Glycerol-3-Phosphate Dehydrogenase 2 Is a Novel Factor H–, Factor H–like Protein 1–, and Plasminogen-Binding Surface Protein of Candida albicans

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Candida albicans uses human complement regulators such as factor H and factor H–like protein 1 (FHL-1) for immune evasion. To define the whole panel of fungal complement-evasion molecules, C. albicans cell extract was absorbed to a factor H–coupled matrix. One 52-kDa protein was eluted and identified by mass spectrometry as glycerol-3-phosphate dehydrogenase 2 (Gpd2). Consequently, Gpd2 was recombinantly expressed and purified. Recombinant Gpd2 binds factor H and FHL-1, mainly via short consensus repeat 7; and binds plasminogen, via lysine residues. The 3 human complement regulators, when attached to candida Gpd2, became functionally active, and the attached host proteins assist in inactivation of the complement cascade or cleave fibrinogen in the extracellular matrix component fibrinogen. Polyclonal Gpd2 antiserum was generated and localized Gpd2 at the surface of C. albicans. In addition, candida Gpd2 bound to human nonphagocytic cells but not to phagocytic U937 cells. Thus, candida Gpd2 is a novel fungal immune evasion protein that binds several human complement regulators and, in addition, binds human cells.

Keywords. C. albicans; complement evasion; human regulators; Gpd2.

Candida albicans is an opportunistic and medically important human pathogen. Infections with Candida can range from superficial to systemic disorders [1, 2]. As the occurrence of resistant C. albicans strains are continuously increasing [3, 4], new prophylactic and therapeutic strategies to treat fungal infections are urgently needed. The identification of novel targets in the form of yeast virulence factors that contribute to pathogenicity provides a relevant platform to define new strategies to fight Candida infections.

The complement system, which is activated by 3 major pathways, forms an immediately acting barrier against invading microbes [5, 6]. The alternative pathway is initiated spontaneously and constantly. The lectin pathway is activated on binding of mannose-binding lectin to mannan and carbohydrate structures on microbial surfaces. The classical pathway is activated via antigen-antibody complexes or by C-reactive protein [7]. Activation of all 3 pathways results in the generation of a C3 convertase that cleaves C3 into C3a and C3b [5, 8, 9]. The cleavage product, C3b, binds particle surfaces, where it acts as opsonin and mediates recognition and phagocytosis by host immune effector cells [10, 11]. C3a has both inflammatory and antimicrobial activity [12, 13].

On human cells, surface complement activation is blocked and controlled by different regulators. Factor H is the major fluid-phase complement regulator that
controls alternative pathway activation at the level of C3. The 150-kDa factor H protein is exclusively composed of 20 repetitive protein domains, termed short consensus repeats (SCRs) [14, 15]. Factor H-like protein 1 (FHL-1) is another alternative-pathway complement regulator and is encoded by an alternatively spliced transcript derived from the factor H gene [16]. Factor H and FHL-1 control complement activation by acting as cofactors for factor I, a serine protease that cleaves C3b into iC3b [16, 17]. Complement factor H–related protein 1 acts downstream of the complement cascade as a regulator of the C5 convertase and further blocks the assembly of terminal complement complex [18].

Pathogenic microbes need to inhibit the efficient and toxic host complement attack to survive and establish an infection. The evasion strategies they use to control complement action are multiple in nature. Currently rather general principles of immune evasion strategies common to, pathogens derived from different phyla are emerging. Human pathogenic fungi such as Candida albicans and Aspergillus fumigatus acquire the human complement regulators factor H, FHL-1, and C4BP from human plasma and bind these factors to their surface [19–23]. Bound to the surface of the pathogen, these human complement regulators maintain their inhibitory functions and block complement activation. Thus, acquisition and localization of human complement regulators at the fungal cell surface allows direct complement inhibition and prevents effective complement-mediated immune attack [21, 24]. Three factor H–binding proteins have been identified from C. albicans: phosphoglycerate mutase 1/complement regulator–acquiring surface protein 1 (Gpm1/CRASP1), pH-regulated antigen 1/CRASP2 (Pra1/CRASP2), and high-affinity glutathione transporter 1 protein (Hgt1p) [25–27]. Gpm1/CRASP1 and Pra1/CRASP2 both bind factor H, FHL-1, and plasminogen. Pra1/CRASP2 binds additional human complement-effector proteins, including C4BP and C3 [11, 23, 25, 27]. In addition to pathogenic fungi, gram-positive bacteria [28, 29], gram-negative bacteria [30, 31], parasites [20], and viruses [32, 33] acquire human complement regulators and use the surface-bound regulators for complement and immune evasion.

