CRD) and amelogenesis imperfect (AI). Mutations in cyclin and CBS domain divalent metal cation transport mediator 4 (*CNNM4*) gene cause JS. Here we described 2 families (3 members) affected by JS. In the first family, JS was caused by the homozygous p.L324P (c.971T>C) missense mutation and the affected patient developed both CRD and AI. In the second family, a specific combination of a compound heterozygous mutation was found – the p.L324P (c.971T>C) missense transition and thenovelp.Y581* (c.1743C>G) nonsense mutation. The proband showed CRD and AI, but her father just developed eye alterations. Together, these findings suggest that the p.L324P mutation in homozygosis induces a complete phenotype with both CRD and AI, but in heterozygosis and in composition with the novel p.Y581*nonsense mutation in *CNNM4* promotes variable clinical expressivity, particularly with lack of dental phenotypes. These different phenotypes could be explained by deletions affecting the proband´s homologous allele, epistasia or interactions with environmental factors leading to residual activity of protein.

Keywords: Jalili syndrome; Cone-rod dystrophy; Amelogenesis imperfecta; *CNNM4;* mutation.

1. Introduction

Jalili syndrome (JS; MIM#217080) is a rare genetic disorder characterized by cone-rode retinal dystrophy (CRD, MIM #120970) and amelogenesis imperfecta (AI, MIM #204700). Both CRD and AI may occur isolately and in combination characterizing JS. CRD is a heterogeneous group of eye disorders that causes degeneration of the photoreceptors of cones and, subsequently, of rods. The clinical characteristics are observed in the first years of life and usually are accompanied by photophobia, loss of central vision, achromatopsia, pendular nystagmus and progressive macular dystrophy (Hamel, 2007). The AI refers to a group of genetic alterations, which interferes in dental enamel formation, affecting its structure and clinical appearance (Aldred et al., 2003).

JS was initially described in a Arab family living in the Gaza strip by Jalili & Smith in 1988 and attually has been reported in 31 families worldwide (Jaouad et al., 2017; Wawrocka et al., 2017). Mutations in the cyclin and CBS domain divalent metal cation transport mediator 4gene (*CNNM4*; MIM #607805), localized in cromossome 2q11.2, are related to JS. *CNNM4* codifies a metal transporter protein belonging to a family of 4 proteins localized on the plasmatic membrane (Garcia et al., 2011). The protein encoded by *CNNM4* is especially involved on magnesium ion transportation and is expressed in several tissues, including retina and dental enamel (Parry et al., 2009; Polok et al., 2009; Yamazaki et al., 2013). In retina, *CNNM4* protein participates on the phototransduction cascade, converting luminous into electrical stimulus, whereas in the dental enamel, *CNNM4* protein is essential for formation of hydroxyapatite during the mineralization process (Parry et al., 2009; Polok et al., 2009).To date, 22 mutations in *CNNM4* have been reported in 108 cases of JS in the world, mainly in Middle Eastean countries (Jaouad et al., 2017; Wawrocka et al., 2017). Here, we report the 2 first families with JS in Brazil. Molecular analysis of the first family identified a previously described homozygous mutation (p.L324P) in exon 1 of

CNNM4 gene. In the second family, affected patients demonstrated acompound heterozygous mutation in *CNNM4*, the p.L324P and the novel nonsense mutationp.Y581*.

2. Clinical description

In this study, we examined two Brazilian families. Family A: a 8-year-old girl, second child of a consanguineous marriage (third cousins), was referred to Stomatology clinic, State University of Montes Claros, Minas Gerais, Brazil, to dental evaluation. At age of 5 years, the proband presented visual impairment and in the ophthalmological evaluation revealed absence of recordable scotopic responses and had a diagnosis of Leber's congenital amaurosis. The best-corrected visual acuity was 20/640 in the right eye and 20/1600 in the left eye with Snellen chart. The refractive error was + 5,00 sphere in both eyes. After 3 years, the patient was reavaluated with the complains of photophobia, pendular nystagmus and achromatopsia (Fig. 1A). Fundus examination showed retinal dystrophy with decreased macular reflex (Fig.1B) and macular pigmentation mobilization (Fig. 1C) were observed.Optical coherence tomography showed a significant reduction in the thickness of the neurosensory retina in the macula and absence of the outer layer of the subfoveal photoreceptors (Fig. 1D), and the electroretinogram pointed out for a complete absence of cone and rod responses (Fig. 1E). The patient complained also about her teeth, and the ophthalmologist referred the patient for dental evaluation. Oral examination revealed a mixed dentition, with generalized diastema and primary and permanent teeth with yellowish discoloration, rough surfaces and conspicuous and irregular defects, characterizing hypoplastic AI (Fig. 1F). No eye and dental abnormalities were observed in other members of this family (Supplementary Fig. 1A).

Family B: the proband with 9-year-old, the only daughter of a non-consanguineous marriage, was referred to Department of Medical Genetics, State University of Campinas,

São Paulo, Brazil, at the request of a pediatric neurologist to genetic analysis, with previus diagnosis of retinal dystrophy. In the examination, the proband reported nystagmus, photophobia and reduced visual acuity (Fig. 2A) since the first years of life. Ophthalmologic examination revealed macular and optic atrophy, decreased retinal thickness and reduced cone responses, but normal rod responses (Fig. 2 B, C, D, E). Oral examination showed generalized yellow to yellowish-brown teeth with rough surfaces and some irregular defects (Fig. 2F). The parents were evaluated and her father (37-year-old) showed strabismus and inability to see clearly with right eye since childhood (Fig. 1G). On ocular examination, proband's father had reduced visual acuity and refractive error of +4.00 in the right eye. Color vision testing and pupillary reflexes were normal, but fundus examination showed generalized depigmentation with bilateral pale disk (Fig. 2 H, I). Optical coherence tomography (OCT) showed thickening of the inner nuclear layer with hyporeflective cysts near the fovea (Fig. 2 J) and the ellipsoid zone appeared to be preserved in both eyes (Fig. 2 K). Optic disc OCT showed diffuse reduction of the nerve fiber layer in both eyes (Fig. 2 L, M). A multifocal electroretinogram was recorded with no abnormalities. Although partially edentulous, the teeth were of normal aspect and consistency (Fig. 2N). The health of the proband's mother was unremarkable (Supplementary Fig. 2A).

