Cross-Reactivity as a Mechanism Linking Infections to Stroke

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The relevance of infections as risk factor for cerebrovascular disease is being increasingly recognized. Nonetheless, the pathogenic link between the two entities remains poorly understood. Consistent with recent advances in medicine, the present work addresses the hypothesis that infection-induced immune responses may affect human proteins associated with stroke. Applying established procedures in bioinformatics, the pathogen antigens and the human proteins were searched for common sequences using pentapeptides as probes. The resulting data demonstrate massive peptide sharing between infectious pathogens—such as Chlamydia pneumoniae, Streptococcus pneumoniae, Tannerella forsythia, Haemophilus influenzae, Influenza A virus, and Cytomegalovirus—and human proteins related to risk of ischemic and hemorrhagic stroke. Moreover, the shared peptides are also evident in a number of epitopes experimentally proven immunopositive in the human host. The present findings suggest cross-reactivity as a potential mechanistic link between infections and stroke.

Keywords: stroke, infections, cross-reactivity, peptides, inflammation

INTRODUCTION

When considered separately from other cardiovascular diseases, stroke ranks fifth among all causes of death (1) and, critically, its incidence is on the rise (2).

The etiology of stroke is multifactorial with various environmental and genetic risk factors. Hypertension, diabetes and insulin resistance, smoking, dyslipidemia, obesity, heavy alcohol consumption, atrial fibrillation, and carotid stenosis are all established and well-investigated modifiable risk factors of stroke (3–5).

Additionally, there is evidence that environmental factors may also increase risk of stroke, including viral and bacterial infections, such as periodontitis (6) and respiratory infections (7), and infection with Chlamydia pneumoniae (8) or Cytomegalovirus (9). However, relatively little is known so far about the role of different pathogens as well as the molecular basis and the mechanisms that potentially link infections to stroke.
Here we set out to investigate whether or not infections can induce immune responses capable of cross-reacting with human proteins that, when altered, have been associated with stroke. Our hypothesis was that immune responses induced by infectious agents might cross-react with crucial stroke-related proteins, thus contributing to the multifactorial pathogenesis of cerebrovascular disease.

To address this hypothesis, we analyzed pathogens, as well as proteins that are known to be associated with increased risk of ischemic and hemorrhagic stroke by searching for common peptides that might underlie cross-reactions.

Specifically, we analyzed antigens from the following pathogens that have been reported to have a possible influence on stroke: the periodontal bacterium *Tannerella forsythia* (10), *Haemophilus influenza* (11), *Streptococcus pneumoniae* (7), *Chlamydia pneumoniae* (8), Influenza A viruses (12, 13), and Human Cytomegalovirus (9).

**METHODS**

We analyzed the amino acid (aa) primary sequence of pathogen antigens (with short name and Uniprot ID in parentheses):

- Surface antigen repeat/outer membrane protein (OMP; UniProtKB: A0A0F7WYE8_CHLPLN) from *Chlamydia pneumoniae*;
- Pneumococcal vaccine antigen A (PVAA;UniProtKB: PVAA_STRR6) from *Streptococcus pneumoniae*;
- Surface antigen BspA (BspA; UniProtKB: O68831_TANFO) from *Tannerella forsythia*;
- Outer membrane antigenic lipoprotein B (LPPB; UniProtKB: LPPB_HAEIN) from *Haemophilus influenzae* (strain ATCC 51907);
- Hemagglutinin (HA H1N1; UniProtKB: HEMA_I34A1) from Influenza A virus (strain A/Puerto Rico/8/1934 H1N1);
- Hemagglutinin (HA H5N1; UniProtKB: HEMA_I96A0) from Influenza A virus (strain A/Goose/Guangdong/1/1996 H5N1);
- Hemagglutinin (HA H3N2; UniProtKB: HEMA_I68A6) from Influenza A virus (strain A/Northern Territory/60/1968 H3N2); and
- 65 kDa phosphoprotein (pp65; UniProtKB: PP65_HCMVM) from Human Cytomegalovirus (HCMV; strain Merlin).

