SLFN14 gene mutations associated with bleeding

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The Schlafen (SLFN) Family

The Schlafen (SLFN) family of genes, which is limited to mammals, was initially discovered in mice by Schwarz et al., investigating the T cell lineage, regulating differentiation and in some instances ablation of growth [1]. In particular, overexpression of SLFN1 resulted in a cell cycle arrest at the G0/G1 stage. Therefore, the family had been named Schlafen, which is translated from German as “to sleep” [1]. Subsequent studies have classified the SLFN family into three distinct subgroups based on gene/protein size and domain homology [2,3]. Ten mouse and six human SLFN genes have been identified to date (Figure 1) [4]. All 10 mouse SLFN proteins possess a core region containing a unique “slfn box” motif with an unknown function. Subgroups II and III contain an extra domain at the C-terminus of the slfn box, conserved by the flanking five amino acid signature (Ser-Trp-Ala ... -Asp-Leu) [SWA ... DL] which appears to be SLFN specific. This was discovered in early characterization of the SLFN family with SLFN3 and SLFN4 (members 3 and 4) possessing a 200 amino acid sequence not found in those in subgroup I [1]. Adjacent to the slfn box is the ‘ATPases associated with diverse cellular activities’ (AAA) domain. Based on protein homology studies, the AAA motif is thought to function similarly to classical AAA domains with the role of ATP/GTP binding in the course of DNA and RNA metabolism and therefore playing a fundamental role in the production and function of SLFN proteins [5,6]. Another protein was discovered with significant similarity to those in subgroup II, extending a further 400 amino acids towards the C-terminus terminal tail. The sequence was aligned by BLAST and the first 570 amino acids were homologous to SLFNs 3 and 4 while the remainder was unique to named SLFN8 leading to the classification of the final SLFN subgroup, III [2]. Additional homologous genes were identified as SLFN5, SLFN9, SLFN10 and SLFN14, whereby the last two coding exons correspond to this unique subgroup. NCBI conserved domain database (CDD) searches revealed significant homology of subgroup III specific extension to motifs typical in superfamily I of RNA/DNA helicases which are known to mediate DNA and RNA metabolism [2]. The same characterization method applies to the human-specific SLFN genes with the absence of any subgroup I members and structural difference in SLFN12L (SLFN12-LIKE). Notably, SLFN5 and SLFN14 are the only SLFN family genes present in both human and mouse. The predicted transcript of SLFN12L is of a similar length to those in subgroup III; however, it also encompasses sequences which are unaccounted for and based on this structural analysis, has not yet been assigned a subgroup. According to length and homology, SLFN14 belongs to subgroup III (Figure 1) [7]. The recently discovered crystal structure of rat SLFN13 (related to human SLFN13 and mouse SLFN8) N-domain gives a functional insight into its ability to cleave RNA acting as an endoribonuclease in inhibiting protein synthesis [8]. Importantly, SLFN13 is the closest parologue for SLFN14 (44% identity and 61% similarity).

SLFN14 in mice is located on chromosome 11 and comprised of five coding exons and in humans is on chromosome 17 with four coding exons [9]. SLFN genes are highly conserved throughout the mammalian species and are located in regions with other genes attributable to T-cell and macrophage development [1]. Early mouse knock-out studies investigating the role of SLFN1 in the immune response showed no phenotype, suggesting potential functional redundancy or minimal contribution of some members in the process [9]. The precise mechanisms of how some of the specific members of the SLFN family cause human disease is still unclear.