3. Methods

This study was approved by the Institutional Review Board (#1.293.651) and written informed consent to participate in the study was obtained from all subjects and guardians of children, in compliance with the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects.

3.1 Genetic analysis of *CNNM4* gene

Genomic DNA was isolated from the buccal mucosa cells as previously described (Aidar and Line, 2007). Direct DNA sequencing of PCR products was performed using the Big Dye Terminator v 3.1 Cycle Sequencing Kit and migrated on *capillary* 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, *USA)*. The *CNNM4* gene was amplified by PCR using primers designed to amplify all 6 exons and their flanking splice junctions (Suplemmentary Table 1).

4. Results

4.1 Genetic analysis

Family A: mutational screening of the affected patient showed a homozygous T to C transition at position 971 (c.971T>C), which lead to the change of a leucine (L) for a proline (P) in the codon 324 (p.L324P). This mutation was observed in her parients, but with a heterozygous pattern. Her brother did not show similar mutation (Supplementary Fig. 1).

Family B: the affected members of this family demonstrated a compound mutation in *CNNM4*: the p.L324P mutation in exon 1 andthe undescribed mutation represented by a C for G substituition at nucleotide 1743 (c.1743C>G), leading to the change of a tyrosine (Tyr, Y) for a stop codon (Stop, *) at position 581 (p.Y581*) (Supplementary Fig. 2). The premature termination at amino acid 581, abolishing the carboxyl-terminal region of *CNNM4* protein.

5. Discussion

Since the first identification of *CNNM4* mutation in JS patients (Jalili and Smith, 1988), 22 different mutations have been reported in patients with this syndrome. Most are

missense mutations, products of single aminoacid substitution, located in the DUF21 and CBS domains of *CNNM4* protein (Jaouad et al., 2017; Wawrocka et al., 2017). Mutations in this region presumably affected the divalent metal transporter function of *CNNM4* protein.This study confirmed that the c.971T>C (p.L324P) transition in *CNNM4*, in homozygosis, is a potentially pathogenic and deleterious mutation. The p.L324P mutation was previously described in a Scottish family and an unreported origin family (Parry et al., 2009; Polok et al., 2009). The patients with 2 copies of the p.L324P mutation displayed the classical combination of CRD and AI. Furthermore, we found the p.L324P mutation segregated in heterozygosis in phenotypically affected patients with JS. These patients carry a compound heterozygous mutation, the other allele bore the undescribed new heterozygous c.1743C>G transversion, leading to a p.Tyr581* (Y581*) mutation on exon 4 of the *CNNM4* gene. At this genotype, the syndrome showed variable clinical expressivity, suggesting that the mutant protein carrying the compound variants is partially active inducing a variable and less severe phenotype than the mutations in homozygosity.

Structurally, *CNNM4* (UniProtKB/Swiss-Prot code Q6P4Q7) is a multidomain protein formed of a DUF21 domain (residues 184-358), that includes a leucine-zipper pattern (residues 188-209) and 4 transmembrane helices (residues 182-204, 239-261, 265-287 and 294-316), a cyclin-box motif domain (residues 548-578), a cyclic nucleotide monophosphate (cNMP)-binding domain (residues 575-695), similar to that usually present in ion channels and cNMP-dependent kinases, and 2 consecutive cystathionine-synthase (CBS) domains (residues 377-438 and 445-511) that presumably exert a regulatory role on its biological activity (Garcia et al., 2011). Among JS mutations found in the literature, the most commun are in DUF21 and CBS domains (Jaouad et al., 2017; Wawrocka et al., 2017). In this study, the family A showed the homozygous mutation in the DUF21 domain and the family B in the CBS domain. To data, no known of the physiological importance of

DUF21 domain, even that point mutations were found in several JS cases. CBS domains are considered to be energy-sensing modules that bind adenosine ligands with different affinities (Scott et al., 2004), although their exact functions are still poorly understood. For instance, CBS domains are involved in the gating of osmoregulatory proteins (Biemans-Oldehinkel et al., 2006), in the transport and binding of Mg2+ (Ishitani et al., 2008), in the modulation of intracellular trafficking of chloride channels (Carr et al., 2003), in nitrate transport (De Angeli et al., 2009) and as internal inhibitors of pyrophosphatase activity (Tuominen et al., 2010). Despite their low degree of sequence similarity, CBS motifs share common structural properties, either isolated or as a domain of larger proteins. Interestingly, mutations in other CBS domain-containing proteins have been associated with several hereditary diseases beyond JS, as retinitis pigmentosa, homocystinuria, familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome, myotonia congenital, idiopathic generalized epilepsy, Dent's disease, osteopetrosis and Bartter syndrome (Ignoul and Eggermont, 2005).

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Figure Legends

Fig. 1. Images of the proband (Family A). (A) Slight facial asymmetry, upper third of the face increased and eyes slightly closed due to photophobia. Right (B) and left (C) eye fundus photography revealing duct-retinal atrophy and presence of macula pigmentation. (D) The optical coherence tomography showed areduction of sensorineural retinal thickness in the macula and absence of the outer layer of subwave photoreceptors. (E) The electroretinogram dipcted absence of rod and cone responses. (F) Mixed dentition with hypoplastic amelogenesis imperfecta and restoration in some teeth.