The primary sequence of pathogen antigens was dissected into partially overlapping pentapeptides with a one-residue-offset: i.e., MFKRI, FKIRI, KRIRR, and so on. Then, each pentapeptide was analyzed for occurrences within a library consisting of primary sequences of human proteins involved in stroke. The human protein library was *a priori* chosen from the UniProtKB Database (https://www.uniprot.org) (14) using the keyword “stroke.” We obtained an unbiased list of 74 human proteins (in)directly associated with stroke (Table S1). Stroke-related proteins are indicated as UniProtKB entry names throughout the present article, except when discussed in detail. The pathogen antigens and the human proteins were searched for common sequences using the pentapeptide as a probe unit because a pentapeptide is an immunobiological determinant sufficient for epitope-paratope interaction and for inducing specific immune responses (15–18).

The immunologic potential of the shared peptides was analyzed using the Immune Epitope Database (IEDB; www.iedb.org) (19). All evaluations were based only on epitopic sequences that had been experimentally validated as immunopositive in the human host.

This linear peptide similarity analysis procedure has been used and described before (20, 21).

**RESULTS**

In a detailed overview, Table 1 shows that 49 out of the 74 human stroke-related proteins share peptide sequences with antigens from pathogens that proved to be (in)directly involved in stroke (6–10). It can be seen that

- The pathogen vs. human peptide overlap is unexpectedly high when considering that the probability for two proteins to share a pentapeptide is 1 out of $20^5$, that is, 0.0000003125 or close to zero.
- The peptide overlap varies widely, with *T. forsythia* BspA and Influenza A HA H3N2 being the pathogen more and less involved in the peptide sharing, respectively.
- The high number of stroke-related proteins involved in the viral peptide overlap precludes a detailed protein-by-protein analysis. However, an example worth noting is the human ATP-binding cassette sub-family C member nine (ABCC9 or SUR2) that shares peptide sequences with all of the pathogen antigens analyzed, with the exception of the Influenza A HA H3N2 virus. ABCC9 is a subunit of ATP-sensitive potassium channels (K$_{ATP}$) that can form cardiac and smooth muscle-type KATP channels with KCNJ11 and mediates neuroprotection (22).

In summary, Table 1 describes a peptide platform that connects the infectious agents under analysis human proteins related to stroke.

Subsequently, in order to define the immunologic potential of the shared peptides, we conducted analyses throughout the peptide immunome cataloged in the Immune Epitope Database (IEDB; www.iedb.org) (19). The search was finalized to identify epitopic sequences corresponding to (or containing) the peptide sequences shared between stroke-related infectious agents and stroke-related human proteins. It was found that a great number of the shared peptides listed in Table 1 are also distributed through hundreds of epitopic sequences with an immunological potential. A list of such epitopic sequences is reported in Table 2.

**CONCLUSION**

Stroke risk appears to be the result of a complex combination of multiple genetic non-modifiable and environmental modifiable factors that can be further classified as either “traditional” or new, “emerging” ones (23). As highlighted by Grau et al. (24, 25), the occurrence of stroke is only partially explained by traditional modifiable cardiovascular
### TABLE 1 | Peptide sharing between pathogen antigens and human proteins that have been associated with stroke.

