VNIVERSITAT DE VALÈNCIA

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IDENTIFICATION OF MOLECULAR AND GENETIC VARIATIONS UNDERLYING SKIN DISEASES

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“Caminante, no hay camino, se hace camino al andar.”

— **Antonio Machado**

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RESUMEN

Las enfermedades de la piel afectan hasta al 70% de la población mundial, representando una carga significativa para los sistemas de salud a nivel global (Hay et al., 2014). Aunque algunas patologías dermatológicas han sido ampliamente estudiadas, muchas permanecen aún poco comprendidas, lo que limita de manera importante el desarrollo de diagnósticos precisos y de tratamientos efectivos. Esta falta de terapias adecuadas repercute directamente en la calidad de vida de los pacientes, ya que la diversidad etiológica de estas enfermedades —que abarca desde condiciones infecciosas, oncológicas y autoinmunes hasta patologías genéticas— genera síntomas que pueden ser intensamente molestos, como picor, dolor, irritación e inflamación. A ello se añaden efectos psicológicos y sociales derivados de la visibilidad de las lesiones cutáneas, que frecuentemente afectan la autoestima, la imagen corporal y las relaciones interpersonales, aumentando el riesgo de ansiedad, depresión y aislamiento social (Verhoeven et al., 2007).

Más allá de los síntomas locales, muchas enfermedades dermatológicas presentan implicaciones sistémicas y pueden estar relacionadas con patologías más graves, como trastornos metabólicos o autoinmunes. Esta interrelación complejiza aún más su abordaje clínico y pone de relieve la necesidad de adoptar un enfoque integral que contemple tanto las manifestaciones cutáneas como las repercusiones generales sobre la salud (Sampaio et al., 2021). En consecuencia, resulta imprescindible profundizar en la comprensión de los mecanismos moleculares y genéticos implicados en estas enfermedades, con el objetivo de desarrollar terapias más eficaces y, potencialmente, soluciones definitivas (Roso-Mares et al., 2024). Bajo esta premisa, el objetivo central de la presente tesis doctoral es identificar variaciones moleculares y genéticas relevantes en patologías dermatológicas raras, aportando conocimiento innovador a un campo todavía en expansión.

En este contexto, una primera parte del trabajo se centró en revisar el papel emergente de los RNAs no codificantes (ncRNAs) en dermatología, prestando especial atención a los microRNAs (miRNAs) y a los long non-coding RNAs (lncRNAs). Estos elementos regulatorios han demostrado contribuir de manera decisiva a la biología cutánea, modulando procesos

fundamentales como la proliferación, diferenciación y apoptosis celular, así como la cicatrización y las respuestas inflamatorias. El análisis de la literatura científica evidenció que los ncRNAs actúan como biomarcadores y firmas moleculares de gran utilidad, capaces de mejorar el diagnóstico, predecir el pronóstico y monitorizar la respuesta a los tratamientos. Asimismo, se identificaron ejemplos en los que funcionan como dianas terapéuticas en distintos contextos patológicos, como el cáncer cutáneo, las enfermedades inflamatorias (psoriasis y dermatitis atópica), los procesos fibróticos y la cicatrización anómala. Una de sus ventajas clínicas más prometedoras radica en que pueden detectarse en fluidos biológicos como sangre, saliva u orina, lo que los convierte en biomarcadores no invasivos de gran valor. Esta revisión permitió concluir que los ncRNAs poseen un potencial transformador en dermatología, tanto en el avance del conocimiento de la fisiopatología de la piel como en el desarrollo de nuevas terapias dirigidas, aunque persisten retos relacionados con su especificidad, entrega y tolerabilidad en la práctica clínica.

La segunda parte del trabajo se focalizó en un caso clínico excepcional que motivó una investigación experimental. Se trató de una paciente diagnosticada con una malformación glomovenosa (GVM) extensa en distribución blaschkoide asociada a polidactilia, una asociación clínica no descrita con anterioridad en la literatura. El análisis genético permitió identificar una mutación germinal en el gen GLMN (c.515C>A, p.Ser172Ter), conocida por su papel en la fisiopatología de las GVM, junto con una mutación somática poco frecuente en CCM2L (c.1505G>A, p.Arg502His), presente únicamente en el tejido afectado. Este hallazgo planteó la hipótesis de que CCM2L podría actuar como un modificador genético de la enfermedad, influyendo en la severidad y en las características fenotípicas observadas.

Para comprobar esta posibilidad, se realizaron estudios funcionales en células endoteliales humanas (HUVECs). Los experimentos demostraron que la variante R502H de CCM2L afecta la capacidad de la proteína para regular la vía MAPK/ERK, lo que se tradujo en un incremento significativo de la proliferación celular. Además, cuando se combinaron alteraciones en GLMN y CCM2L, se observó un efecto aditivo sobre el crecimiento celular, reforzando la hipótesis de una interacción funcional entre ambos genes en la patogénesis de la enfermedad.

En conjunto, estos resultados no solo amplían el espectro clínico y genético de las malformaciones glomovenosas, sino que también sugieren un papel de CCM2L como modulador de la expresión fenotípica en estas patologías raras. De este modo, la presente investigación contribuye con un hallazgo innovador sobre el mecanismo molecular implicado en una enfermedad rara genética como son las GVM y establece una base sólida para el diseño de futuros estudios orientados al desarrollo de nuevas estrategias diagnósticas y terapéuticas.

SUMMARY

Skin diseases affect up to 70% of the global population, representing a significant burden on healthcare systems worldwide (Hay et al., 2014). Although some dermatological conditions have been extensively studied, many remain poorly understood, which greatly limits the development of accurate diagnoses and effective treatments. This lack of adequate therapies directly impacts patients' quality of life, since the etiological diversity of these conditions—ranging from infectious, oncological, and autoimmune disorders to genetic pathologies—produces intensely distressing symptoms such as itching, pain, irritation, and inflammation. Added to this are the psychological and social effects derived from the visibility of skin lesions, which often affect self-esteem, body image, and interpersonal relationships, thereby increasing the risk of anxiety, depression, and social isolation (Verhoeven et al., 2007).

Beyond local symptoms, many dermatological diseases present systemic implications and may be associated with more severe conditions, such as metabolic or autoimmune disorders. This interrelationship further complicates their clinical management and highlights the need for a comprehensive approach that considers both cutaneous manifestations and their broader health consequences (Sampaio et al., 2021). Consequently, it is essential to deepen our understanding of the molecular and genetic mechanisms underlying these diseases, with the goal of developing more effective therapies and, potentially, definitive solutions (Roso-Mares et al., 2024). Under this premise, the central objective of this doctoral thesis is to identify relevant molecular and genetic variations in rare dermatological disorders, contributing innovative knowledge to a field that is still expanding.

In this context, the first part of the thesis focused on reviewing the emerging role of non-coding RNAs (ncRNAs) in dermatology, with particular attention to microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). These regulatory elements have been shown to play a decisive role in skin biology, modulating fundamental processes such as cell proliferation, differentiation, and apoptosis, as well as wound healing and inflammatory responses. A review of the scientific literature revealed that ncRNAs act as highly useful biomarkers and molecular signatures, capable of improving diagnosis, predicting prognosis, and monitoring treatment response. Moreover, examples have been identified in which they serve as therapeutic targets in various

pathological contexts, such as skin cancer, inflammatory diseases (psoriasis and atopic dermatitis), fibrotic processes, and abnormal wound healing. One of their most promising clinical advantages lies in their detectability in biological fluids such as blood, saliva, or urine, making them valuable non-invasive biomarkers. This review concluded that ncRNAs hold a transformative potential in dermatology, both for advancing our understanding of skin pathophysiology and for developing new targeted therapies, although challenges remain regarding their specificity, delivery, and clinical tolerability.

The second part of the work focused on an exceptional clinical case that motivated an experimental investigation. It involved a patient diagnosed with an extensive blaschkoid glomuvenous malformation (GVM) associated with polydactyly, a clinical association not previously reported in the literature. Genetic analysis identified a germline mutation in the GLMN gene (c.515C>A, p.Ser172Ter), known for its role in GVM pathophysiology, together with a rare somatic mutation in CCM2L (c.1505G>A, p.Arg502His), found exclusively in the affected tissue. This discovery raised the hypothesis that CCM2L might act as a genetic modifier of the disease, influencing its severity and phenotypic characteristics.

To test this possibility, functional studies were conducted in human umbilical vein endothelial cells (HUVECs). The experiments demonstrated that the R502H variant of CCM2L impairs the protein's ability to regulate the MAPK/ERK pathway, resulting in a significant increase in cell proliferation. Furthermore, when GLMN and CCM2L alterations were combined, an additive effect on cell growth was observed, reinforcing the hypothesis of a functional interaction between both genes in the pathogenesis of the disease.

Taken together, these findings not only expand the clinical and genetic spectrum of GVMs but also suggest a role for CCM2L as a modulator of phenotypic expression in these rare disorders. In this way, the present research contributes an innovative finding on the molecular mechanism involved in a rare genetic disease such as GVM and establishes a solid foundation for the design of future studies aimed at developing new diagnostic and therapeutic strategies.

RESUM

Les malalties de la pell afecten fins al 70% de la població mundial, representant una càrrega significativa per als sistemes de salut a escala global (Hay et al., 2014). Encara que algunes patologies dermatològiques han sigut àmpliament estudiades, moltes continuen poc compreses, la qual cosa limita de manera important el desenvolupament de diagnòstics precisos i de tractaments efectius. Aquesta falta de teràpies adequades repercutix directament en la qualitat de vida dels pacients, ja que la diversitat etiológica d'aquestes malalties —que abasta des de condicions infeccioses, oncològiques i autoimmunes fins a patologies genètiques— genera símptomes que poden ser intensament molestos, com picor, dolor, irritació i inflamació. A això s'afegixen efectes psicològics i socials derivats de la visibilitat de les lesions cutànies, que sovint afecten l'autoestima, la imatge corporal i les relacions interpersonals, incrementant el risc d'ansietat, depressió i aïllament social (Verhoeven et al., 2007).

Més enllà dels símptomes locals, moltes malalties dermatològiques presenten implicacions sistèmiques i poden estar relacionades amb patologies més greus, com trastorns metabòlics o autoimmunes. Aquesta interrelació encara més complexitza el seu abordatge clínic i posa de relleu la necessitat d'adoptar un enfocament integral que contemple tant les manifestacions cutànies com les repercussions generals sobre la salut (Sampaio et al., 2021). En conseqüència, resulta imprescindible aprofundir en la comprensió dels mecanismes moleculars i genètics implicats en aquestes malalties, amb l'objectiu de desenvolupar teràpies més eficaces i, potencialment, solucions definitives (Roso-Mares et al., 2024). Sota aquesta premissa, l'objectiu central de la present tesi doctoral és identificar variacions moleculars i genètiques rellevants en patologies dermatològiques rares, aportant coneixement innovador a un camp encara en expansió.

En aquest context, una primera part del treball es va centrar a revisar el paper emergent dels RNAs no codificants (ncRNAs) en dermatologia, amb especial atenció als microRNAs (miRNAs) i als long non-coding RNAs (lncRNAs). Aquests elements reguladors han demostrat contribuir de manera decisiva a la biologia cutània, modulant processos fonamentals com la proliferació, diferenciació i apoptosi cel·lular, així com la cicatrització i les respostes inflamatories. L'anàlisi de la literatura científica va evidenciar que els ncRNAs actuen com a biomarcadors i firmes

moleculars de gran utilitat, capaços de millorar el diagnòstic, predir el pronòstic i monitorar la resposta als tractaments. Així mateix, es van identificar exemples en què funcionen com a dianes terapèutiques en diversos contextos patològics, com el càncer cutani, les malalties inflamatòries (psoriasi i dermatitis atòpica), els processos fibròtics i la cicatrització anòmala. Un dels seus avantatges clínics més prometedors radica en què poden detectar-se en fluids biològics com sang, saliva o orina, la qual cosa els convertix en biomarcadors no invasius de gran valor. Aquesta revisió va permetre concloure que els ncRNAs posseïxen un potencial transformador en dermatologia, tant en l'avenç del coneixement de la fisiopatologia de la pell com en el desenvolupament de noves teràpies dirigides, encara que persisteixen reptes relacionats amb la seua especificitat, entrega i tolerabilitat en la pràctica clínica.

La segona part del treball es va focalitzar en un cas clínic excepcional que va motivar una investigació experimental. Es tractava d'una pacient diagnosticada amb una malformació glomuvenosa (GVM) extensa en distribució blaschkoide associada a polidactília, una associació clínica no descrita amb anterioritat en la literatura. L'anàlisi genètic va permetre identificar una mutació germinal en el gen GLMN (c.515C>A, p.Ser172Ter), coneguda pel seu paper en la fisiopatologia de les GVM, juntament amb una mutació somàtica poc freqüent en CCM2L (c.1505G>A, p.Arg502His), present únicament en el teixit afectat. Aquest descobriment va plantejar la hipòtesi que CCM2L podria actuar com un modificador genètic de la malaltia, influint en la severitat i en les característiques fenotípiques observades.

Per a comprovar aquesta possibilitat, es van realitzar estudis funcionals en cèl·lules endotelials humanes (HUVECs). Els experiments van demostrar que la variant R502H de CCM2L afecta la capacitat de la proteïna per a regular la via MAPK/ERK, la qual cosa es va traduir en un increment significatiu de la proliferació cel·lular. A més, quan es van combinar alteracions en GLMN i CCM2L, es va observar un efecte additiu sobre el creixement cel·lular, reforçant la hipòtesi d'una interacció funcional entre ambdós gens en la patogènesi de la malaltia.

En conjunt, aquests resultats no sols amplien l'espectre clínic i genètic de les malformacions glomuvenoses, sinó que també suggerixen un paper de CCM2L com a modulador de l'expressió fenotípica en aquestes patologies rares. D'aquesta manera, la present investigació contribuïx amb una troballa innovadora sobre el mecanisme molecular implicat en una malaltia rara

genètica com són les GVM i establix una base sòlida per al disseny de futurs estudis orientats al desenvolupament de noves estratègies diagnòstiques i terapèutiques.

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RESUMEN DE LA TESIS DOCTORAL

IDENTIFICACIÓN DE LAS VARIACIONES MOLECULARES Y GENÉTICAS DE LAS ENFERMEDADES DE LA PIEL

Introducción

Las enfermedades cutáneas afectan a entre uno y dos tercios de la población mundial, lo que las convierte en algunas de las afecciones más prevalentes en la salud humana. Más allá de su alta incidencia, las enfermedades dermatológicas imponen una carga significativa tanto a los pacientes como a los sistemas sanitarios. A menudo se manifiestan con lesiones visibles que no solo provocan síntomas físicos como prurito, dolor, inflamación e irritación crónica, sino que también generan profundos efectos psicosociales, incluyendo disminución de la autoestima, ansiedad, depresión y aislamiento social.

La complejidad de las enfermedades cutáneas radica en la diversidad de sus causas, que abarcan desde trastornos infecciosos, autoinmunes y neoplásicos hasta síndromes hereditarios. Además, muchas enfermedades dermatológicas presentan asociaciones sistémicas, vinculando las manifestaciones cutáneas con patologías más amplias, como trastornos metabólicos o autoinmunes. Estas características subrayan la importancia de investigar los mecanismos moleculares y genéticos que subyacen a las enfermedades de la piel, con el objetivo último de mejorar su diagnóstico, pronóstico y tratamiento.

El avance de la biología molecular y de las tecnologías genómicas de alto rendimiento ha abierto nuevas vías para descifrar las complejas redes que regulan la fisiología y la patología cutáneas. Entre estos descubrimientos, los ARN no codificantes (ncRNA), que anteriormente se consideraban “ruido” transcripcional, han emergido como moléculas reguladoras clave. Aunque solo el 2% del genoma humano codifica proteínas, se han identificado más de 27 000 ncRNAs, muchos de los cuales participan en procesos directamente relevantes para la dermatología: diferenciación epidérmica, cicatrización, fibrosis, respuestas inflamatorias y oncogénesis.

Los microARNs (miRNAs) y los ARN largos no codificantes (lncRNAs) han demostrado ejercer profundos efectos reguladores, modulando la expresión génica tanto a nivel transcripcional como postranscripcional. Su capacidad para actuar como biomarcadores e incluso como dianas terapéuticas los sitúa a la vanguardia de la dermatología de precisión. Es especialmente destacable su estabilidad en fluidos biológicos y sus patrones de expresión específicos de tejido, que los convierten en candidatos prometedores para diagnósticos no invasivos.

No obstante, si bien la investigación en ncRNAs ha transformado el marco conceptual de la dermatología, también ha puesto de relieve importantes desafíos para su traslación clínica. Entre ellos destacan la necesidad de garantizar la especificidad terapéutica, diseñar sistemas de liberación eficaces para tratamientos basados en ARN y minimizar los efectos inmunológicos adversos. Su potencial resulta innegable: desde el melanoma hasta la psoriasis, y desde la dermatitis atópica hasta las enfermedades cutáneas fibróticas, los ncRNAs aportan nuevas perspectivas sobre los mecanismos patogénicos y abren la puerta a estrategias terapéuticas innovadoras y dirigidas.

Dentro de este panorama más amplio, las malformaciones glomuvenosas (GVMs) representan un modelo ideal para explorar la intersección entre genética y dermatología clínica. Las GVMs son anomalías vasculares raras, generalmente congénitas, caracterizadas por canales venosos anómalos y cúmulos de células glómicas. Aunque benignas, pueden ser desfigurantes y dolorosas, afectando de forma significativa la calidad de vida. Tradicionalmente mal clasificadas como glomangiomas, las GVMs se reconocen hoy como una entidad clínica distinta con una base genética única.

El descubrimiento de mutaciones en el gen glomulin (GLMN) estableció los fundamentos de la genética molecular de las GVMs. Sin embargo, la frecuente variabilidad en la gravedad clínica, incluso entre individuos portadores de la misma mutación en GLMN, sugiere que deben existir modificadores adicionales que influyen en la expresión de la enfermedad. Esta observación plantea preguntas cruciales: ¿podrían otros genes cooperar con GLMN para definir el fenotipo de las GVMs? Y, de ser así, ¿podrían estos nuevos actores revelar nuevas dianas terapéuticas?

A partir de esta premisa, la hipótesis central de la tesis plantea que, además de las mutaciones en GLMN, podrían existir alteraciones somáticas en genes reguladores de la función endotelial

—como CCM2L— que actúan como modificadores del fenotipo, contribuyendo a la variabilidad clínica y al incremento de la proliferación celular observado en las GVMs.

La presente tesis se concibe para poner a prueba esta hipótesis. Integrando una revisión sistemática, el análisis genético molecular y ensayos funcionales experimentales, este trabajo busca identificar tanto contribuyentes moleculares conocidos como nuevos en las enfermedades cutáneas, particularmente en las

A través de este enfoque integrador, la tesis demuestra no solo el papel central de GLMN, sino también la implicación de CCM2L (Cerebral Cavernous Malformation 2-Like), un gen no vinculado previamente a las GVMs. El descubrimiento de la mutación CCM2L R502H en tejido afectado, y su efecto demostrado sobre la proliferación endotelial a través de la vía MAPK/ERK, amplía la etiología genética de las GVMs más allá de glomulin. Este hallazgo representa un avance significativo en la comprensión de la patogénesis molecular de las malformaciones vasculares y subraya el valor de combinar la secuenciación genómica con la validación funcional en dermatología traslacional.

La hipótesis de este trabajo propone que mutaciones adicionales en genes relacionados con la vasculatura, como CCM2L, pueden actuar como modificadores genéticos que influyen en la gravedad y la distribución de las malformaciones glomuvenosas más allá de las mutaciones canónicas en GLMN. Los análisis moleculares avanzados pueden ayudar a esclarecer su contribución patogénica.

Objetivos

Objetivo principal

Esta tesis doctoral tiene como objetivo avanzar en la comprensión de los mecanismos moleculares y genéticos implicados en las enfermedades cutáneas, con especial énfasis en el papel de los ARN no codificantes (ncRNAs) y en la contribución de las mutaciones somáticas a la patogénesis de las malformaciones glomuvenosas (GVMs). Asimismo, pretende identificar nuevas estrategias terapéuticas mediante el análisis de dianas moleculares y alteraciones genéticas relevantes asociadas a estas condiciones.

Objetivos específicos

- Identificar y analizar posibles dianas moleculares para el desarrollo de nuevas terapias dirigidas a las GVMs, explorando los mecanismos patogénicos subyacentes y las vías de señalización implicadas en la enfermedad.

- Identificar y caracterizar nuevas mutaciones somáticas en el tejido afectado por GVMs y evaluar su papel en la patogénesis de la enfermedad, con el fin de ampliar el conocimiento actual sobre su etiología genética. Para ello se incluye:

    - La confirmación de la presencia de la mutación en muestras de tejido afectado mediante secuenciación del genoma completo (WGS).

    - La evaluación del impacto funcional de la mutación en células endoteliales de vena umbilical humana (HUVECs), concretamente su efecto sobre la proliferación celular.

    - La investigación de cómo la mutación en CCM2L altera la vía de señalización MAPK/ERK y contribuye a la hiperproliferación celular.

    - La contextualización de estos hallazgos en relación con las mutaciones previamente descritas en GLMN y su vínculo con la fisiopatología de las GVMs.

- La discusión de la relevancia clínica de los resultados y su posible aplicación en el desarrollo de nuevas estrategias diagnósticas o terapéuticas para las GVMs.

Métodos

Revisión sistemática

- **Revisión de los ARN no codificantes en dermatología**

Con el fin de contextualizar el papel de los ncRNAs, se realizó una revisión bibliográfica centrada en sus funciones biológicas en la fisiología y patología de la piel. Los artículos científicos se recuperaron de las bases de datos PubMed y Web of Science, sin restricción temporal estricta, utilizando términos como “non-coding RNA”, “microRNA”, “long non-coding RNA”, “skin” y “dermatology”.

Las publicaciones fueron seleccionadas en función de su relevancia para las enfermedades dermatológicas, prestando especial atención a los estudios centrados en la comprensión de los mecanismos biológicos y moleculares y a sus aplicaciones traslacionales. Los resultados se sintetizaron para ofrecer una visión general de los ncRNAs como biomarcadores, firmas moleculares y posibles dianas terapéuticas en dermatología. La revisión sistemática de los ncRNAs realizada como parte de este trabajo se ha publicado en la revista Human Genetics, lo que avala su calidad y pertinencia en el ámbito de la dermatología molecular.

(Roso-Mares, A., Andújar, I., Díaz Corpas, T. et al. Non-coding RNAs as skin disease biomarkers, molecular signatures, and therapeutic targets. Hum. Genet. 143, 801–812 (2024). https://doi.org/10.1007/s00439-023-02588-4)

- **Revisión de las dianas moleculares en las malformaciones glomuvenosas**

Se llevó a cabo una segunda revisión sistemática con el objetivo de identificar las vías genéticas y moleculares relevantes para las GVMs. Se efectuaron búsquedas en PubMed, Web of Science y Embase, empleando los términos “glomuvenous malformation” y “glomulovenous”, restringidos a artículos en inglés o español publicados entre enero de 1994 y diciembre de 2023.

Tras eliminar duplicados y estudios no pertinentes, se incluyeron aquellos que describían mutaciones genéticas, mecanismos moleculares y enfoques terapéuticos. Esta revisión resumió el papel establecido de las mutaciones en GLMN como principal impulsor de las GVMs e integró

evidencia adicional sobre la participación de las vías TGF-β, PI3K/AKT/mTOR, NOTCH, RAS-MAPK/ERK y TEK/TIE2, así como el potencial uso de la secuenciación de nueva generación y la biopsia líquida.

Caso clínico y obtención de muestras

La investigación experimental se inició con la evaluación de una paciente que presentaba una amplia distribución blaschkoide de GVMs asociada a polidactilia, una presentación clínica inusual no descrita previamente en la literatura. Se obtuvieron biopsias cutáneas de tejido lesional y no lesional bajo el correspondiente consentimiento informado y la aprobación ética pertinente.

Este enfoque pareado permitió distinguir las mutaciones germinales de las somáticas, siendo estas últimas las esperadas exclusivamente en el tejido afectado.

Secuenciación y análisis de variantes

Se realizó secuenciación del exoma completo (WES) a partir de ADN extraído de las muestras lesionales y no lesionales. El filtrado bioinformático priorizó las variantes raras y con potencial efecto sobre proteínas, coherentes con un papel patogénico.

De un conjunto inicial de 616 mutaciones, se identificaron cuatro genes candidatos: GDF10, C3, CCM2L y DLG3. Entre ellos, CCM2L fue priorizado en función de su predicha deleteriedad y de su plausibilidad biológica.

Estudios funcionales en células endoteliales

Con el objetivo de investigar el potencial patogénico de las variantes candidatas, se desarrolló una serie de experimentos en células endoteliales de vena umbilical humana (HUVECs), consideradas un modelo biológicamente relevante para el estudio del desarrollo vascular y de la proliferación endotelial.

Silenciamiento génico

El silenciamiento génico se utilizó inicialmente para determinar si la reducción en la expresión de los genes candidatos afectaba el comportamiento endotelial. Se diseñaron ARNs de horquilla corta (shRNAs) dirigidos específicamente contra CCM2L y los otros genes seleccionados.

Estas secuencias se clonaron en plásmidos pLKO, posteriormente introducidos en HUVECs mediante transfección. Tras el proceso, las células se recuperaron bajo condiciones de cultivo adecuadas antes de someterse a los ensayos experimentales posteriores.

La eficiencia del silenciamiento se validó mediante PCR cuantitativa (qPCR). El ARN se extrajo de las células transfectadas, se retrotranscribió a ADNc y se analizó con cebadores específicos. Una reducción significativa de los niveles de transcrito confirmó el silenciamiento exitoso, garantizando que los cambios fenotípicos observados se debían a la supresión dirigida y no a efectos inespecíficos.

Sobreexpresión génica

De forma complementaria, se realizaron experimentos de sobreexpresión para estudiar los efectos del aumento de la actividad de CCM2L. Se clonaron tanto la secuencia silvestre como la variante mutante R502H identificada en la paciente en vectores de expresión lentiviral.

Los lentivirus se produjeron en líneas celulares empaquetadoras y se usaron para infectar HUVECs. Para garantizar una expresión estable y sostenida, se aplicó selección con puromicina, eliminando las células no infectadas y enriqueciendo aquellas con el constructo integrado.

La expresión estable de las formas silvestre y mutante se confirmó mediante qPCR y Western blot, generando un sistema controlado para comparar directamente los efectos de la mutación R502H frente a la proteína normal bajo condiciones experimentales idénticas.

Ensayos de proliferación y migración celular

Para cuantificar los efectos biológicos de las perturbaciones génicas, se realizaron ensayos de proliferación y migración.

La proliferación celular se midió mediante el ensayo Alamar Blue®, que se basa en la reducción de resazurina a resorufina como indicador de actividad metabólica y viabilidad celular. Las células transfectadas o transducidas se sembraron a densidades iguales y se monitorizó su crecimiento diariamente durante seis días. La fluorescencia se cuantificó con un lector de placas, proporcionando datos comparativos de las tasas de proliferación.

La migración celular se evaluó mediante el ensayo de cicatrización ("scratch assay"). Se realizaron heridas mecánicas en monocapas confluentes de HUVECs utilizando una punta de pipeta. Se adquirieron imágenes en intervalos definidos y se cuantificó la velocidad de cierre de la herida mediante análisis de imagen. Este método permitió evaluar cómo el silenciamiento o la sobreexpresión génica influían en la motilidad endotelial, un proceso esencial para el desarrollo y remodelado vascular.

Análisis proteico y de vías de señalización

El Western blot se empleó para investigar si la mutación R502H alteraba la estabilidad proteica o la señalización intracelular. Se prepararon lisados celulares de HUVECs expresando la forma silvestre o mutante de CCM2L y se separaron por SDS-PAGE. Posteriormente, se transfirieron a membranas y se incubaron con anticuerpos específicos frente a CCM2L y ERK fosforilado (p-ERK), un efector clave de la vía MAPK.

La fosforilación de ERK se eligió como marcador por su papel central en la regulación de la proliferación celular y por los resultados preliminares que sugerían que la mutación podría potenciar la señalización de crecimiento.

Para evaluar la estabilidad proteica, las células se trataron con cicloheximida, un inhibidor de la síntesis proteica. Se recogieron muestras en distintos intervalos temporales y se analizaron mediante Western blot, comparando la tasa de degradación entre las formas silvestre y R502H de la proteína.

Modelado estructural

Para complementar los ensayos celulares, se realizaron estudios de modelado estructural *in silico*. Dado que no existen estructuras experimentales resueltas para CCM2L, se empleó AlphaFold para generar predicciones tridimensionales de las formas silvestre y mutante. Los modelos se visualizaron y compararon con UCSF ChimeraX, centrándose en la región que rodea al residuo 502, analizando los patrones de enlaces de hidrógeno y la distribución de carga superficial que podrían explicar las diferencias de estabilidad observadas in vitro.

Este análisis se consideró exploratorio, dado que las predicciones no reemplazan los datos estructurales experimentales y no pueden reflejar completamente la flexibilidad conformacional ni las modificaciones postraduccionales. Por tanto, los resultados se interpretaron con cautela, como una aproximación preliminar para orientar futuras investigaciones.

Análisis estadístico

Todos los experimentos se realizaron por duplicado o triplicado, y los datos se analizaron mediante ANOVA y *T-test*, según correspondiera. Se consideró estadísticamente significativa una $p < 0,05$.

La combinación de enfoques genéticos, funcionales y estructurales proporcionó un marco sólido para identificar y validar la contribución patogénica de las mutaciones en CCM2L en las GVMs.