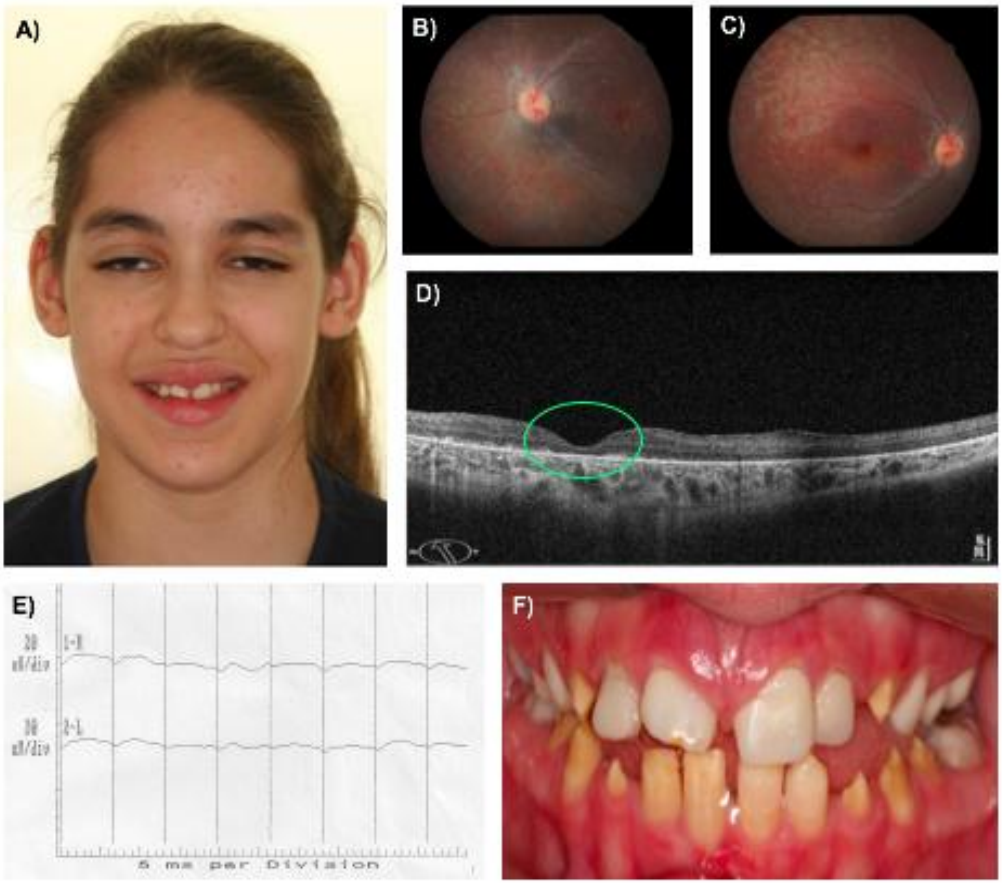
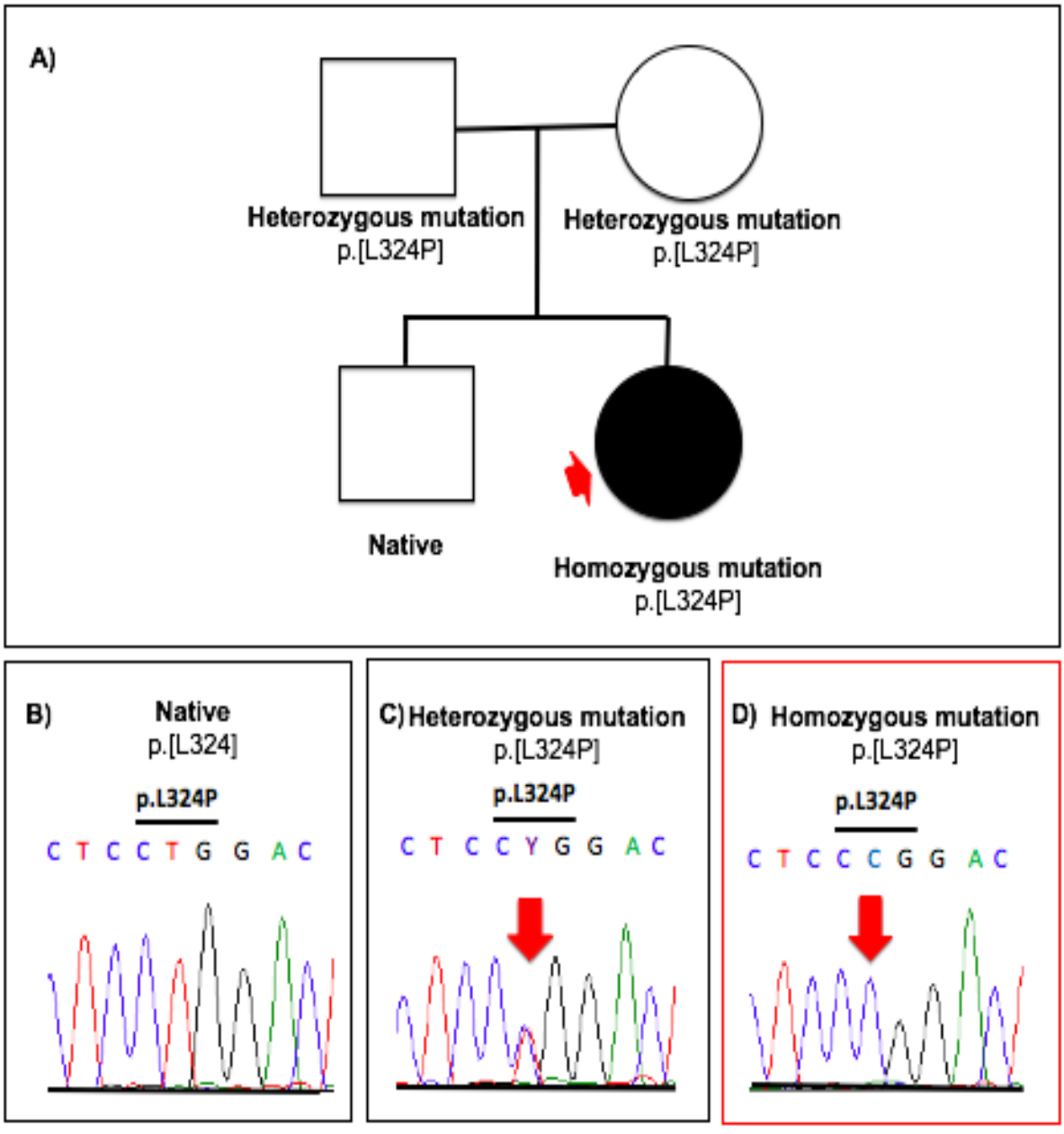
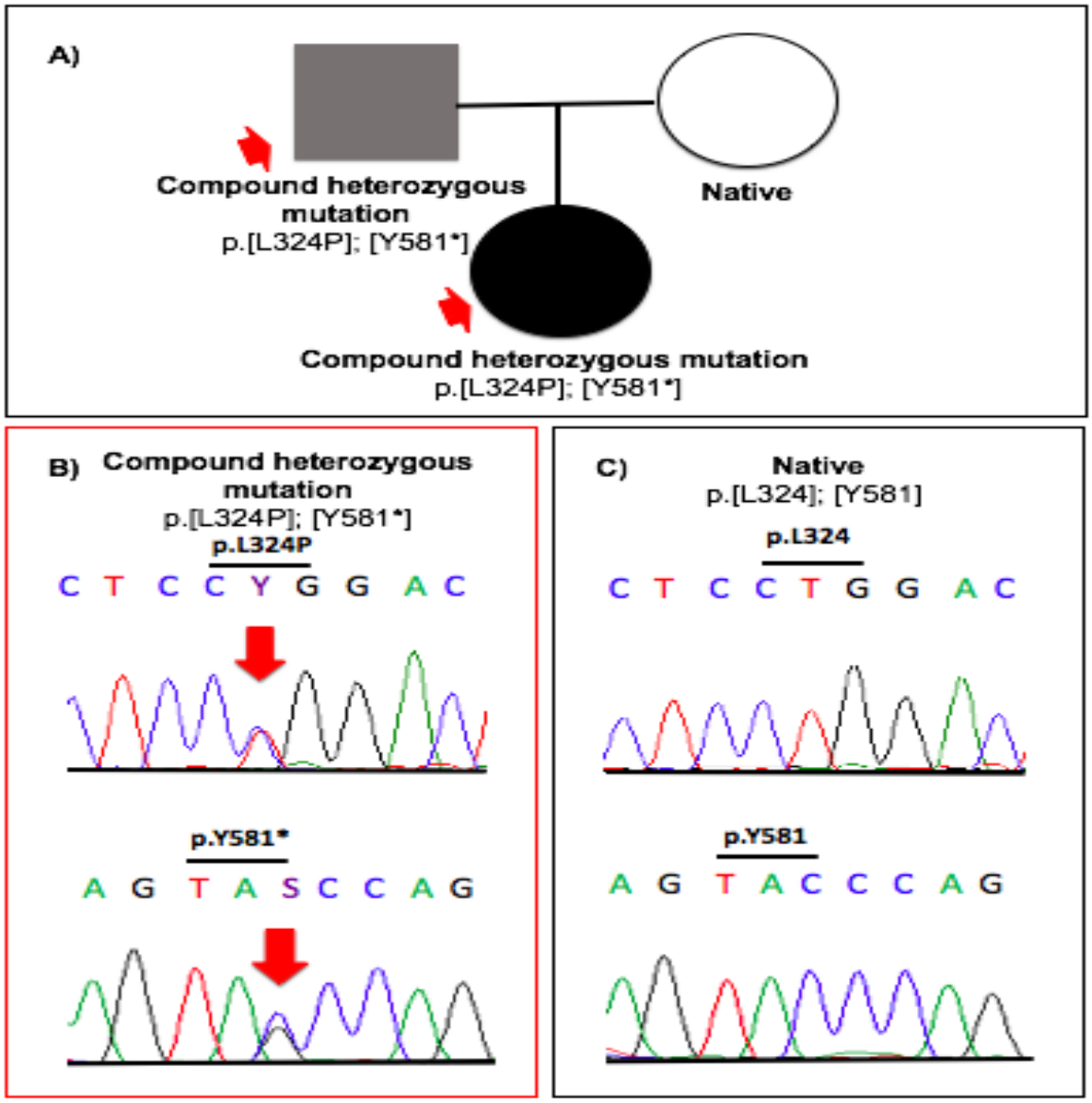