| Shared peptides\(^{a,b}\) | Human protein involved in the peptide\(^{b,c}\) |
|-----------------------------|-----------------------------------------------|
| **C. pneumoniae OMP:**     |                                               |
| ITNYL                       | **ABCC9.** ATP-binding cassette sub-family C member 9 |
| RKFLL                       | **CCM2.** Cerebral cavernous malformations 2 protein |
| RKFLL: KGFS                   | **CCM2L.** Cerebral cavernous malformations 2 protein-like |
| ASSVLD; LEHNOQ                | **CSF1R.** Macrophage colony-stimulating factor 1 receptor |
| IALHL                       | **DAPK1.** Death-associated protein kinase 1 |
| SEKGT                       | **FA5.** Coagulation factor V |
| PTTLQ                       | **GNAO.** Guanine nucleotide-binding protein G(i) subunit alpha |
| EGPCC                       | **HTRA1.** Serine protease HTRA1 |
| NTTAE                       | **KCNE2.** Potassium voltage-gated channel subfamily E member 2 |
| GFRCGL; LRSSA                | **NOTC3.** Neurogenic locus notch homolog protein 3 |
| VSAAG                       | **NU155.** Nuclear pore complex protein Nup155 |
| SGLOGG                      | **PARD1.** Protein disintegrin and Tumor necrosis factor receptor |
| SGNQV                       | **PAWR.** PRKC apoptosis WT1 regulator protein |
| GYFAS                       | **PDE4D.** cAMP-specific 3',5'-cyclic phosphodiesterase 4D |
| DSSPR; SPRTP                 | **RN213.** E3 ubiquitin-protein ligase RNF213 |
| **S. pneumoniae PVAA:**    |                                               |
| LAMQY                       | **ABCC9.** ATP-binding cassette sub-family C member 9 |
| TVAPL; VAPLL                 | **KCNA5.** Potassium voltage-gated channel subfamily A member 5 |
| AQNGK                       | **KLOK.** Klotho |
| SASGS                       | **LMNA.** Prelamin-A/C |
| LVLAV                       | **NMDE2.** Glutamate receptor ionotrophic, NMDA 2B |
| IOTLTL                      | **NU5M.** NADH-ubiquinone oxidoreductase chain 5 |
| **T. forsythia BspA:**     |                                               |
| AWTAR; SGKT                  | **ABCC9.** ATP-binding cassette sub-family C member 9 |
| GLQTL; LTITN                 | **B1L.** Bax inhibitor 1 |
| TSLAL                       | **CO4A2.** Collagen alpha-2(I) chain |
| APQRA                       | **COQ8A.** Atypical kinase COQ8A, mitochondrial |
| GKKAV                       | **CX53.** Gap junction alpha-5 protein |
| IFVST                       | **GATA5.** Transcription factor GATA-5 |
| NCGAL                       | **GATA6.** Transcription factor GATA-6 |
| HSLQCS                      | **IL4.** Interleukin-4 |
| LGATA; GATAQ                 | **ITIH4.** Inter-alpha-trypsin inhibitor heavy chain H4 |
| DALTT                       | **KCNQ1.** Potassium voltage-gated channel subfamily Q member 1 |
| AGGAL; VTTIG                 | **KRIIT1.** Krev interaction trapped protein 1 |
| TAPDA                       | **LYAM3.** P-selectin |
| EGPFAL                      | **NMDE2.** Glutamate receptor ionotrophic, NMDA 2B |
| VTNQI                       | **NOTC3.** Neurogenic locus notch homolog protein 3 |
| DGVNT; SGTTG                | **SCN4B.** Sodium channel subunit beta-4 |
| GLFLLL                      | **SCN5A.** Sodium channel protein type 5 subunit alpha |
| TLPNNS                      | **SYLM.** Probable leucine—tRNA ligase, mitochondrial |
| TLPDQ; VTLPN                 | **ZFHX3.** Zinc finger homeobox protein 3 |
| LDPDALL; LTLSA; SGLITS; TLPDA |                                               |
| **H. influenzae LPPB:**    |                                               |
| TSNFP; GIDIS                 | **ABCC9.** ATP-binding cassette sub-family C member 9 |
| LLLPL                       | **ACE.** Angiotensin-converting enzyme |
| SFLLLL; TTTVS                | **ANP.** Natriuretic peptides A |
| AQPAP                       | **CSF1R.** Macrophage colony-stimulating factor 1 receptor |
| ILVAD                       | **ENPP4.** Bis(5'-adenosyl)-triphosphatase ENPP4 |
| VTSSV                       | **GATA6.** Transcription factor GATA-6 |