SLFN14 Mutations in Patients with Platelet Disorders and Bleeding

Platelet function disorders are associated with excessive bleeding and with the advent of next-generation sequencing (NGS) including whole exome sequencing (WES) the genetic impact of affected individuals can be evaluated. Initially, three extended families
were investigated in the Genotyping and Phenotyping of Platelets Study, with three heterozygous single nucleotide variations in SLFN14 resulting in missense mutations of consecutive amino acids in the AAA domain [10]. The following year, an additional family was discovered with another missense mutation in the same region (R223W) and in 2019, Saes et al., reported a further patient with an alternative base change at nucleotide c.657 yet still resulting in the K219N mutation (Table I and Figure 1) [11,12]. All four mutations were inherited in an autosomal dominant fashion whereby amino acid changes are conserved across all mammalian species, except for V220D. The R223W variant is the only variant out of these families to be reported on gnomAD (genome aggregation database) with a frequency of 6.5e-6. This highlights variant rarity amongst different populations and confinement to the families described. All patients in these reported families were heterozygous for the mutations and therefore phenotypically defined by platelet-type bleeding disorder 20 (BDPLT20) which is specific to heterozygous mutations in the SLFN14 gene. The absence of homozygous patients in the families suggests the mutant allele in each variant group has significant influence over the wild type. Indeed, heterozygous SLFN14 protein expression was reduced by 65%-80% when compared to controls and in platelet spreading assays there is a reduction of proplatelet extensions from developing megakaryocytes [10,11]. This apparent dominant negative effect on overall protein expression can impact the maturation of megakaryocytes, affecting differentiation and production of fully functional platelets [10,11]. In order to decipher the role of SLFN14 in platelet biogenesis, it has been demonstrated that SLFN14 acts as an endoribonuclease in controlling gene expression [13,14]. SLFN14 is strongly overexpressed in rabbit
Table I. SLFN14 variants reported to date in patients with inherited macrothrombocytopenia, platelet-type bleeding disorder 20 (BDPLT20).

| Patient | Genomic Variant | Protein effect | Variant type | Inheritance | Platelet count (x10^9/L) | Mean platelet volume (MPV fl) | ISTH BAT Score | Aggregation/ Secretion Defect | Reference |
|---------|-----------------|----------------|--------------|-------------|--------------------------|-------------------------------|----------------|-----------------------------|-----------|
| A; III 2 | c.659 T > A | p.V220D | Missense | Het | 140 | 9.1 | 5 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| A; III 3 | c.659 T > A | p.V220D | Missense | Het | 74 | 10.4 | 10 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| A; IV 2 | c.659 T > A | p.V220D | Missense | Het | 110 | 9.3 | 13 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| A; IV 4* | c.659 T > A | p.V220D | Missense | Het | 100 | 11.1 | 22 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| A; IV 5 | c.659 T > A | p.V220D | Missense | Het | 116 | 11.2 | 21 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| B; I 2 | c.657 A > T | p.K219N | Missense | Het | 83 | 11.9 | 13 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| B; II 3* | c.657 A > T | p.K219N | Missense | Het | 68 | 11.9 | 20 | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| C; II 2* | c.652 A > G | p.K218E | Missense | Het | 89 | 13.0 | NA | ADP, Collagen and PAR-1-activating peptide/ATP | [10] |
| D; II 1 | c.667 C > T | p.R223W | Missense | Het | 87 | 12.1 | 5 | NA/NA | [11] |
| D; II 2 | c.667 C > T | p.R223W | Missense | Het | 91 | 21.0 | 2 | NA/NA | [11] |
| D; III 3* | c.667 C > T | p.R223W | Missense | Het | 79 | 12.3 | 9 | NA/NA | [11] |
| E; I 1* | c.657 A > C | p.K219N | Missense | Het | NR | NR | NR | ADP, Collagen and PAR-1-activating peptide/ATP | [12] |

All patients identified with variants in the SLFN14 gene and affected by inherited bleeding. *: Proband in family case; Het: Heterozygous inheritance pattern; International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (BAT) score; NA: Not Available for in vitro study; NR: Not Reported in publication. Thrombocytopenia was defined as platelet count <150x10^9/L.

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slFN14 cause inherited macrothrombocytopenia characterized by enlarged platelets and moderate to severe bleeding phenotypes.

Three out of the five families with slFN14 genetic variants show reduced aggregation to agonists ADP, PAR-1 and Collagen, decreased ATP secretion and dominant inheritance pattern.

Endoribonuclease activity of slFN14 contributes to platelet formation by regulating translation process during their maturation.

The exact role of slFN14 in platelet biogenesis and how mutations within its AAA domain contribute to inherited thrombocytopenia remain to be explored.

Declaration of interest
The authors report no conflict of interest.