Fig. 2. Images of the proband and his father (Family B). (A) Slight facial asymmetry and upper third of the face increased. Right (B) and left (C) eye fundus photography revealing macular and optic atrophy. (D, E) Electroretinogram showing normal of rod response (D) and absence of cone response (E). (F) Mixed dentition with hypoplastic amelogenesis imperfecta. (G) Photograph of the proband's father revealed strabismus. (H, I) Fundus photograph showing generalized depigmentation with bilateral pale disk in both eyes. (J) Optical coherence tomography showing thickening of the inner nuclear layer near with hyporeflective cysts near the fovea and (K) the ellipsoid zone preserved. (L, M) Optical coherence tomography of optic disc showing diffuse reduction of nerve fiber layer in both eyes. (N) Photograph of mouth showing teeth with intact dental enamel.

Supplementary Figure 1. Family A heredogram and identification of missense mutation in *CNNM4* gene.(A) Pedigree of the studied family. Filled symbols represent affected individuals and open symbols represent unaffected individuals. (B, C, D) Representative electropherogram of DNA sequencing from the proband and her family, showing the proband's brother with native sequence (B), the proband's parents with heterozygous p.L324P (C) and the proband with homozygous p.L324P mutation (D) in *CNNM4*.



Supplementary Figure 2. Family B heredogram and sequence analysis of *CNNM4* gene.(A) Pedigree of the studied family. Black and grey filled symbols represent affected individuals and open symbols represent unaffected individuals. (B) Representative electropherogram of DNA sequencing from the proband and her father with a c.971T>C, CTG>CCG transversion, which results in a p.Leu324Pro (L324P) mutation in the 1 exon and a c.1743C>G, TAC>TAG transversion, with results in a p.Tyr581* (Y581*) mutation in 4 exon of*CNNM4*. (C) The proband's mother showed native sequence.



Supplementary table 1: Primers of *CNNM4* exons.