(Continued)
TABLE 1 | Continued

| Shared peptides<sup>a,b</sup> | Human protein involved in the peptide Sharing <sup>b,c</sup> |
|-------------------------------|-----------------------------|
| GNLII | ITH4. Inter-alpha-trypsin inhibitor heavy chain H4 |
| PGANG; SGSRG | KCNAS5. Potassium voltage-gated channel subfamily A member 5 |
| APDYS; PYDSK; DYSKI; TYTPG | KRTT1. Krev interaction trapped protein 1 |
| SNVGG; SPSVP | NU155. Nuclear pore complex protein Nup155 |
| AYLAG | PDE3A. cGMP-inhibited 3′,5′-cyclic phosphodiesterase A |
| LLPLS; AYLAG; VTSSV; QEVKA | RN213. E3 ubiquitin-protein ligase RNF213 |
| GPIKS | SCN5A. Sodium channel protein type 5 subunit alpha |
| KKFL | SYLM. Probable leucine–IFNA ligase, mitochondrial |

Influenza A HA H1N1:

| LAVK | ADA2. Adenosine deaminase 2 |
| LLVSL | ATP6. ATP synthase subunit a |
| ENAVV | ABCC9. ATP-binding cassette sub-family C member 9 |
| ASSLV | PDE3A. cGMP-inhibited 3′,5′-cyclic phosphodiesterase A |
| AELLV, ELLLV, LLVLL, LVLLV | DAPK1. Death-associated protein kinase 1 |
| TVLEK; YSVSV; QTPLG; FLDIW | RN213. E3 ubiquitin-protein ligase RNF213 |
| TSNAS | NMDE2. Glutamate receptor ionotropic, NMDA 2B |
| CALAA | GAS6. Growth arrest-specific protein 6 |
| LLVLL; YAADO; KVDGV | KLOT. Klotho |
| EELRE | LMNA. Prelamin-A/C |
| YSEE | ZFHX3. Zinc finger homeobox protein 3 |

Influenza A HA H5N1:

| LLAIV | AL5AP. Arachidonate 5-lipoxygenase-activating protein |
| LLLAI | ABCC9. ATP-binding cassette sub-family C member 9 |
| AQDIL; ISGVK | PDE4D. cAMP-specific 3′,5′-cyclic phosphodiesterase 4D |
| LLLAI | CYTC. Cystatin-C |
| ORLVVP; AELLV, ELLLV | DAPK1. Death-associated protein kinase 1 |
| ILEKT, UKHLL, VSSAC | RN213. E3 ubiquitin-protein ligase RNF213 |
| EGGWQ | KLOT. Klotho |
| SLALA | NUSM. NADH-ubiquinone oxidoreductase chain 5 |
| KIVLL; LVLAT | NU155. Nuclear pore complex protein Nup155 |
| ARLNR; SIYST | KCNQ1. Potassium voltage-gated channel subfamily KQT member 1 |
| VSSAC | SCN1B. Sodium channel subunit beta-1 |
| VPEWS | TBX5. T-box transcription factor TBX5 |
| SVAGW | S19A2. Thiamine transporter 1 |
| SLALA | GATA5. Transcription factor GATA-5 |

Influenza A HA H3N2:

| GGSNA; AELLV | DAPK1. Death-associated protein kinase 1 |
| INSN | SAMH1. Deoxyxynucleoside triphosphate triphosphohydrolase SAMHD1 |
| KITYG | MYL4. Myosin light chain 4 |
| LLGDP | KCNAS5. Potassium voltage-gated channel subfamily A member 5 |
| ISFAI | HTRA1. Serine protease HTRA1 |
| VLNV | SCN3B. Sodium channel subunit beta-3 |

<sup>a</sup> References in Table S1 (Supplementary Material).

<sup>b</sup> Viral/bacterial antigens are described under Methods. Further details at https://www.uniprot.org (1–4).

<sup>c</sup> Multiple occurrences in bold.

<sup>d</sup> Human proteins given as UniProt entry and name. Further details at https://www.uniprot.org (1–4).

Risk factors, such as increasing blood pressure, cigarette smoking, and diabetes mellitus. Most importantly, infectious diseases appear to play a key role in contributing to the risk of stroke and are to be counted among “emerging” modifiable risk factors that receive increasing scientific interest (6–10, 23, 26, 27).