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References
1. Schwarz DA, Katayama CD, Hedrick SM. Schlafen, a new family of growth regulatory genes that affect thymocyte development. Immunity 1998;9(5):657–668.
2. Geserick P, Kaiser F, Klemm U, Kaufmann SHE, Zerrahn J. Modulation of T cell development and activation by novel members of the Schlafen (slfn) gene family harbouring an RNA helicase-like motif. Int Immunol 2004;16(10):1535–1548. doi:10.1093/intimm/dxh155
3. Neumann B, Zhao L, Murphy K, Gonda TJ. Subcellular localization of the Schlafen protein family. Biochem Biophys Res Commun 2008;370(1):62–66. doi:10.1016/j.bbrc.2008.03.032
4. Mavrommatis E, Arslan AD, Sassano A, Hua Y, Kroczynska B, Plataniotis LC. Expression and regulatory effects of murine Schlafen (Slfn) genes in malignant melanoma and renal cell carcinoma. J Biol Chem 2013;288(46):33006–33015. doi:10.1074/jbc.M113.460741
5. Hanson PL, Whiteheart SW. AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol 2005;6:519. doi:10.1038/nrm1684
6. Lupas AN, Martin J. AAA proteins. Curr Opin Struct Biol 2002;12(6):746–753.
7. Liu F, Zhou P, Wang Q, Zhang M, Li D. The Schlafen family: complex roles in different cell types and virus replication. Cell Biol Int 2018;42(1):2–8. doi:10.1002/cibi.20778
8. Yang J-Y, Deng X-Y, Li Y-S, Ma X-C, Feng J-X, Yu B, Chen Y, Luo Y-L, Wang X, Chen M-L, et al. Structure of Schlafen13 reveals a new class of tRNA/rRNA- targeting RNase engaged in translational control. Nat Commun 2018;9(1):1165. doi:10.1038/s41467-018-03544-x
9. Bustos O, Naik S, Ayers G, Casola C, Perez-Lamigueiro MA, Chippindale PT, Pritham EJ, de la Casa-Espartero E. Evolution of the Schlafen genes, a gene family associated with embryonic lethality, meiotic drive, immune processes and orthopoxvirus virulence. Gene 2009;447(1):1–11. doi:10.1016/j.gene.2009.07.006
10. Fletcher SJ, Johnson B, Lowe GC, Ben D, Drake S, Lordkipanidze M, Guitt IS, Dawood B, Rivera J, Simpson MA, et al. slFN14 mutations underlie thrombocytopenia with excessive bleeding and platelet secretion defects. J Clin Invest 2015;125(9):3600–3605. doi:10.1172/JCI80347
11. Marconi C, Di Buduo CA, Barozzi S, Palombo F, Pardini S, Zaninetti C, Pippucci T, Noris P, Balduini A, Seri M, et al. slFN14-related thrombocytopenia: identification within a large series of patients with inherited thrombocytopenia. Thromb Haemost 2016;115(5):1076–1079. doi:10.1160/TH15-11-0884
12. Saes JL, Simons A, de Munnik SA, Nijziel MR, Blijlevens NMA, van der Reijden BA, Smit Y, Brons PP, van Heerde WL, et al. Whole exome sequencing in the diagnostic workup of patients with a bleeding diathesis. Haemophilia 2019;25(1):127–135. doi:10.1111/hae.13638
13. Pisarev VP, Muslimov IA, Tcherepanov A, Pisarev AV. Characterization of novel ribosome-associated endoribonuclease SLFN14 from rabbit reticulocytes. Biochemistry 2015;54(21):3286–3301. doi:10.1021/acs.biochem.5b00302
14. Fletcher SJ, Pisarev VP, Khan AO, Tcherepanov A, Morgan NV, Pisarev AV. Role of the novel endoribonuclease SLFN14 and its disease-causing mutations in ribosomal degradation. Rna 2018;24(7):939–949. doi:10.1261/rna.066415.118
15. Ricciardi S, Miluzio A, Brina D, Clarke K, Bonomo M, Aiolfi R, Guidotti LG, Falciani F, Bisio F. Eukaryotic translation initiation factor 6 is a novel regulator of reactive oxygen species-dependent megakaryocyte maturation. J Thromb Haemost 2015;13(11):2108–2118. doi:10.1111/jth.13150
16. Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. Blood 2010;115(16):3196–3205. doi:10.1182/blood-2009-10-78129