Resultados

El análisis del exoma completo reveló una nueva variante somática en CCM2L, potencialmente relevante para la patogénesis de las GVMs.

Se realizó secuenciación del exoma completo (WES) sobre ADN extraído de tejido lesional y no lesional de la paciente. Este análisis pareado permitió identificar variantes somáticas, distinguiendo aquellas presentes únicamente en la piel afectada.

Los datos de secuenciación revelaron un total de 616 variantes exclusivas del tejido lesional. Dado el elevado número de posibles mutaciones, se aplicó una estrategia de filtrado rigurosa para priorizar aquellas con mayor probabilidad de contribuir a la patogénesis. El primer paso consistió en excluir las variantes comunes presentes en bases de datos públicas como gnomAD, de modo que el análisis se centrara en variantes raras, más plausiblemente patogénicas en una enfermedad poco frecuente como la malformación glomuvenosa.

Tras los distintos pasos de filtrado, cuatro genes emergieron como los candidatos más sólidos para análisis posterior: GDF10, C3, CCM2L y DLG3. Todos ellos presentan funciones conocidas o plausibles en procesos biológicos relevantes para el desarrollo vascular o la regulación endotelial.

GDF10 codifica el factor de diferenciación del crecimiento 10, miembro de la superfamilia TGF-β, implicado en el remodelado tisular.

C3 codifica el componente del complemento 3, elemento central del sistema inmune innato, con funciones descritas en angiogénesis y activación endotelial.

DLG3 codifica una proteína de andamiaje sináptico, estudiada principalmente en contextos neuronales, pero con potencial participación en complejos de señalización celular.

Entre estos candidatos, CCM2L se priorizó como el más relevante desde el punto de vista biológico. La variante identificada correspondía a una mutación de pérdida de sentido *(missense)* en el residuo 502, que sustituía una arginina por histidina (R502H). Este residuo se localiza en el dominio C-terminal de la proteína, una región funcionalmente importante.

Las predicciones *in silico* clasificaron la mutación como deletérea, con alta probabilidad de alterar la función proteica. Es relevante señalar que CCM2L es un parálogo de CCM2, gen implicado en la formación de malformaciones cavernomatosas cerebrales, otro trastorno vascular caracterizado por la alteración del desarrollo de los vasos sanguíneos. La relación funcional entre CCM2 y CCM2L en la regulación de la estabilidad endotelial justificó su selección prioritaria para la validación experimental.

La alteración de CCM2L modifica la proliferación y la migración de las células endoteliales.

Con el fin de evaluar el papel potencial de CCM2L en la biología endotelial y analizar las consecuencias funcionales de la variante R502H, se llevaron a cabo una serie de experimentos en células endoteliales de vena umbilical humana (HUVECs), modelo ampliamente utilizado para el estudio del desarrollo vascular, la proliferación y la migración celular.

El silenciamiento de CCM2L reduce la proliferación endotelial y aumenta la migración celular.

El silenciamiento de CCM2L mediante shRNA se confirmó por qPCR, mostrando una reducción significativa de los niveles de CCM2L respecto a los controles.

Los ensayos funcionales demostraron que la disminución de CCM2L se asoció con una reducción de la proliferación celular a lo largo de varios días de cultivo (ensayo Alamar Blue®). En contraste, los ensayos de cicatrización evidenciaron que las células con expresión reducida de CCM2L presentaban una mayor capacidad migratoria, cerrando la herida con mayor rapidez que los controles.

Estos resultados indican que CCM2L favorece la proliferación endotelial y limita la migración, de modo que su pérdida desplaza el comportamiento celular hacia un fenotipo más migratorio.

La mutación R502H en CCM2L potencia la proliferación endotelial en comparación con la proteína silvestre.

Para complementar los resultados del silenciamiento, se generaron líneas celulares que sobreexpresaban CCM2L silvestre o su variante mutante R502H. La expresión estable se confirmó mediante selección con puromicina y análisis por qPCR.

La sobreexpresión de CCM2L silvestre produjo un incremento modesto pero constante de la proliferación respecto a los controles no infectados. En cambio, la sobreexpresión de la variante R502H provocó un aumento significativamente mayor de la proliferación, lo que sugiere que la mutación potencia los efectos proliferativos de la proteína.

Los ensayos de migración realizados en paralelo no mostraron diferencias sustanciales entre la forma silvestre y la mutante, lo que indica que la mutación R502H afecta principalmente a la capacidad proliferativa más que a la motilidad celular.

La mutación R502H en CCM2L confiere una mayor estabilidad a la proteína en comparación con la forma silvestre.

El ensayo de estabilidad proteica reveló que la mutación R502H en CCM2L se asociaba con niveles proteicos más elevados respecto a la forma silvestre (WT), lo que sugiere que la proteína mutante presenta una vida media más prolongada y una menor tasa de degradación (Figura 9a y 9b).

Estos resultados indican que la sustitución del residuo de arginina por histidina podría inducir cambios conformacionales que reducen la susceptibilidad de la proteína a la degradación, contribuyendo así a su mayor estabilidad y, en consecuencia, a la activación sostenida de la señalización proliferativa observada en las células endoteliales.

La mutación R502H en CCM2L reduce su capacidad para inhibir la señalización MAPK/ERK, provocando un aumento de la fosforilación de ERK.

El análisis comparativo de la expresión de CCM2L silvestre y de su variante R502H en HUVECs mostró que la proteína mutante presentaba una capacidad reducida para reprimir la señalización de la vía MAPK/ERK, evidenciada por un aumento de los niveles de ERK fosforilado (p-ERK) en las células que expresaban la variante R502H en comparación con la forma silvestre.

Este incremento en la activación de ERK sugiere que la mutación altera la regulación negativa fisiológica ejercida por CCM2L sobre esta vía, favoreciendo una mayor proliferación endotelial.

Para explorar si la disrupción simultánea de CCM2L y GLMN producía efectos celulares diferenciales, se llevaron a cabo ensayos de silenciamiento individual y combinado en HUVECs.

El silenciamiento simultáneo de GLMN y CCM2L incrementa la proliferación endotelial, sugiriendo un efecto funcional interdependiente entre ambos genes.

Dado que la paciente presentaba una mutación germinal en GLMN y una mutación somática en CCM2L, se diseñaron experimentos de doble silenciamiento para explorar la posible interacción entre ambos genes en las células endoteliales.

Las HUVECs se transdujeron con siRNAs dirigidos a GLMN, CCM2L o a ambos simultáneamente. Los ensayos de proliferación mostraron que el doble silenciamiento aumentó la proliferación celular de forma más marcada que el silenciamiento de GLMN por separado, mientras que la tasa de migración no mostró diferencias significativas respecto al control.

Cabe señalar que la depleción mediada por siRNA no reproduce de manera exacta la pérdida de función heterocigota observada clínicamente, por lo que los resultados deben interpretarse con cautela.

En conjunto, estos hallazgos indican que GLMN y CCM2L ejercen efectos interactivos y complejos sobre el comportamiento endotelial, y respaldan una función biológica relevante de la variante CCM2L c.1505G>A (p.Arg502His) en las células endoteliales vasculares.

Discusión

Esta tesis doctoral aporta nuevos conocimientos sobre la base molecular y genética de las enfermedades cutáneas, con especial énfasis en las malformaciones glomuvenosas (GVMs). Mediante la combinación de revisión bibliográfica y trabajo experimental original, el estudio aborda tres ejes principales: el papel de los ARN no codificantes en dermatología, la exploración de las vías moleculares implicadas en las GVMs, y el descubrimiento de una nueva mutación somática en CCM2L que amplía la etiología genética de esta enfermedad más allá de las mutaciones clásicas en GLMN.

La primera fase de la investigación se dedicó a revisar la función de los ncRNAs en dermatología. Los lncRNAs y microRNAs emergieron como reguladores esenciales de la biología cutánea, al influir en la proliferación, diferenciación, inflamación, cicatrización y tumorigenesis. Su expresión, altamente específica de tejido y tipo celular, junto con su estabilidad en fluidos biológicos, los convierte en biomarcadores idóneos para el diagnóstico y seguimiento de enfermedades dermatológicas. Entre los ejemplos destacados, los lncRNAs PRANCR y DANCR regulan la autorrenovación y diferenciación epidérmica, mientras que los microRNAs miR-203 y miR-21 participan en la proliferación de queratinocitos y en las vías inflamatorias.

Estos hallazgos subrayan el potencial de los ncRNAs como herramientas diagnósticas y terapéuticas, aunque también revelan los retos traslacionales asociados a su aplicación clínica, como la especificidad de las intervenciones, los métodos de administración y la posible inmunogenicidad. No obstante, el cuerpo de evidencia disponible consolida a los ncRNAs como un pilar de la dermatología de precisión, proporcionando el marco conceptual que inspiró las fases posteriores de la tesis.

En este contexto en evolución, un caso clínico se convirtió en el eje del trabajo experimental. La paciente presentaba una extensa distribución blaschkoide de lesiones vasculares asociada a polidactilia, un fenotipo inusual y grave diagnosticado finalmente como malformación glomuvenosa. Este caso motivó la realización de una revisión centrada en las GVMs para situar las observaciones dentro del marco genético y molecular de las anomalías vasculares.

La revisión confirmó que las mutaciones en GLMN son el principal impulsor genético de las GVMs, conforme al modelo clásico de “doble impacto” (two-hit model), que implica la herencia de un alelo mutado y la posterior inactivación somática del alelo silvestre en el tejido lesional. Sin embargo, la variabilidad clínica observada incluso entre pacientes con la misma mutación sugiere la existencia de factores moleculares adicionales que modulan la gravedad y la extensión de la enfermedad.

Asimismo, la revisión destacó varias vías de señalización relevantes para la biología vascular, aún no asociadas directamente a GVMs pero con potencial papel patogénico. Entre ellas destacan TGF-β y PI3K/AKT/mTOR, implicadas en la diferenciación del músculo liso vascular y la angiogénesis, respectivamente; y NOTCH y RAS-MAPK/ERK, esenciales para la función endotelial y la estabilidad vascular. La evidencia derivada de otras anomalías vasculares, junto con el desarrollo de terapias dirigidas —como los inhibidores de PI3K (alpelisib) y de mTOR (sirolimus)—, subraya la importancia de situar las GVMs dentro de un marco molecular más amplio.

Sobre esta base, la investigación experimental abordó el caso clínico atípico que había originado la revisión. La secuenciación del exoma completo (WES) de tejido lesional y no lesional permitió identificar 616 variantes únicas, de las cuales GDF10, C3, CCM2L y DLG3 se priorizaron para análisis funcional. Entre ellas, CCM2L destacó por su potencial patogénico y su similitud funcional con CCM2, gen previamente implicado en malformaciones vasculares cerebrales.

Los estudios funcionales en HUVECs aportaron evidencias sólidas sobre el papel patogénico de la mutación R502H en CCM2L. La sobreexpresión de la proteína mutante incrementó significativamente la proliferación endotelial respecto a la forma silvestre, efecto acompañado de un aumento de la fosforilación de ERK y de una mayor estabilidad proteica. Por el contrario, el silenciamiento de CCM2L redujo la proliferación y modificó la dinámica de migración, reforzando su función reguladora. De forma notable, el doble silenciamiento de GLMN y CCM2L produjo efectos aditivos, lo que sugiere una interacción funcional cooperativa entre ambos genes.

El modelado estructural confirmó que la sustitución R502H genera cambios conformacionales sutiles que estabilizan la proteína, proporcionando una explicación mecanicista coherente con

los resultados experimentales. En conjunto, estos hallazgos establecen a CCM2L como un nuevo contribuyente en la patogénesis de las GVMs, y explican la severidad atípica del fenotipo observado en la paciente. La coexistencia de una mutación germinal en GLMN y una mutación somática en CCM2L ilustra cómo los genes modificadores pueden potenciar la gravedad del cuadro clínico y explicar la variabilidad fenotípica no atribuible únicamente a GLMN.

Las implicaciones de este hallazgo son relevantes. La inclusión de CCM2L en los paneles diagnósticos genéticos podría mejorar la identificación de pacientes con presentaciones atípicas o graves. Además, la activación demostrada de la vía MAPK/ERK sugiere que los inhibidores de esta cascada, ya disponibles en oncología, podrían constituir estrategias terapéuticas prometedoras para las GVMs.

La posibilidad de dirigir terapias hacia la vía MAPK/ERK en malformaciones glomovenosas abre una nueva línea de investigación con implicaciones clínicas considerables. Fármacos ya aprobados en otras indicaciones oncológicas, como inhibidores de MEK o ERK, podrían ser reposicionados y evaluados en modelos preclínicos de GVMs, aprovechando su perfil farmacocinético y la experiencia acumulada en seguridad. Este tipo de estrategias de reposicionamiento, junto con el uso de biomarcadores moleculares para monitorizar la respuesta, constituye un paso esencial hacia el desarrollo de tratamientos más selectivos, menos invasivos y potencialmente más eficaces.

Más allá de CCM2L, estos resultados deben situarse dentro del panorama genético de las malformaciones vasculares. En la última década, la secuenciación de nueva generación ha revelado que los distintos subtipos de anomalías vasculares suelen estar impulsados por vías convergentes, aunque mediadas por genes diferentes. En las malformaciones venosas, las mutaciones activadoras en TEK (TIE2) y PIK3CA son de las más frecuentes y activan la vía PI3K/AKT/mTOR, promoviendo la supervivencia y el crecimiento endotelial. En las malformaciones arteriovenosas, se han identificado mutaciones somáticas en KRAS y MAP2K1, implicando directamente la vía RAS/MAPK. La identificación de la mutación CCM2L R502H, que estabiliza la proteína y potencia la fosforilación de ERK, sitúa a las GVMs dentro de este grupo de anomalías vasculares impulsadas por la señalización MAPK.

Esta convergencia molecular tiene consecuencias terapéuticas directas. El reconocimiento de que las anomalías vasculares comparten vías patogénicas recurrentes y farmacológicamente modulables ha empezado a transformar su tratamiento. Inhibidores como alpelisib (PI3K) o sirolimus (mTOR) han mostrado eficacia en distintos tipos de malformaciones, mientras que inhibidores de MEK se están evaluando en lesiones con mutaciones en MAP2K1. La demostración de activación de MAPK/ERK en GVMs con mutaciones en CCM2L abre la posibilidad de explorar estrategias terapéuticas similares. Aunque las GVMs son lesiones benignas, sus formas extensas o segmentarias pueden ser dolorosas, desfigurantes y refractarias al tratamiento convencional, lo que subraya la necesidad de opciones terapéuticas innovadoras.

La inclusión de CCM2L en los estudios genéticos de anomalías vasculares tendría también valor diagnóstico. Muchos pacientes con sospecha clínica de GVMs resultan negativos para mutaciones en GLMN, y la incorporación de nuevos genes podría aumentar el rendimiento diagnóstico y permitir una clasificación más precisa. Además, reconocer el papel de genes modificadores como CCM2L ayuda a explicar la variabilidad fenotípica y aporta información útil para el pronóstico y el asesoramiento genético.

Pese a la solidez de los resultados, deben reconocerse ciertas limitaciones. Los hallazgos genéticos y funcionales derivan del análisis de un solo caso, lo que requiere validación en cohortes más amplias para establecer la prevalencia y relevancia de las mutaciones en CCM2L. Aunque las HUVECs constituyen un modelo adecuado para la biología endotelial, no reproducen completamente la complejidad del entorno vascular in vivo. Además, aunque este estudio demostró la asociación entre la mutación R502H y la activación de la vía MAPK/ERK, el mecanismo bioquímico exacto por el cual la mutación estabiliza la proteína permanece por esclarecerse.

Las futuras líneas de investigación deberían incluir la ampliación del cribado genético en pacientes con GVMs, el desarrollo de modelos animales que permitan estudiar las consecuencias funcionales de las mutaciones en CCM2L, y la evaluación preclínica de inhibidores de MAPK/ERK como posibles tratamientos. Paralelamente, será fundamental

continuar explorando las otras vías moleculares destacadas en la revisión, para comprender la heterogeneidad genética y patogénica de las GVMs.

Este caso clínico y su investigación han sido publicados en JAAD Case Reports bajo el título: “Glomulin and cerebral cavernous malformations 2 protein-like mutations in an extensive blaschkoid glomuvenous malformation with polydactyly” (Roso-Mares, A., et al. JAAD Case Reports. 2025;63:139–142).

En conjunto, esta tesis realiza una contribución amplia e integradora al campo de la genética dermatológica. Más allá de consolidar el conocimiento existente, esta investigación aporta un nuevo marco interpretativo sobre la interacción entre genes estructurales y moduladores en las malformaciones cutáneo-vasculares, concretamente en las GVMs. Propone una hipótesis inédita en la literatura dermatológica: que las mutaciones somáticas en genes reguladores de la función endotelial, como CCM2L, pueden actuar como modificadores del fenotipo asociado a GLMN, contribuyendo a la variabilidad clínica observada. Este planteamiento no solo amplía la etiología genética de las GVMs, sino que también introduce un nuevo eje de estudio en el campo de la biología vascular cutánea.

De manera particular, los resultados obtenidos confirman la hipótesis planteada de que CCM2L puede desempeñar un papel relevante en las GVMs, al menos en el contexto de este caso clínico concreto. Este hallazgo sugiere que CCM2L podría actuar como gen modificador, modulando la proliferación endotelial y contribuyendo al fenotipo observado en presencia de mutaciones en GLMN. Cabe destacar que este es el primer estudio que vincula CCM2L con una enfermedad cutánea, lo que abre nuevas perspectivas para el análisis de su función en la homeostasis vascular de la piel.

En su conjunto, el trabajo sienta las bases para estrategias diagnósticas y terapéuticas más precisas en dermatología y establece un nuevo punto de partida para la investigación traslacional en genética cutánea, orientada a la validación de genes modificadores, la generación de modelos funcionales y el desarrollo de terapias personalizadas basadas en la caracterización molecular de cada paciente.

Conclusiones

- La revisión bibliográfica respalda la hipótesis de que varios lncRNAs —como PRANCR (proliferación), H19 (angiogénesis) y Zeb2-AS1 (inflamación)— modulan procesos cutáneos clave, lo que los posiciona como biomarcadores y potenciales dianas terapéuticas en enfermedades vasculares de la piel.

- La vía PI3K/AKT/mTOR, esencial para la angiogénesis, podría influir indirectamente en la fisiopatología de las GVMs. Sin embargo, hasta la fecha no se han descrito mutaciones ni datos funcionales que confirmen su implicación directa. Los inhibidores de PI3K (alpelisib) y de mTOR (sirolimus) se están evaluando en malformaciones venosas de flujo lento, aunque aún no existen resultados específicos para GVMs.

- Este trabajo describe por primera vez la mutación GLMN c.515C>A (p.Ser172Ter) en ausencia de una segunda mutación somática en el mismo gen. Este codón de parada prematuro implica una pérdida de función de GLMN y refuerza su papel central en la patogénesis de las GVMs. No obstante, los resultados sugieren que los mecanismos patogénicos de las GVMs se extienden más allá de las mutaciones en GLMN.

- El análisis genómico identificó la mutación somática CCM2L c.1505G>A (p.Arg502His), que activa la vía MAPK/ERK y aumenta la proliferación endotelial, lo que indica que CCM2L actúa como un gen modificador en las GVMs.

- Se propone que CCM2L regula la proliferación celular en las malformaciones vasculares asociadas a GLMN, contribuyendo a la variabilidad fenotípica observada entre pacientes. La identificación de variantes adicionales en CCM2L y la evaluación de inhibidores dirigidos a esta vía representan pasos clave hacia el desarrollo de terapias personalizadas para las GVMs.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
SMA type 1	Spinal Muscular Atrophy type 1
ASO	Antisense Oligonucleotide
AUC	Area Under the Curve
BANCR	BRAF-Activated Non-Coding RNA
BCA	Bicinchoninic Acid Assay
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
BRBN	Blue Rubber Bleb Nevus Syndrome
C3	Complement Component 3
CARF	Collaborator of ARF (cell growth regulator protein)
CCM	Cerebral Cavernous Malformations
CCM2L	Cerebral Cavernous Malformation 2-like
CHX	Cycloheximide
CMLM	Primary Cutaneous Microcystic Lymphatic Malformation
COVID19	Coronavirus Disease 2019
DA	Atopic Dermatitis
DANCR	Differentiation Antagonizing Non-Protein Coding RNA
DGCR8	Gene coding for a component of the microprocessor complex of miRNA
DLG3	Discs Large MAGUK Scaffold Protein 3
DLL4	Delta-like 4, Notch ligand
DMD	Duchenne Muscular Dystrophy
DNA	Deoxyribonucleic Acid
ECs	Endothelial Cells
ERK	Extracellular signal-Regulated Kinase
EZH2	Enhancer of Zeste Homolog 2
FDA	U.S. Food and Drug Administration
FKBP	FK506 Binding Protein
GDF10	Growth Differentiation Factor 10
GLMN	Glomulin Gene
GTPase	Enzyme that hydrolyzes GTP
GVMs	Glomuvenous Malformations
HOTAIR	HOX Transcript Antisense Intergenic RNA
HUVECs	Human Umbilical Vein Endothelial Cells
LB	Liquid Biopsy
IL	Interleukin
LINC-PINT	Long Intergenic Non-Coding RNA, P53 Induced Transcript
MAPK	Mitogen-Activated Protein Kinase
MEK	MAPK/ERK Kinase
MF	Mycosis Fungoides
MITF	Microphthalmia-Associated Transcription Factor
ML	Lymphatic Malformations
MRX34	MicroRNA-34a encapsulated in lipid nanoparticles
MV	Venous Malformations
NGS	Next-Generation Sequencing
NOTCH	Notch Receptor, signaling pathway involved in angiogenesis

OXPHOS	Oxidative Phosphorylation
PBS	Phosphate Buffered Saline
PDGFRB	Platelet-Derived Growth Factor Receptor Beta
PDL	Pulsed Dye Laser
PGC1α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PRANCR	Proliferation-Associated Non-Coding RNA
RAC1	Rho Family GTPase Member
RAF	Rapidly Accelerated Fibrosarcoma (kinase protein family)
RIPA	Radioimmunoprecipitation Assay Buffer
RNA	Ribonucleic Acid
SAMMSON	Survival Associated Mitochondrial Melanoma-Specific Oncogenic Non-Coding RNA
SCF	Skp1-CUL1-F-box Protein Complex
SMA	Smooth Muscle Alpha-Actin
SMN	Survival Motor Neuron
SSc	Systemic Sclerosis
TEK	Tyrosine Kinase Receptor Tie2
TGF-β	Transforming Growth Factor Beta
TLR	Toll-Like Receptor
UCSD	University of California, San Diego
UCSF	University of California, San Francisco
VEGF	Vascular Endothelial Growth Factor
XRN2	5'-3' Exoribonuclease 2
eRNAs	Enhancer RNAs
gRNA	Guide RNA
lncRNA	Long Non-Coding RNA
mRNA	Messenger RNA
miRNA/miR	MicroRNA
microRNA 203	MicroRNA-203, involved in epigenetic regulation
ncRNA	Non-Coding RNA
oncomiR	Oncogenic MicroRNA
piRNAs	PIWI-Interacting RNAs
qPCR	Quantitative Polymerase Chain Reaction
shRNA	Short Hairpin RNA
shortRNA	Short RNA
snoRNAs	Small Nucleolar RNAs
vSMC	Vascular Smooth Muscle Cells

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LIST OF TABLES

1. INTRODUCTION

A. NON-CODING RNAS AS SKIN DISEASE BIOMARKERS, MOLECULAR SIGNATURES, AND THERAPEUTIC TARGETS.

The genetic sequence that encodes proteins collectively accounts for less than 2% of the human genome. The remaining ~98% is termed the 'non-coding genome' and was traditionally thought to have less biomedical relevance. However, genome-wide association studies have found that most disease-associated loci reside in non-coding regions (Zhu, Tazearslan and Suh, 2017). One of the key functional elements of the non-coding genome are non-coding RNAs (ncRNAs), which are transcribed RNA genes that are not translated into proteins (Rao, 2017; Rederstorff, 2021). The human genome contains > 27,000 ncRNAs, comparable to the ~20,000 genes encoding for proteins (*GENCODE - The GENCODE Project*, 2022).

Accumulating experimental evidence demonstrates that ncRNAs play roles in a wide range of biological mechanisms(Zhu, Tazearslan and Suh, 2017). Perturbations to ncRNAs are linked to over 500 different traits and diseases including neurological disorders, cardiovascular diseases, metabolic syndrome, autoimmune diseases, and cancer(Bao *et al.*, 2019). This recognition has sparked interest in studying the potential of ncRNAs as biomarkers and therapeutic target (Matsui y Corey 2017).

A.1. Classifications and functions of ncRNAs

Despite being named after the one function they do not perform, ncRNAs have diverse features and perform a broad array of functions including gene expression regulation, cellular signaling, genome organization, and cell differentiation(Wang, 2018). Several excellent recent reviews have discussed the molecular mechanisms of ncRNAs (Bayraktar *et al.*, 2023; Loganathan and Doss C, 2023; Mattick *et al.*, 2023).

Noncoding RNAs can be categorized based on length, structure, and/or function. Traditionally, those longer than 200 nucleotides are known as long non-coding RNAs (lncRNAs) and those under 200 nucleotides are referred as short noncoding RNAs (Loganathan and Doss C, 2023). Some ncRNAs are designated by functions or features such as enhancer-associated RNAs (eRNAs), circular RNAs (circRNAs), small nucleolar RNAs (snoRNAs), and Piwi-interacting RNAs (piRNAs). A ncRNA can belong to multiple categories: for instance, an enhancer-associated RNA can be a long noncoding RNA, and a Piwi-interacting RNA is a short noncoding RNA.

A.2. Roles of ncRNAs in the skin

In the skin, ncRNAs influence cellular proliferation, differentiation, apoptosis, and senescence, as well as tissue-level processes such as hair growth and wound healing(Cai *et al.*, 2020; Khan *et al.*, 2020; Otten *et al.*, 2023). Here, we briefly summarize key roles of lncRNAs and miRNAs in cutaneous biology.

A.2.1. Long noncoding RNAs (lncRNAs)

LncRNAs encompass a diverse group of ncRNAs that are present in the transcriptomes of a large diversity of species. There are tens of thousands of lncRNAs expressed from the human genome. The recent release of GENCODE recognizes 19,928 lncRNAs (*GENCODE - The GENCODE Project*, 2022), while LNCipedia, a public database of human lncRNAs, contains 127,802 transcripts and 56,946 genes as of June 2023 (Volders *et al.*, 2015).

In the skin, lncRNAs participate in epidermal homeostasis, wound healing, fibrosis, inflammation, aging, as well as progression of skin diseases and cancer (Hombach and Kretz, 2013; Rusek and Krasowska, 2021). The progenitor-renewal-associated lncRNA, PRANCR, affects stemness of epidermal progenitors as well as epithelial stratification through control of cell cycle regulators and mRNA splicing of differentiation-associated genes (Cai *et al.*, 2020; Otten *et al.*, 2021, 2023). The differentiation-antagonizing noncoding RNA, DANCR, is expressed in epidermal progenitors and opposes premature differentiation of epidermal stem cells through interaction with histone-modifying proteins (Zhang *et al.*, 2021). These lncRNAs exemplify the participation of ncRNAs within a regulatory network that governs the balance between progenitor cells in the basal layer and differentiated cells forming the overlying layers.

The integral role of lncRNAs in normal skin function has been accompanied by the discovery of their roles in disease processes. One example is the antisense transcript Zeb2-AS1, which impacts epithelial-mesenchymal transition through downregulation of E-cadherin mRNA and protein levels (Mahboobeh *et al.*, 2020), and the lncRNA H19, which promotes wound healing in diabetic foot ulcers by recruiting serum response factor, increasing connective tissue growth factor, and activating the MAPK signaling pathway (Li *et al.*, 2020).

A.2.2. MicroRNAs (miRNAs)

MicroRNAs are 21–25 nucleotides in length and regulate post-transcriptional silencing of target genes through complementarity to target messenger RNAs. A single miRNA can interact with multiple mRNAs by sequence-specific base pairing, often within the 3′ untranslated region (UTR) of the target mRNA (Lu and Rothenberg, 2018). Many miRNA originate from within an intron of a protein-coding gene (Løvendorf and Skov, 2015). More than 2000 miRNAs have been identified in humans and many display conservation between species (Kozomara, Birgaoanu and Griffiths-Jones, 2019; Laganà, 2019).

A significant role for miRNAs in the skin was demonstrated by the experimental knockout of Dicer and DGCR8, two enzymes required for the generation of mature miRNAs. Knockout in mouse skin resulted in defective hair formation and a hyperproliferative epidermis, establishing a function for miRNAs in skin and its appendages (Andl *et al.*, 2006). One well-characterized example, microRNA 203 (miR-203), is expressed in suprabasal epidermal layers and suppresses keratinocyte replication in cells that have exited the basal compartment. miR-203 promotes the degradation of p63mRNA, a master epithelial transcription factor expressed abundantly in basal proliferative keratinocytes that supports epidermal stem cell function but is downregulated in suprabasal layers. A reciprocal expression pattern between p63 (basal) and miR-203 (suprabasal) sets the demarcation between basal layer proliferation and post-mitotic suprabasal layer differentiation. The regulatory function of miR-203 and other miRNAs in keratinocyte proliferation is also reflected in their roles in wound healing, psoriasis, and cancers. After the skin sustains a wound, keratinocytes near the wound edge replicate to generate new cells that migrate to fill the wound gap. Sets of miRNAs influence the different phases of skin repair including inflammation (miR-146a, miR-155, miR132, miR-21, miR-125b, miR-223), proliferation

(miR-21, miR-132, miR-31, miR-99 family) and remodeling (miR29a/b/c and miR-192) (Meng *et al.*, 2018). miR-203, as introduced previously, participates in wound re-epithelialization by controlling the expression of target proteins responsible for keratinocyte proliferation and migration (Viticchiè *et al.*, 2012).