CNNM4	Primer	Product length	°C*
Exon 1	F1: CCACCTTAAGCGACTGTACC R1: TCAGGTTGCTGGAGATGTTG	513	52
	F2: ATGGCATCATCTTCGTGTCC R2: GCAGCACCGTAATTAGGAGAA	390	57
	F3: CAAGGACTCACTGCTCTTCAT R3: ATTGTGAGGGAGGTGTTGAC	282	59
	F4: ACCTCCCTCACAATCCTTCTA R4: CTCCGTCACCTTCAACATCTC	285	57
	F5: CTTCAACACCATGTCGGAGATA R5: GGGAACTACATCTGGCCTTAC	245	59
Exon 2	F1: CCCATCCTGGTGACTTATGAC R1: CAGCTAGGGACACCAAGTTT	319	57
Exon 3	F1: TCCAGTCTCTTCCTAAGTCCTC R1: GTGACCGTATTTCCACCAAGA	241	57
Exon 4	F1: ACAGCAGTGATCGAGCTTTAG R1: AGCAGGTGGGAGAGAGG	318	59
Exon 5	F1: CCCACTGTCCCTTGTTCCT R1: CATGCTCAGTTCTGGTGTCG	270	57
Exon 6	F1: CTCTCAACTCACAGCCTCAATC R1: GGACTAACAGGTTCCCTCTCT	380	59

*Annealing temperature

5 CONCLUSÕES

- Através das avaliações clínicas, imaginológicas e de fotografias realizadas nas duas famílias, confirmou-se a presença de amelogênese imperfeita do tipo hipoplásica nas duas filhas (Família A e B), sendo este tipo de amelogênese imperfeita já manifestada em outras famílias reportadas. Revelou-se também ausência de amelogênese imperfeita no pai da família B,variação fenotípica ainda não relatada em estudos anteriores, levando a suspeitar de penetrância incompleta no gene *CNNM4*.

- As avaliações oculares realizadas através de exames clínico e oftalmológico, vieram corroborar nos membros afetados das duas famílias (A e B), presença de alterações oculares, caracterizando distrofia de cones e bastonetese presença de lesão macular do tipo 2, alterações já descritas em outras famílias estudadas.

- Os achados obtidos nas avaliações clínicas e genéticas vieram confirmar a presença de mutações no gene *CNNM4* com variação fenotípica-genotípica, sugerindo que os eventos epistaticos, os efeitos ambientais ou a atividade restante da proteína podem modular as variações clínicas e a gravidade da síndrome de Jalili.

- Diante das confirmações oculares, dental e genética, estas duas famílias caracterizam os primeiros casos descritos da síndrome de Jalili no Brasil.

6 CONSIDERAÇÕES FINAIS

- A partir dessa pesquisa, enfatiza-se a importância de trabalho multidisciplinar entre dentista e oftalmologista para diagnóstico adequado da sindrome de Jalili.

- O tratamento odontológico multidisciplinar (endodontia, prótese, dentística, ortodontia) permitirá que os pacientes sintam menos as consequências da amelogênese imperfeita, através de cuidados preventivos, reabilitação estética e funcional, proporcionando melhora da mastigação, redução da hipersensibilidade dentinária, refletindo positivamente na autoestima do paciente. Enquanto o tratamento oftalmológico possibilitará o uso de métodos para que os pacientes possam enxergar com a porcentagem de visão que possui, além de diminuir os efeitos da fotofobia apresentada.

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APÊNDICE

APÊNDICE A - Termo de Consentimento Livre e Esclarecido

Estudo: Correlação fenótipo-genótipo na síndrome de Jalili.

Responsável: Hercílio Martelli Júnior

Antes de aceitar a realização desta pesquisa, é importante a explicação e compreensão sobre os procedimentos propostos. Esta declaração descreve o objetivo, os procedimentos, benefícios, riscos, desconfortos e precauções do estudo. Nenhuma garantia ou promessa pode ser feita sobre os resultados do estudo.

1- Objetivo: Avaliar as características clínicas, imaginológicas e genéticas em uma família acometida pela síndrome de Jalili.

2- Metodologia/procedimentos: O presente estudo será em familiares portadores de alterações clínicas médicas e odontológicas. Serão realizadas avaliações médicas e odontológicas. Os pacientes com alterações dentárias e ou médicas deverão ir a Montes Claros, para realizar exames específicos da visão e radiografias dentárias. Serão realizadas fotografias das regiões que apresentarem alteração. Também será coletado saliva dos pacientes para estudo genético.

3- Justificativa: Em decorrência da raridade e falta de conhecimento médico sobre a síndrome de Jalili e suas repercussões orgânicas, destaca-se a importância de se estudar esta anomalia em todos os seus aspectos, de modo a auxiliar no diagnóstico, tratamento e aconselhamento genético.

4- Benefícios: Diagnóstico, aconselhamento genético e possível tratamento dos pacientes acometidos, além de descobrir novas informações sobre esta síndrome.

5- Desconfortos e riscos: Os pacientes com alterações dentárias e ou médicas deverão ir a Montes Claros, para realizar exames específicos da visão e radiografias dentárias, mas todas as medidas de biossegurança serão tomadas para amenizar os desconfortos e riscos possíveis.

6- Danos: Nenhum dano está previsto. Os participantes desta pesquisa não terão nenhuma despesa com exames e viagens decorrentes desta pesquisa.

7- Metodologia/procedimentos alternativos disponíveis: Nenhum

8- Confidencialidade das informações: Pretende-se divulgar os resultados desta pesquisa no meio científico, mas não incluirão informações e nem imagens que permitam a identificação dos participantes.

9- Compensação/indenização: Nenhum

10 - Consentimento:

Li e entendi as informações precedentes. Tive oportunidade de fazer perguntas e todas as minhas dúvidas foram respondidas a contento. Este formulário está sendo assinado voluntariamente por mim, indicando meu consentimento para a realização desta pesquisa, até que eu decida o contrário. Receberei uma cópia assinada deste consentimento. Qualquer dúvida, poderei entrar em contato com o responsável por esta pesquisa, Hercílio Martelli Junior, através dos telefones (038) 8687-2275 e (38) 3224-8372.