Searching for possible immunopathogenic links between infection and risk of stroke, the present study aimed to analyze
| 1 | 2 | 1 | 2 | 1 | 2 |
|---|---|---|---|---|---|
| 58129 | SGLTSlf | 459109 | sLLPLShv | 554097 | lvesyLTPDGrii |
| 66225 | tSQLTSi | 466105 | tglplLVAV | 554098 | lvesyLTPDGriiK |
| 68617 | tSQLTSi | 467909 | LLLLLRiev | 554195 | mtfHINSGKvp |
| 69631 | vLVLGIal | 47133 | SPRTPpvil | 555093 | ogDGKLVKaLk |
| 79809 | ELVLLVenerlid | 47166 | tAELLVL | 555672 | rslrtqELERE |
| 113324 | dgFLWdmYnELVLL | 475091 | akefNTTAei | 557456 | vvesyLTPDGrii |
| 113533 | idlwfnELVLL | 476488 | avIDGVNTl | 563134 | eaeVELLVKh |
| 150977 | eeaklnreei | 478710 | GLTTIlknv | 570114 | aELLVshga |
| 151075 | ynAELLVLenerlid | 479848 | neLVLAdy | 571918 | EELREaaw |
| 151076 | ypgdfidYELREeq | 482780 | nryASSLV | 573110 | haCALAAsw |
| 163409 | serLalFSSA | 483921 | pykLVoNVLATg | 575661 | kqapgLATAG |
| 164690 | gvdtGIDIShsdf | 485150 | rySGNQVlf | 577318 | eskKSFLcic |
| 164772 | vLLVSLgai | 487836 | TVLEKyfryl | 578014 | sprtspLpLl |
| 190442 | hELLVLvkkaq | 489284 | vLLVSLdyqgmlp | 581635 | aLKHLLsy |
| 194133 | llaAWTARa | 489926 | ypASSLVvv | 584143 | lldksVAPLL |
| 196781 | silEKTsiay | 490154 | anaALLPLj | 585672 | kgVSAAGilke |
| 213534 | kssGKTTrik | 492412 | irfTVLEKl | 587804 | rLKHLLsy |
| 223189 | ldLLLPLnl | 492430 | irfSGTTGqm | 589061 | rLKHLLsy |
| 223510 | peLLLPL | 492987 | lepsorALLPLl | 590120 | slsSPSVP |
| 223880 | seeLLLPL | 493222 | mpeyasCLLLPLl | 590444 | tveSSEGTk |
| 224543 | fLPLLSlf | 497010 | tpvyLGATAgmrll | 591020 | srskVLLV |
| 262701 | yrtLLVLs | 499610 | plLLVSLw | 591635 | eEELREyrv |
| 240338 | sASSLVkidSlv | 499947 | qroqLVSll | 594849 | krELREK |
| 243935 | FLDWynha | 506316 | kpplLVLmnr | 601366 | rTLPDGthel |
| 409699 | gpprlLPLL | 513877 | trALLPLU | 602564 | fAPDYSsrl |
| 423950 | alLKHLSy | 514637 | eitTVLEKI | 603166 | rTPDGLthel |
| 424543 | fLPLLSlf | 519070 | tpvyLGATAgmrll | 605701 | tAPAFinn |
| 426499 | riltAVARTy | 504703 | clyLLAl | 606516 | fsslLPLShl |
| 430136 | plspfLDPDdny | 505049 | editLKT | 607194 | laELReNw |
| 430260 | rtVLEKtry | 505187 | fastVAPLLef | 610338 | qEVAKAlt |
| 435575 | rlLPLLj | 505268 | flaTRGTSTli | 612086 | crLKHLLsy |
| 435576 | rlLPLLj | 507273 | mptLPLLl | 613909 | rnvkEVEL |
| 436234 | aptGPKSIdlm | 507343 | mtLPLLl | 615815 | evAEVll |
| 437494 | gAGGALVhrd | 507484 | paSPSVPi | 616957 | glvnSGLTSv |
| 437792 | gSGQLGltdk | 509413 | assPSPVlj | 617652 | rLKHLLsy |
| 440682 | SPRTpvsvip | 511858 | arrSLALArpKssdvy | 617652 | rLKHLLsy |
| 441210 | TVLEKyel | 518869 | inhvSVAGWvsgd | 620564 | fAPDYSsrl |
| 442404 | apaAPQRAl | 519272 | ilTEkVspdfre | 620828 | qhyekSGNOV |
| 443560 | erySGNQVfl | 520276 | kqelLVSL | 621193 | rnvkEVEL |
| 444320 | gslLPLSek | 521984 | lqagAGALQvhr | 624191 | asfapiSFLak |
| 445722 | kplpFRTTGli | 523925 | qaAGALQvhrsvir | 626581 | glvnSGLTSv |
| 448068 | nrVPPTGi | 532960 | mPTGIney | 627346 | crAGALsl |
| 448661 | SPRTDPpff | 535616 | ELVLKqkhpseirf | 627740 | qAPDYSsrl |
| 448662 | SPRTpdpdkhal | 537116 | rikveELVLEK | 628038 | crAGALsl |
| 448918 | sryLPLLs | 541075 | alpAGALqo | 628737 | vPVTGyey |
| 449666 | VSAAGKvgl | 544699 | eEELREkay | 629959 | alvhLLVSl |
| 453784 | flpLLLV | 544699 | EELREkay | 632600 | tvPDLVLl |
| 456288 | LLLAILpivh | 544699 | rsgaAGALp | 633968 | tvPDLVLl |
| 456316 | LLLLPlpl | 545442 | srwpLLPLl | 634370 | tvPDLVLl |
| 457304 | nltLVSL | 548311 | llGDPDmrav | 636430 | mPTGIney |
| 457363 | pekTLSAU | 551494 | esyLTPDGrii | 637221 | stdLLVLsL |
| 458901 | nAEELVirv | 553367 | kqfipxlyELVLL | 639941 | tvPDLVLl |