In skin cancers, miRNAs regulate cancer-associated biological processes at the steps of cancer cell proliferation, angiogenesis, invasion, and metastasis (Durante *et al.*, 2021). Some miRNAs have features of tumor suppressors, such as miR-203 and miRNA-451a in cutaneous basal cell carcinoma (Viticchiè *et al.*, 2012). By contrast, other miRNAs have been proposed to act as oncogenes—also called oncomiRs—and are overexpressed in cancers (Svoronos, Engelman and Slack, 2016). MiR-125b is an oncomiR overexpressed in epitelial tumors that promotes initiation and malignant progression of skin tumors in mice (Zhang, Ge and Fuchs, 2014).

A.3. Importance of ncRNAs in Dermatology

Skin diseases are among the most prevalent human medical conditions, affecting between 30 and 70% of the population (Hay *et al.*, 2014). Skin conditions can arise from genetic, autoimmune, neoplastic, inflammatory, and infectious causes. However, the causes of many skin conditions are still unknown, and treatment of even the most common skin conditions remains challenging in some cases. The emergence of biologic therapies including IL-4/13 inhibitors for atopic dermatitis (Le Floc'h *et al.*, 2020) and IL-17/23 inhibitors for psoriasis (Heath *et al.*, 2019) represents a major advance for patients suffering from these conditions and highlight the benefit of identifying skin disease mechanisms and generating targeted therapies.

In this context, Dermatology continues to have a need for better diagnostic, prognostic, and therapeutic tools. A survey from the National Psoriasis Foundation was found that half of the patients that suffer from psoriasis in the US are dissatisfied with the current available treatments (Armstrong *et al.*, 2013). In addition, many skin conditions can share clinical and histopathological features, which complicates prompt diagnosis and treatment. For example, mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma; its early stage typically manifests as infiltrative patches or plaques with fine scale. However, by appearance it

can be hard to distinguish from eczema or psoriasis (Niu *et al.*, 2021), which can lead to undesirable delays in diagnosis.

Dermatology may experience an evolution from labeling conditions in a global disease category ("eczema") to an approach of identifying specific molecular perturbations affecting individuals. Genetic alterations affecting epidermal homeostasis and differentiation collectively result in over 100 human skin diseases (Lopez-Pajares *et al.*, 2013), as well as skin aging and senescence (Soheilifar *et al.*, 2022). Determining the relationship between ncRNAs and skin diseases has raised the potential for considering the role of ncRNAs as skin diagnostics and therapeutics.

A.4. ncRNAs as biomarkers and molecular signatures

Biomarkers are measurable indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Bethesda, 2001). An example of a clinical biomarker is hemoglobin A1c, which serves as an indicator of serum glucose levels over the preceding several months. A molecular signature shares qualities with a biomarker and is defined as a set of features—which may include DNA, RNA, proteins, or other molecules—that can be assessed as a predictor for a phenotype of interest.

Biomarkers and molecular signatures have potential to improve the practice of clinical dermatology. Diagnosis of some skin conditions remains challenging even with a comprehensive clinical history and histopathology (Dai, Machan and Fraga, 2014). In these cases, biomarkers and molecular signatures hold promise in guiding an accurate diagnosis. Single-cell profiling was recently shown to provide a better classification of inflammatory skin diseases that were challenging to diagnose using only clinical and histological data (Liu *et al.*, 2012). In addition to improved diagnosis, there is hope that molecular profiling will improve our ability to track the progress and prognosis of skin diseases (Ma *et al.*, 2017). This goal has led to research in developing biomarkers to follow the clinical course of patients with atopic dermatitis and psoriasis (Renert-Yuval *et al.*, 2021; Ramessur *et al.*, 2022).

Noncoding RNAs have unique potential as biomarkers and molecular signatures (Yi, Friedland and McGee, 2022). LncRNAs display high cell-, tissue- and tumor-specific expression patterns

which are a desirable feature to accurately classify subclasses of tumors or predict responses to treatments (Huarte, 2015). In addition, miRNAs, lncRNAs, and circRNAs are detectable in body fluids such as blood, urine, and saliva, making them useful assay markers that can be measured without the need for invasive procedures (Pardini *et al.*, 2019; Yang and Wang, 2021). The stability of ncRNAs is conferred by the fact that they are often released into the circulation bound to proteins and packaged in extracellular vesicles that protect them against degradation (Kalra, Drummen and Mathivanan, 2016; Fabbiano *et al.*, 2020; Garcia-Martin *et al.*, 2022).

LncRNAs have shown specific potential as prognostic markers (Cheetham *et al.*, 2013). High expression of the lncRNA HOTAIR is correlated with poor outcomes in patients with breast cancer or esophageal squamous cell carcinoma and with tumor recurrence in hepatocellular carcinoma patients following liver transplantation (Gupta *et al.*, 2010; Yang *et al.*, 2011; Li *et al.*, 2013). One study addressed the prognostic value of lncRNAs in cutaneous melanoma by integrating lncRNA expression profiles from the Cancer Genome Atlas database and matching clinical information from a cohort of 225 subjects with melanoma (Ma *et al.*, 2017). The results suggested a prognostic value of six lncRNAs (LINC01260, HCP5, PIGBOS1, RP11-247L20.4, CTA-292E10.6 and CTB-113P19.5). The cohort was classified in high-risk group and low-risk group depending on the result of a risk score calculated based on these 6 lncRNAs. The survival of patients in the low-risk group was 81.8% at 36 months and 77.7% at 60 months which compared with 50.9% and 26.6%, respectively, in the high-risk group. The area under the curve (AUC) of the six-lncRNA signature for survival prediction was 0.726 at 36 months of OS and 0.825 at 60 months of OS. Overall, the lncRNA signature risk score showed a predictive association with decreased survival (Ma *et al.*, 2017). LncRNA expression has been studied for their diagnostic and prognostic potential in other types of skin cancers as well (Chen *et al.*, 2020; Wang *et al.*, 2020).

Among the classes of ncRNAs, miRNA expression signatures have been the most extensively studied. Their potential as disease signatures is biologically linked to their known involvement in cell differentiation, growth, migration, and apoptosis. Moreover, miRNAs are present not only in the intracellular space, but also in extracellular spaces such as serum, urine, and saliva that make them accessible for assays (Borgia *et al.*, 2022). MiR-155, miR-223, miR-21, and miR-146a levels

have been found to be high in the inflammatory skin diseases acne vulgaris (AV) and hidradenitis suppurativa (HS) (Borgia *et al.*, 2022). Consistent with this, miR-146a was identified as regulator of skin inflammation and inducer of sebocyte proliferation by inducing the TLR1/2 and 4 activated SZ95 sebocytes (Rebane *et al.*, 2014). This offers a biological connection between the miRNA and disease process of AV and HS, both of which histologically involve inflammation of the pilosebaceous unit. miR-155 and miR-146a have been proposed as candidate diagnostic markers in AD (Sonkoly *et al.*, 2010). miR-155 is a proinflammatory factor in epithelial cells and a regulator of apoptosis that modulates CTLA-4 and favors the chronic stimulation of a Th1 response. miR-155 correlates positively with disease severity, Th17 cell abundance, RORgt mRNA expression, and IL-17 mRNA expression and plasma concentration (Sonkoly *et al.*, 2010). Expression of miR-146a in AD is elevated in keratinocytes and chronic lesional skin and inhibits the expression of chemoattractant molecules such as CCL5 and CCL8 (Rebane *et al.*, 2014). Collectively, studies have highlighted the roles of miRNAs in atopic dermatitis (AD) (Yu *et al.*, 2020) and underscore the potential of ncRNAs as biomarkers and molecular signatures. Ongoing validation studies will be needed to determine if these ncRNA signatures are robust and to translate ncRNA biomarkers into clinical practice.

A.5. ncRNAs as tools and targets

A.5.1. RNAs as therapeutic targets

Noncoding RNAs are a new class of targets for disease therapy and expand the potential range of druggable targets beyond proteins and cells (Yu *et al.*, 2019). The biological mechanisms and functions performed by ncRNAs may provide a valuable approach to addressing the problem of disease relapse and resistance to standard treatments (Matsui and Corey, 2017). Noncoding RNAs can be targeted using nucleic acid-based drugs, small-molecule inhibitors, and gene-editing methods (Arun, Diermeier and Spector, 2018). One important observation is that some ncRNAs display enriched expression in cancer tissues and also contribute to carcinogenesis, providing the potential for disease cell specificity (Wang *et al.*, 2019).

A.5.2. Melanoma as a case study for targeting lncRNAs

Malignant melanoma (MM) is the 5th and 6th most common cancer in men and women, respectively. Early-stage**,** localized melanoma has 5-year survival rates above 90% but regionally invasive and metastasized disease both confer a significantly poorer prognosis (NIH - Cancer Stat Facts, 2022). MM is characterized by one of the highest somatic mutation rates observed amongst cancer types(Moran *et al.*, 2018).

SAMMSON (*survival-associated mitochondrial melanoma-specific oncogenic ncRNA*) is a lncRNA highly expressed in melanoma (Goding, 2016; Leucci *et al.*, 2016). This lncRNA promotes an increase in rRNA maturation and protein synthesis in the cell cytosol and mitochondria by modulating the localization of CARF, an RNA-binding protein sequestering XRN2 in the nucleoplasm and limiting nucleolar rRNA maturation. SAMMSON promotes the formation of an aberrant RNA–protein complex in the cytoplasm containing CARF and p32, a mitochondrial protein required for the processing of mitochondrial rRNAs. By boosting translation, SAMMSON ultimately confers a growth advantage to immortalized cells (Vendramin *et al.*, 2018). The initial discovery of SAMMSON (Leucci *et al.*, 2016) and identifying its highly specific expression in melanoma made it an attractive potential therapeutic target (Huarte, 2015; Goding, 2016).

Activating mutations in BRAF kinase are the most common genetic alterations in melanoma dependent on the RAF/MEK/ERK pathway (Haq *et al.*, 2013). BRAF activation suppresses the levels of MITF and PGC1α which are regulators of mitochondrial metabolism leading to a suppressed oxidative phosphorylation gene expression and mitocondrial density in melanoma. (Haq *et al.*, 2013). BRAF inhibitors, such as vemurafenib, can have a strong anti-tumor effect in patients with oncogenic BRAF-driven melanomas (Han *et al.*, 2021). However, a drawback to vemurafenib monotherapy is the common and rapid development of drug resistance (Kim and Cohen, 2016).

A recent study found that a SAMMSON-CARF-p53 signaling axis regulates the adaptive resistance of mutant BRAF melanoma cells to RAF inhibitors (Han *et al.*, 2021). SAMMSON was found to contribute to resistance to RAF inhibitors by protecting melanoma cells against drug-induced cell death by metabolic reprogramming: it modifies the process of OXPHOS through

reduction of the mitochondrial fraction of p32 (De Paepe, Lefever and Mestdagh, 2018), which diminishes their vulnerability as a target (Han *et al.*, 2021). However, melanoma cells that acquire resistance to BRAF inhibitors remain addicted to SAMMSON expression. A recent study showed that SAMMSON was rapidly upregulated following inhibition of ERK signaling in mutant BRAF melanoma cells. Importantly, experimental depletion of SAMMSON enhanced the sensitivity to RAF inhibitors in mutant BRAF melanoma cells in vivo and in vitro (Han *et al.*, 2021). As a result, SAMMSON is a promising target in patients with BRAF inhibitor resistance and provides a novel approach to treat relapsed patients (Goding, 2016; Leucci *et al.*, 2016).

SAMMSON illustrates the principle that RNA-based therapies may serve as valuable alternative therapeutic targets to address or prevent drug resistance to conventional medications (Yu *et al.*, 2019). RNA-based therapies can provide a pathway to treat targets currently considered "undruggable" and may also have the versatility to allow re-targeting of mutated targets (Yu *et al.*, 2019; Zhang *et al.*, 2022).

One well-known target in cancer is p53, the most frequently inactivated gene across all cancer types (Zhang *et al.*, 2022). It has generally been considered a challenging protein to target (Duffy and Crown, 2021) despite its central role in oncogenesis (Zhou, Hao and Lu, 2019). In this context, the identification of the long intergenic non-protein coding RNA, p53-induced transcript (LINC-PINT) has sparked interest in its potential as an alternative way to manipulate the biological impact of p53 on cancers. LINC-PINT displays features of a tumor suppressor lncRNA in human malignancies including melanoma. LINC-PINT attenuates cancer cell proliferation, apoptosis, stemness, migration, endothelial-mesenchymal transition, and invasion (He et al. 2021). It is downregulated in melanoma tissues and cell lines (Huang *et al.*, 2019; Xu *et al.*, 2019), and its lower expression in melanoma is correlated with poorer overall and disease-free survival (Xu *et al.*, 2019). Studies show that LINC-PINT overexpression inhibits melanoma cell proliferation by downregulating BANCR (Huang *et al.*, 2019) and/or suppressing cell proliferation and migration via recruiting EZH2 to the promoter of cell cycle related genes (Xu *et al.*, 2019).

Overall, while conventional drugs and RNA-based therapies have some similarities in their applications, mechanisms of action, and delivery methods, they each also have distinctive

features in their potential to treating diseases. A comparison of key features of conventional vs. RNA-based therapies is presented in Table 1.

Table 1. Features of conventional treatments compared to RNA-based or RNA-targeting therapies.

	Conventional Treatments	RNA-Based Therapies
Mechanism of Action	Interact with other proteins (e.g., receptors) to activate or inhibit function.	Target or deliver RNA molecules into cells to modulate cellular or tissue activity.
Target Specificity	Usually target a specific protein or molecular pathway.	Can target DNA, RNA, and/or proteins.
Administration	Mature delivery systems including oral, intravenous, inhaled, topical, and others.	It can be challenging to deliver RNA molecules at therapeutic levels.
Safety	Established history of safety testing.	Not fully understood, unintended effects and adverse immune events are an ongoing consideration.
Applications	Wide range of diseases, but some disease-specific proteins are difficult to target.	Unique potential for congenital and germline disorders, cancers, and other conditions where targeting genomic DNA or RNA is desirable.

A.6. Fibrotic diseases as a case study for targeting miRNAs

Fibrotic diseases are difficult to treat with existing methods and new therapies are in great need (Griffin *et al.*, 2022). Scleroderma, or systemic sclerosis (SSc), is an autoimmune fibrotic disorder characterized by excessive collagen deposition that can affect multiple organs including the skin, lungs, heart, and kidney. The mortality rate is greater in SSc than in the general population with the leading causes of death being interstitial lung disease and pulmonary arterial hypertension (Bergamasco *et al.*, 2019).

Noncoding RNAs may have a role in the pathogenesis and treatment of scleroderma. Up and down regulation of miRNAs involved in fibrosis has been identified in blood and tissue samples from patients with SSc (Szabo *et al.*, 2021). There has been particular interest in targeting miRNAs for scleroderma because it is generally regarded to have a complex, multifactorial origin and may benefit from treatments that elicit a multipronged response. MicroRNAs can impact multiple genes and pathways, and can theoretically provide a broader yet still disease-specific response (Winkle *et al.*, 2021). In one study, miR-155 was found to be upregulated in skin fibroblasts from patients with SSc and localized scleroderma as well as in experimental skin fibrosis models. A cholesterol-conjugated miR-155 antagomir was developed as an approach to block miR-155 activity in the skin. Topical administration of the miR-155 antagomir led to the downregulation of both Wnt/β-catenin and Akt signaling, both known contributors to fibrosis,

and successfully led to inhibition of sclerotic features in murine skin models. These results highlighted the potential for miR-155 as a potential therapeutic target and are representative of the potential for miRNAs to treat complex conditions (Yan *et al.*, 2016).

A.7. RNA and nucleic acid-based therapeutic tools.

RNA and other nucleic acid-based therapeutics offer the possibility to expand the range of therapeutic molecular targets. Approved drugs are estimated to target less than 700 human proteins and some estimate that only about 3000 of ~20,000 human proteins are considered druggable (Dammes and Peer, 2020). The adaptability of nucleic acid-based drugs is appealing: The recent example of rapid generation and deployment of mRNA vaccines against mutated strains of novel coronavirus-19 (COVID19) (Barbier et al., 2022; Rohner et al., 2022) demonstrate the flexibility of nucleic acid therapies that can be nimbly engineered to widen their applicability.

Nucleic acid-based therapies include RNA aptamers that block protein targets; guide RNAs (gRNAs) that directly edit gene sequences; antisense RNAs (asRNAs) that can block protein translation; and miRNAs and siRNAs that target mRNAs. (Yu *et al.*, 2019). The interest in leveraging noncoding RNAs has expanded as investigators better understand the roles ofncRNA in diseases such as psoriasis (Abdallah *et al.*, 2023), vitiligo (Brahmbhatt *et al.*, 2021), wound healing and keloid formation (Guo *et al.*, 2017; Meng *et al.*, 2018; Tsai and Ogawa, 2019; Jiang *et al.*, 2020), dermatomyositis (Horsburgh *et al.*, 2017) and skin cancers (Goding, 2016; Leucci *et al.*, 2016; Xu *et al.*, 2019; Zhu *et al.*, 2020; Durante *et al.*, 2021).

As of early 2023, there are 32 ongoing trials testing antisense oligonucleotides, 39 testing siRNAs, 3 testing shRNAs and 1 testing miRNA mimics in active clinical trials (ClinicalTrials.gov). The first miRNA mimic utilized in clinical trials in the skin was the miRNA-29b mimic (Remlarsen) in 2019. MiR-29 regulates multiple molecular processes involved in the fibrotic response. Its dysregulated expression has been implicated in scar formation and scleroderma. In a Phase II, double-blind, placebo-controlled study to investigate its efficacy, safety, and tolerability, investigators performed intradermal injection of miR-29b mimics or inhibitors in mice, rat, and rabbit models as well as in human skin fibroblasts in vitro and in human volunteers. Administration of remlarsen reduced biomarkers of fibrosis, including deposition of collagen and

expression of fibrosis-related proteoglycans and cytokines, while miR-29b antagonists induced them. In human wounds, remlarsen repressed collagen expression and the development of fibroplasia in incisional skin wound biopsies evaluated by a blinded board-certified pathologist, and significantly decreased both the depth and area of fibroplasia compared to placebo. These results provided encouraging evidence that remlarsen could help prevent formation of hypertrophic scars and keloids, and may also be a potential therapy to inhibit cutaneous fibrosis in conditions such as scleroderma (Gallant-Behm *et al.*, 2019).

In Table 2 we summarize the major classes of RNA-based therapies, their functions, and the current stage of their development in human clinical trials or clinical practice. We provide examples of drugs that are either in development or already approved for clinical use for disease, with an emphasis on skin conditions, but also including some non-cutaneous diseases. Of the skin treatments included in Table 2, oblimersen is the only agent that has reached Phase III trials. It is an ASO designed to target BCL-2 in patients with advanced malignant melanoma. Although trials did not find significant effects on overall survival (Frantz, 2004; Bedikian *et al.*, 2006), understanding the factors contributing to unsuccessful results has provided guidance for improved RNA-based therapies for advanced melanoma, as described in Gagliardi and Ashizawa (Gagliardi and Ashizawa, 2022).

Table 2. RNA and nucleic acid-based therapeutics.

Name	Description	Function	Clinical Trials	Example
Antisense Oligonucleotides (ASOs)	Single-stranded DNA molecules with full complementarity to a specific messenger RNA (mRNA).	Block protein translation, degrade mRNA, or modify pre-mRNA splicing.	Yes, Phase III	Oblimersen (G3139, Bcl-2 antisense oligonucleotide) – Melanoma
Small Interfering RNA (siRNA)	Single- or double-stranded RNA generated by Dicer from double-stranded RNA.	Gene silencing and reduction of specific protein expression via the RISC/AGO-2 pathway.	Yes, Phase II	BMT 101 (cp-asiRNA) – Hypertrophic scar
Short Hairpin RNA (shRNA)	Hairpin-shaped RNA structures recognized by the RNA interference machinery, processed into active siRNAs.	Gene silencing (more effective than equivalent siRNAs); bi-shRNA has greater silencing efficacy.	Yes, Phase I	Vigil™ (rhGMCSF and bi-shRNA furin) – Ewing sarcoma, non-small cell lung cancer, liver cancer
miRNA Mimics	Synthetic double-stranded RNA fragments similar to endogenous miRNAs.	Mimic the function of an endogenous miRNA.	Yes, Phase II	Remlarsen (miR-29 mimic) – Keloid
AntimiRs	Modified ASOs (2-O-methyl) conjugated with cholesterol at the 3' end.	Inhibit miRNA function.	Yes, Phase II	Miravirsen (anti-miR-122) – Hepatitis C
miRNA Sponges	Artificial transcripts with multiple binding sites for miRNAs.	Inhibit or sequester one or more miRNAs.	No	-
miRNA Masking ASOs	Modified (2′-O-methyl) 22-nt antisense oligoribonucleotides against a target mRNA for an endogenous miRNA of interest.	Hide the miRNA binding site on the target gene; specific and safe therapeutic strategy.	No	-
Circular RNAs (circRNAs)	Covalently closed 3′-5′ loops without 5′ cap or 3′ poly(A) tail.	Modulation of pathological native circRNAs through therapeutic silencing or ectopic expression/engineering of artificial circRNAs with desired molecular effects.	In development	circRNA-Uck2 in Acute Myocardial Infarction
lncRNA Therapies	RNA transcripts ≥200 nucleotides	Expected expansion of targets for RNA interference (RNAi) and CRISPR	No	-
Others: ssRNA	Uncapped single-stranded RNA with polyU repeats, complexed with a polymeric carrier formed by cationic peptides cross-linked with disulfide bonds	Immunostimulatory activity. CV8102 – Activation of TLR7/8/RIG-1	Yes, Phase I	CV8102 RNA-based adjuvant. Intratumoral injection. Melanoma, squamous cell carcinoma of the skin or head/neck, adenoid cystic carcinoma

In other fields of medicine, RNA-directed therapies are FDA-approved and in active use. For example, nurinersen and eteplirsen are approved for the treatment of spinal muscular atrophy (SMA) and Duchenne muscular dystrophy (DMD), respectively(Abreu and Waldrop, 2021). Nurinersen is a 2-methoxyethyl modified antisense oligonucleotide (ASO) designed to target an intronic splicing silencer to alter splicing of exon 7 in the survival motor neuron-2 (SMN2) pre-mRNA. Successful modulating of SMN2 splicing generates functional SMN protein that compensates for the genetic loss-of-function mutation in SMN1. Nurinersen is delivered by intrathecal injection. In a multicenter Phase 3 randomized, double-blind, sham-controlled trial of nusinersen in infants with Type 1 SMA treated at a mean age of 7.9 weeks, 41% of treated infants showed improvement in motor milestones on a standardized neurological exam compared with 0% in the group control (Finkel *et al.*, 2017).

Eteplirsen is a morpholino ASO that has modifications to its ribose sugar to inhibit degradation from nucleases and improve target affinity. It promotes exon 51 exclusion of the DMD gene and restores the reading frame in individuals with DMD that have a specific subset of nonsense mutations that are amenable to this therapy. Clinical trials demonstrated a modest increase of dystrophin expression, attenuation of respiratory decline, and improved ambulation in the majority of treated individuals when compared with historical controls (Aartsma-Rus *et al.*, 2009).

These examples show some of the advantages of RNA-based therapies compared with conventional drugs. In these cases, a detailed understanding of the patient's genotype is required to know whether the treatment is appropriate. Although there are important ethical considerations to resolve, the principle of nucleic acid-based therapies also raises the possibility of directly modifying genomic DNA. In theory, this capability could enable the treatment of genetic diseases at early stages, before the development of symptoms or the progression to severe stages. In some cases, it is hoped that genome therapy will potentially eradicate the disease completely and durably.

A.8. Challenges to RNA therapies

Three key challenges to the development and use of RNA-based therapies are the issues of specificity, delivery, and tolerability.

Specificity

As is the case with all drugs, a major consideration is not only the on-target effects of the drug but also the undesired on- and off-target effects (Winkle et al., 2021). Adverse effects have been observed with RNA therapeutics, especially when administered systematically. The first-in-human clinical trial of MRX34, a miR-34a mimic, was targeted for patients with advanced solid tumors. Unfortunately, the drug resulted in severe immune-mediated toxicities, which resulted in four patient deaths in expansion cohorts. MRX34 is a synthetic, double-stranded miR-34a mimic encapsulated in a liposomal nanoparticle (Hong et al., 2020) that was found to affect gene expression in leukocytes in addition to the target tumor cells, and led to complement activation (Hart et al., 2020). These unwanted effects likely mediated the adverse immune toxicity and were not seen in pre-clinical studies in non-human primates. These outcomes indicate that careful testing and ongoing innovation of appropriate chemical modifications, delivery vehicles, and dose optimization will be necessary to maximize the specificity of RNA therapies.

One additional consideration is that miRNAs are known to target multiple different mRNAs, which can mediate unintended off-target effects but could also be viewed as a potential positive feature. In some diseases, a miRNA therapeutic could theoretically target multiple beneficial pathways or molecular targets at once. In this way, the breadth of miRNAs and their targets are both an opportunity as well as an additional level of complexity. Because the effect on each target is significantly dependent on dosing of the miRNA mimic or inhibitor, this will represent a major challenge to applying miRNA-related therapeutics effectively (Winkle *et al.*, 2021).

Delivery

RNA molecules are larger and more complex macromolecules compared to most conventional drugs. Their size and unstable nature are obstacles to delivery across cellular plasma membranes (Dammes and Peer, 2020). Many tissues and blood serum contain abundant RNAses that rapidly degrade unprotected RNAs (Yu et al., 2019), requiring strategies to maintain

RNA stability. Engineered solutions to these problems include the development of vesicle delivery systems or bacteriophage-bacterial minicell delivery vehicles (Winkle et al., 2021). xosomes are membranous particles that are naturally secreted by a cell into the extracellular space (Garcia-Martin et al., 2022). These vesicles can carry a cargo of DNA, RNA, proteins, lipids, and metabolites in a manner that is protected from the extracellular environment, making them an attractive method for engineering the delivery of exogenous nucleic acid cargo (Garcia-Martin et al., 2022).

Exosomes have increasingly been recognized as a mode of cell-to-cell communication, in which the cargo is transferred from a donor to a recipient cell, leading to changes in gene expression and cellular behavior in ways that impact health and disease (Garcia-Martin *et al.*, 2022). The Vesiclepedia database includes more 10,000 entries for non-coding RNAs (Winkle *et al.*, 2021), illustrating that ncRNAs are natural passengers in exosomes. In addition to exosomes, other promising delivery strategies are under investigation that include cationic lipids, viral or bacterial agents, and cell-penetrating peptides (Winkle *et al.*, 2021). Delivery technologies will be vital to the success of RNA-based therapies. In the case of the skin, topical and injectable methods are feasible but for visceral organs this will offer a greater challenge.

Tolerability

A general concern for drugs is the issue of tolerability and the issue of allergic potential. Therapeutic RNAs can be recognized as foreign and evoke an immune response. One well-defined mechanism is the recognition of foreign RNAs by pathogen-associated molecular pattern receptors such as Toll-like receptors (TLRs), which can stimulate an adverse immune response. There have been advancements addressing the immunogenicity of RNA therapeutics, but this obstacle remains substantial. Potential solutions to address this issue are focused on reducing the immunogenic and toxic response. The development of a database that allows the screening of miRNAs that target TLRs or of exact immune adverse reactions for each tested RNA therapeutic would be a suitable tool to check before starting any clinical trial.

Conversely, the use of a limited but active drug dose over a prolonged time or combining RNA therapeutics with other drugs could minimize immunogenic effects. Additionally, the development of bioengineered RNA, such as short antisense RNAs with less than 21 nucleotides

('tiny' LNAs) to avoid the activation of TLR, using small mRNA inhibitors or targeting ncRNAs with interaction element blockers or structural element lockers, are other approaches under active development (Winkle *et al.*, 2021).

LncRNA have emerged as key regulators of cutaneous physiology and a range of dermatoses, making them plausible candidates to modulate the penetrance and clinical heterogeneity of GVMs. Building on this premise, our project was initially designed to explore the potential modulatory role of lncRNAs in GVMs. However, to obtain a more comprehensive and unbiased view of the disease's molecular landscape, we incorporated extensive genomic analyses. This integrative approach—combining a critical review of lncRNAs with a next-generation genetic study—allowed us to reframe our research questions and consider additional putative modifier genes, thereby opening the door to an in-depth examination of the pathogenic mechanisms involved in GVMs. On this foundation, the second part of the Introduction focuses on reviewing the current state of knowledge regarding GVMs, the major signaling pathways implicated in their pathogenesis, and the identification of molecular and genetic variation underlying these and other cutaneous diseases.

B. IDENTIFICATION OF MOLECULAR TARGETS FOR NEW THERAPEUTICS IN GLOMUVENOUS MALFORMATIONS (GVMs).

Glomuvenous malformations (GVMs) are cutaneous vascular hyperproliferations that manifest as hyperkeratotic pink to violaceous nodules. The lesions exhibit a distinctive cobblestone-like appearance and demonstrate tenderness with pressure (Amyere *et al.*, 2013; Skowronek *et al.*, 2020; Schiffmann *et al.*, 2021). Generally, GMVs are either congenital or present during childhood (Nutsathapana and Tresukosol, 2021). Its prevalence is uncertain, and comprises approximately 5% of all venous malformations, with an estimated incidence of 1 in 10,000. The true prevalence may be higher due to unreported cases (Skowronek *et al.*, 2020).