Nome do participante	Data	Assinatura do responsável

Nome da testemunha	Data	Assinatura da testemunha

Nome do coordenador da pesquisa	Data	Assinatura do coordenador da pesquisa

ANEXOS

ANEXO A - Parecer do Comitê de Ética em Pesquisa - Projeto: Correlação fenótipo-genótipo na síndrome de Jalili

Este estudo foi conduzido de acordo com os preceitos determinados pela resolução 466/12 do Conselho Nacional de Saúde do Ministério da Saúde, e pela resolução CFO 179/93 do Código de Ética Profissional Odontológico. Este projeto de pesquisa foi submetido e aprovado pelo Comitê de Ética em Pesquisa da Universidade Estadual de Montes Clarossob parecer nº1.293.651. Além disso, todos os participantes ou responsáveis legais pelo mesmo assinarão um Termo de Consentimento de Esclarecimento Livre (Apêndice A) concordando em participar da investigação científica e serão informados oralmente e em linguagem compreensível dos objetivos e justificativa do estudo, bem como do direito de desistirem da pesquisa a qualquer momento. Medidas protetoras contra radiação serão usadas durante os exames imaginológicos. Serão também garantidos a privacidade, confidencialidade e anonimato das informações coletadas. Somente a partir da obtenção do consentimento, foram realizadas as entrevistas e consultas.

Os menores de idade ficaram sob a responsabilidade de seus pais ou responsáveis, sendo a participação autorizada pelos mesmos.

UNIVERSIDADE ESTADUAL DEMONTES CLAROS -UNIMONTES

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Correlação fenótipo-genótipo na síndrome de Jalili

Pesquisador: LUCIANO SÓLIA NÁSSER

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP)

Versão:4

CAAE: 45864815.3.0000.5146

Instituição Proponente: Universidade Estadual de Montes Claros - UNIMONTES

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER
Número do Parecer: 1.293.651

Apresentação do Projeto:

A pesquisa trata-se de estudo quantitativo e descritivo com o objetivo de avaliar as características clínicas, imaginológicas e genéticas em uma família acometida pela síndrome de Jalili. Serão estudados todos os membros de uma família previamente identificada com características sugestivas

de síndrome de Jalili, residente no município de Porteirinha, localizada no norte de Minas Gerais, a 174 Km de Montes Claros. Os pacientes serão submetidos a avaliações oftalmológicas e odontológicas, além de fotografias. Aqueles com alterações serão encaminhados para avaliações oftalmológicas, odontológicas e tomografias orofaciais. Também será coletada saliva dos pacientes para avaliação genética.

Objetivo da Pesquisa:

Avaliar e descrever as características clínicas, imaginológicas, genéticas e o padrão de herança em uma nova família acometida pela síndrome de Jalili.

Avaliação dos Riscos e Benefícios:

Riscos: Os pacientes com alterações dentárias e ou médicas deverão realizar exames específicos da visão e tomografias dentárias, mas todas as medidas de biossegurança serão tomadas para amenizar os desconfortos e riscos possíveis. Para o exame oftalmológico, serão pingadas 3 gotas de colírio tropicamida em cada olho a fim de dilatar a pupila.

Benefícios: Quanto aos benefícios, informações de diagnóstico, aconselhamento genético e tratamento dos pacientes acometidos, com correção dos erros de refração com óculos feitos por ofalmologista treinado em baixa visão e reabilitação da estética dentária com cirurgião-dentista experiente em amelogênese imperfeita.

Maior conhecimento acerca da síndrome.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante acerca da síndrome de Jalili.

Considerações sobre os Termos de apresentação obrigatória:

Adequados.

Recomendações:

Apresentação de relatório final por meio da plataforma Brasil, em "enviar notificação".

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Considerações Finais a critério do CEP:

O projeto respeita os preceitos éticos da pesquisa em seres humanos, sendo assim somos favoráveis à aprovação do mesmo.

Endereço: Av. Dr. Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089 **UF:** MG**Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

UNIVERSIDADE ESTADUAL DEMONTES CLAROS – UNIMONTES

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_526455.pdf	21/09/2015 13:22:59		Aceito
Folha de Rosto	Folha_de_rosto.pdf	21/09/2015 13:22:21	LUCIANO SÓLIA NÁSSER	Aceito
Outros	Termo de assentimento S. Jalili- CEP-digitalizado Násse-projeto S. Jalili sem Rx 09-08-15.pdf	09/08/2015 17:53:40		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo de consentimento livre e esclarecido- projeto S. Jalili 2015 sem Rx 09-08-15.pdf	09/08/2015 17:51:45		Aceito
Projeto Detalhado / Brochura Investigador	Projeto sindrome de Jalili 2015- Célia Maia- word 3ª correção sem Rx.docx	09/08/2015 17:50:39		Aceito
Outros	Fonte de recursos projeto S. Jalili-CEP 2015 igitalizado.pdf	27/05/2015 22:34:42		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

MONTES CLAROS, 23 de Outubro de 2015

_____

Assinado por:

Ana Augusta Maciel de Souza

(Coordenador)

ANEXO B – Ficha de exame odontológico

Unimontes	**PRONTUÁRIO ODONTOLÓGICO - EXAME SEMIOLÓGICO**		
1. Identificação		**Observação Importante**	
1.1. Identificação do Paciente			
Nome:			
Sexo: M ( ) F ( )	Data de Nascimento://	Idade:	Telefone: ( )
Rua:			Bairro:Vila Guará
Cidade:		Procedência:( ) Zona Rural ( ) Zona Urbana	
Naturalidade:		Nacionalidade:	
Pai:		Mãe:	
Local de Trabalho:		Ocupação:	
1.2. Identificação do Aluno			
Nome:			
Disciplina:	Período:	Data:/ /	
Professor(es) responsável(eis):			
2. Anamnese			
Queixa principal ou motivo da consulta (Q.P.):			
História da moléstia atual: (H.M.A):			

Experiências anteriores de tratamento odontológico (sim ou não, último tratamento, tipo, problemas com anestesia, aceitação):
Hábitos: (fumo, bebida alcoólica, higiene bucal, alimentares, drogas ilícitas, onicofagia, etc):

Centro de Ciências Biológicas e da Saúde **Departamento de Odontologia- ☎3229:8020 e 3229:8294**

