

## Chapter 3

# **The human orthologue of murine *Mpzl3* with predicted adhesive and immune functions is a potential candidate gene for immune-related hereditary hair loss**

This chapter is based on:

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## Abstract

We have recently reported a mutation within the conserved immunoglobulin V-type domain of the predicted adhesion protein MPZL3 (MIM 611707) in rough coat (*rc*) mice with severe skin abnormalities and cyclic and progressive hair loss. In this study, we analyzed the human orthologue gene MPZL3 on chromosome 11q23.3, to test the hypothesis that mutated MPZL3 might be a candidate for similar symptoms in humans. Data were integrated from the Ensembl, NCBI and USCS genome informatics databases, protein structure modeling was carried out using THMM and EBI Inter Pro Scan softwares, domain structure and potential posttranslational modification sites were identified with CBS servers and 3D structure was generated with the Swiss-Model server. Results show that the predicted MPZL3 protein is highly conserved in mammals, has two transmembrane motifs flanking an extracellular Ig-like domain and is expressed in immune cells among others. The R100Q mutation identified in *rc* mice is within the Ig-domain recognition loop known for functions in T-cell receptors and cell adhesion. Further support for an immune related role is that the homologous Myelin Protein Zero and EVA1 function in cell adhesion and immune response. Based on the results of the *rc* mouse study, 3D structure, homology predictions, comprehensive NCBI Entrez database analyses of multiple polymorphisms and mutations within the human MPZL3 gene, and its cell and tissue expression pattern, we postulate that homologous or compound heterozygous mutations of MPZL3 might be involved in immune mediated human hereditary disorders presenting with hair loss.

## Introduction

The rough coat (*rc*) mutation arose spontaneously (Dickie, 1966) and homozygous *rc* mice show in both sexes growth retardation, cyclic and progressive hair loss, and sebaceous gland hypertrophy due to sebocyte hyperplasia which results in increased lipid synthesis and clumped hair (rough coat) (Hayashi et al., 2004). In the hepatic sinusoids of the liver of *rc* mice, we noted increased numbers of erythrocytes, lymphocytes, polymorphonuclear leukocytes (neutrophils) and enlarged Kupffer cells compared to normal controls. By one year of age, 60% of the *rc* mice develop spontaneous and persistent ulcerated lesions on the ventral skin of the neck (Cao et al., 2007). At the ulcer sites, localized extensive granulation tissue formations were present with neutrophilic, mastocytic and lymphoplasmacytic dermatitis (Hayashi et al., 2004). The *rc* locus was previously mapped to 32.0 cM on mouse chromosome 9 (Eicher EM, 1977). Subsequently, we defined and reduced the mapping interval for this locus to 246kb, which contains 11 candidate genes. Using DNA sequence analysis of all coding exons and flanking splice sites within this region, we identified a single missense mutation in the Myelin Protein Zero Like 3 (*Mpzl3*) gene (MIM 611707) that resulted in an R100Q substitution within the conserved immunoglobulin (Ig)

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Hs  MQQ-RGAAGSRGCALFPLLGLVLFQGVYIVFSLEIRADAHVRGVGEKIKLKCTFKSTSDVTD
Pt  MQQ-RGAAGSRGCALFPLLGLVLFQGVYIVFSLEIHADAHVRGVGEKIKLKCTFKSTSDVTD
Mm  MQLARGTVGGRGALFPLLSILVVQGARIVLSLEISADAHVRGVGEKIKLKCTFKSSSDVTD
Md  VKYFGFLYL-GLYAVLSMEIKADAHVRGYIGESIKLKCTFRSSSPVSD
Gg  LSGAGAAGMRGGEVVAYLGLWLLPSGICGALSLDIKTSPEVRGVGEQIVLKCSFKSSFPITE
Dr  MMDVNRSAFKGLLWCVFFTSGLC--MSVWSPAEVSVVSGSAVSLSCSFSSSSRVTS