Plaque-type GVMs are characterized by their congenital origin, extensive distribution, and progressive involvement after birth. These lesions typically exhibit subtle, flat, pink, bluish, or

dual-toned characteristics, often darkening with time. They may be misdiagnosed as capillary malformations, early vascular malformations (VM), early hemangiomas, blue nevi or even blue rubber bleb nevus syndrome (BRBN) (Mallory *et al.*, 2006; Ramessur *et al.*, 2023). Lesions can present with partial atrophy upon birth, which makes accurate identification particularly challenging. Moreover, GVMs with a plaque-like appearance are difficult to treat. They respond poorly to sclerotherapy or embolization, and surgical interventions may leave disfiguring scars (Moreno-Arrones *et al.*, 2018).

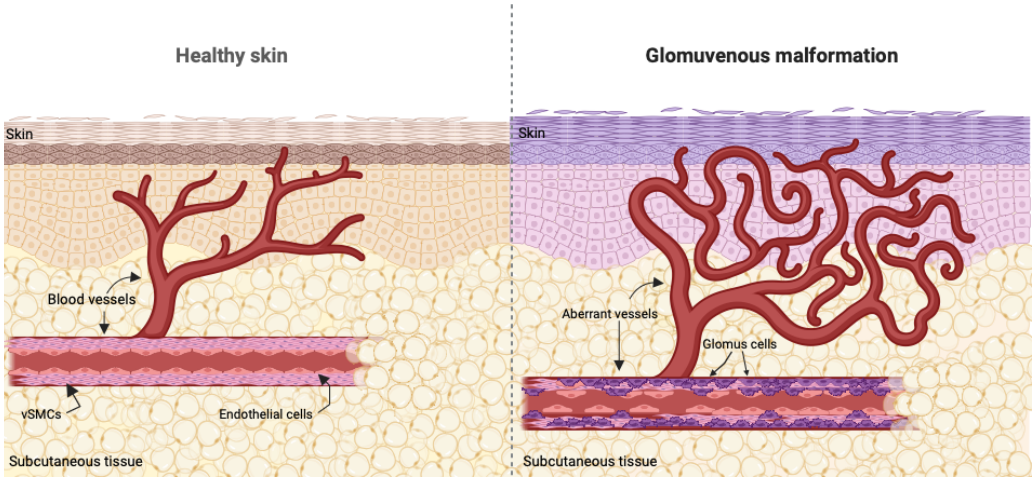


***Figure 1**. Comparative illustration of healthy skin and aberrant vessel in GVM. Schematic comparison between healthy skin with normal blood vessels (left) and an aberrant blood vessel typical of glomuvenous malformations (GVM) (right).*

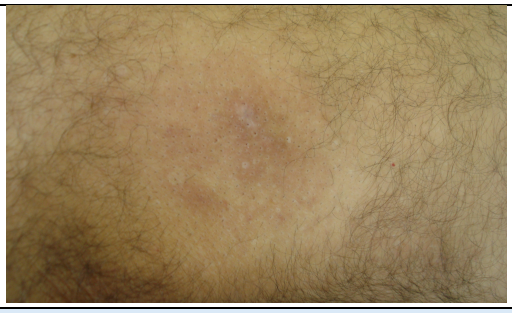
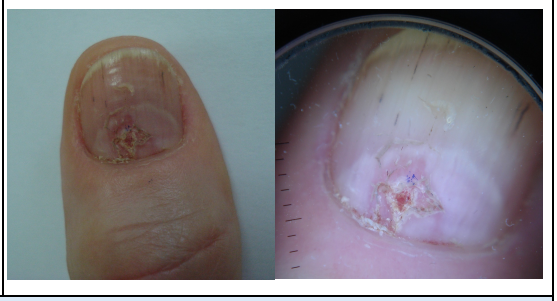
Although classified as benign, GVMs can significantly impact the quality of life for affected patients. They can cause emotional distress when GVMs affect conspicuous areas and can develop complications such as bleeding or infection. Complicating the clinical picture even further, GVMs can present with a multifocal distribution, which amplifies the morbidity of the condition even further (Amyere *et al.*, 2013).

B.1. Glomuvenous malformations vs glomangiomas

GVMs have been historically referred to as glomangiomas; however, the most updated understanding recognizes GVMs as a distinct entity (Brouillard *et al.*, 2002; Boon *et al.*, 2004; Borroni *et al.*, 2014) based on clinical presentation, behavior, and histopathological features (Table 1). GVMs are characterized by a multifocal distribution of hyperkeratotic purplish cobblestone-like lesions, often exhibiting a congenital or childhood onset.

In contrast, glomangiomas, usually solitary lesions, present as bluish or reddish nodules, typically appearing in adulthood and commonly found in the subungual area and distal portions of extremities. GVMs do not exhibit the characteristics of true neoplasms. The latest International Society for the Study of Vascular Anomalies (ISSVA) 2018 classification categorizes glomuvenous malformations under “venous malformations” (Kunimoto, Yamamoto and Jinnin, 2022).

Tabla 3. *Differences between GVMs and glomangiomas.*

	GVM (multiple variant type)	Glomangioma (Glomus tumor, single lesion)
Origin	Loss of function mutation in GLMN gene (Ch. 1p22.1 -OMIM 601749-).	Trauma. Idiopathic.
Onset	Frequently inherited, few cases sporadic.	Sporadic.
Time of onset	Congenital/childhood.	Adult.
Clinical presentation	Multifocal. Hyperkeratotic purplish cobblestone-like lesion. Pink plaque-type at birth.	Solitary. Solitary bluish or reddish nodule usually at the nail bed. Triad: paroxysmal pain, local tenderness, and sensitivity to cold.
Histology	Larger and more irregular vascular spaces, coated by few layers of glomus cells, and is not encapsulated. The glomus cell is small, with an eosinophilic cytoplasm and rounded or oval central nuclei.	Usually encapsulated and has more glomus cells coating the vascular lumina. Without important vascular component.
Location	High phenotypic variability. Commonly found in the limbs.	Subungual area most common and distal portions of extremities.
Growth pattern	Grow slowly over time and can expand in size and change coloration.	Tend to remain relatively small, ranging from a few millimeters to a few centimeters in diameter.
Treatment options	Treatment options include surgical excision, laser therapy, embolization, or sclerotherapy. However, complete removal may not always be possible due to the complex nature of GVMs and their potential involvement of surrounding structures.	Surgical excision is the primary treatment approach. Laser therapy or sclerotherapy may be considered for smaller lesions.
Image		

**Images obtained from the Department of Dermatology, Hospital Dr. Peset, Valencia, Spain.*

The histopathology of GVMs shows irregular and dilated thin vascular channels and intermingled nerve fibers within the dermis and subcutaneous fat, with involvement of mucosal or other

tissues being rare (Brouillard *et al.*, 2002; Nutsathapana and Tresukosol, 2021). These channels are lined by endothelial cells and accompanied by solid strands and nests of glomus cells as depicted in Figure 1 (Borroni *et al.*, 2014). Glomus cells, distinctive to GVMs, are vascular smooth muscle cells (vSMCs) that have undergone aberrant differentiation originating from the glomus body, a specialized neuromyoarterial receptor predominantly situated in the reticular dermis (Nutsathapana and Tresukosol, 2021). Aberrant glomus cells in GVMs can alter sensory perception.

Notably, half of the patients demonstrate allodynia in response to external pressure, despite the absence of spontaneous pain under baseline conditions (Boon et al., 2004). In a GVM, a few layers of glomus cells typically encircle these vascular channels, distinguishing it from a glomus tumor proper, where vessels may be less conspicuous, and glomus cells are more numerous (Table 1). The core area of the malformation, where glomus cells and abnormal veins are concentrated, typically displays the most notable abnormalities. In contrast, concurrent alterations in the adjacent vasculature may also be noted, unlike the encapsulated nature commonly seen in glomus tumors (Nikhil et al., 2022).

Immunohistochemistry analysis has revealed distinctive characteristics of glomus cells, indicating their unique identity. Glomus cells express smooth muscle cell (SMC) α-actin and vimentin, while conspicuously lacking desmin, a marker typically associated with differentiated muscle cells (Borroni et al., 2014; Moreno-Arrones et al., 2018). Moreover, glomus cells exhibit negative staining for the von Willebrand factor and the S-100 neuronal marker (Brouillard et al., 2020). The absence of desmin and the negative expression of von Willebrand factor and S-100 represent the hallmark immunophenotype of glomus cells. Additionally, GVMs characteristically present with normal D-dimer levels, in contrast to other vascular anomalies that typically show elevated D-dimer levels. This distinction allows for the detection of concealed vascular malformations and helps differentiate GVMs from other multifocal venous lesions (Dompmartin et al., 2009).

Surgical excision is the prevailing treatment for GVM, but laser therapy has been advocated as an alternative approach (Shah et al., 2021). A retrospective study of 17 patients treated with dual-

wavelength pulse dye laser (PDL) and Nd:YAG revealed a 94% reduction in GVM size with minimal adverse events (Moreno-Arrones et al., 2018). A case report of a 6-month-old patient supported the safety and efficacy of combined PDL and Nd:YAG therapy for surgically non-amenable lesions (Nguyen, Boon and Vikkula, 2014). While the literature on laser applications is limited, preliminary findings suggest potential roles for argon-carbon dioxide in superficial lesions and PDL for pain relief (Shah et al., 2021). The 1064nm Nd:YAG laser has also been applied, but carries a higher risk of scarring and ulceration (Moreno-Arrones et al., 2018).

Interventions for GVM primarily focus on the destruction of overgrown vascular tissue. However, a greater understanding of the genetic basis of these disorders raises the potential for using targeted biological medical therapies. Ideally, medical strategies would aim to halt progression and complications or even reverse its appearance from an early age. Because of its rarity, GVMs have been challenging to study in large numbers. In this review, we synthesize the scientific literature on GVMs to describe the most updated understanding of the genes, signaling pathways, and molecular mechanisms that contribute to this vascular disorder.

B.2. Molecular pathways involved in the pathogenesis of GVM

B.2.1. Involvement of Glomulin (GLMN) in the pathogenesis of GVM

a. GLMN is an established etiological factor

Loss-of-function mutations in the glomulin gene (GLMN), also known as FAP68 or FAP48, have been definitively identified as the principal genetic cause of GVM (Boon *et al.*, 1999; Grisendi *et al.*, 2001; Brouillard *et al.*, 2002; Nguyen, Boon and Vikkula, 2014). GLMN is expressed in endothelial cells (ECs) and vSMCs (Queisser *et al.*, 2021). The vascular changes typically suggest that the GLMN gene plays a pivotal role in angiogenesis, especially in the context of vSMC development. While GLMN is believed to contribute to vascular smooth muscle differentiation, the specific mechanism linking its dysfunction to the observed phenotype remains unclear. Despite the widespread distribution of GLMN expression, GVM exclusively manifests with cutaneous lesions (Ramessur *et al.*, 2023)

Patients with GVM usually exhibit an autosomal dominant inheritance pattern with incomplete penetrance. Affected individuals inherit a loss-of-function GLMN allele, and acquire a somatic loss of the wild-type allele in the affected tissue (Tron *et al.*, 2012). Mutations have been identified across the entire gene with no genotype-phenotype correlation (Brouillard and Vikkula, 2007). It has been hypothesized that the "second hit" may underlie the phenotypic diversity of GVM. Glomuvenous malformations have the highest number reported of somatic second-hit mutations among VMs (Fereydooni, Dardik and Nassiri, 2019). An alternative possibility is that additional modifier mutations may trigger or influence the severity of GVMs.

In support of the second hit hypothesis, one study uncovered 16 somatic mutations, primarily through acquired uniparental isodisomy (aUPID) involving chromosome 1p. These aUPID breakpoints reside within a high-DNA-flexibility region rich in A and T nucleotides (1p13.1–1p12), indicative of a fragile site (Amyere *et al.*, 2013). These findings suggest a genetic scenario in which the inherited GLMN variant in 1p22.1 becomes homozygous in affected tissues without genetic material loss.

Though multiple mutations in GLMN can lead to loss of function, approximately 70% of documented GVM cases are comprised of eight mutations: 157delAAGAA (40.7%), 108C>A (9.3%), 1179delCAA (8.1%), 421insT and 738insT (4.65% each), 554delA+556delCCT (3.5%), and 107insG and IVS5-1(G>A) (2.3% each). This observation allows for relatively efficient first-pass screening for gene mutations (Brouillard *et al.*, 2013). Recent published cases have shown additional, less common GLMN mutations, which include: c.1299+1G>A, c.1319G > A, p. Trp440*, 053274.2c.1214 + 2T> C, c.743dup, p.Leu248Phefs14, c.971dupT; p.Leu324Phefs19, and c.108C>A; p.Cys36* (Table 2). Remarkably, GLMN is unique in composition, lacking paralogous counterparts. The deficiency of the GLMN protein is a critical factor in GVMs, and this deficiency cannot be alleviated or lessened by the presence or absence of other proteins (Aerts *et al.*, 2007).

Table 4. *Publications within the last five years that presented cases of GVM.*

Publication	Skowronek D, et al.	Couselo-Rodríguez C, et al.		Farquhar K, et al.	Nutsathapana N, et al.	Gil F, et al.	McMahon MH, et al.	Schiffmann ML, et al.	Esteves M, et al.	Ramessur R, et al.
Subjects	3	2		1	1	1	2	1	1	1
Onset	Birth - Increased when adults	10 yo	Birth	Birth	10 yo	Puberty	Childhood 6 yo	14 yo - Increased with age and pregnancy	Adolescence	Childhood
Genetic diagnostic tools	NGS gene panel analysis, Sanger sequencing	Sanger seq.	Sanger seq.	-	-		Gene panel testing		Genetic analysis	Targeted genetic sequencing
Familial	3 generations	2 generations	2 generations	Multiple generations	Sporadic	1 generation Sibling w/ lesions	1 generation Siblings	2 generations	3 generations	4 generations
Affected gene	heterozygous variant in GLMN	GLMN	GLMN	heterozygous variant in GLMN	-	heterozygous variant in GLMN	heterozygous variant in GLMN	heterozygous variant in GLMN	heterozygous variant in GLMN	heterozygous variant in GLMN
Mutation	ENST00000370360.8 c.1299+1G>A	NM_053274.2: c.1319G > A, p.Trp440*	NM_053274.2 c.1214 + 2T> C	c.743dup, p.Leu248Phefs*14	-	c.971dupT; p.Leu324Phefs*19	c.108C>A; p.Cys36*	c.108C>A; p.Cys36*	c.971dup; p.Leu324Phefs*19	c.1720C>T in exon 19 Of GLMN
Localization of lesion	Eyebrow, jaw, arm, foream, abdominal, chest wall, back, neck	Ankle, trunk	Shoulder and trunk	Facial	Wrist, ankle, back	Upper and lower limbs, trunk, and face	Foot and lower back	Arms and legs	Face, arm, and back	Middle finger, neck, arms, thighs,a nkles and toes.
Comments				Associated with deep intracranial cavernoma and developmental venous anomaly		Sclerotherapy with growing concentrations of polidocanol		Sclerotherapy with 0.5% polidocanol		
Year	2020	2022		2021	2021	2019	2022	2021	2019	2023

b. GLMN as a regulator of TGF-β signaling pathway

GLMN is a regulator of the SCF (Skp1-CUL1-F-box protein) E3 ubiquitin-protein ligase complex and is involved in vSMCs differentiation through alteration of TGF-β signaling (McIntyre *et al.*, 2004). TGF-β regulates SMC development, embryogenesis, immunity, and tissue repair (Williams *et al.*, 2021). The immunophilin FK506-binding protein (FKBP) 12 binds and inhibits TGF-β R-I (Bonner and Boulianne, 2017). This could lead to the hypothesis that GLMN may play a part in the mTOR signaling pathway (Uebelhoer, Boon and Vikkula, 2012). Possible targets for GVM therapy might thus be the TGF-b-signaling pathway and modulators of mTOR.

The FKBPs are targets of immunosuppressive agents such as FK506 (tacrolimus) and rapamycin (sirolimus), and through inhibition of T-cell activation and proliferation and they may regulate GLMN (Bonner and Boulianne, 2017). Initially, it was reported that GLMN binds FKBP12 (Chambraud *et al.*, 1996), but the interaction of GLMN with FKBP12 has been described as comparatively weak, while FKBP12.6 and FKBP51 have emerged as novel and stronger binding partners. These interactions could potentially significantly impact the regulation of the SCF E3 ubiquitin-protein ligase complex, and thus on the molecular pathway of glomuvenous malformations.

A prospective phase II study was conducted to assess the efficacy and safety of sirolimus in patients with extensive or complex slow-flow vascular malformations (Hammer *et al.*, 2018). The study found that sirolimus was effective in treating extensive lymphatic malformations (LM), venous malformations (VM), and/or complex malformations that were refractory to conventional treatments and it was well-tolerated. Several ongoing clinical trials are investigating the use of sirolimus in congenital venous malformations (Table 5). However, patients with GVMs were not included in these studies.

c. GLMN role in PI3K/AKT/mTOR pathway

The interactions between FKBPs and GLMN, along with the promising results from clinical trials, highlight the significance of the PI3K/AKT/mTOR pathway in vascular anomalies. This pathway is crucial for cell cycle regulation, proliferation, and migration, and is often termed the "anti-

apoptosis pathway" and is essential for normal blood vessel development during embryogenesis (Karar and Maity, 2011).

Recent investigations have explored the therapeutic potential of alpelisib, an FDA-approved PI3K inhibitor primarily indicated for the management of advanced breast cancer distinguished by the presence of PIK3CA mutations and hormone receptor negativity (André *et al.*, 2019). The study showed promising outcomes for alpelisib in treatment of vascular malformations associated with TEK or PIK3CA mutations. In a cohort of eighteen patients, treatment led to improved overall quality of life, with mean PedsQL total scores in children increasing by ~20 points (scale 0–100) and adult SF-36 physical functioning scores improving by ~15 points (Sterba *et al.*, 2023). The results underscore the potential for alpelisib to deliver significant therapeutic efficacy while maintaining a favorable safety profile (Sterba *et al.*, 2023). A clinical trial is underway to assess the utility of alpelisib in pediatric and adult patients with lymphatic malformations associated with a PIK3CA Mutation (EPIK-L1).

GLMN exerts regulatory control over the functional dynamics of the RBX1-CUL1-containing SCF^{Fbw7} complex (Duda *et al.*, 2012). Reduced levels of Rbx1 and Rbx1-associated cullins have been observed in GLMN-deficient cells and tissues as well as in GVM specimens (Duda *et al.*, 2012; Tron *et al.*, 2012). The depletion of Glmn activity results in an increased turnover rate of Fbw7, which serves as the substrate receptor for CUL1-RING-ligase (CRL1). Consequently, this hampers the efficacy of the $CRL1^{Fbw7}$ complex in mediating the degradation of its target proteins, Cyclin E and c-Myc, due to an inhibition of its E3 ubiquitin ligase activity (Tron et al., 2012). Investigations have reported elevated levels of Cyclin E and c-Myc within GLMN-knockout and knockdown cell lines, as well as in teratomas, mouse embryonic tissues, and GVM lesions, providing substantive evidence for the contributory role of disrupted Fbw7 homeostasis in GVM development (Tron et al., 2012).

Besides, GLMN interacts with the inactive, non-phosphorylated form of the hepatocyte growth factor (HGF) receptor c-Met with PI3K downstream target p70S6K inducing vascular smooth muscle cell migration and angiogenesis (Palmieri et al., 2021). And GLMN might also have some

protective effects against macrophage cell death induced by NLR inflammasome activation (Suzuki *et al.*, 2014; Brouillard *et al.*, 2020)

Table 5. *Summary of ongoing clinical trials focused on testing innovative therapies for the treatment of vascular malformations*

Last update posted	Title	Drug	Vascular Malformation inclusion criteria	Status	Phase 3	Results	Investigators
1/29/21	Treatment of Congenital Vascular Malformations Using Sirolimus: Improving Quality of Life (Sirolimus)	Sirolimus	Congenital venous malformation, or lymphatic malformation or combined	Unknown	Phase 3	No posted	**RadMaroeska Loo, te, Dr.,Radboud University Medical Centeboud University Medical Center**
11/10/21	Efficacy and Safety of Rapamycin to Complex Vascular Anomalies in Pediatric Patients	Rapamycin	Kaposiform Hemangioendotheliomas, Tufted Angioma or complicated vascular malformation	Recruiting	Phase 3	No posted	**Yang Lihua, Zhujiang Hospital**
3/15/22	Efficacy and Safety of Sirolimus to Vascular Anomalies	Sirolimus	Kaposiform Hemangioendotheliomas without Kasabach-Merritt Phenomenon, Tufted Angioma without Kasabach-Merritt Phenomenon, Capillary Malformations, Lymphatic Malformations, Venous Malformations, Capillary-Venous Malformation (CVM), Capillary-Lymphatic Malformation (CLM), Lymphatic-Venous Malformation (LVM), Capillary-Lymphatic-Venous Malformation (CLVM), Multifocal Lymphangiomatosis and Thrombocytopenia (MLT)	Completed	Not applicable	No posted	**West China Hospital**
2/24/23	Efficacy and Safety of Sirolimus in Vascular Anomalies That Are Refractory to Standard Care	Sirolimus	Patients with complex vascular anomalies that are refractory to standard care	Recruiting	Phase 3	No posted	**Laurence M Boon, MD, PhD. Cliniques universitaires Saint-Luc**
5/10/23	Topical Sirolimus in Cutaneous Lymphatic Malformations	Sirolimus	Diagnosis of primary cutaneous microcystic lymphatic malformation (CMLM) confirmed by histopathological or dermoscopic examination, with or without an underlying malformation or a syndromic malformation (Protée syndrome for instance), responsible for impairment (oozing, bleeding and/or pain)	Recruiting	Phase 2	No posted	**Annabel Maruani. University Hospital, Tours**
4/16/19	Onreltea (Brimonidine) Gel In Pediatric Patients With Capillary Malformations	Brimonidine 0.33% gel	diagnosis of facial capillary malformation (port-wine stain, PWS) made by a dermatologist	Terminated	Phase 3	No posted	**Elena Pope, MD. The Hospital for Sick Children, Ontario, Canada**
11/30/23	Alpelisib in Pediatric and Adult Patients With Lymphatic Malformations Associated With a PIK3CA Mutation. (EPIK-L1)	Alpelisib	PIK3CA-mutated lymphatic malformations (LyM)	Not yet recruiting	Phase 2/3 multi-center study	No posted	**Novartis Pharmaceuticals**

B.2.2. Possible Alternative Pathways in the Pathogenesis of Glomulovenous Malformations

Mutations in the GLMN gene represent the main genetic cause of GVMs. However, we hypothesize that the dysfunction in these malformations may be modulated by additional alterations in molecular pathways involved in vascular homeostasis. Therefore, we have explored potential signaling mechanisms related to blood vessel development and stability that may be dysregulated in GVMs. These mechanisms could contribute to their pathophysiology or act as disease modifiers.

a. TIE-2 (TEK)/Angioprotein Pathway

The TIE-2 receptor tyrosine kinase was the first gene associated with inherited VM development and is a key regulator of vascular development and stability (Joussen et al., 2021). This receptor is located on chromosome 9p21 and is specific for endothelial cells (Palmieri et al., 2021). Mutations in the angiopoietin receptor TIE-2/TEK have been identified as the cause for different cutaneous vascular malformations such as sporadic VM, cutaneomucosal venous malformation (VMCM) with autosomal dominant inheritance, and BRBN (Limaye et al., 2009; Wouters et al., 2010; Soblet et al., 2017; McMahon, Tahir and Balasubramanian, 2022).

GVM demonstrates a clinical presentation reminiscent of these cutaneous vascular malformations. Instances of GVM have been documented wherein the cutaneous manifestation closely mirrors that of BRBN (Ramessur et al., 2023). However, BRBN represents a pathological entity fraught with potentially life-threatening internal vascular complications, underscoring the imperative of accurate diagnosis. Diagnosis of GVM via a genetic blood test can avoid long-term surveillance testing and anxiety around the risk of gastrointestinal bleeding (McMahon, Tahir and Balasubramanian, 2022). Hereditary mucocutaneous venous malformations, arising from mutations in the angiopoietin receptor TIE-2, manifest with a relative deficiency of appropriately differentiated vSMCs. In contrast, GVMs consist of dilated vein-like conduits harboring varying quantities of undifferentiated vSMCs, specifically glomus cells, within their endothelial walls (McIntyre et al., 2004).

A hypothesis posited the potential synergy between VMGLOM and GLMN in the regulation of angiogenesis, concomitant with the TIE-2 signaling pathway (Brouillard et al., 2002; Yadav et al., 2013). However, no studies have been identified to substantiate or experimentally validate this hypothesis. Exploring and confirming this conjecture could significantly contribute to therapeutic research focusing on these pathological conditions.

b. NOTCH signaling

The NOTCH signaling pathway is pivotal in vascular development and homeostasis, exerting multifaceted control over EC behavior, embryonic vasculature formation, and the differentiation of vSMCs (Zhou *et al.*, 2022). NOTCH4 and its ligand DLL4 expression on ECs orchestrates vascular dynamics, activating NF-κB signaling to enhance VEGF secretion and regulate EC behavior through DLL4-mediated NOTCH and VEGFA-VEGFR2 signaling(Pitulescu *et al.*, 2017). Deficiencies in NOTCH signaling, as evidenced by studies in mice, lead to embryonic vasculature defects, specifically inducing EC sprouting and proliferation (Pitulescu *et al.*, 2017; Nadeem *et al.*, 2020). In-depth insights into the intricate interplay of NOTCH signaling within the context of vascular EC can be found in a recent work (Akil *et al.*, 2021).

Additionally, NOTCH signaling plays a crucial role in the development of vSMCs, and inhibiting NOTCH signaling in neural crest cells, particularly NOTCH2 and NOTCH3, results in vascular dysplasia, and aortic defects (Campos *et al.*, 2002; Boucher *et al.*, 2013). Downstream factors PAX1, SCX, and SOX9, regulated by NOTCH signaling, control progenitor cell differentiation towards vSMCs. Additionally, NOTCH signaling responds to laminar flow, contributing to vascular balance, as ECs sense and transform mechanical forces into intracellular signals (Zhou *et al.*, 2022).

Despite a notable scarcity of studies investigating NOTCH pathway alterations in GVM, recent research reveals NOTCH gene rearrangements in 54% of glomus tumor patients and NOTCH3 mutations in both glomus tumors and select pericytic tumors, studied alongside PDGFRB gene mutations (Agaram *et al.*, 2020; Iwamura *et al.*, 2023). Interestingly, study outcomes caution against relying on PDGFRB or NOTCH3 mutations for effective subclassification of pericytic tumors due to their indistinct presence (Iwamura *et al.*, 2023). Despite GVM not being formally

categorized as pericytic tumors, the shared characteristics between glomus tumors and GVM may indicate a potential involvement of the NOTCH pathway in GVM pathogenesis, prompting a need for further research to enhance understanding and potentially develop treatments for GVM. This exploration is particularly relevant given the ongoing investigation of several NOTCH-targeted therapies, with DLL4-targeted therapies showing promising results in preclinical studies (Zhou *et al.*, 2022).

c. RAS-MAPK/ERK pathway, RhoA/ROCK pathway, CCM1, CCM2, and CCM3 genes

Certain pathways and genes have been explored in the context of GVMs, yet clear causal associations remain elusive. The RAS-MAPK/ERK pathway, a pivotal regulator of cellular proliferation and differentiation, has not been explicitly linked to GVMs. However, the observation of neurofibromin loss in glomus cells raises the possibility of secondary effects on this pathway, rather than indicating a primary pathogenic role. Neurofibromin, encoded by the NF1 gene, acts as a negative regulator of the RAS pathway (Bergoug *et al.*, 2020).

In the exploration of genetic factors, mutations in the CCM1, CCM2, and CCM3 genes have been linked to cerebral cavernous malformations (Queisser *et al.*, 2021). The adaptor complex comprised of CCM1, CCM2, and CCM3 proteins interact with MAP3K3 as well as with the small GTPase RAC1 (Ras-related C3 botulinum toxin substrate 1), loss of this complex induces MAP3K3 (Queisser *et al.*, 2021). Furthermore, this protein complex plays a crucial role in controlling endothelial barrier function by suppressing RhoA-ROCK activity downstream of cell–cell adherens junctions. Loss of CCM leads to heightened RhoA–ROCK activity, resulting in elevated stress fiber formation and the disturbance of adherens junctions, thereby compromising endothelial integrity (Brouillard *et al.*, 2020). This pathway influences vascular morphogenesis through the regulation of contractility and migration, crucial in processes such as angiogenesis and vascular morphogenesis. As research continues, a deeper understanding of these pathways and genetic influences may unveil new therapeutic targets for GVMs.

B.3. Tools for identifying potential therapeutic targets in GVM

Genetic profiling is essential for the accurate diagnosis of GVMs (Bisdorff-Bresson, Eyries and Boccara, 2021). While deep sequencing technologies have facilitated the unbiased detection of mutations in the clinical setting, their accessibility and availability remain limited. In many cases, targeted Sanger sequencing or the use of specific gene panels represent more affordable and feasible alternatives for clinical practice. However, these approaches carry the risk of lower sensitivity and may miss mutations in genes not included in the panels.

An additional challenge in detecting mutations in GVMs lies in their somatic mosaic nature, meaning that mutations may be present only in a subset of cells within the affected tissues. This requires higher sequencing depth to ensure accurate identification of the causal mutation in patient samples (Li *et al.*, 2023).