O&M – 11.01

Estado emocional e psíquico: (stress, ansiedade, depressão, psicose, etc):
Alergias:
Sofre de distúrbios cardiovasculares:Sim ( ) Não ( )
Alteração Pressão arterial?
Portador de prótese arterial?
Portador de prótese cardíaca?
- Outros:
Alterações circulatórias:
Sofre de algum distúrbio sangüíneo? Anemia ( ) Coagulopatia ( ) Outros:
Apresenta histórico de febre reumática? Sim ( ) Não ( )
Sofre de algum distúrbio respiratório? Sim ( ) Não ( )
Bronquite: Asma:
- Outros:
Sofre de algum distúrbio gastrointestinal?
Gastrite: Úlcera:
Hepatite: Cirrose:
Sofre de algum distúrbio renal? Sim ( ) Não ( )
- Qual?
Você é diabético? Sim ( ) Não ( )

Teve ou tem doença infecto-contagiosa? Sim ( ) Não ( )
- Qual?
Qual é o seu tipo sangüíneo?
História Pregressa Pessoal (HP)
Você está em tratamento médico, ou fez tratamento nos últimos 12 meses (época e motivo)?
Uso atual de medicamentos ou nos últimos 12 meses:
História Familiar
Pais (vivos ou não, causa mortis):
Número de irmãos:
Irmãos falecidos (número e causa):
Doenças comuns na família: Diabetes Melitus ( ) Hipertensão Arterial ( ) Câncer ( )
- Outras:
Doenças contagiosas:Sim ( ) Não ( ) Qual?
História Social(Moradia, Trabalho, Alimentação, Saneamento)

Outras informações:	Polegar
Assinatura do Paciente:	
3. Exame Físico	
EXAME OBJETIVO GERAL	
P.A: Temp.: Pulso:	
Respiração: Fácies: Cor da Pele:	
EXAME OBJETIVO ESPECIAL	
ATM:	
Linfonodos:	
Lábios:	
Mucosas:	
Soalho Bucal:	
Palato:	
Orofaringe:	
Glândulas e Ductos Salivares:	
Língua:	
Gengiva:	
Cálculo sub ou supra:	
Higiene Bucal:	
Hábitos Nocivos:	
Portador de Aparelho Protético ou Ortodôntico:	

Oclusão:
Alterações Encontradas (Local e Descrição):dentes amarelados com muita sensibilidade, ausência de esmalte:
OBSERVAÇÕES:
1ª REVISÃO SEMIOLÓGICA:
Ao ser revista a história clínica do paciente e após exame físico e avaliação dos resultados dos exames complementares foram observadas as seguintes alterações:

ALTERAÇÕES CLÍNICAS ENCONTRADAS

DENTE	**ALTERAÇÃO CLÍNICA**
18	
17	
16	
15 – 55	
14 – 54	
13 – 53	
12 – 52	
11 – 51	
21 – 51	
22 – 62	
23 – 63	
24 – 64	
25 – 65	

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35 – 75	
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33 – 73	
32 – 72	
31 – 71	
41 – 81	
42 – 82	
43 – 83	
44 – 84	
45 – 85	
46	
47	
48	
Exames complementares pedidos:	

<table>
<tr><td colspan="4">Exames radiológicos:</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">Outros exames:</td></tr>
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<tr><td colspan="4"></td></tr>
<tr><td rowspan="2">Data:
___/09___/_2014_____</td><td>Aluno Examinador:</td><td rowspan="2">Professor(es):</td><td></td></tr>
<tr><td>Aluno Auxiliar:</td><td></td></tr>
</table>

ANEXO C- Normas para publicação do periódico European Journal of Medical Genetic.

EUROPEAN JOURNAL OF MEDICAL GENETICS

DESCRIPTION

The *European Journal of Medical Genetics* (EJMG) is a peer-reviewed journal that publishes articles in English on various aspects of **human** and **medical genetics** and of the **genetics** of experimental models.

Original clinical and experimental research articles, short clinical reports, review articles and letters to the editor are welcome on topics such as :

- Dysmorphology and syndrome delineation
- Molecular genetics and molecular cytogenetics of inherited disorders
- Clinical applications of genomics and nextgen sequencing technologies
- Syndromal cancer genetics
- Behavioral genetics
- Community genetics
- Fetal pathology and prenatal diagnosis
- Genetic counseling

AUDIENCE

Researchers, Geneticists, Cytogeneticists.

IMPACT FACTOR

2016: 2.137 © Thomson Reuters Journal Citation Reports 2017

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INTRODUCTION

BEFORE YOU BEGIN

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Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

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You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

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To verify originality, your article may be checked by the originality detection software iThenticate. See also:

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Categories of manuscript

The following categories of articles can be proposed to European Journal of Medical Genetics:

Editorial comments: These manuscripts are solicited by the Editors.

Review: Authors are advised to discuss review proposals with the Editors before submitting it. There is no specific format for reviews.

Experimental research: Results of experimental investigations related to medical genetics, such as cell biology and animal models of human disorders, technology-oriented research, bioinformatics research... In experimental research articles, the experimental data are the most important elements of the work.

Clinical research: Results of original works on any aspects of Medical Genetics, including: delineation of new syndromes with demonstrated genetic, genomic cause reports of series of patients, reports on biochemical, genetic or genomic data in relation with patient(s), exome based studies with functional data, genetic epidemiology or association studies for monogenetic or genomic disorders and developmental anomalies, In clinical research articles, the experimental data are the support to clinical investigations.

Clinical Reports: Report of significant new findings in a single patient, a single pedigree, or a small series of patients with a known disorder.

Exome Reports: Report of abnormal exome sequence results usually found in a single pedigree (trio or sibship), with their associated clinical data, including cases for which functional studies are not possible. An Exome Report typically covers patients with well described, recognizable phenotype (known entities or "new" syndromes) and one or several candidate sequence variations predicted to be pathogenic, patients with a known clinical diagnosis yet unexpected NGS results. The main goal of this section is to allow rapid dissemination of possible genotype-phenotype correlations without the necessity to have strong functional evidences, or to wait for data replication.
Genetic forum: The manuscripts of this section may address the practice of medical genetics, and all its correlates: ethics, social issues, organization, perspectives... or propose innovative hypotheses on any topics related to human genetics there is no specific format for Genetic forum articles.
Letter to the Editor: Letter to Editor provides a mean of communication between the authors and readers. Although not original research per se, a Letter may provide new insight, make corrections, offer alternate theories, or request clarification about content printed in the journal. An author can answer to a Letter by the same mean.