Column 1: Epitopes listed according to the IEDB-ID number. Epitope details and references at www.immuneepitope.org [19]. Column 2: Sequences common to pathogen antigens and human proteins related to stroke are indicated in capital letters.
the potential immunologic relationship between pathogens and human proteins that, when altered, have been associated with risk of stroke. In line with our hypothesis, we found that immune cross-reactions between infectious pathogens and human stroke-related proteins might occur, thus increasing the risk of stroke (see Tables 1, 2).

The immunologically relevant peptide sharing reported in the present study depicts a complex scenario. Some potential molecular targets of cross-reactions are proteins belonging to the cardiovascular system, thus possibly directly accounting for cerebrovascular damage. Other possible targets are proteins of the immune system, thus suggesting mechanisms resulting in immune dysregulation which could lead to cerebrovascular damage.

An example of the first type of potential targets are ion-channels, particularly potassium (K\(^+\)) and sodium (Na\(^+\)) channels (ABCC9, KCNE2, KCNA5, KCNQ1, SCN4B, SCN5A, SCN1B, SCN3B, see Table 1). Accordingly, a growing body of evidence points to the involvement of cardiac K\(^+\) and Na\(^+\) channel dysfunction (cardiac channelopathies as a result of genetic mutations and/or inflammatory mechanisms) in the pathogenesis of atrial fibrillation (AF), an established risk factor for stroke (28, 29). Moreover, autoantibodies targeting ion-channels may be involved in cardiac arrhythmias (30). In light of the potential cross-reactivity suggested by the observed peptide sharing, AF and subsequent stroke could result from antibodies primarily targeting epitopes of infective agents but also cross-reacting with cardiac ion channels. For instance, activating antibodies could lead to a gain-of-function of K\(^+\)-channels and inhibiting antibodies to a loss-of-function of Na\(^+\)-channels. This could promote re-entry or increase susceptibility to early and/or delayed afterdepolarizations, two mechanisms that can generate AF (31, 32).