Optimizing access to deep clinical sequencing could significantly improve diagnostic sensitivity in GVMs. A promising strategy in this context is the use of liquid biopsy (LB) based on next-generation sequencing (NGS) for the analysis of cell-free DNA (cfDNA). This technique has proven useful for early cancer detection and monitoring, bypassing the spatial and temporal limitations of conventional tissue biopsies (Palmieri *et al.*, 2021; Li *et al.*, 2023). Its application in the study of vascular malformations, which share somatic mutations with some tumors, suggests its potential in the diagnosis and monitoring of GVMs.

NGS-LB has enabled the identification of somatic activating mutations p.T1010I and p.D1028N in the MET gene, previously not associated with vascular malformations, but involved in signaling pathways altered in both hereditary vascular malformations and GVMs (Palmieri *et al.*, 2021). These findings highlight the potential of this technology to detect somatic mosaic mutations at exceptionally low frequencies, which could help characterize vascular phenotypes.

In addition, deep sequencing can generate genetic data that allow for the identification of modifier mutations, which may influence disease severity or progression. Integrating these advanced tools into clinical practice could not only improve diagnostic accuracy but also support the development of targeted therapeutic strategies for GVMs.

Significant advances have increased our understanding of GVMs and the vital functional role of GLMN in disease pathogenesis. However, the factors responsible for the variable penetrance of the condition, the range of dysregulated cellular signaling pathways, and their potential for therapeutic targeting have not yet been fully explored. Next-generation sequencing will facilitate a better understanding of the molecular landscape of GVMs and allow for the testing of therapeutic options to prevent complications or halt disease progression.

These advances in GVM research and molecular tools highlight the importance of studying clinical cases that can provide further insights into the underlying mechanisms of the disease. In this context, we describe the case of a patient with an uncommon clinical presentation of GVM, whose detailed phenotypic characterization may help broaden our understanding of the clinical and genetic variability of this condition.

HYPOTHESIS

The hypothesis of this work proposes that additional mutations in vascular-related genes, such as CCM2L, may function as genetic modifiers that influence the severity and distribution of glomuvenous malformations beyond canonical GLMN mutations. Advanced molecular analyses can help elucidate their pathogenic contribution.

2. AIM AND OBJECTIVES

GENERAL AIM

The aim of this doctoral thesis is to advance the understanding of the molecular and genetic mechanisms involved in skin diseases, with a particular emphasis on the role of ncRNAs and the contribution of somatic mutations to the pathogenesis of GVMs. Furthermore, the thesis seeks to identify novel therapeutic strategies through the analysis of molecular targets and relevant genetic alterations associated with these conditions.

SPECIFIC OBJECTIVES

1. To identify and analyze potential molecular targets for the development of new therapies for GVMs by exploring the underlying pathogenic mechanisms and the signaling pathways implicated in the disease.
2. To identify and characterize novel somatic mutations in tissue affected by GVMs and assess their role in disease pathogenesis, with the aim of expanding current knowledge on the genetic etiology of GVMs. This includes:
    - Confirming the presence of the mutation in affected tissue samples through whole genome sequencing (WGS).
    - Evaluating the functional impact of the mutation in human umbilical vein endothelial cells (HUVECs), specifically its effect on cellular proliferation.
    - Investigating how the mutation in CCM2L alters the MAPK/ERK signaling pathway and contributes to cellular hyperproliferation.
    - Contextualizing these findings in relation to previously reported GLMN mutations and their link to the pathophysiology of GVMs.
    - Discussing the clinical relevance of these findings and their potential application in developing new diagnostic or therapeutic strategies for GVMs.

3. MATERIALS AND METHODS

3.1. Review of the literature

Review of Non-Coding RNAs in Dermatology

To contextualize the role of ncRNAs, a literature review was conducted focusing on their biological functions in skin physiology and pathology. Scientific articles were retrieved from PubMed and Web of Science, with no strict time restriction, using terms such as “non-coding RNA”, “microRNA”, “long non-coding RNA”, “skin”, and “dermatology”. Publications were screened for relevance to dermatological diseases, with emphasis on mechanistic studies and translational applications.

Review of Molecular Targets in Glomuvenous Malformations

A second review was performed to identify molecular and genetic pathways relevant to GVMs. Searches in PubMed, Web of Science, and Embase included the terms “glomuvenous malformation” and “glomulovenous”, restricted to articles in English or Spanish published between January 1994 and December 2023. After removal of duplicates and non-relevant items, studies describing genetic mutations, molecular mechanisms, and therapeutic approaches were included.

3.2. Clinical report

We describe the case of a two-week-old female patient who, at birth, presented with cutaneous lesions consistent with an extensive glomuvenous malformation (GVM) and right unilateral preaxial polydactyly. Physical examination revealed a large violaceous plaque extending from the right inguinal region to the right foot, following a pattern characteristic of Blaschko's lines. In the most distal region, a sixth toe was observed on the right foot, as illustrated in Figures 2a and 2b. Additionally, an oval-shaped violaceous plaque measuring approximately 3 cm in longitudinal diameter and 1.5 cm in transverse diameter was identified on the lower right scapular area, also consistent with the diagnosis (Figure 2c).

The patient was born at term via spontaneous vaginal delivery, with no reported complications during pregnancy and no placental abnormalities. No additional pathological findings were noted on physical examination, and there was no family history of GVM.

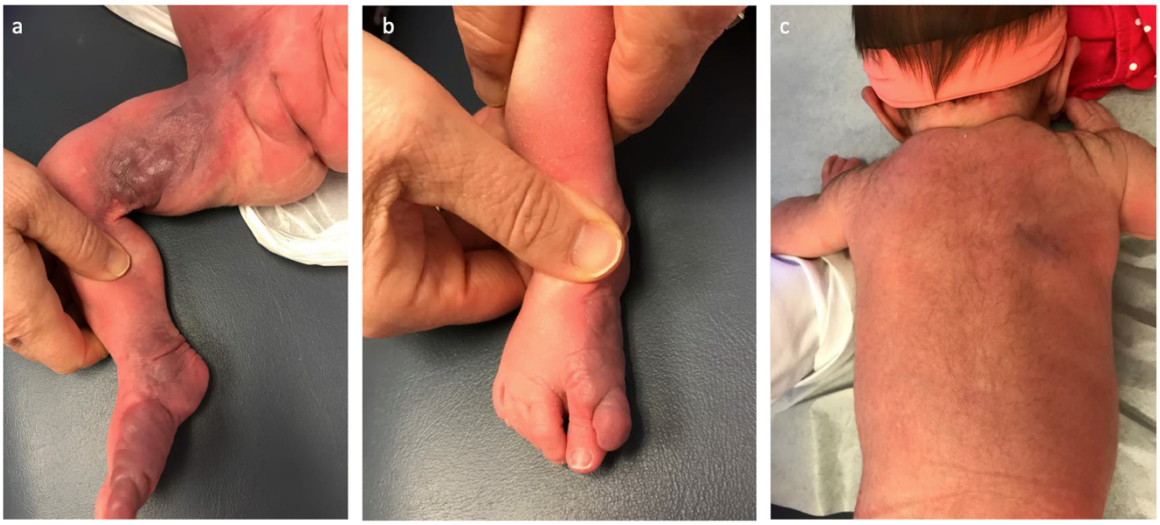


***Figure 2**. Images showing the congenital lesions of the patient under study. (a) Irregular violaceous plaque extending from the right inguinal region to the right foot. (b) Image of the right foot showing a sixth toe medial to the first toe, with tissue consistent with glomuvenous malformations (GVM). (c) Oval-shaped violaceous plaque on the right scapular region.*

Magnetic resonance imaging revealed plaque-like abnormalities affecting the skin and soft tissues, extending from the anterior pelvic wall to the right lower extremity (Figure 3).

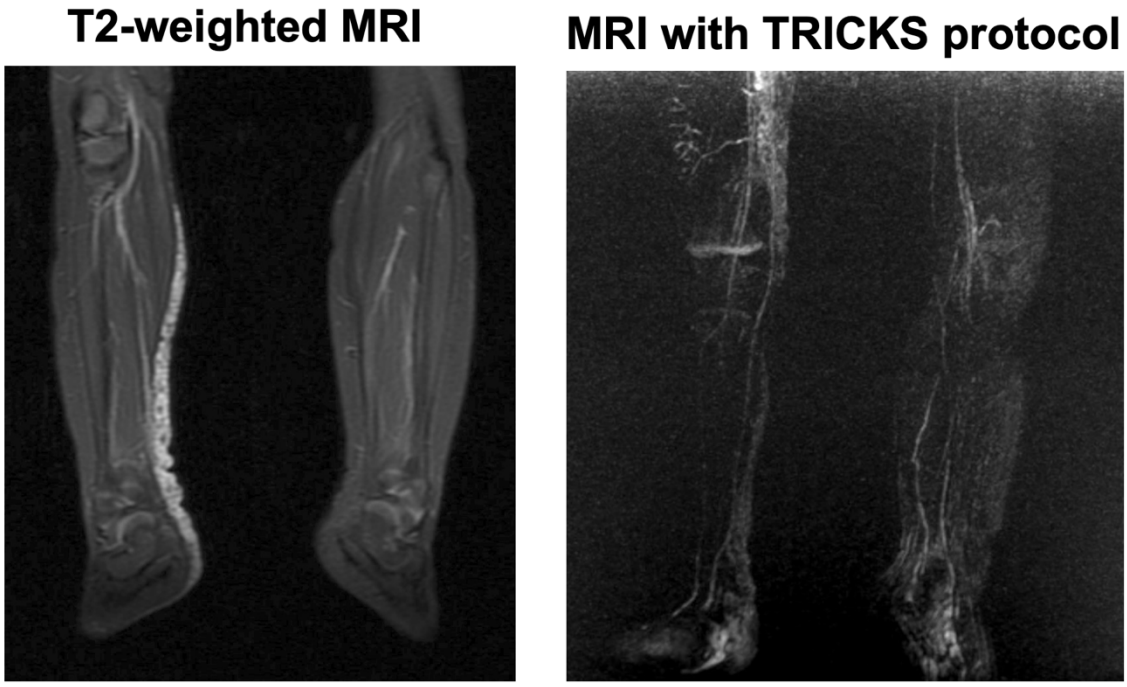


***Figure 3**. Radiological images of the lesion obtained by magnetic resonance imaging (MRI). Left: MRI shows T2 hyperintense vascular channels along the medial-posterior region of the right lower leg. Right: Magnetic resonance angiography (MRA) using the TRICKS protocol reveals delayed-phase enhancement of the vascular lesion, findings consistent with a GVM.*

The blaschkoid distribution of the GVM suggested the possibility of a postzygotic somatic mutation (Wasilewska *et al.*, 2022). With approval from the UC San Diego IRB and after obtaining informed consent from the patient's parents, we performed whole-exome sequencing (WES) on genomic DNA extracted from both affected and unaffected skin.

Genetic analysis revealed the presence of a previously unreported mutation, GLMN c.515C>A, p.Ser172Ter, with a variant allele frequency close to 50%, suggesting a heterozygous loss-of-function pattern. Since the allele frequency was similar in both the affected and control tissue, no clear secondary inactivation event of GLMN was identified in the lesional skin.

To our knowledge, the association between polydactyly and GVM has not been previously described, raising the possibility of an additional genetic alteration contributing to this clinical manifestation.

3.2.1. Study design

This work follows a single-case study design supported by subsequent translational research. Given the rarity of glomuvenous malformations and the unusual phenotype observed, this approach enables an in-depth investigation of potential molecular contributors to disease presentation. As an inherent limitation, conclusions derived from a single patient cannot be broadly generalized to the wider population, and statistical validation is not possible. Nonetheless, the combination of detailed clinical documentation, comprehensive genomic analysis, and functional assays performed in endothelial cells strengthens the biological plausibility of the findings. Although the results should be interpreted with caution, the extensive research performed provides valuable insight and supports the generation of new hypotheses for future studies.

3.2.2. Ethical Approval and Informed Consent

Ethical approval for the clinical case, diagnostic procedures, and research use of biological samples was granted by the UC San Diego Institutional Review Board (IRB) on January 27, 2022. Written informed consent for clinical evaluation and research participation was obtained from the patient's parents (legal guardians), in accordance with CEIm requirements. In addition, written consent authorizing the publication of clinical photographs was obtained and signed by the parents on August 26, 2021. The acquisition, processing, and dissemination of all clinical and imaging data were conducted in accordance with institutional policies, applicable regulations, and international ethical guidelines for research involving human subjects.

3.3. Histology and Immunohistochemistry

Histological and immunohistochemical analyses were performed on tissue sections obtained from the patient's skin lesion. The tissue was fixed in 10% formalin and embedded in paraffin. Subsequently, 4 µm-thick sections were cut using a microtome and mounted on treated glass slides.

Hematoxylin and Eosin (H&E) Staining

The sections were deparaffinized and rehydrated through a graded series of xylene and alcohol baths. Staining was performed with hematoxylin for 5 minutes, followed by rinsing in running tap water and differentiation in hydrochloric acid-alcohol. The sections were then stained with eosin for 1 minute, dehydrated through an ascending alcohol series, and mounted with a permanent mounting medium. This staining allowed for the evaluation of overall tissue architecture and cellular morphology.

Immunohistochemistry for CD34 and SMA

To detect endothelial and smooth muscle cells, primary antibodies against CD34 and smooth muscle actin (SMA) were used. Following deparaffinization and rehydration, antigen retrieval was performed by incubating the slides in citrate buffer (pH 6.0) in a microwave oven for 20 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide, and sections were incubated with blocking serum. The slides were then incubated overnight at 4 °C with primary antibodies: anti-CD34 (1:100 dilution) and anti-SMA (1:200 dilution). The following day, a peroxidase-conjugated secondary antibody was applied, and detection was carried out using diaminobenzidine (DAB). Finally, sections were counterstained with hematoxylin, dehydrated, and mounted.

3.4. Sequencing and analysis

Biopsies were obtained from both lesional and non-lesional skin areas of the patient diagnosed with glomuvenous malformations. Whole-exome sequencing (WES) was performed using standard protocols. High-quality DNA samples were sequenced on an Illumina HiSeq platform to achieve comprehensive genome coverage.

Single nucleotide variants (SNVs) and insertions/deletions were identified using Mutect2, a tool within the Genome Analysis Toolkit (GATK). The analysis involved comparing the non-lesional

area (control) with the lesional area (case) to identify genetic differences. The resulting variants were filtered using Microsoft Excel.

The initial comparison identified 616 mutations. To prioritize variants for further study, we applied filters to identify potentially deleterious mutations based on criteria such as mutation type, conservation, and predicted impact on protein function. Additionally, variants were evaluated using the Combined Annotation Dependent Depletion (CADD) score and the PHRED quality score to assess the likelihood of pathogenicity (Dompmartin *et al.*, 2009).

From the filtered list, 46 mutations were considered potentially deleterious. A more detailed screening was conducted based on the known functions of the genes involved in vascular and skin biology. This led to the selection of four candidate mutations for further investigation in the following genes: GDF10, C3, CCM2L, and DLG3 (Table 2).

3.5. Short hairpin RNAs (ShRNA) Design

Since the selected mutations result in gene loss-of-function, four shRNAs were designed for each gene to silence their expression in the study cells: human umbilical vein endothelial cells (HUVECs). The shRNAs were designed using the GPP Web Portal from the Broad Institute, resulting in a total of 16 shRNAs for the four target genes.

Primer pairs for each gene were designed using the Primer-BLAST tool from the U.S. National Library of Medicine. The sequences of the primers are detailed in Table 6. The shRNAs designed for each gene were inserted into the pLKO plasmid using a transformation protocol with competent E. coli cells (MIX&GO competent cells from Zymo Research).

Table 6. *Plasmid and primer sequences used in the study.*

qPCR *primers*		
REAGENT	**SOURCE**	**IDENTIFICATOR**
CCM2L-FWD	Eton Bioscience	AGCCCCGACGCCTACTG
CCM2L-REV	Eton Bioscience	ACTCTGGTCCCCGTAGATGA
C3-FWD	Eton Bioscience	GCTGCTCCTGCTACTAACCC
C3-REV	Eton Bioscience	ACCATGGTCTCCTCGCTCT
DLG3-FWD	Eton Bioscience	TGATGAACAGCAGCATGAGC
DLG3-REV	Eton Bioscience	CCTGCCACCACTCATCATCA
GDF10-FWD	Eton Bioscience	GGGCAACACGGTCCGC
GDF10-REV	Eton Bioscience	GAGTAGAAGTGGAAAGTGGCCG
ShRNA *primers*		
REAGENT	**SOURCE**	**IDENTIFICATOR**
1. C3-sh1-Forward	Eton Bioscience	CCGGTCTACGAAGCTCATGAATATACTCGAGTATATTCATGAGCTTCGTAGATTTTTG
2. C3-sh1-Reverse	Eton Bioscience	AATTCAAAAATCTACGAAGCTCATGAATATACTCGAGTATATTCATGAGCTTCGTAGA
3. C3-sh2-Forward	Eton Bioscience	CCGGTGCAGGAGGCTAAAGATATTTCTCGAGAAATATCTTTAGCCTCCTGCATTTTTG
4. C3-sh2-Reverse	Eton Bioscience	AATTCAAAAATGCAGGAGGCTAAAGATATTTCTCGAGAAATATCTTTAGCCTCCTGCA
5. C3-sh3-Forward	Eton Bioscience	CCGGTCAACTCACCTGTAATAAATTCTCGAGAATTTATTACAGGTGAGTTGATTTTTG
6. C3-sh3-Reverse	Eton Bioscience	AATTCAAAAATCAACTCACCTGTAATAAATTCTCGAGAATTTATTACAGGTGAGTTGA
7. C3-sh4-Forward	Eton Bioscience	CCGGCAGATAAAGCTTCAGTTATATCTCGAGATATAACTGAAGCTTTATCTGTTTTTG
8. C3-sh4-Reverse	Eton Bioscience	AATTCAAAAACAGATAAAGCTTCAGTTATATCTCGAGATATAACTGAAGCTTTATCTG
9. GDF10-sh1-Forward	Eton Bioscience	CCGGACGGAAAGAACTCTGTTTAAACTCGAGTTTAAACAGAGTTCTTTCCGTTTTTTG
10. GDF10-sh1-Reverse	Eton Bioscience	AATTCAAAAAACGGAAAGAACTCTGTTTAAACTCGAGTTTAAACAGAGTTCTTTCCGT
11. GDF10-sh2-Forward	Eton Bioscience	CCGGTCGACCAGAAGGCCGTGTATTCTCGAGAATACACGGCCTTCTGGTCGATTTTTG
12. GDF10-sh2-Reverse	Eton Bioscience	AATTCAAAAATCGACCAGAAGGCCGTGTATTCTCGAGAATACACGGCCTTCTGGTCGA
13. GDF10-sh3-Forward	Eton Bioscience	CCGGGGCTGGAATGAATGGATAATCCTCGAGGATTATCCATTCATTCCAGCCTTTTTG
14. GDF10-sh3-Reverse	Eton Bioscience	AATTCAAAAAGGCTGGAATGAATGGATAATCCTCGAGGATTATCCATTCATTCCAGCC
15. GDF10-sh4-Forward	Eton Bioscience	CCGGACTGTAGATTGTGTATGTTATCTCGAGATAACATACACAATCTACAGTTTTTTG
16. GDF10-sh4-Reverse	Eton Bioscience	AATTCAAAAAACTGTAGATTGTGTATGTTATCTCGAGATAACATACACAATCTACAGT
17. CCM2L-sh1-Forward	Eton Bioscience	CCGGAGCTGCTGCAGCTCCTTTAATCTCGAGATTAAAGGAGCTGCAGCAGCTTTTTTG
18. CCM2L-sh1-Reverse	Eton Bioscience	AATTCAAAAAAGCTGCTGCAGCTCCTTTAATCTCGAGATTAAAGGAGCTGCAGCAGCT
19. CCM2L-sh2-Forward	Eton Bioscience	CCGGATCTCCTGATTCTCCTTATATCTCGAGATATAAGGAGAATCAGGAGATTTTTTG
20. CCM2L-sh2-Reverse	Eton Bioscience	AATTCAAAAAATCTCCTGATTCTCCTTATATCTCGAGATATAAGGAGAATCAGGAGAT
21. CCM2L-sh3-Forward	Eton Bioscience	CCGGCCCGATAAGAGCTCAATAAATCTCGAGATTTATTGAGCTCTTATCGGGTTTTTG
22. CCM2L-sh3-Reverse	Eton Bioscience	AATTCAAAAACCCGATAAGAGCTCAATAAATCTCGAGATTTATTGAGCTCTTATCGGG

23. CCM2L-sh4-Forward	Eton Bioscience	CCGGGGACATTTCACCATGGAATATCTCGAGATATTCCATGGTGAAATGTCCTTTTTG
24. CCM2L-sh4-Reverse	Eton Bioscience	AATTCAAAAAGGACATTTCACCATGGAATATCTCGAGATATTCCATGGTGAAATGTCC
25. DLG3-sh1-Forward	Eton Bioscience	CCGGTCTCCGAATTTCCACATAAATCTCGAGATTTATGTGGAAATTCGGAGATTTTTG
26. DLG3-sh1-Reverse	Eton Bioscience	AATTCAAAAATCTCCGAATTTCCACATAAATCTCGAGATTTATGTGGAAATTCGGAGA
27. DLG3-sh2-Forward	Eton Bioscience	CCGGCTCACTGGAAGAGATTTATAACTCGAGTTATAAATCTCTTCCAGTGAGTTTTTG
28. DLG3-sh2-Reverse	Eton Bioscience	AATTCAAAAACTCACTGGAAGAGATTTATAACTCGAGTTATAAATCTCTTCCAGTGAG
29. DLG3-sh3-Forward	Eton Bioscience	CCGGGAATGAGGCTAACTGTATAAACTCGAGTTTATACAGTTAGCCTCATTCTTTTTG
30. DLG3-sh3-Reverse	Eton Bioscience	AATTCAAAAAGAATGAGGCTAACTGTATAAACTCGAGTTTATACAGTTAGCCTCATTC
31. DLG3-sh4-Forward	Eton Bioscience	CCGGGTCTACCCATGAATAAATAAACTCGAGTTTATTTATTCATGGGTAGACTTTTTG
32. DLG3-sh4-Reverse	Eton Bioscience	AATTCAAAAAGTCTACCCATGAATAAATAAACTCGAGTTTATTTATTCATGGGTAGAC

DNA extraction was performed using the QIAprep Spin Miniprep Kit, and sequencing was carried out to verify the correct insertion of the shRNAs into the plasmid.

Once the plasmids were validated, a quantitative polymerase chain reaction (qPCR) assay was conducted to determine which shRNAs effectively suppressed the expression of the selected genes (Table 7).

3.6. Design of CCM2L overexpression plasmids

The wild-type (CCM2L-WT) and mutant (CCM2L-R502H) sequences were cloned into the pLEX lentiviral vector fused to a hemagglutinin (HA) tag. The CCM2L-WT and CCM2L-R502H sequences were amplified by PCR, purified, and digested along with the pLEX vector using appropriate restriction enzymes. The digested products were purified and ligated, followed by transformation into competent E. coli cells.

Positive clones were identified by colony PCR, restriction digestion, and sequencing. Plasmid DNA from verified clones was prepared using a miniprep kit, ensuring the correct incorporation of the sequences for subsequent detection with HA antibodies.

3.7. Lentivirus production with 293T cells and PEI (polyethylenimine)

Lentivirus was generated using 293T cells and PEI. Transfection mixtures were prepared with the lentiviral plasmids designed for this study (primers detailed in Table 2), along with pUG-MDG and pCMV-Δ8.91, Opti-MEM, and PEI. These mixtures were added to 293T cells, which were then incubated under standard culture conditions at 37°C. (Process shown in a scheme in Figure 4).

Lentiviral supernatants were collected 72 hours after transfection and mixed with Lenti-X Concentrator (Takara) at a 1:3 volume ratio, followed by incubation at 4 °C for at least 1 hour. The mixture was then centrifuged at 2000 × g for 1 hour at 4 °C. After removing the supernatant, the lentiviral pellet was resuspended in cold phosphate-buffered saline (PBS) and aliquoted for storage at -20 °C.

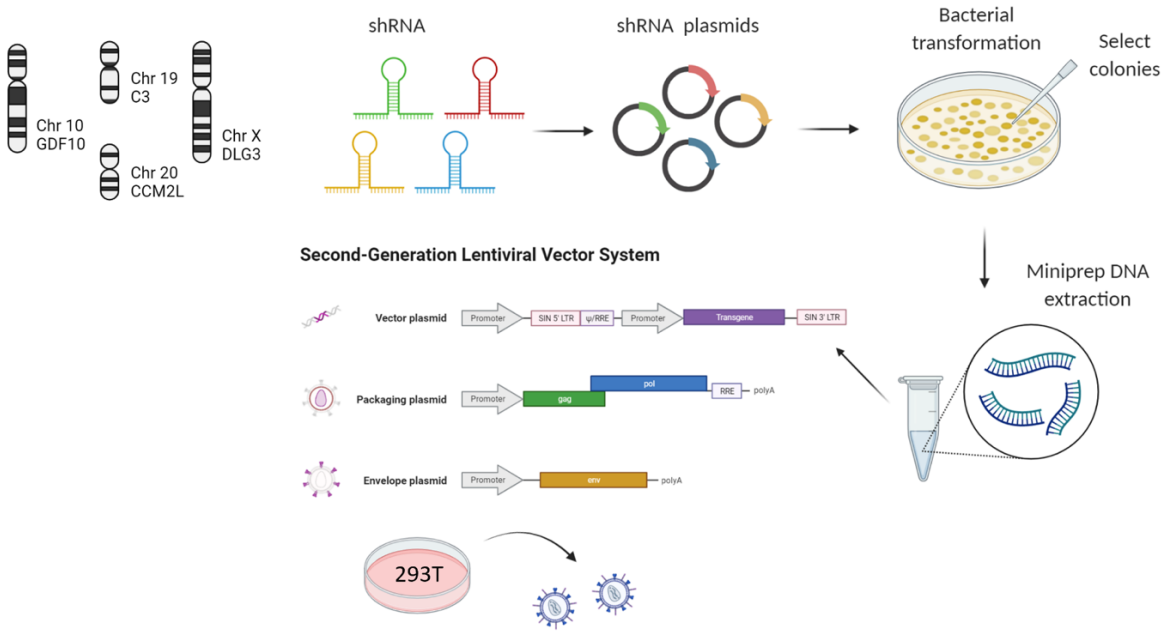


Figure 4. *Schematic of the shRNA Design and Lentivirus Production Process.*

3.8. HUVEC (Human Umbilical Vein Endothelial Cells) infection

The primary cells used in our study were HUVECs. Cell infection was carried out in a suspension of 5×10^5 HUVECs with polybrene (PB), by adding 50 µl of a 30× concentration of each designed lentivirus. Scrambled (SCR) lentivirus was used as a knockdown control, and an empty vector was used as the overexpression control. Infected cells were selected using puromycin, as the lentiviral constructs contained a puromycin resistance gene.

3.9. siRNA knowdown in HUVECs

To investigate the functional role of the candidate genes in endothelial cells, gene silencing was performed using siRNA in HUVECs. siRNAs targeting GLMN, CCM2L, and both genes combined (GLMN + CCM2L; double knockdown) were used, along with a scrambled siRNA as a control. Cells were seeded in 24-well plates (30,000–40,000 cells/well, depending on the assay) and transfected with 6 pmol of siRNA using Lipofectamine™ RNAiMAX in Opti-MEM medium. After 48 hours of incubation, knockdown efficiency was assessed by RT-qPCR using gene-specific primers and the ΔΔCt method. Only conditions showing >50% reduction in gene expression were used for further functional assays.

3.10.Alamar Blue® Cell Proliferation Assay

The Alamar Blue® assay is a colorimetric and fluorometric method used to assess cell viability and proliferation based on metabolic activity. The assay employs resazurin, a non-toxic, cell-permeable blue dye that is reduced to the fluorescent and pink-colored compound resorufin by metabolically active cells.

In this study, the assay was used to evaluate the impact of gene knockdown or overexpression on the proliferation of HUVECs. Cells were subjected to either lentiviral transduction (for overexpression or shRNA-mediated knockdown) or siRNA transfection and subsequently seeded in 24-well plates.

At designated time points (day 0, day 3, and day 6 post-treatment), 10% v/v of Alamar Blue® reagent (Thermo Fisher Scientific) was added directly to the culture medium. The cells were then incubated at 37 °C in a humidified incubator with 5% CO_2 for 4 hours.

After incubation, fluorescence was measured using a microplate reader (excitation at 560 nm, emission at 590 nm). Background fluorescence from control wells containing only medium and reagent (no cells) was subtracted. The resulting fluorescence intensity is proportional to the number of viable, metabolically active cells and reflects changes in proliferation.

The assay was performed in triplicate for each condition. Results were normalized to baseline values (day 0) to determine fold changes in cell proliferation over time.

3.11. Migration assay

HUVECs were infected with the designed lentiviruses or transfected with siRNAs in the case of the double knockdown condition. Subsequently, a cell migration analysis was performed using a wound healing assay. Treated HUVECs were cultured in 20 mm diameter wells, and a scratch was made in each well using a 200 µl pipette tip to create a cell-free area. Wound closure progression was monitored at 2-hour intervals over 24 hours using ImageJ software. The wound closure rate was then calculated based on the measured data.

3.12. RNA isolation and Quantitative Reverse Transcription PCR (RT-qPCR)

For both infected and non-infected HUVECs, samples were collected in RLT buffer (Qiagen). RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen). The extracted RNA was then reverse transcribed into cDNA using the iScript Reverse Transcription Supermix (Bio-Rad).