Dissemination of information

Rare/unreported genomic aberrations: Rare variants detected by array technologies, should be entered whenever possible in one of the existing databases for chromosomal aberrations (for instance: DECIPHER, ClinVar or ECARUCA). Please mention the accession number in your manuscript. Entering gene variations in one of the existing databases prior to acceptance is mandatory. Most database include a possibility to submit result and to keep them hidden till publication of the article, allowing mutation to remain confidential during reviewing process.
Rare/unreported sequence variations All sequence variations should be submitted to a general database (for example: ClinVar, dbSNP or to a locus-specific database. Directories of locus-specific databases are available at HGVS or LSDB).This submission is necessary for novel and for confirmatory data : it is important that all unpublished data is submitted to public databases. The manuscript should indicate to which public database the data of the study were submitted, and include, when possible, the accession numbers. The information must be inserted in the Results section. When accession numbers for large number of variations must be listed, this information should be added as Supplementary online material.

New submissions

Online submission Manuscripts should be submitted via the journals online submission page at https://www.evise.com/profile/api/navigate/EJMG. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail. Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. It is important that the file be saved in the native, editable format of the word processor used. The system automatically converts your files to a single PDF file, which is used in the peer-review process. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication).
We recommend to use whenever possible the formatting rules that are described below. To avoid unnecessary errors, you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor. Tables may be included in the manuscript of submitted in separate files. Figures are submitted as separate files. Captions of Tables and Figures are included in the main file.
Layout: Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. Do not use the word processor options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very like that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). See also the section on Electronic artwork.
Typing: Manuscript are typed with 11 or 12 fonts, with double spaced lines. The text should be in single-column format.. Pages must be numbered. Lines are not numbered (this will be done automatically when the pdf submission is created).
Headings and subheadings: Use caps letters only when necessary (first letter of the title, first letter of family names, acronyms and other commonly accepted abbreviations. You may use bigger or bold font for readability. Headings and subheadings are not numbered.
Figures and Tables: They are consecutively numbered in Arabic numerals, following the order of appearance in the text, and should be referred to in the text by their number (Fig. 1; Table 1).
Style preferences: The preferred spelling for a word is its American usage. English usage is acceptable but must be consistent in the manuscript.

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Nomenclature and terminology

Genetic nomenclature:
Description of genes and proteins must fit with to the official HGNC (HUGO nomenclature committee) gene symbols. Typographic rules for gene and protein symbols: human gene symbols are italicized, with all letters in uppercase (e.g., SHH, for sonic hedgehog) and follow the HUGO Gene Nomenclature Committee guidelines. Protein designations are not italicized and all letters are in uppercase (SHH). For other model organisms, refer to species-specific nomenclature standards. See Wain HM et al. Guidelines for Human Gene Nomenclature. Genomics 2002, 79:464- 470. Cytogenetic variations are reported following the most recent International System for Human Cytogenetic Nomenclature (ISCN 2013). Always mention the reference genome when reporting array data with nucleotide positions. All mutations and variations must be described at DNA-level. When descriptions at RNA or protein level are given in the text, including Title and Abstract. All mutations and variations must be reported in accordance with the online recommendations of the Human Genome Variation Society(HGVS: http://varnomen.hgvs.org/) and use the free access Mutalyzer website(https://www.mutalyzer.nl) to check the syntax you used for variant description.
Genomic DNA-level: Always use the g.prefix.If you present personal data, always mention the genome of reference.
Coding DNA-level Always use the c.prefix.For personal data, the DNA reference sequence from the RefSeq database must be mentioned with both database accession and version number (example: NM_001199954.1)

Protein level Always use the p. prefix and three letter AA code (example: p.Lys76Asn).Descriptions at protein level may only be given in addition to a description at DNA level. Genbank protein reference sequence must be mentioned (example: AAA51567.1 or NP_001186883.1) Mention clearly in the text when the description at protein level is given without any experimental evidence (i.e. no RNA nor protein analysis). Alternatively, in absence of experimental evidence, you may use HGVS recommendation and describe protein variation between brackets, for example: p.(Arg22Ser).
See detailed recommendations in Taschner and Den Dunnen, Hum Mutat 2011 32:507-511. and the updates on HGVS website.
Descriptive terminology for dysmorphology: If the manuscript reports specific morphological features of human subjects or other developemental anomalies, authors should use the Human Phenotype Ontology (HPO) which provides a structured, comprehensive and well-defined set of descriptors. Alternatively, for craniofacial features, hands and/or feet, the authors may use the Elements of Morphology: Standard Terminology (Am J Med Genet 2009, 149A (1) : 1-127).

Clinical description of patients

The term "case report" is not accepted, and patient should not be identified by their initials or file number. Patients described in a manuscript should be regarded with sensitivity. Stigmatizing terms should be avoided. As often as possible, refer to pictures with relevant features : facial dysmorphism is better illustrated by a picture than by an extensive description. Please provide sex, ethnicity, parental age, biometry at birth and gestational age, biometry at last investigation. For each biometric value, give the correspondant z-score in standard deviations give pertinent family information.

General organization of a manuscript

Layout:
A manuscript must be provided to EJMG as one single word processor document. Figures are submitted separately. Each manuscript generally contain the following sections, in this order.
Title page: Includes title, authors, affiliation, corresponding author
Abstract
Keywords

Main Text: in articles, it is divided by several headings and subheadings:(see below for templates specific to each type of manuscript)
Acknowledgments
Accession numbers (if any)
Figure Titles and Legends
Tables(with a title and legend for each)
References
Supplemental data:(if any):A list of the titles of each file included submitted as Supplemental Data (this section do not include the supplemental data themselves).
Title: Keep it concise and focus the main message. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
Authors and affiliation:
Names: First forename is given in extenso, followed if necessary by the initials of other forenames, and by the family name. When there is more than one affiliation, the author's family name is followed by one or several superscript letters indicating their affiliation (John R. Doea,b,,). Degrees or titles are not mentioned. The superscript letters do not have to be sequentially ordered, to allow more structured affiliation section (see next paragraph).
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Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

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