Hs  KLTIDWTYRPPSSSHTVSIFHYQSFQYPTTAGTFRDRISWVGNVYKGDASISISNPTIKDNGT
Pt  KLTIDWTYRPPSSSRTVSIFHYQSFQYPTTAGTFRDRISWVGNVYKGDASISISNPTIKDNGT
Mm  KLTIDWTYRPPSSSRTESIFHYQSFQYPTTAGTFRDRISWAGNVYKGDASISISNPTLKDNGT
Md  KLTIDWTYRQPTSSRIEPIFHYQSFQYPTTAGTFRDRISWVGDVYRGDASISISNPTMRDNGT
Gg  SLTIDWTYRPLTGGLMETVFHYQSVAYPASVGRFKDRISWVGNAAKGDASIAIQSPSVSDNGT
Dr  LMSVDWKFKPQSGGPAKLFFFHSSVAYPVEDERFRGRVKWTGSPSGGDASIQLLNATQNDNGT

Hs  FSCAVKNPPDVHHNIPMTELTVTERGFGTMLSSVALLSILVFVPSAVVALLLVRMGRKAAGL
Pt  FSCAVKNPPDVHHNIPMTELTVTERGFGTMLSSVALLSILVFVPSAVVALLLVRMGRKAAGL
Mm  FSCAVKNPPDVYHNIPLTELTVTERGFGTMLSSVALLSILVFVPSAVVILLLVRMGRKATGV
Md  FSCSVKNPPDVHHNIPMTELVTVTERGFAIQLSSAALLSILVFIPSAVVVLLLVRMGRKSAGL
Gg  FICSVKNPPDVYHNIPQTVLIVTERGFSFQLTSVTLLSIVVFIPSTVVVLLLVRMGRKSGVI
Dr  FSCSVRNPPDIQNGTAQTVLTVTPKRVSLTLDVGLLVCVGPSAVVTLLLSRICCCEGP

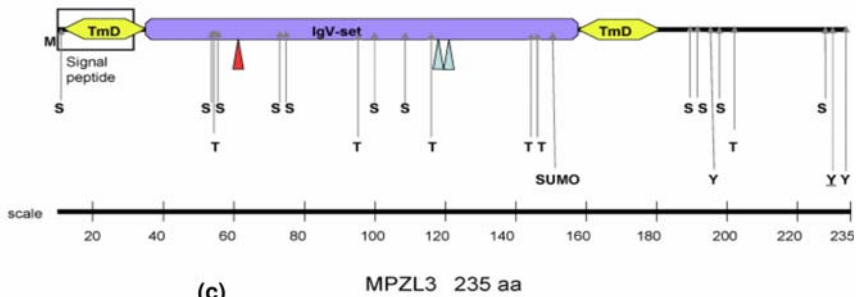
Hs  KKRSRSGYKKSSIEVSDDTDQEEEEACMARLCVRCAECLDSYEE--TY
Pt  KKRSRSGYKKSSIEVSDDTDQEEEEACMARLCVRCAECLDSYEE--TY
Mm  QKRSRSGYKKSSIEVSDETDQEDSNDCMTRLCVRCAECLDSYEEEEAY
Md  KKKSRSGYKKSSIEVSDDTDQEDEDCMRRFCTRCAECLDSYEE--AY
Gg  KEKKRPSCKSSVE---ESESEYTDNCMGRLKTWCLNCVDTDEED--PY
Dr  PALQHHSPIEVIAGEEYFYTQTQHKHSACCCYFKDSEYDEDEYMADKLHEHTITESQC
    
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Compare	Identities	Positives	Gaps
Hs-Pt	233/235 (99%)	233/235 (99%)	0/235 (0%)
Hs-Mm	205/237 (86%)	214/237 (90%)	2/237 (1%)
Hs-Md	175/221 (79%)	195/221 (88%)	1/221 (0%)
Hs-Gg	127/232 (55%)	163/232 (70%)	3/232 (1%)
Hs-Dr	71/238 (30%)	102/238 (43%)	4/238 (2%)