To assess CCM2L overexpression, RT-qPCR was performed using the iTaq Universal SYBR Green Supermix (Bio-Rad) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The specific primers used are listed in Table 6.

3.13. Western blot

Protein Extraction and Quantification

Total protein was extracted from HUVECs under various experimental conditions. Cells were first washed with cold phosphate-buffered saline (PBS) and lysed in cold RIPA buffer (200 µL per 6 cm culture dish). The cells were scraped using a cell scraper and gently resuspended by pipetting. The lysates were transferred to pre-chilled Eppendorf tubes and sonicated for 5 minutes using 30-second on/off intervals at high power, while maintained on ice. After sonication, the samples were centrifuged at 12,000 rpm for 10 minutes at 4 °C. The resulting supernatant containing total protein was collected into a new pre-chilled tube, and the pellet was discarded.

Protein concentration was measured using the Pierce BCA Protein Assay Kit (23225, Thermo Fisher Scientific). Equal amounts of protein (3 µg in 20 µL total volume) were loaded into each well of a NuPAGE 4–12% gel. Electrophoresis was performed at 100 V for 1 hour, followed by transfer of proteins onto nitrocellulose membranes (MilliporeSigma).

Membranes were incubated overnight at 4 °C with appropriate primary antibodies. The next day, membranes were incubated with fluorescently labeled anti-rabbit or anti-mouse secondary antibodies and visualized using the Odyssey imaging system. Band intensities were quantified using ImageJ and Image Studio (Odyssey) software.

Two Western blot-based assays were performed to evaluate protein stability and MAPK/ERK pathway activation:

1. Protein Stability Assay with Cycloheximide (CHX)

To assess the stability of CCM2L protein, HUVECs were infected with lentiviruses encoding CCM2L wild type (WT), CCM2L R502H, or an empty vector control. Protein synthesis was inhibited by treating the cells with 300 µL of cycloheximide (CHX). Samples were collected at 0-

, 4-, 8-, and 24-hours post-treatment. Western blotting was performed using anti-HA and β-actin antibodies to evaluate CCM2L protein degradation over time (Figure 8).

2. MAPK/ERK Pathway Activation Assay

To investigate whether the MAPK/ERK pathway is involved in the cellular response, HUVECs were cultured under the same conditions as above. Protein extracts were analyzed by Western blot using antibodies specific to phosphorylated MAPK/ERK, total MAPK/ERK, and β-tubulin as a loading control. The objective was to evaluate pathway activation in cells overexpressing CCM2L WT, CCM2L R502H, or empty vector (Figure 8).

3.14. Statistical Analysis

All experiments were conducted in replicates (at least 3 times), and data were analyzed using ANOVA and t-tests as appropriate. A p-value < 0.05 was considered statistically significant.

3.15. CCM2L 3D Structure Visualization

The 3D structure of the CCM2L-WT protein and the CCM2L-R502H mutation was predicted using UCSF ChimeraX and AlphaFold. UCSF ChimeraX, a molecular visualization software, was used for the initial handling and visualization of the protein structures, while AlphaFold, an advanced artificial intelligence system developed by DeepMind, was employed to predict accurate 3D models from amino acid sequences.

The WT CCM2L sequence served as the reference structure, while the CCM2L-R502H mutation—characterized by the substitution of arginine with histidine at position 502—was selected for investigation. Amino acid sequences were input into the AlphaFold prediction pipeline to generate 3D structural models, which were subsequently analyzed using UCSF ChimeraX for visual inspection and comparison.

The impact of the mutation on protein conformation and stability was assessed through comparative analysis and structural visualization. Validation was performed using quality

assessment metrics and comparison with available experimental data, in accordance with ethical guidelines and data usage policies.

4. RESULTS

4.1. Histology and Immunohistochemistry

Histological analysis of the affected skin revealed dilated venous vessels in the dermis (Figure 5), lined by uniform cuboidal cells. Immunohistochemical staining showed positive CD34 expression in the vessels, while the cuboidal lining cells expressed smooth muscle actin α (SMA+), findings consistent with an extensive plaque-type glomuvenous malformation (GVM) associated with preaxial polydactyly.

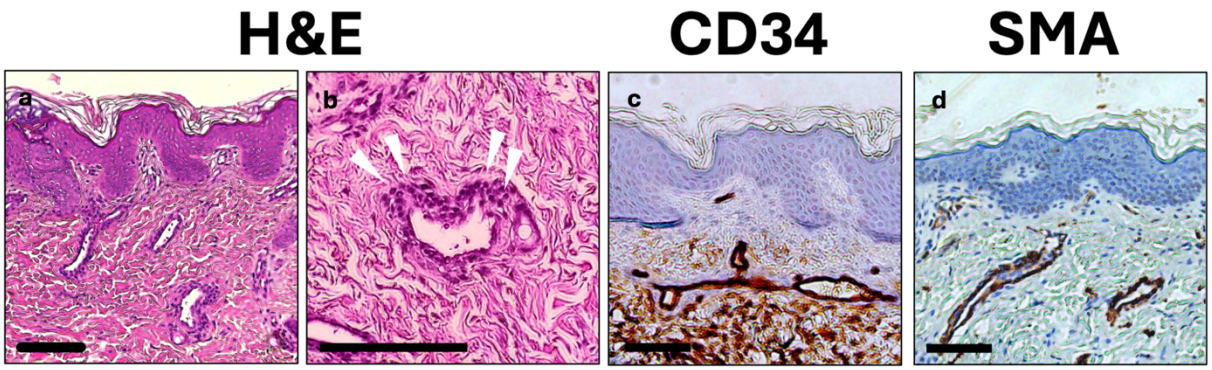


***Figure 5**. Images a and b show hematoxylin and eosin (H&E) staining; glomus cells are indicated by white arrowheads. The second image is shown at higher magnification. Image c presents immunohistochemical staining with the CD34 marker, specifically highlighting the blood vessel regions. Image d shows staining with smooth muscle actin α (SMA), allowing visualization of smooth muscle cells within the blood vessels, indicating the presence of glomus cells—a characteristic feature of GVMs.*

4.2. Potentially deleterious Mutations GDF10, C3, CCM2L y DLG3

When comparing lesional and non-lesional skin samples from the patient shown in Figure 2, we identified 616 mutations in the lesional skin. Subsequent filtering revealed 46 potentially deleterious mutations. The key mutations selected for further investigation were located in the genes GDF10, C3, CCM2L, and DLG3 (Table 7). Among them, the mutations in DLG3 and GDF10

were gain-of-stop-codon mutations in GVM, while the C3 mutation was a non-synonymous single-nucleotide variant (SNV) involving a C-to-T substitution at position 6694567 [rs6694567C/T] (Ref. GRCh37.p13 Primary Assembly). The study of C3 was discontinued due to inconclusive results following qPCR analysis (Figure 6).

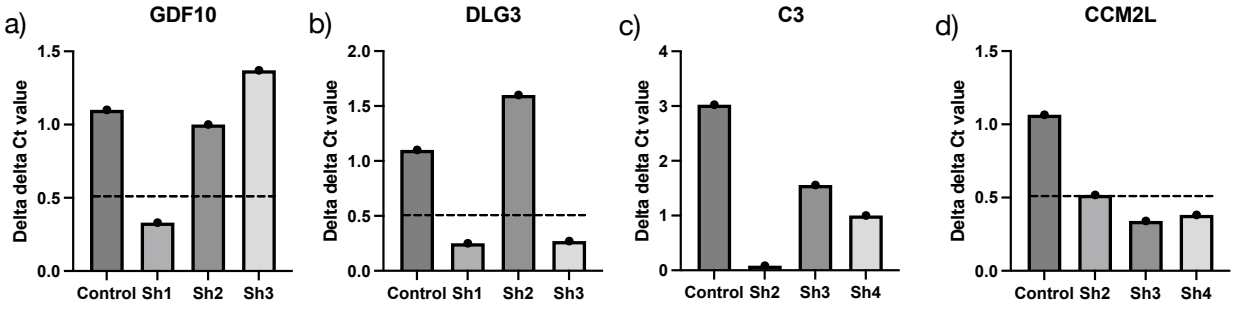


***Figure 6**. qPCR results showing gene knockdown efficiency. The dashed line marks the 0.5 value; shRNAs with values below this threshold were selected for achieving more than 50% gene silencing.*

The CCM2L mutation (NC_000020.10, location Ch20 20q11.21, Ref. GRCh37.p13 Primary Assembly, Gene ID 140706) was a non-synonymous single nucleotide variant (SNV) involving a G-to-A substitution at position 30618906 [rs867616586 G/A]. CCM2L c.1505G>A, p.Arg502His (CCM2L-R502H). See the information gathered for each gene considered in the investigation in Table 7.

***Table 7.** Selected genes for the study.*

Gen	GDF10	C3	DLG3	CCM2L
***Cromosomal position**	chr10	chr19	chrX	chr20
****** Gene region**	exonic	exonic	exonic	exonic
******* Variant classification**	stopgain	nonsynonymous SNV	stopgain	nonsynonymous SNV
§CADD score	8.043065	3.724187	2.021058	6.494174
†PHRED score	40	25.9	19.9	36
‡Probabilidad de ser perjudicial	YES	YES	YES	YES
‖ Prob. Deleterious	X	YES	YES	YES
¶Illness association	X	YES	X	X
⊥Skin disease association	YES	X	YES	YES

∃Summary NCBI	Tumor suppressor	Classical and alternative complement activation pathways.	Tumor suppressor	MAPK signaling cascade and cerebral cavernous malformations
χSkin expression (Mean RPKM)	0.153 ± 0.062	24.064 ± 7.594	2.611 ± 0.564	4.022 ± 0.984

* **Chromosomal position:** Chromosome where the gene is located.

** **Gene region:** Location or type of mutation within the gene.

*** **Variant classification:** Type of mutation associated with the gene.

§ **CADD Score:** Combined Annotation Dependent Depletion score estimating the deleteriousness of the variant.

†PHRED score: Scaled quality score representing the likelihood of a variant being functionally damaging.

‖**Prob. Deleterious:** Indicates whether the mutation is likely to be harmful.

¶**Illness association:** Whether the gene has been linked to any human disease.

⊥**Skin disease association:** Whether the gene has specifically been associated with dermatological conditions.

Tumor suppression/growth association: Indicates whether the gene is involved in tumor suppression or cell growth regulation.

∃**Summary NCBI:** Functional summary of the gene from the NCBI gene database.

χSkin expression (Mean RPKM): Average expression level of the gene in skin tissue, measured in RPKM (Reads Per Kilobase of transcript per Million mapped reads).

The genes included in Table 7 were selected based on various criteria relevant to the study. For each gene, detailed information was compiled, including chromosomal position, the region or type of identified mutation, and variant classification. Additionally, the likelihood of the mutation being deleterious was evaluated using the CADD score.

Their association with diseases in general—and specifically with skin-related conditions—was analyzed. The genes' involvement in processes such as tumor suppression or growth, as well as in cellular proliferation and differentiation, was also considered. To complement this information, a descriptive summary from the National Center for Biotechnology Information (NCBI) was included, along with data on gene expression levels in skin tissue, measured in RPKM units.

This comprehensive compilation of features allowed for the prioritization of genes of interest based on their biological and clinical relevance in the context of the study.

4.3. CCM2L *knockdown* partially reduced cell proliferation and improved cell migration in HUVECs

As previously mentioned, C3 knockdown resulted in inconclusive qPCR results and was therefore excluded from further analyses (Figure 6). Optimal shRNAs for gene knockdown were identified by qPCR (Figure 6) and used in subsequent assays.

Knockdown of DLG3 and GDF10 in HUVECs showed no growth over time. The proliferation assay results for GDF10-sh1, DLG3-sh1, and DLG3-sh3 displayed a flat line, indicating no proliferation. These findings were inconsistent with the expected GVM phenotype, suggesting that these genes do not positively regulate GVM. Due to these opposing results, GDF10 and DLG3 were excluded from further analysis.

CCM2L knockdown in HUVECs did not result in a statistically significant decrease in cell proliferation compared to the control, but it did show an increase relative to the other gene knockdowns (Figure 7a).

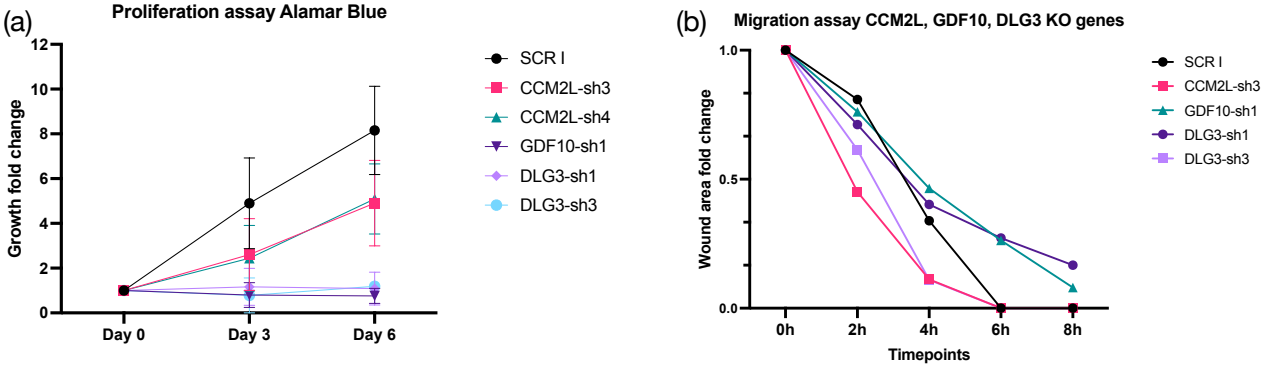


Figure 7. *Cell Proliferation and Migration Results in Silenced Genes. (a) Graph showing the results of the cell proliferation assay following gene knockdown in HUVECs. (b) Graph displaying the results of the cell migration assay following gene knockdown in HUVECs*

Wound healing assays, which evaluate cell migration, revealed that GDF10-sh1 and DLG3-sh1 exhibited delayed wound closure compared to the control. However, DLG3-sh3 showed faster

wound closure, similar to the results observed with CCM2L-sh3. This inconsistency in the DLG3 results led to the decision to exclude DLG3 from further analysis (Figure 7b).

In contrast, CCM2L knockdown cells demonstrated improved wound closure compared to the SCR control. CCM2L knockdown in HUVECs resulted in partially reduced cell proliferation and enhanced cell migration, indicating a complex regulatory role in these processes. Consequently, further studies were conducted focusing on the mutated CCM2L gene.

4.4. CCM2L-R502H resulted in increased proliferation of HUVECs but did not affect cell migration

To determine the impact of the mutation observed in the patient with GVM, we overexpressed both the wild-type (WT) and mutated (R502H) versions of *CCM2L* in HUVECs. Overexpression of both *CCM2L* WT and *CCM2L* R502H led to elevated proliferation rates compared to the control group. Notably, the *CCM2L* R502H mutation exhibited an even greater increase in proliferation, suggesting a potential pro-proliferative effect of this mutation in HUVECs (Figure 8a).

However, no significant differences in cell migration were observed between overexpression of *CCM2L* WT, *CCM2L*R502H, or the empty vector control (Figure 8b).

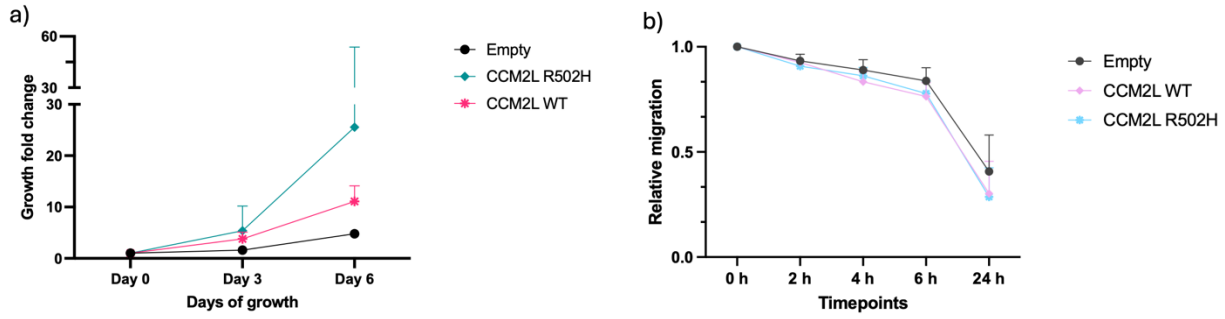


***Figure 8**. Cell Proliferation and Migration Results in Overexpressed Genes. (a) Graph showing the results of the cell proliferation assay following overexpression of CCM2L WT and CCM2L R502H in HUVECs. (b) Graph displaying the results of the cell migration assay following overexpression of CCM2L WT and CCM2L R502H in HUVECs.*

4.5. CCM2L-R502H increases protein stability and enhances MAPK/ERK activation in HUVECs

The protein stability assay revealed that the CCM2L R502H mutation resulted in higher protein levels compared to the WT, suggesting increased stability of the mutated protein (Figure 9 (a) and (b)).

To further investigate the underlying mechanisms of the observed proliferative effects, we examined the activation of the MAPK/ERK pathway. Western blot analysis showed that phospho-ERK expression decreased in cells overexpressing CCM2L WT or CCM2L R502H compared to the empty vector control. Notably, the CCM2L R502H mutation exhibited higher levels of phospho-ERK compared to the WT (Figure 9 (c) and (d)). These findings suggest that the CCM2L-R502H mutation enhances activation of the MAPK/ERK pathway relative to the WT, which may contribute to the increased proliferation observed in HUVECs.

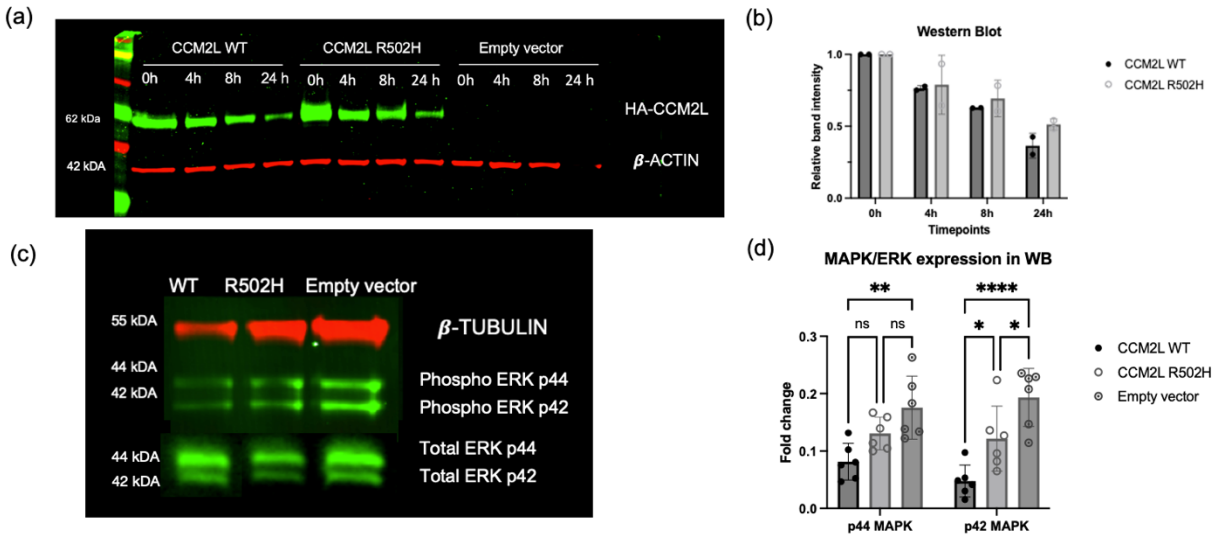


Figure 9. *Western Blot Result Images. (a) Western blot image of HA-CCM2L and β-actin loading control in protein lysates from HUVECs overexpressing CCM2L-R502H and CCM2L-WT, treated with 300 µg/mL cycloheximide (CHX) to assess protein stability over different time points. (b) Graph showing the relative expression of HA-CCM2L at different time points based on band intensity quantification. (c) Relative expression levels of phospho-ERK (p42 and p44) were compared by quantifying band intensities after overexpressing CCM2L-WT, CCM2L-R502H, and an empty*

*vector as control in HUVECs. (d) Western blot image showing phospho-ERK band intensities relative to β-tubulin expression. Statistical significance levels are indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.*

4.6. Doble knockdown of CCM2L y GLMN increased proliferation without affecting HUVEC migration

To examine whether the combined alteration of CCM2L and GLMN would produce distinct cellular effects, we performed individual and combined knockdown experiments (Figure 10).

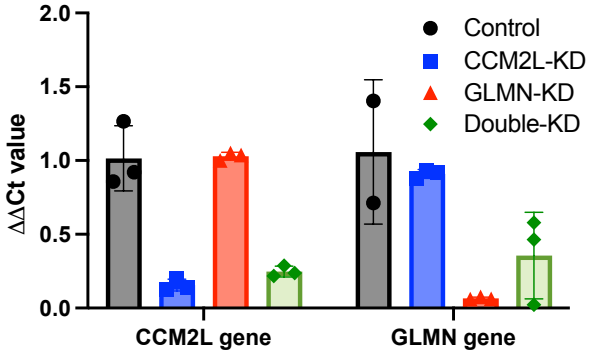


***Figure 10**. Knockdown Efficiency of siRNAs Targeting CCM2L, GLMN, and Both Genes Simultaneously in HUVECs. Quantitative PCR was performed to measure mRNA expression levels of CCM2L and GLMN following siRNA transfection. Expression levels were normalized to GAPDH and analyzed using the ΔΔCT method. Bars represent the mean ± standard deviation (SD) of ΔΔCT values from three biological replicates per condition. CCM2L-KD and GLMN-KD each show efficient knockdown of their respective targets, while the double knockdown condition ("Double-KD") demonstrates simultaneous suppression of both CCM2L and GLMN transcripts.*

Double knockdown increased HUVEC proliferation to a greater extent than individual *GLMN* knockdown (Figure 11a). In contrast, the double knockdown did not alter HUVEC migration speed compared to the control (Figure 11b).

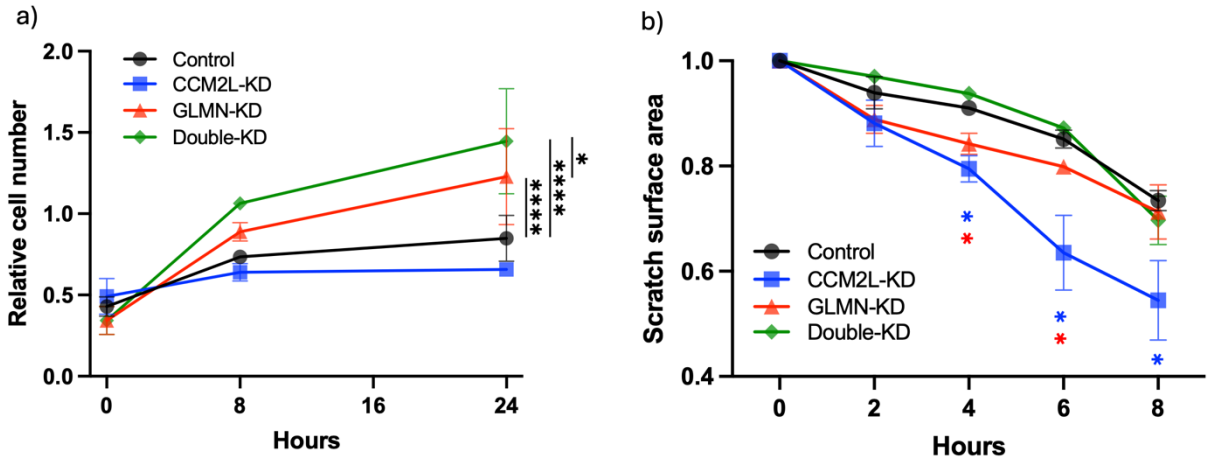


Figure 11. *Results of Double Knockdown of CCM2L and GLMN. (a) Proliferation assay in HUVECs. Growth curves of cells with CCM2L knockdown (CCM2L-KD), GLMN knockdown (GLMN-KD), double knockdown (double KD), and scrambled siRNA control. At 24 hours, GLMN-KD and double KD cells showed significantly increased proliferation compared to the control (****P < 0.0001). The double knockdown also showed greater proliferation than both CCM2L-KD (****P < 0.0001) and GLMN-KD (P = 0.0395), indicating a synergistic effect (two-way ANOVA with post hoc comparisons). (b) Migration assay in HUVECs after siRNA transfection. Wound closure was measured at 0, 2, 4, 6, and 8 hours in control, CCM2L-KD, GLMN-KD, and double KD cells. Two-way repeated measures ANOVA showed significant effects of time (****P < 0.0001), siRNA condition (P = 0.0002), and their interaction (****P < 0.0001). At 4 hours, both CCM2L-KD and GLMN-KD cells migrated significantly faster than the control (*P < 0.05). In contrast, the double knockdown migrated significantly slower than the individual knockdowns (*P < 0.05), but not significantly different from the control, indicating that combined knockdown of CCM2L and GLMN reverses the enhanced migration observed in the individual knockdowns.*

It is worth noting that siRNA-mediated knockdown may not accurately replicate the heterozygous loss-of-function observed clinically. These results suggest that GLMN and CCM2L exert interactive and complex effects on HUVEC behavior and support a biological role for the CCM2L c.1505G>A variant in vascular endothelial cells.

4.7. The 3D structure of CCM2L with the R502H mutation shows minimal changes according to current prediction tools

A comparative analysis of the 3D structures of the CCM2L protein in its wild-type (WT) form and its R502H mutant variant was conducted using ChimeraX, a tool developed by the University of California, San Francisco (UCSF) (Meng et al., 2023). The 3D structure of CCM2L WT was

obtained from the AlphaFold Protein Structure Database, and the R502H mutation was modeled using AlphaFold prediction. Figure 12 shows a close-up of residue 502 in the WT protein, where an arginine (R) is present. In comparison, the structure of the mutant variant shows that histidine (H) replaces arginine at the same position, highlighting local modifications in the three-dimensional structure attributable to this substitution.

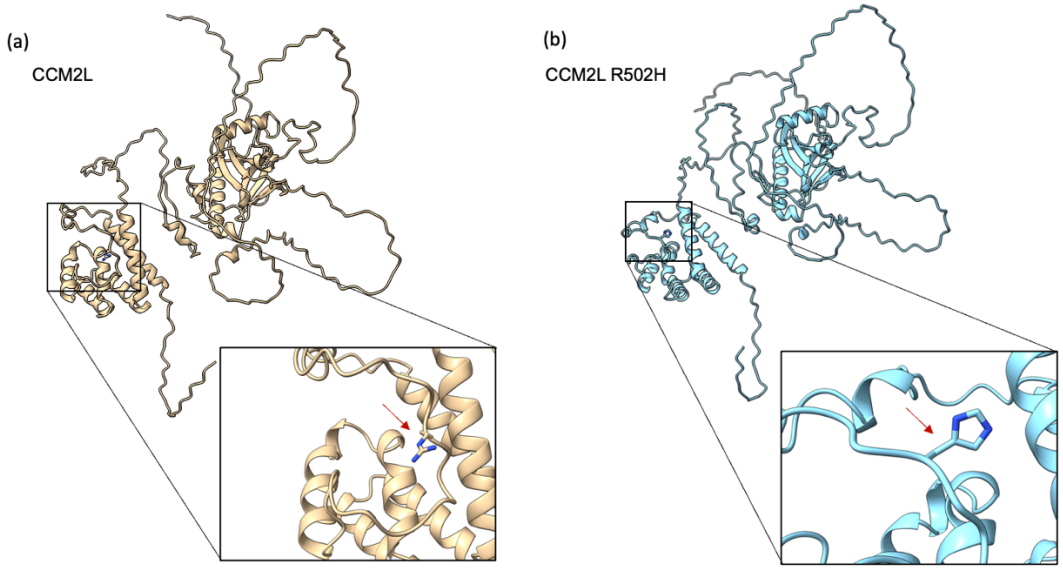


Figure 12. *3D Structure Visualization of the Protein Using UCSF ChimeraX. (a) Visualization of CCM2L WT, accompanied by a magnified image highlighting the amino acid at position 502, which is arginine (R). (b) Visualization of the CCM2L protein with the R502H mutation, along with a close-up view of position 502, where histidine (H) replaces the original amino acid.*

The analysis of the R502H mutation in the CCM2L protein revealed a root-mean-square deviation (RMSD) of 11.634 Å between the predicted structures of CCM2L WT and CCM2L R502H, calculated by aligning 4,360 atoms from each structure. This value, which reflects the average distance between equivalent atoms in the aligned structures (Coutsias and Wester, 2019), suggests differences in the overall conformation of the two proteins, indicating a structural impact of the R502H mutation.

However, the accuracy of the model used for this prediction is limited, preventing definitive conclusions. The pLDDT confidence score generated by AlphaFold indicates low reliability in the region spanning residues 490–520, highlighted in orange in the corresponding track. This region

includes residue 502, reinforcing the uncertainty about structural accuracy in this specific area, as shown in Figure 13.