The second class of potential targets includes proteins that actively modulate the inflammatory response, such as cytokines and colony-stimulating factor receptors (IL-4, macrophage colony-stimulating factor receptor 1, see Table 1) (33, 34). IL-4, for example, is a well-investigated tolerogenic cytokine that is able to suppress inflammatory responses and organ-specific autoimmunity in both animal models and humans (35, 36). It is then conceivable that autoantibodies downregulating the function of these proteins can promote inflammatory responses, thus increasing the risk of cerebrovascular damage and stroke. Indeed, inflammatory responses appear to be crucial in the pathogenesis of stroke by inducing atherosclerosis progression, pro-thrombotic activation, and AF—among other mechanisms (37). Inflammation can therefore be considered as one key factor underpinning the relationship between classical stroke risk factors and comorbidities. It appears that not just single infections, but overall infectious burden from multiple agents predicts stroke incidence. Moreover, poor outcome may be proportional to systemic inflammatory burden both in patients and experimental models. For instance, Influenza and Streptococcus infection seem to contribute to stroke incidence and outcome, and evidence from experimental models indicate that blocking inflammatory processes might be an effective prevention strategy (38, 39).

The increasingly recognized relevance of inflammation in stroke is consistent with a possible role of peptide sharing-based cross-reactivity as contributing factors to cerebrovascular damage. In fact, the past two decades of immunologic research have radically changed the way we think of inflammation and innate immunity. It is now known that innate immune responses can “specifically” drive the following adaptive responses through recognition of pathogen-associated molecular patterns (PAMPs) (40, 41). That is to say that peptide epitopes, cell-wall components, and other PAMPs activate immune cells already from the very first stages of immune reactions and drive inflammation. Indeed, there are examples of cross-reactivity between host and pathogen-associated molecular patterns: identical inflamasomes and toll-like receptors (TLRs) recognizing molecular fingerprints of both pathogens (the PAMPs) and injured host cells (so-called danger-associated molecular patterns; DAMPs). For instance, both bacteria LPS and HMGB1 from injured host cells activate TLR4, with consequent inflammation in various tissues including the brain (41, 42). TLR- and inflamasome-dependent pathways seem to be important drivers of inflammation, vascular disease, and reportedly contribute to stroke outcome (43, 44).

Our preliminary results underline the importance of further experimental efforts to define the molecular basis through which microbial infections might contribute to an increased risk of stroke (45–50). Future studies should evaluate immunoreactivity against the peptides shared by infectious pathogens and human stroke-related proteins in sera from stroke patients. Possibly, such serological analyses could also help identify specific markers predicting a higher risk of stroke and might therefore be useful to design preventive strategies following an infection. The ultimate translational relevance of our finding lies in the possibility of adopting effective individualized primary and secondary preventive strategies in patients at risk for stroke after infections. Generic hygienic measure, as well as antibiotic prophylaxis and vaccination campaigns have already been proposed and tested with contrasting results (37). Identifying and stratifying patients according to individual biomarker profiles would allow to personalize treatment for each patient, thus possibly increasing overall efficacy.

Until now, stroke is a leading cause of preventable death and adult disability (1–5, 47–52), but preventive strategies mostly concentrate on traditional cardiovascular risk factors (53–56). Moreover, “cryptogenic” stroke (i.e., ischemic stroke with no obvious cause) poses a challenge in terms of primary and secondary prevention (57, 58).

Given the burden of cerebrovascular disease, and the potential to identify immunological markers that may then serve as prognostic indicators of risk of cerebrovascular damage after an infection, our results justify further intensive research on the cross-reactive link between infections and risk of stroke.
AUTHOR CONTRIBUTIONS

GL formulated the hypothesis, analyzed the data and wrote the manuscript. GL, AF, and BS interpreted the data, revised and finalized the manuscript.

FUNDING

We acknowledge support for the Article Processing Charge from the DFG (German Research Foundation, 393148499) and the Open Access Publication Fund of the University of Greifswald and from the Deutsche Forschungsgemeinschaft to AF (SFB1315 TP B03).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00469/full#supplementary-material
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Conflict of Interest Statement: AF received consulting fees from Bayer and Novartis and honoraria for oral presentations from Novartis, Boehringer-Ingelheim, Lilly, and Biogen Idec (unrelated to current research).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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