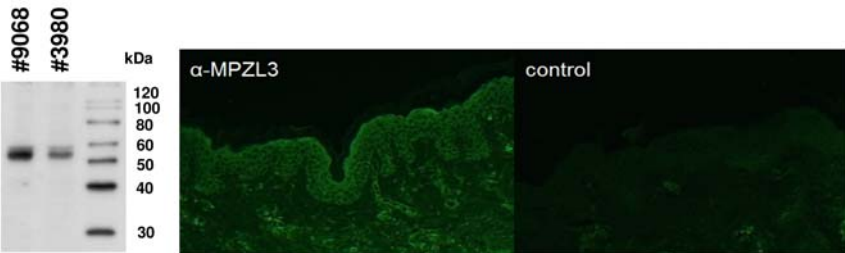
**Figure 1.** Different level of *MPZL3* amino acid sequence homologies in mammals and lower vertebrates. The identical residues (identities) are highlighted with yellow background, the conservative substitutions (positives) are highlighted with blue background. Dashes (-) indicate deletions. Bold letters show the conserved Immunoglobulin V-type domain. The red highlighted R indicates the R100Q mutation found in *rc* mice. Hs: *Homo sapiens*, Pt: *Pan troglodytes*, Mm: *Mus musculus*, Md: *Monodelphis domestica*, Gg: *Gallus gallus*, Dr: *Danio rerio*.

V-type domain of the predicted *MPZL3* protein (Cao et al., 2007) (Chapter 2). In this study, towards understanding the normal and pathological function of human *MPZL3*, we analyzed its gene structure along with polymorphisms and mutations, tissue expression pattern and functional domains using genome database information, homology modeling and protein structure predictions.

a)



b)



**Figure 2.** (a) MPZL3 domain structure and potential posttranslational modification sites. Empty rectangle: signal peptide, yellow hexagons: transmembrane (TmD) domains; purple hexagon: immunoglobulin V-type domain; blue triangles: potential N-glycosylation sites; red triangle: potential O-glycosylation site; purple arrow, predicted sumoylation site; grey arrows, potential phosphorylation sites; S: Ser; T: Thr; Y: Tyr. The underlined Y is a predicted sulphated tyrosine. (b) Western blot analysis of cultured primary human fibroblasts (donors #9068 and #3980) using affinity-purified anti-MPZL3 antibodies (1:500). (c) Immunofluorescent detection of MPZL3 (1:100) in human skin. Controls were incubated with normal goat serum instead of anti-MPZL3.

## Results

Ensembl database analysis identified orthologues of the MPZL3 protein in mammals with 88–99% similarity that declined in non-mammalian vertebrates (Figure 1). The R100Q substitution we identified in *rc* mice lies within the conserved Ig V-type domain of MPZL3 and affects a residue conserved in all vertebrate species.

The human gene *MPZL3* at chromosome band 11q23.3 is highly homologous to the mouse *Mpzl3* gene including genomic context, exon/intron organization, nucleotide and predicted amino acid sequences. Analysis of the predicted 235 amino acid MPZL3 protein using TMHMM (Krogh et al., 2001; TMHMM) and EBI InterProScan software revealed two transmembrane motifs at amino acid positions 12–34 and 159–181 that flank the extracellular Ig-like domain (position 31–148, Figure 2a). Structural modeling placed the R100Q mutation within the recognition loop of the Ig-like domain known for roles in T cell receptors, cell–cell recognition and cell

adhesion (Teichmann and Chothia, 2000). The Human Protein Reference Database biological process prediction suggested an immune response role for *MPZL3* (Peri, 2003)

The NCBI Entrez SNP database contained numerous single nucleotide polymorphisms (SNPs) within the *MPZL3* gene. Two synonymous SNPs fall within the conserved Ig V-type domain at amino acid positions 63 and 137. A frame shift mutation outside the Ig V-type domain is at amino acid 150 -/A. Three non-synonymous mutations, M155V, V168G and V172M substitutions were in exon 4 and a D228V substitution in exon 6. As the mouse and human genes are orthologues with 84.5% identity at the nucleotide and 86.8% identity at the amino acid level that reaches 93.3% within the Ig V-type conserved domain (Cao et al., 2007), it is suggested that humans with mutations within the conserved Ig V-type domain may develop symptoms similar to the *rc* mouse.