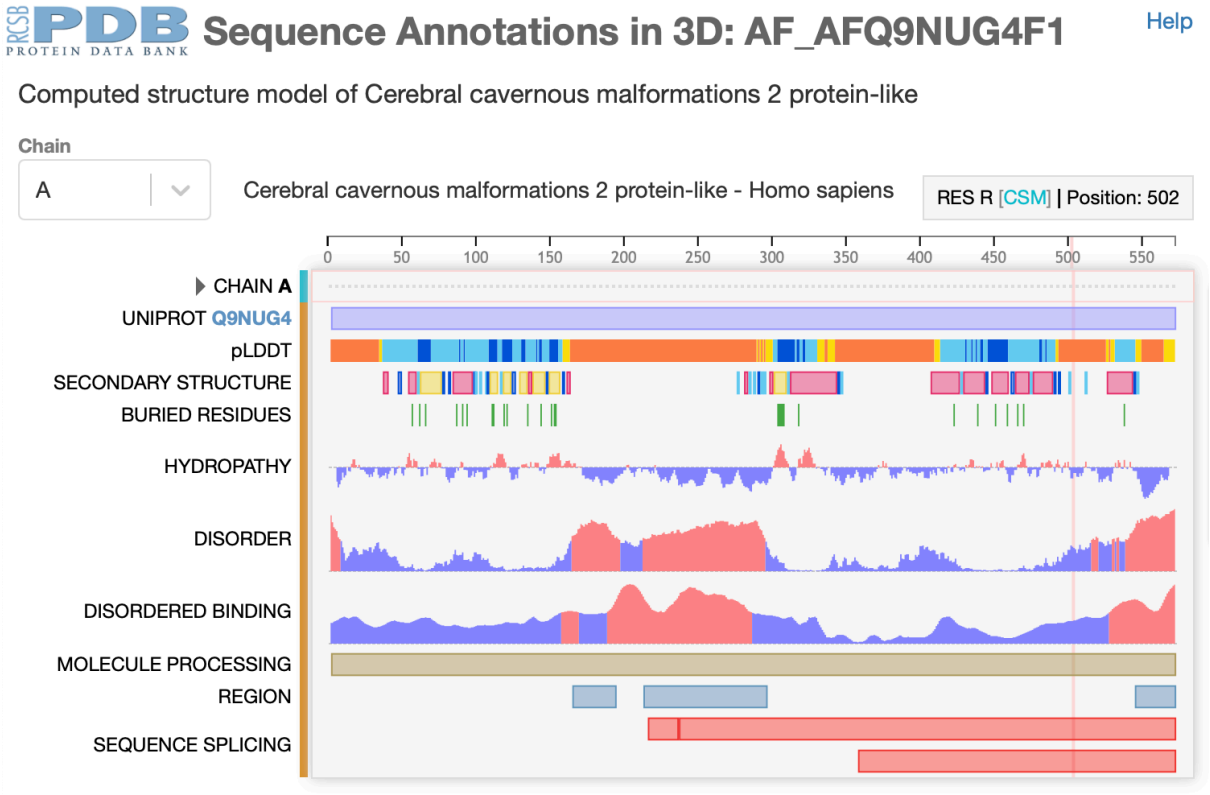


Figure 13. *Sequence Annotations of the Three-Dimensional Representation of the Computational Structural Model AF_AFQ9NUG4F1 of the CCM2L Protein Generated by AlphaFold. The confidence index (pLDDT) is visualized using a color scale: blue (high confidence), yellow (intermediate confidence), and orange (low confidence). The position of residue 502 is marked with a red line, indicating a region of low confidence according to the pLDDT score, highlighting the limited structural prediction accuracy in this area.*

Currently, no complete experimental structure for *CCM2L* is available in the Protein Data Bank (PDB). However, several fragments of related proteins are accessible, including:

- **4YKC**: Crystal structure of the C-terminal adaptor domain of cerebral cavernous malformation protein 2 (CCM2).
- **4WJ7**: PTB domain of CCM2 in complex with KRIT1 NPxY/F3.
- **4FQN**: Crystal structure of the Harmonin Homology Domain (HHD) of CCM2.
- **4Y5O**: CCM2 HHD in complex with the NPB1 region of MEKK3.
- **4YKD**: Crystal structure of the truncated C-terminal adaptor domain of CCM2.
- **4YL6**: Crystal structure of the truncated C-terminal adaptor domain of CCM2 in complex with an internal helix of MEKK3.

Fragment 4YKC encompasses the region containing residue 502 and shares approximately 50% sequence identity with *CCM2L*. However, structural disorder is observed around the mutation site, preventing accurate determination of its structure from experimental data. Therefore, any structural calculations or interpretations based on these models cannot be considered conclusive due to the lack of reliable information in this critical region.

5. DISCUSSION

The etiopathogenesis of GVMs is still not completely understood. Current literature does not offer conclusive explanations nor consistently identifies mutations beyond GLMN, leaving open the possibility that additional genetic variants may be involved in their development. In light of this uncertainty and having access to tissue samples from a patient with GVM, we conducted a genetic study to further explore the molecular mechanisms underlying this condition.

In this context, we investigated the genetic basis of a glomuvenous malformation in a two-week-old female patient with an atypical phenotypic presentation that included preaxial polydactyly. Following comprehensive mutational analysis, we identified a mutation in GLMN (c.515C>A, p.Ser172Ter), which represents a previously unreported variant. Since GLMN is the gene that is most commonly associated with GVMs (Brouillard et al., 2002, 2020; Brouillard, 2005), this finding confirms the molecular diagnosis of the patient and expands the known mutational spectrum of this gene. The GLMN c.515C>A, p.Ser172Ter mutation is particularly relevant, as it introduces a premature stop codon, likely resulting in a loss of protein function. Although it has been reported that GLMN mutations cause GVMs regardless of whether they affect the coding sequence (Brouillard *et al.*, 2002). Our findings provide new scientific insight, as no association between GLMN mutations and polydactyly has been previously reported. This suggests that this variant may play a key role in the patient's phenotypic presentation.

According to the existing literature, most GVM cases follow a "two-hit" model (Brouillard et al., 2002). This second-hit hypothesis proposes that a first inherited germline mutation is complemented by a second somatic alteration in specific cells, leading to complete loss of function of the affected gene only in certain regions of the body. This dual genetic alteration could explain the localized appearance of lesions in patients with mutations in vascular regulatory or tumor suppressor genes, despite the systemic presence of the germline mutation. Therefore, we searched for a second somatic mutation in GLMN in the affected skin of our patient. However, we found no evidence of an additional somatic lesion in GLMN. This finding led us to hypothesize that other genes might be involved in GVM pathogenesis and prompted us to explore additional genetic factors that could modulate disease presentation.

To this end, we performed whole-exome sequencing (WES) on both affected and unaffected skin, identifying 616 somatic variants in the lesion. After comprehensive functional filtering, we prioritized 46 potentially pathogenic mutations. Of these, four genes were selected for functional analysis as possible candidates: GDF10, described as a tumor suppressor; C3, a key component of the classical complement pathway associated with various skin diseases; DLG3, also linked to tumor suppressor functions; and CCM2L, a gene involved in vascular integrity regulation.

The results of in vitro cell migration and proliferation assays in HUVECs following knockdown of these four genes using lentiviral vectors led to the exclusion of C3 due to technical issues related to insufficient knockdown efficiency, as well as GDF10 and DLG3, whose results were inconclusive. However, CCM2L knockdown partially reduced cell proliferation while increasing migration speed, suggesting a regulatory role in endothelial behavior. As a result, we selected this gene as a potential modulator in GVM pathogenesis.

CCM2L is a regulator of vascular integrity previously implicated in cerebral cavernous malformations (CCMs) and cardiovascular development (Zheng *et al.*, 2012; Choi *et al.*, 2021). CCMs, also known as cavernous angiomas, are low-flow hemorrhagic vascular lesions of the central nervous system that affect up to 0.5% of the population (Fischer *et al.*, 2013). They represent a major cause of stroke, cerebral hemorrhage, and neurological deficits in young individuals. CCMs are inherited as an autosomal dominant disorder caused by loss-of-function mutations in one of three genes: CCM1 (KRIT1), CCM2 (Malcavernin, Osm), or CCM3 (PDCD10). The CCM genes encode non-homologous cytoplasmic proteins that form a single signaling complex (Choi *et al.*, 2021).

In this context, CCM2L is a paralog of CCM2 that is predominantly expressed in endothelial cells (ECs). CCM2L competes with CCM2 for binding to CCM1, exerting an antagonistic function to that of CCM2 during vascular development. Increased CCM2L expression may represent a compensatory mechanism in the pathogenesis of CCMs, while its loss exacerbates lesion formation in CCM2-deficient mouse models through enhanced MAP3K3-KLF signaling (Choi *et al.*, 2021). In addition, in the context of cardiovascular development, CCM2L is selectively expressed in endothelial cells during periods of active cardiovascular growth. CCM2L

competitively blocks the stabilizing signals mediated by CCM2 at the biochemical, cellular, and organismal levels (Zheng *et al.*, 2012). Loss of CCM2L reduces the expression of endocardial growth factors and impairs tumor growth and wound healing. This evidence supports a fundamental role for CCM2L in the coordinated regulation of vascular stability and growth during cardiovascular development and in postnatal stages (Zheng *et al.*, 2012).

Together, these observations underscore the critical role of CCM2L in regulating vascular stability in both the central nervous system and the cardiovascular context. However, its involvement in vascular malformations of the skin had not been previously reported. The exclusive detection of the CCM2L c.1505G>A, p.Arg502His (CCM2L-R502H) variant in the affected skin tissue of our patient, together with in vitro functional results demonstrating its impact on endothelial proliferation, suggests that CCM2L may play a previously unrecognized role in the pathogenesis of GVMs. This discovery expands the functional scope of CCM2L beyond the neurovascular and cardiovascular systems, proposing it as a potential modulatory gene in dermatological contexts as well.

This prompted the experimental evaluation of its specific function in human endothelial cells (HUVECs). To this end, the CCM2L-R502H variant was transfected into HUVECs and compared with CCM2L knockdown and control cells. Subsequently, to investigate a potential functional interaction with GLMN, double gene knockdown experiments were conducted. These assays showed that the simultaneous depletion of both genes significantly increased cell proliferation compared to GLMN knockdown alone, suggesting an interdependent effect on endothelial dynamics.

It is worth noting that siRNA-mediated depletion may not accurately replicate the heterozygous loss of function observed clinically. Nevertheless, taken together, these findings support the hypothesis that CCM2L may be modulating the vascular phenotype observed in our patient and represent the first functional evidence linking this gene to cutaneous vascular malformations.

To explore the underlying mechanisms, we performed a protein stability Western blot and observed elevated levels of CCM2L in the R502H mutant compared to CCM2L-WT, suggesting

a possible increase in protein stability. We also assessed the activation of the MAPK/ERK pathway, given its role in cell proliferation and the maintenance of vascular integrity (Sun et al., 2015). We found that overexpression of CCM2L-R502H in HUVECs significantly increased phospho-ERK levels, indicating sustained activation of this pathway. Previous studies have proposed that CCM2L acts as a negative regulator of the MAPK/ERK signaling pathway in murine models (Zheng *et al.*, 2012). In this context, our findings suggest that the R502H mutation interferes with the inhibitory function of CCM2L, generating a gain-of-function effect that promotes cellular hyperproliferation. This mechanism could be contributing to the phenotype observed in our patient, positioning CCM2L as a potential modulatory gene in the pathogenesis of GVMs.

To integrate these findings, we propose a mechanistic model in which, in the absence of a second mutation in GLMN, the CCM2L-R502H variant acts as a functional modulator of cell proliferation and MAPK/ERK signaling pathway activation, thereby contributing to the development of the vascular phenotype observed in our patient (Figure 14).

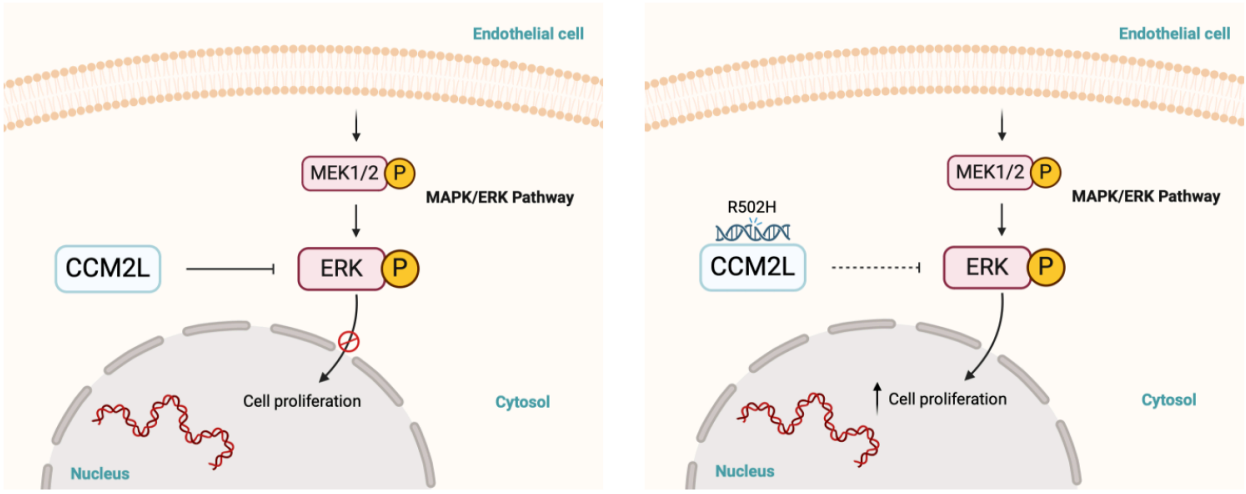


Figure 14. *Schematic Hypothesis of the Impact of the CCM2L-R502H Mutation on the MAPK/ERK Signaling Pathway in the Context of GVMs.*

The 3D structural analysis of the CCM2L protein revealed a root-mean-square deviation (RMSD) of 11.634 Å between the predicted structure of the wild-type form and the R502H variant, calculated from the alignment of 4,360 equivalent atoms. This value suggests that the mutation induces a relevant conformational change in the overall architecture of the protein.

However, the region surrounding residue 502 showed low confidence in the computational models generated, limiting precise interpretation of its local structure. This uncertainty is further supported by the absence of a complete experimental structure for CCM2L in the Protein Data Bank, as well as by the structural disorder observed in available homologous proteins such as CCM2. Taken together, these findings suggest a potential structural impact of the R502H mutation, although additional experimental validation is required to confirm its effect on protein function.

Despite these findings, our study presents several limitations that should be taken into consideration. First, its single-case design inherently restricts the generalizability of the observations and precludes statistical validation. As a result, the conclusions should be interpreted with caution. Nevertheless, the rarity of the phenotype, together with the integration

of genomic data and functional analyses, increases the relevance of these observations and supports their potential value in generating hypotheses for future research.

Second, the absence of an additional somatic mutation in GLMN in our patient raises the possibility that undetected genetic events—such as structural variants or mutations in non-coding regions—may have been missed in our WES analysis. This loss could potentially result from mechanisms such as uniparental isodisomy of the GLMN genomic region, as reported in other studies (Amyere et al., 2013).

Third, the limited amount of tissue obtained through biopsy did not allow for additional experiments that could have helped validate this hypothesis. Second, although the CCM2L-R502H variant showed a functional impact on cell proliferation and MAPK/ERK activation in HUVECs, we cannot rule out the possibility that it is a passenger mutation without a direct pathogenic role. However, its rarity in the general population (allele frequency < 0.0001 in dbSNP) suggests that it is not a common variant without functional relevance, and therefore additional studies will be necessary to establish its causal impact on GVMs.

A final consideration is that, the functions of CCM2L appear to be context-dependent, potentially varying according to cell type or the signaling pathways involved. This functional complexity limits the direct extrapolation of our findings to other pathophysiological contexts and underscores the need for complementary studies to clarify the specific role of CCM2L in the pathogenesis of GVMs.

Future studies should further investigate the interaction between GLMN and CCM2L in the context of vascular malformations. Particularly, it will be important to assess how variants in CCM2L may influence the phenotypic manifestation of GVMs and whether their alteration could explain the presence of polydactyly in our patient.

Additionally, expanding the genetic analysis to a larger cohort of patients with GVM will help determine the prevalence of CCM2L mutations and their potential role as a modifier gene in these

vascular anomalies. It would also be relevant to explore whether activation of the MAPK/ERK pathway represents a potential therapeutic target in GVM cases involving CCM2L alterations.

In conclusion, this study represents the first functional evidence linking a variant in CCM2L to cutaneous vascular malformations in humans, whose association with congenital skin lesions had not been previously described. Additionally, this work presents the first published case of GVM associated with polydactyly and reports for the first time the germline mutation GLMN c.515C>A, p.Ser172Ter, which had not been previously documented in the literature.

It is also the first time CCM2L has been implicated in the pathogenesis of a cutaneous vascular disease, and the first report of the somatic variant CCM2L c.1505G>A, p.Arg502His in a human GVM sample. These findings not only contribute new insights into the genetic mechanisms underlying GVMs but also raise the possibility that CCM2L may act as a modulatory gene in the absence of a second somatic mutation in GLMN.

This hypothesis opens new avenues for exploring alternative pathogenic pathways in congenital vascular malformations and underscores the importance of incorporating complementary functional analyses in the study of rare genetic variants. Therefore, these results expand current knowledge of the genetic determinants of GVMs and prompt a reconsideration of the candidate gene spectrum involved in their pathogenesis.

6. CONCLUSIONS

1. The literature review supports the hypothesis that several lncRNAs — PRANCR (proliferation), H19 (angiogenesis), and Zeb2-AS1 (inflammation) — modulate key cutaneous processes, positioning them as biomarkers and potential therapeutic targets in vascular skin diseases.
2. The PI3K/AKT/mTOR pathway, essential for angiogenesis, may indirectly influence the pathophysiology of GVMs; however, to date, no mutations or functional data have confirmed a direct involvement. Inhibitors such as sirolimus and alpelisib are being evaluated in slow-flow venous malformations, but trials have yet to report specific results for GVMs.
3. We report for the first time the GLMN c.515C>A (p.Ser172Ter) mutation without a second somatic mutation in the same gene. This premature stop codon implies a loss of GLMN function and reinforces its central role in GVM pathogenesis; however, we suggest that the pathogenic mechanisms of GVMs extend beyond GLMN mutations.
4. Genomic analysis identified the somatic mutation CCM2L c.1505G>A (p.Arg502His), which activates the MAPK/ERK pathway and enhances endothelial cell proliferation, suggesting that CCM2L acts as a modifier gene in GVMs.
5. We propose that CCM2L regulates cell proliferation in vascular malformations associated with GLMN, contributing to the observed phenotypic variability. The identification of additional variants in CCM2L and the evaluation of inhibitors targeting this pathway represent key steps toward personalized therapies for GVM.

7. REFERENCES

Aartsma-Rus, A. *et al.* (2009) 'Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations', *Human Mutation*, 30(3), pp. 293–299. Available at: https://doi.org/10.1002/humu.20918.

Abdallah, H.Y. *et al.* (2023) 'Expression Signature of Immune-Related MicroRNAs in Autoimmune Skin Disease: Psoriasis and Vitiligo Insights', *Molecular Diagnosis & Therapy*, 27(3), pp. 405–423. Available at: https://doi.org/10.1007/s40291-023-00646-1.

Abreu, N.J. and Waldrop, M.A. (2021) 'Overview of gene therapy in spinal muscular atrophy and Duchenne muscular dystrophy', *Pediatric Pulmonology*, 56(4), pp. 710–720. Available at: https://doi.org/10.1002/ppul.25055.

Aerts, V. *et al.* (2007) 'GLMN (Glomulin)', *Atlas of Genetics and Cytogenetics in Oncology and Haematology* [Preprint]. Available at: http://atlasgeneticsoncology.org/gene/43022/glmn-(glomulin).

Agaram, N.P. *et al.* (2020) 'A Molecular Reappraisal of Glomus Tumors and Related Pericytic Neoplasms With Emphasis on NOTCH-gene Fusions', *American Journal of Surgical Pathology*, 44(11), pp. 1556–1562. Available at: https://doi.org/10.1097/PAS.0000000000001531.

Akil, A. *et al.* (2021) 'Notch Signaling in Vascular Endothelial Cells, Angiogenesis, and Tumor Progression: An Update and Prospective', *Frontiers in Cell and Developmental Biology*, 9, p. 642352. Available at: https://doi.org/10.3389/fcell.2021.642352.

Amyere, M. *et al.* (2013) 'Somatic Uniparental Isodisomy Explains Multifocality of Glomuvenous Malformations', *The American Journal of Human Genetics*, 92(2), pp. 188–196. Available at: https://doi.org/10.1016/j.ajhg.2012.12.017.

Andl, T. *et al.* (2006) 'The miRNA-Processing Enzyme Dicer Is Essential for the Morphogenesis and Maintenance of Hair Follicles', *Current Biology*, 16(10), pp. 1041–1049. Available at: https://doi.org/10.1016/j.cub.2006.04.005.

André, F. *et al.* (2019) 'Alpelisib for *PIK3CA* -Mutated, Hormone Receptor–Positive Advanced Breast Cancer', *New England Journal of Medicine*, 380(20), pp. 1929–1940. Available at: https://doi.org/10.1056/NEJMoa1813904.

Armstrong, A.W. *et al.* (2013) 'Undertreatment, Treatment Trends, and Treatment Dissatisfaction Among Patients With Psoriasis and Psoriatic Arthritis in the United States: Findings From the National Psoriasis Foundation Surveys, 2003-2011', *JAMA Dermatology*, 149(10), p. 1180. Available at: https://doi.org/10.1001/jamadermatol.2013.5264.

Arun, G., Diermeier, S.D. and Spector, D.L. (2018) 'Therapeutic Targeting of Long Non-Coding RNAs in Cancer', *Trends in Molecular Medicine*, 24(3), pp. 257–277. Available at: https://doi.org/10.1016/j.molmed.2018.01.001.

Bao, Z. *et al.* (2019) 'LncRNADisease 2.0: an updated database of long non-coding RNA-associated diseases', *Nucleic Acids Research*, 47(D1), pp. D1034–D1037. Available at: https://doi.org/10.1093/nar/gky905.

Barbier, A.J. *et al.* (2022) 'The clinical progress of mRNA vaccines and immunotherapies', *Nature Biotechnology*, 40(6), pp. 840–854. Available at: https://doi.org/10.1038/s41587-022-01294-2.

Bayraktar, E. *et al.* (2023) 'Targeting miRNAs and Other Non-Coding RNAs as a Therapeutic Approach: An Update', *Non-Coding RNA*, 9(2), p. 27. Available at: https://doi.org/10.3390/ncrna9020027.

Bedikian, A.Y. *et al.* (2006) 'Bcl-2 Antisense (oblimersen sodium) Plus Dacarbazine in Patients With Advanced Melanoma: The Oblimersen Melanoma Study Group', *Journal of Clinical Oncology*, 24(29), pp. 4738–4745. Available at: https://doi.org/10.1200/JCO.2006.06.0483.

Bergamasco, A. *et al.* (2019) 'Epidemiology of systemic sclerosis and systemic sclerosis-associated interstitial lung disease', *Clinical Epidemiology*, Volume 11, pp. 257–273. Available at: https://doi.org/10.2147/CLEP.S191418.

Bergoug, M. *et al.* (2020) 'Neurofibromin Structure, Functions and Regulation', *Cells*, 9(11), p. 2365. Available at: https://doi.org/10.3390/cells9112365.

Bethesda (2001) 'Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework', *Clinical Pharmacology & Therapeutics*, 69(3), pp. 89–95. Available at: https://doi.org/10.1067/mcp.2001.113989.

Bisdorff-Bresson, A., Eyries, M. and Boccara, O. (2021) 'Congenital vascular lesions, could MAPK and PI3K inhibitors pave the way to new therapies?', *Current Opinion in Oncology*, 33(2), pp. 95–100. Available at: https://doi.org/10.1097/CCO.0000000000000712.

Bonner, J.M. and Boulianne, G.L. (2017) 'Diverse structures, functions and uses of FK506 binding proteins', *Cellular Signalling*, 38, pp. 97–105. Available at: https://doi.org/10.1016/j.cellsig.2017.06.013.

Boon, L.M. *et al.* (1999) 'A Gene for Inherited Cutaneous Venous Anomalies ("Glomangiomas") Localizes to Chromosome 1p21-22', *The American Journal of Human Genetics*, 65(1), pp. 125–133. Available at: https://doi.org/10.1086/302450.

Boon, L.M. *et al.* (2004) 'Glomuvenous Malformation (Glomangioma) and Venous Malformation: Distinct Clinicopathologic and Genetic Entities', *Archives of Dermatology*, 140(8). Available at: https://doi.org/10.1001/archderm.140.8.971.

Borgia, F. *et al.* (2022) 'MicroRNA Cross-Involvement in Acne Vulgaris and Hidradenitis Suppurativa: A Literature Review', *International Journal of Molecular Sciences*, 23(6), p. 3241. Available at: https://doi.org/10.3390/ijms23063241.

Borroni, R.G. *et al.* (2014) 'Glomuvenous malformations with smooth muscle and eccrine glands: unusual histopathologic features in a familial setting', *Journal of Cutaneous Pathology*, 41(3), pp. 308–315. Available at: https://doi.org/10.1111/cup.12283.

Boucher, J.M. *et al.* (2013) 'A Receptor-Specific Function for Notch2 in Mediating Vascular Smooth Muscle Cell Growth Arrest Through Cyclin-dependent Kinase Inhibitor 1B', *Circulation Research*, 113(8), pp. 975–985. Available at: https://doi.org/10.1161/CIRCRESAHA.113.301272.

Brahmbhatt, H.D. *et al.* (2021) 'The long noncoding RNA *MALAT1* suppresses miR-211 to confer protection from ultraviolet-mediated DNA damage in vitiligo epidermis by upregulating sirtuin 1*',

British Journal of Dermatology, 184(6), pp. 1132–1142. Available at: https://doi.org/10.1111/bjd.19666.

Brouillard, P. *et al.* (2002) 'Mutations in a Novel Factor, Glomulin, Are Responsible for Glomuvenous Malformations ("Glomangiomas")', *The American Journal of Human Genetics*, 70(4), pp. 866–874. Available at: https://doi.org/10.1086/339492.

Brouillard, P. *et al.* (2013) 'Genotypes and Phenotypes of 162 Families with a ***Glomulin*** Mutation', *Molecular Syndromology*, 4(4), pp. 157–164. Available at: https://doi.org/10.1159/000348675.

Brouillard, P. *et al.* (2020) 'Disorders of the Venous System', in *Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics*. Elsevier, pp. 251–260. Available at: https://doi.org/10.1016/B978-0-12-812532-8.00009-4.

Brouillard, P. and Vikkula, M. (2007) 'Genetic causes of vascular malformations', *Human Molecular Genetics*, 16(R2), pp. R140–R149. Available at: https://doi.org/10.1093/hmg/ddm211.

Cai, P. *et al.* (2020) 'A genome-wide long noncoding RNA CRISPRi screen identifies *PRANCR* as a novel regulator of epidermal homeostasis', *Genome Research*, 30(1), pp. 22–34. Available at: https://doi.org/10.1101/gr.251561.119.

Campos, A.H. *et al.* (2002) 'Determinants of Notch-3 Receptor Expression and Signaling in Vascular Smooth Muscle Cells: Implications in Cell-Cycle Regulation', *Circulation Research*, 91(11), pp. 999–1006. Available at: https://doi.org/10.1161/01.RES.0000044944.99984.25.

Chambraud, B. *et al.* (1996) 'FAP48, a New Protein That Forms Specific Complexes with Both Immunophilins FKBP59 and FKBP12', *Journal of Biological Chemistry*, 271(51), pp. 32923–32929. Available at: https://doi.org/10.1074/jbc.271.51.32923.

Cheetham, S.W. *et al.* (2013) 'Long noncoding RNAs and the genetics of cancer', *British Journal of Cancer*, 108(12), pp. 2419–2425. Available at: https://doi.org/10.1038/bjc.2013.233.

Chen, S. *et al.* (2020) 'The Current State of Research Regarding the Role of Non-Coding RNAs in Cutaneous Squamous Cell Carcinoma', *OncoTargets and Therapy*, Volume 13, pp. 13151–13158. Available at: https://doi.org/10.2147/OTT.S271346.

Choi, J.P. *et al.* (2021) 'CCM2L (Cerebral Cavernous Malformation 2 Like) Deletion Aggravates Cerebral Cavernous Malformation Through Map3k3-KLF Signaling Pathway', *Stroke*, 52(4), pp. 1428–1436. Available at: https://doi.org/10.1161/STROKEAHA.120.031523.

Coutsias, E.A. and Wester, M.J. (2019) 'RMSD and Symmetry', *Journal of Computational Chemistry*, 40(15), pp. 1496–1508. Available at: https://doi.org/10.1002/jcc.25802.

Dai, H., Machan, M. and Fraga, G.R. (2014) 'How Accurate Are the Suggested Diagnoses on Biopsy Requisitions for Inflammatory Skin Disease? A Retrospective Study of 348 Cases', *The American Journal of Dermatopathology*, 36(4), pp. 298–302. Available at: https://doi.org/10.1097/DAD.0b013e3182a2d7d3.

Dammes, N. and Peer, D. (2020) 'Paving the Road for RNA Therapeutics', *Trends in Pharmacological Sciences*, 41(10), pp. 755–775. Available at: https://doi.org/10.1016/j.tips.2020.08.004.

De Paepe, B., Lefever, S. and Mestdagh, P. (2018) 'How long noncoding RNAs enforce their will on mitochondrial activity: regulation of mitochondrial respiration, reactive oxygen species production, apoptosis, and metabolic reprogramming in cancer', *Current Genetics*, 64(1), pp. 163–172. Available at: https://doi.org/10.1007/s00294-017-0744-1.

Dompmartin, A. *et al.* (2009) 'Elevated D-dimer Level in the Differential Diagnosis of Venous Malformations', *Archives of Dermatology*, 145(11). Available at: https://doi.org/10.1001/archdermatol.2009.296.

Duffy, M.J. and Crown, J. (2021) 'Drugging "undruggable" genes for cancer treatment: Are we making progress?', *International Journal of Cancer*, 148(1), pp. 8–17. Available at: https://doi.org/10.1002/ijc.33197.

Durante, G. *et al.* (2021) 'Non-coding RNA dysregulation in skin cancers', *Essays in Biochemistry*. Edited by C.C. Hon, 65(4), pp. 641–655. Available at: https://doi.org/10.1042/EBC20200048.

Fabbiano, F. *et al.* (2020) 'RNA packaging into extracellular vesicles: An orchestra of RNA-binding proteins?', *Journal of Extracellular Vesicles*, 10(2). Available at: https://doi.org/10.1002/jev2.12043.

Fereydooni, A., Dardik, A. and Nassiri, N. (2019) 'Molecular changes associated with vascular malformations', *Journal of Vascular Surgery*, 70(1), pp. 314-326.e1. Available at: https://doi.org/10.1016/j.jvs.2018.12.033.

Finkel, R.S. *et al.* (2017) 'Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy', *New England Journal of Medicine*, 377(18), pp. 1723–1732. Available at: https://doi.org/10.1056/NEJMoa1702752.

Fischer, A. *et al.* (2013) 'Cerebral cavernous malformations: from CCM genes to endothelial cell homeostasis', *Trends in Molecular Medicine*, 19(5), pp. 302–308. Available at: https://doi.org/10.1016/j.molmed.2013.02.004.

Frantz, S. (2004) 'Lessons learnt from Genasense's failure'.

Gagliardi, M. and Ashizawa, A.T. (2022) 'Making Sense of Antisense Oligonucleotide Therapeutics Targeting Bcl-2', *Pharmaceutics*, 14(1), p. 97. Available at: https://doi.org/10.3390/pharmaceutics14010097.

Gallant-Behm, C.L. *et al.* (2019) 'A MicroRNA-29 Mimic (Remlarsen) Represses Extracellular Matrix Expression and Fibroplasia in the Skin', *Journal of Investigative Dermatology*, 139(5), pp. 1073–1081. Available at: https://doi.org/10.1016/j.jid.2018.11.007.

Garcia-Martin, R. *et al.* (2022) 'MicroRNA sequence codes for small extracellular vesicle release and cellular retention', *Nature*, 601(7893), pp. 446–451. Available at: https://doi.org/10.1038/s41586-021-04234-3.

GENCODE - The GENCODE Project (2022). Available at: https://www.gencodegenes.org/pages/gencode.html (Accessed: 28 October 2022).

Goding, C.R. (2016) 'Targeting the lncRNA SAMMSON Reveals Metabolic Vulnerability in Melanoma', *Cancer Cell*, 29(5), pp. 619–621. Available at: https://doi.org/10.1016/j.ccell.2016.04.010.

Griffin, M.F. *et al.* (2022) 'The role of Wnt signaling in skin fibrosis', *Medicinal Research Reviews*, 42(1), pp. 615–628. Available at: https://doi.org/10.1002/med.21853.

Grisendi, S. *et al.* (2001) 'Ligand-regulated Binding of FAP68 to the Hepatocyte Growth Factor Receptor', *Journal of Biological Chemistry*, 276(49), pp. 46632–46638. Available at: https://doi.org/10.1074/jbc.M104323200.

Guo, L. *et al.* (2017) 'MicroRNA expression signature and the therapeutic effect of the microRNA-21 antagomir in hypertrophic scarring', *Molecular Medicine Reports*, 15(3), pp. 1211–1221. Available at: https://doi.org/10.3892/mmr.2017.6104.

Gupta, R.A. *et al.* (2010) 'Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis', *Nature*, 464(7291), pp. 1071–1076. Available at: https://doi.org/10.1038/nature08975.

Hammer, J. *et al.* (2018) 'Sirolimus is efficacious in treatment for extensive and/or complex slow-flow vascular malformations: a monocentric prospective phase II study', *Orphanet Journal of Rare Diseases*, 13(1), p. 191. Available at: https://doi.org/10.1186/s13023-018-0934-z.

Han, S. *et al.* (2021) 'LncRNA SAMMSON Mediates Adaptive Resistance to RAF Inhibition in BRAF-Mutant Melanoma Cells', *Cancer Research*, 81(11), pp. 2918–2929. Available at: https://doi.org/10.1158/0008-5472.CAN-20-3145.

Haq, R. *et al.* (2013) 'Oncogenic BRAF Regulates Oxidative Metabolism via PGC1α and MITF', *Cancer Cell*, 23(3), pp. 302–315. Available at: https://doi.org/10.1016/j.ccr.2013.02.003.

Hart, M. *et al.* (2020) 'Wrinkle in the plan: miR-34a-5p impacts chemokine signaling by modulating CXCL10/CXCL11/CXCR3-axis in $CD4^+$, $CD8^+$ T cells, and M1 macrophages', *Journal for ImmunoTherapy of Cancer*, 8(2), p. e001617. Available at: https://doi.org/10.1136/jitc-2020-001617.

Hay, R.J. *et al.* (2014) 'The Global Burden of Skin Disease in 2010: An Analysis of the Prevalence and Impact of Skin Conditions', *Journal of Investigative Dermatology*, 134(6), pp. 1527–1534. Available at: https://doi.org/10.1038/jid.2013.446.

Heath, M.S. *et al.* (2019) 'Pharmacotherapeutic strategies for standard treatment-resistant psoriasis', *Expert Opinion on Pharmacotherapy*, 20(4), pp. 443–454. Available at: https://doi.org/10.1080/14656566.2018.1559819.

Hombach, S. and Kretz, M. (2013) 'The non-coding skin: Exploring the roles of long non-coding RNAs in epidermal homeostasis and disease: Review essay', *BioEssays*, 35(12), pp. 1093–1100. Available at: https://doi.org/10.1002/bies.201300068.

Hong, D.S. *et al.* (2020) 'Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours', *British Journal of Cancer*, 122(11), pp. 1630–1637. Available at: https://doi.org/10.1038/s41416-020-0802-1.

Horsburgh, S. *et al.* (2017) 'MicroRNAs in the skin: role in development, homoeostasis and regeneration', *Clinical Science*, 131(15), pp. 1923–1940. Available at: https://doi.org/10.1042/CS20170039.

Huang, Q. *et al.* (2019) 'lncRNA LINC-PINT is downregulated in melanoma and regulates cell proliferation by downregulating lncRNA BANCR', *Oncology Letters* [Preprint]. Available at: https://doi.org/10.3892/ol.2019.10631.

Huarte, M. (2015) 'The emerging role of lncRNAs in cancer', *Nature Medicine*, 21(11), pp. 1253–1261. Available at: https://doi.org/10.1038/nm.3981.

Iwamura, R. *et al.* (2023) 'PDGFRB and NOTCH3 Mutations are Detectable in a Wider Range of Pericytic Tumors, Including Myopericytomas, Angioleiomyomas, Glomus Tumors, and Their Combined Tumors', *Modern Pathology*, 36(3), p. 100070. Available at: https://doi.org/10.1016/j.modpat.2022.100070.

Jiang, B. *et al.* (2020) 'Down-regulation of long non-coding RNA HOTAIR promotes angiogenesis via regulating miR-126/SCEL pathways in burn wound healing', *Cell Death & Disease*, 11(1), p. 61. Available at: https://doi.org/10.1038/s41419-020-2247-0.

Joussen, A.M. *et al.* (2021) 'Angiopoietin/Tie2 signalling and its role in retinal and choroidal vascular diseases: a review of preclinical data', *Eye*, 35(5), pp. 1305–1316. Available at: https://doi.org/10.1038/s41433-020-01377-x.

Kalra, H., Drummen, G. and Mathivanan, S. (2016) 'Focus on Extracellular Vesicles: Introducing the Next Small Big Thing', *International Journal of Molecular Sciences*, 17(2), p. 170. Available at: https://doi.org/10.3390/ijms17020170.

Karar, J. and Maity, A. (2011) 'PI3K/AKT/mTOR Pathway in Angiogenesis', *Frontiers in Molecular Neuroscience*, 4. Available at: https://doi.org/10.3389/fnmol.2011.00051.

Khan, A.Q. *et al.* (2020) 'Role of non-coding RNAs in the progression and resistance of cutaneous malignancies and autoimmune diseases', *Seminars in Cancer Biology*, p. S1044579X20301589. Available at: https://doi.org/10.1016/j.semcancer.2020.07.003.

Kim, A. and Cohen, M.S. (2016) 'The discovery of vemurafenib for the treatment of BRAF-mutated metastatic melanoma', *Expert Opinion on Drug Discovery*, 11(9), pp. 907–916. Available at: https://doi.org/10.1080/17460441.2016.1201057.

Kunimoto, K., Yamamoto, Y. and Jinnin, M. (2022) 'ISSVA Classification of Vascular Anomalies and Molecular Biology', *International Journal of Molecular Sciences*, 23(4), p. 2358. Available at: https://doi.org/10.3390/ijms23042358.

Le Floc'h, A. *et al.* (2020) 'Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Rα antibody, is required to broadly inhibit type 2 inflammation', *Allergy*, 75(5), pp. 1188–1204. Available at: https://doi.org/10.1111/all.14151.

Leucci, E. *et al.* (2016) 'Melanoma addiction to the long non-coding RNA SAMMSON', *Nature*, 531(7595), pp. 518–522. Available at: https://doi.org/10.1038/nature17161.

Li, D. *et al.* (2023) 'Genomic profiling informs diagnoses and treatment in vascular anomalies', *Nature Medicine*, 29(6), pp. 1530–1539. Available at: https://doi.org/10.1038/s41591-023-02364-x.

Li, H. *et al.* (2020) 'Identification of potential key mRNAs and LncRNAs for psoriasis by bioinformatic analysis using weighted gene co-expression network analysis', *Molecular Genetics and Genomics*, 295(3), pp. 741–749. Available at: https://doi.org/10.1007/s00438-020-01654-0.

Li, X. *et al.* (2013) 'Long non-coding RNA HOTAIR, a driver of malignancy, predicts negative prognosis and exhibits oncogenic activity in oesophageal squamous cell carcinoma', *British Journal of Cancer*, 109(8), pp. 2266–2278. Available at: https://doi.org/10.1038/bjc.2013.548.

Liao, P., Satten, G.A. and Hu, Y.-J. (2017) 'PhredEM: a phred-score-informed genotype-calling approach for next-generation sequencing studies', *Genetic Epidemiology*, 41(5), pp. 375–387. Available at: https://doi.org/10.1002/gepi.22048.

Liu, Y. *et al.* (2012) 'miRNA Expression Profiles in Keloid Tissue and Corresponding Normal Skin Tissue', *Aesthetic Plastic Surgery*, 36(1), pp. 193–201. Available at: https://doi.org/10.1007/s00266-011-9773-1.

Loganathan, T. and Doss C, G.P. (2023) 'Non-coding RNAs in human health and disease: potential function as biomarkers and therapeutic targets', *Functional & Integrative Genomics*, 23(1), p. 33. Available at: https://doi.org/10.1007/s10142-022-00947-4.

Lopez-Pajares, V. *et al.* (2013) 'Genetic pathways in disorders of epidermal differentiation', *Trends in Genetics*, 29(1), pp. 31–40. Available at: https://doi.org/10.1016/j.tig.2012.10.005.

Løvendorf, M.B. and Skov, L. (2015) 'miRNAs in inflammatory skin diseases and their clinical implications', *Expert Review of Clinical Immunology*, 11(4), pp. 467–477. Available at: https://doi.org/10.1586/1744666X.2015.1020301.

Lu, T.X. and Rothenberg, M.E. (2018) 'MicroRNA', *Journal of Allergy and Clinical Immunology*, 141(4), pp. 1202–1207. Available at: https://doi.org/10.1016/j.jaci.2017.08.034.

Ma, X. *et al.* (2017) 'Expression profiles analysis of long non-coding RNAs identified novel lncRNA biomarkers with predictive value in outcome of cutaneous melanoma', *Oncotarget*, 8(44), pp. 77761–77770. Available at: https://doi.org/10.18632/oncotarget.20780.

Mahboobeh, Z. *et al.* (2020) 'LNCRNA ZEB2-AS1 : A promising biomarker in human cancers', *IUBMB Life*, 72(9), pp. 1891–1899. Available at: https://doi.org/10.1002/iub.2338.

Mallory, S.B. *et al.* (2006) 'Congenital Plaque-Type Glomuvenous Malformations Presenting in Childhood', *Archives of Dermatology*, 142(7). Available at: https://doi.org/10.1001/archderm.142.7.892.

Matsui, M. and Corey, D.R. (2017) 'Non-coding RNAs as drug targets', *Nature Reviews Drug Discovery*, 16(3), pp. 167–179. Available at: https://doi.org/10.1038/nrd.2016.117.

Mattick, J.S. *et al.* (2023) 'Long non-coding RNAs: definitions, functions, challenges and recommendations', *Nature Reviews Molecular Cell Biology*, 24(6), pp. 430–447. Available at: https://doi.org/10.1038/s41580-022-00566-8.

McIntyre, B.A.S. *et al.* (2004) 'Glomulin is predominantly expressed in vascular smooth muscle cells in the embryonic and adult mouse', *Gene Expression Patterns*, 4(3), pp. 351–358. Available at: https://doi.org/10.1016/j.modgep.2003.09.007.

McMahon, M.H., Tahir, N. and Balasubramanian, M. (2022) '*GLMN* causing vascular malformations: the clinical and genetic differentiation of cutaneous venous malformations', *BMJ Case Reports*, 15(6), p. e246114. Available at: https://doi.org/10.1136/bcr-2021-246114.

Meng, E.C. *et al.* (2023) 'UCSF CHIMERAX : Tools for structure building and analysis', *Protein Science*, 32(11), p. e4792. Available at: https://doi.org/10.1002/pro.4792.

Meng, Z. *et al.* (2018) 'miRNA delivery for skin wound healing', *Advanced Drug Delivery Reviews*, 129, pp. 308–318. Available at: https://doi.org/10.1016/j.addr.2017.12.011.

Moran, B. *et al.* (2018) 'Epigenetics of malignant melanoma', *Seminars in Cancer Biology*, 51, pp. 80–88. Available at: https://doi.org/10.1016/j.semcancer.2017.10.006.

Moreno-Arrones, O.M. *et al.* (2018) 'Glomuvenous malformations: dual PDL-Nd:YAG laser approach', *Lasers in Medical Science*, 33(9), pp. 2007–2010. Available at: https://doi.org/10.1007/s10103-018-2493-x.

Nguyen, H.-L., Boon, L.M. and Vikkula, M. (2014) 'Genetics of vascular malformations', *Seminars in Pediatric Surgery*, 23(4), pp. 221–226. Available at: https://doi.org/10.1053/j.sempedsurg.2014.06.014.

NIH - Cancer Stat Facts (2022) *Melanoma of the Skin - Cancer Stat Facts*, *SEER*. Available at: https://seer.cancer.gov/statfacts/html/melan.html (Accessed: 21 March 2023).

Nikhil, C. *et al.* (2022) 'Glomus tumor: A case series study of 30 cases and review of the literature', *Journal of Orthopaedic Association of South Indian States*, 19(1), p. 39. Available at: https://doi.org/10.4103/joasis.joasis_37_21.

Niu, Z. *et al.* (2021) 'The value of high-frequency ultrasonography in the differential diagnosis of early mycosis fungoides and inflammatory skin diseases: A case-control study', *Skin Research and Technology*, 27(3), pp. 453–460. Available at: https://doi.org/10.1111/srt.12945.

Nutsathapana, N. and Tresukosol, P. (2021) 'Glomuvenous Malformations: A Case Report and Review'.

Otten, A.B.C. *et al.* (2021) *The noncoding RNA* PRANCR *regulates splicing of* Fibronectin-1 *to control keratinocyte proliferation and migration*. preprint. Cell Biology. Available at: https://doi.org/10.1101/2021.06.22.449364.

Otten, A.B.C. *et al.* (2023) 'The long noncoding RNA PRANCR is associated with alternative splicing of fibronectin-1 in keratinocytes', *Journal of Investigative Dermatology*, p. S0022202X23001732. Available at: https://doi.org/10.1016/j.jid.2023.01.038.

Palmieri, M. *et al.* (2021) 'MET somatic activating mutations are responsible for lymphovenous malformation and can be identified using cell-free DNA next generation sequencing liquid biopsy', *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, 9(3), pp. 740–744. Available at: https://doi.org/10.1016/j.jvsv.2020.07.015.

Pardini, B. *et al.* (2019) 'Noncoding RNAs in Extracellular Fluids as Cancer Biomarkers: The New Frontier of Liquid Biopsies', *Cancers*, 11(8), p. 1170. Available at: https://doi.org/10.3390/cancers11081170.

Pitulescu, M.E. *et al.* (2017) 'Dll4 and Notch signalling couples sprouting angiogenesis and artery formation', *Nature Cell Biology*, 19(8), pp. 915–927. Available at: https://doi.org/10.1038/ncb3555.

Queisser, A. *et al.* (2021) 'Genetic Basis and Therapies for Vascular Anomalies', *Circulation Research*, 129(1), pp. 155–173. Available at: https://doi.org/10.1161/CIRCRESAHA.121.318145.

Ramessur, R. *et al.* (2022) 'Biomarkers of disease progression in people with psoriasis: a scoping review*', *British Journal of Dermatology*, 187(4), pp. 481–493. Available at: https://doi.org/10.1111/bjd.21627.

Ramessur, R. *et al.* (2023) 'Glomovenous malformation secondary to a heterozygous nonsense variant in *GLMN*: a clinical mimicker of blue rubber bleb naevus syndrome', *Clinical and Experimental Dermatology*, 49(1), pp. 108–110. Available at: https://doi.org/10.1093/ced/llad302.

Rao, M.R.S. (ed.) (2017) *Long Non Coding RNA Biology*. Singapore: Springer Singapore (Advances in Experimental Medicine and Biology). Available at: https://doi.org/10.1007/978-981-10-5203-3.

Rebane, A. *et al.* (2014) 'MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes', *Journal of Allergy and Clinical Immunology*, 134(4), pp. 836-847.e11. Available at: https://doi.org/10.1016/j.jaci.2014.05.022.

Rederstorff, M. (ed.) (2021) *Small Non-Coding RNAs: Methods and Protocols*. New York, NY: Springer US (Methods in Molecular Biology). Available at: https://doi.org/10.1007/978-1-0716-1386-3.

Renert-Yuval, Y. *et al.* (2021) 'Biomarkers in atopic dermatitis—a review on behalf of the International Eczema Council', *Journal of Allergy and Clinical Immunology*, 147(4), pp. 1174-1190.e1. Available at: https://doi.org/10.1016/j.jaci.2021.01.013.

Rentzsch, P. *et al.* (2021) 'CADD-Splice—improving genome-wide variant effect prediction using deep learning-derived splice scores', *Genome Medicine*, 13(1), p. 31. Available at: https://doi.org/10.1186/s13073-021-00835-9.

Rohner, E. *et al.* (2022) 'Unlocking the promise of mRNA therapeutics', *Nature Biotechnology* [Preprint]. Available at: https://doi.org/10.1038/s41587-022-01491-z.

Roso-Mares, A. *et al.* (2024) 'Non-coding RNAs as skin disease biomarkers, molecular signatures, and therapeutic targets', *Human Genetics*, 143(7), pp. 801–812. Available at: https://doi.org/10.1007/s00439-023-02588-4.

Rusek, M. and Krasowska, D. (2021) 'Non-Coding RNA in Systemic Sclerosis: A Valuable Tool for Translational and Personalized Medicine', *Genes*, 12(9), p. 1296. Available at: https://doi.org/10.3390/genes12091296.

Sampaio, A.L. *et al.* (2021) 'Skin manifestations associated with systemic diseases – Part I', *Anais Brasileiros de Dermatologia*, 96(6), pp. 655–671. Available at: https://doi.org/10.1016/j.abd.2021.02.008.

Schiffmann, M. -L. *et al.* (2021) 'Sclerotherapy in a rare case of glomuvenous malformation (GVM) with severe skin manifestation', *Journal of the European Academy of Dermatology and Venereology*, 35(9). Available at: https://doi.org/10.1111/jdv.17295.

Shah, A. *et al.* (2021) 'Surgical and non-surgical treatment modalities for glomuvenous malformations', *Dermatology Online Journal*, 27(7). Available at: https://doi.org/10.5070/D327754380.

Skowronek, D. *et al.* (2020) 'Identification and characterization of a GLMN splice site variant in a family with glomuvenous malformations', *European Journal of Dermatology*, 30(2), pp. 179–181. Available at: https://doi.org/10.1684/ejd.2020.3716.

Soheilifar, M.H. *et al.* (2022) 'Non-coding RNAs in photoaging-related mechanisms: a new paradigm in skin health', *Biogerontology*, 23(3), pp. 289–306. Available at: https://doi.org/10.1007/s10522-022-09966-x.

Sonkoly, E. *et al.* (2010) 'MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte–associated antigen 4', *Journal of Allergy and Clinical Immunology*, 126(3), pp. 581-589.e20. Available at: https://doi.org/10.1016/j.jaci.2010.05.045.

Sterba, M. *et al.* (2023) 'Targeted treatment of severe vascular malformations harboring PIK3CA and TEK mutations with alpelisib is highly effective with limited toxicity', *Scientific Reports*, 13(1), p. 10499. Available at: https://doi.org/10.1038/s41598-023-37468-4.

Sun, Y. *et al.* (2015) 'Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis', *Journal of Receptors and Signal Transduction*, 35(6), pp. 600–604. Available at: https://doi.org/10.3109/10799893.2015.1030412.

Svoronos, A.A., Engelman, D.M. and Slack, F.J. (2016) 'OncomiR or Tumor Suppressor? The Duplicity of MicroRNAs in Cancer', *Cancer Research*, 76(13), pp. 3666–3670. Available at: https://doi.org/10.1158/0008-5472.CAN-16-0359.

Szabo, I. *et al.* (2021) 'Novel Concepts in Systemic Sclerosis Pathogenesis: Role for miRNAs', *Biomedicines*, 9(10), p. 1471. Available at: https://doi.org/10.3390/biomedicines9101471.

Tron, A.E. *et al.* (2012) 'The Glomuvenous Malformation Protein Glomulin Binds Rbx1 and Regulates Cullin RING Ligase-Mediated Turnover of Fbw7', *Molecular Cell*, 46(1), pp. 67–78. Available at: https://doi.org/10.1016/j.molcel.2012.02.005.

Tsai, C.-H. and Ogawa, R. (2019) 'Keloid research: current status and future directions', *Scars, Burns & Healing*, 5, p. 205951311986865. Available at: https://doi.org/10.1177/2059513119868659.

Uebelhoer, M., Boon, L.M. and Vikkula, M. (2012) 'Vascular Anomalies: From Genetics toward Models for Therapeutic Trials', *Cold Spring Harbor Perspectives in Medicine*, 2(8), pp. a009688–a009688. Available at: https://doi.org/10.1101/cshperspect.a009688.

Vendramin, R. *et al.* (2018) 'SAMMSON fosters cancer cell fitness by concertedly enhancing mitochondrial and cytosolic translation', *Nature Structural & Molecular Biology*, 25(11), pp. 1035–1046. Available at: https://doi.org/10.1038/s41594-018-0143-4.

Verhoeven, E. *et al.* (2007) 'Psychosocial well-being of patients with skin diseases in general practice', *Journal of the European Academy of Dermatology and Venereology*, 21(5), pp. 662–668. Available at: https://doi.org/10.1111/j.1468-3083.2007.02049.x.

Viticchiè, G. *et al.* (2012) 'MicroRNA-203 contributes to skin re-epithelialization', *Cell Death & Disease*, 3(11), pp. e435–e435. Available at: https://doi.org/10.1038/cddis.2012.174.

Volders, P.-J. *et al.* (2015) 'An update on LNCipedia: a database for annotated human lncRNA sequences', *Nucleic Acids Research*, 43(D1), pp. D174–D180. Available at: https://doi.org/10.1093/nar/gku1060.

Wang, W.-T. *et al.* (2019) 'Noncoding RNAs in cancer therapy resistance and targeted drug development', *Journal of Hematology & Oncology*, 12(1), p. 55. Available at: https://doi.org/10.1186/s13045-019-0748-z.

Wang, Y. *et al.* (2020) 'The Roles of lncRNA in Cutaneous Squamous Cell Carcinoma', *Frontiers in Oncology*, 10, p. 158. Available at: https://doi.org/10.3389/fonc.2020.00158.

Wang, Z. (2018) 'Diverse roles of regulatory non-coding RNAs', *Journal of Molecular Cell Biology*, 10(2), pp. 91–92. Available at: https://doi.org/10.1093/jmcb/mjy026.

Wasilewska, K. *et al.* (2022) 'Postzygotic mutations and where to find them – Recent advances and future implications in the field of non-neoplastic somatic mosaicism', *Mutation Research/Reviews in Mutation Research*, 790, p. 108426. Available at: https://doi.org/10.1016/j.mrrev.2022.108426.

Williams, E. *et al.* (2021) 'ALK2 Receptor Kinase Association with FKBP12.6 Is Structurally Conserved with the ALK2-FKBP12 Complex', *Biomedicines*, 9(2), p. 129. Available at: https://doi.org/10.3390/biomedicines9020129.

Winkle, M. *et al.* (2021) 'Noncoding RNA therapeutics — challenges and potential solutions', *Nature Reviews Drug Discovery*, 20(8), pp. 629–651. Available at: https://doi.org/10.1038/s41573-021-00219-z.

Xu, Y. *et al.* (2019) 'Long Non-coding RNA LINC-PINT Suppresses Cell Proliferation and Migration of Melanoma via Recruiting EZH2', *Frontiers in Cell and Developmental Biology*, 7, p. 350. Available at: https://doi.org/10.3389/fcell.2019.00350.

Yadav, P. *et al.* (2013) 'Vascular Anomalies of the Head and Neck: A Review of Genetics', *Seminars in Ophthalmology*, 28(5–6), pp. 257–266. Available at: https://doi.org/10.3109/08820538.2013.825279.

Yan, Q. *et al.* (2016) 'Targeting miR-155 to Treat Experimental Scleroderma', *Scientific Reports*, 6(1), p. 20314. Available at: https://doi.org/10.1038/srep20314.

Yang, R. and Wang, R.C. (2021) 'Research Techniques Made Simple: Studying Circular RNA in Skin Diseases', *Journal of Investigative Dermatology*, 141(10), pp. 2313-2319.e1. Available at: https://doi.org/10.1016/j.jid.2021.07.156.

Yang, Z. *et al.* (2011) 'Overexpression of Long Non-coding RNA HOTAIR Predicts Tumor Recurrence in Hepatocellular Carcinoma Patients Following Liver Transplantation', *Annals of Surgical Oncology*, 18(5), pp. 1243–1250. Available at: https://doi.org/10.1245/s10434-011-1581-y.

Yi, J.Z., Friedland, M.H. and McGee, J.S. (2022) 'Epigenetics and precision medicine in skin diseases', in *Epigenetics in Precision Medicine*. Elsevier, pp. 193–221. Available at: https://doi.org/10.1016/B978-0-12-823008-4.00003-2.

Yu, A.-M. *et al.* (2019) 'RNA therapy: Are we using the right molecules?', *Pharmacology & Therapeutics*, 196, pp. 91–104. Available at: https://doi.org/10.1016/j.pharmthera.2018.11.011.

Yu, X. *et al.* (2020) 'MicroRNAs in atopic dermatitis: A systematic review', *Journal of Cellular and Molecular Medicine*, 24(11), pp. 5966–5972. Available at: https://doi.org/10.1111/jcmm.15208.

Zhang, G. *et al.* (2022) 'Strategies for targeting undruggable targets', *Expert Opinion on Drug Discovery*, 17(1), pp. 55–69. Available at: https://doi.org/10.1080/17460441.2021.1969359.

Zhang, L., Ge, Y. and Fuchs, E. (2014) '*miR-125b* can enhance skin tumor initiation and promote malignant progression by repressing differentiation and prolonging cell survival', *Genes & Development*, 28(22), pp. 2532–2546. Available at: https://doi.org/10.1101/gad.248377.114.

Zhang, Z. *et al.* (2021) 'Emerging role of lncRNA DANCR in progenitor cells: beyond cancer', pp. 1399–1409.

Zheng, X. *et al.* (2012) 'Dynamic Regulation of the Cerebral Cavernous Malformation Pathway Controls Vascular Stability and Growth', *Developmental Cell*, 23(2), pp. 342–355. Available at: https://doi.org/10.1016/j.devcel.2012.06.004.

Zhou, B. *et al.* (2022) 'Notch signaling pathway: architecture, disease, and therapeutics', *Signal Transduction and Targeted Therapy*, 7(1), p. 95. Available at: https://doi.org/10.1038/s41392-022-00934-y.

Zhou, X., Hao, Q. and Lu, H. (2019) 'Mutant p53 in cancer therapy—the barrier or the path', *Journal of Molecular Cell Biology*, 11(4), pp. 293–305. Available at: https://doi.org/10.1093/jmcb/mjy072.

Zhu, Y., Tazearslan, C. and Suh, Y. (2017) 'Challenges and progress in interpretation of non-coding genetic variants associated with human disease', *Experimental Biology and Medicine*, 242(13), pp. 1325–1334. Available at: https://doi.org/10.1177/1535370217713750.

Zhu, Z. *et al.* (2020) 'The comprehensive detection of miRNA, lncRNA, and circRNA in regulation of mouse melanocyte and skin development', *Biological Research*, 53(1), p. 4. Available at: https://doi.org/10.1186/s40659-020-0272-1.