Our RT-PCR studies detected *Mpzl3* expression in multiple mouse tissues and immunostaining localized *MPZL3* in the epidermis and hair follicles (Cao et al., 2007). Based on EST counts in NCBI, Unigene database human *MPZL3* expression was similarly detected in brain, esophagus, heart, kidney, liver, lung, muscle, spleen, and in addition in blood, colon, eye, lymph node, mammary gland, mouth, ovary, parathyroid, pharynx, pituitary gland, prostate, stomach, testis, uterus and vascular tissues. EMBL-EBI Array Express microarray database analysis showed *MPZL3* expression in dendritic, CD4 and CD8 central memory and effector T cells.

Western blot analysis of *MPZL3* detected a 54 kDa and a fainter 56 kDa band in cultured primary human dermal fibroblasts, which may result from dimerization and/or from posttranslational modifications of *MPZL3* (predicted MW 25.98 kDa, Figure 2b). Using immunohistochemistry, we localized *MPZL3* in similar regions of the human skin (Figure 2c) as in the mouse skin (Cao et al., 2007) including hair follicles (not shown).

## Conclusion

This study demonstrated that the *MPZL3* Ig V-type adhesive domain is extracellular flanked by transmembrane domains, a domain motif found in proteins with immune function. Expression of *MPZL3* in immune cells supports its potential immune-related role. Furthermore, the evolutionary closely related protein (Ensembl Gene Tree View analysis data) Epithelial V-like antigen 1 (EVA1) plays roles in cell adhesion and thymus and lymphocyte development (Guttinger et al., 1998). The results suggest that the *MPZL3* protein may be involved in immune function and mutations within the conserved V-type domain may contribute to human disorders with immune-system deficiencies.

Significant association exists between mutations causing skin and hair follicle abnormalities and immunological defects (Hayashi et al., 2004; Yu et al., 2008). Hair follicles have properties of an immune privileged/protected site (Gilhar and

Kalish, 2006). Human hair loss disorders including alopecia areata, represent a breakdown in immune privilege with subsequent destruction of the hair follicle by T lymphocytes (Gilhar et al., 2005; Martinez-Mir et al., 2007). Ig-domain containing adhesion molecules play important roles in homing of lymphocytes to inflammation sites (Gilhar and Kalish, 2006). *PVRL1* located on the same chromosome as *MPZL3*, encodes a membrane adhesion protein with one V-like and two C-like extracellular Ig domains and is known to be associated with hereditary alopecia (Suzuki et al., 2000). Alopecia areata affects adults and children of both sexes with a lifetime risk of approximately 1.7%. While its exact molecular mechanism is not known, it is considered an autoimmune disease (Freyschmidt-Paul et al., 2001) and likely a polygenic disorder similar to other autoimmune diseases (McDonagh and Tazi-Ahnini, 2002). Based on our results, expression and mutational analysis of *MPZL3* in patients and families may provide valuable information towards understanding the involvement of *MPZL3* in hereditary alopecia.

## Methods

The NCBI (<http://www.ncbi.nlm.nih.gov/>), Ensembl (<http://www.ensembl.org/index.html>), and UCSC Genome Bioinformatics (<http://genome.ucsc.edu/>) databases were used for gene structure and sequence analyses. EST confirmed protein sequence was used to predict the protein structure with TMHMM (Krogh et al., 2001; TMHMM) (<http://www.cbs.dtu.dk/services/TMHMM/>) and EBI InterProScan softwares (<http://www.ebi.ac.uk/InterProScan/>). The template sequence that showed the highest sequence identity to *MPZL3* was 1NEU (Myelin Po protein precursor) and was selected for 3D homology modeling by the 3D jury consensus method (Ginalski et al., 2003) using the fold recognition server BioInfoBank Meta Server (Metaserver1) (<http://bioinfo.pl/meta/>). The 3D structure generation was carried out through the Swiss-Model server (Guex, 1997) (<http://swissmodel.expasy.org/SWISS-MODEL.html>) and visualization was performed using the Swiss-PDBViewer (Guex, 1997) program (<http://expasy.org/spdbv/>). *MPZL3* domain structure and potential posttranslational modification sites identified with EBI-InterProScan (<http://www.ebi.ac.uk/Tools/InterProScan/>) and CBS servers (Blom et al., 1999; Gupta R, 2004; Julenius et al., 2005), SUMOplot™ Analysis Program (Xue et al., 2006).

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