Given that several pathogens express and use multiple host complement-regulator-binding proteins to avoid complement-mediated attack, we aimed to identify additional factor H–binding Candida proteins and here identify the glycerol 3-phosphate-dehydrogenase 2 (Gpd2) as a novel factor H–binding protein in C. albicans.

METHODS

C. albicans Strains and Growth Conditions

The C. albicans wild-type strain SC5314 [34] was cultivated in yeast extract peptone dextrose medium at 30°C. Hyphal growth was induced in Roswell Park Memorial Institute 1640 liquid medium (BioWhittaker, Lonza) at 37°C for 1.5 hours. Yeast cells were collected by centrifugation and counted with a hemocytometer (Fein-Optik, Bad Blankenburg, Germany).

Identification of Factor H–Binding Protein

C. albicans cell extracts were prepared as described [35]. Briefly, C. albicans yeast cells (1 × 10^10 cells/mL) were mixed with glass beads, cooled at −80°C for 60 seconds, and disrupted by a Micro Smash beads cell disrupter for 120 seconds at 2300 g. The samples were then cooled at −80°C for 5 minutes, and the disruption cycle was repeated, for a total of 10 cycles. C. albicans cell extracts were loaded to a factor H–coupled HiTrap NHS-activated HP column (GE Healthcare). After washing, the bound protein was eluted with elution buffer (containing 0.1 mol/mL glycine and 0.5 mol/mL NaCl, pH 2.7) and further identified by mass spectrometry. One factor H–binding protein identified was Gpd2.

Expression and Purification of Recombinant Proteins

The C. albicans GPD2 gene was amplified by polymerase chain reaction (PCR), using genomic DNA from strain SC5314 and primers SI (5′-CGGGTACCAATGACATCCTTCGCATATCCAA-3′) and S2 (5′-TTTCTAGAGGTTCAGTACCTTCTCAATAATTGT-3′); KpnI and XbaI restriction sites are underlined. The resulting DNA fragment contained the complete CaGPD2 coding region, which was flanked by KpnI and XbaI restriction sites. The PCR product was subcloned into Escherichia coli vector pCR4Blunt-TOPO (Invitrogen), amplified, isolated, and subsequently cloned into the KpnI and XbaI sites of the Pichia pastoris vector pPICZαB (Invitrogen). Gpd2 was recombinantly expressed as a His-tagged protein in P. pastoris strain X33. FHL-1 (SCRs 1–7) and recombinant deletion constructs of factor H were expressed and purified as described elsewhere [27, 36].

Binding Assays

Gpd2, bovine serum albumin, or gelatin (0.5 μg/well in 50 μL carbonate-bicarbonate buffer) were immobilized onto a microtiter plate (MaxiSorb, Nunc) at 4°C overnight. After blocking with 0.2% gelatin (Merck), factor H, various factor H deletion mutants, plasminogen (Chromogenix), or buffer was added (1 μg/well) and incubated for 1.5 hours at room temperature. Bound protein was detected by specific antiseraum, followed by a horseradish peroxidase–conjugated secondary antiseraum (Dako). Reaction development was assayed by adding o-phenylenediamine dihydrochloride (Sigma) or tetramethylbenzidine (Sigma) and stopped by 2M sulfuric acid. The final color was measured at an optical density of 492 nm or 450 nm, respectively. Ligand affinity blotting was performed as described elsewhere to detect the interaction between factor H and Gpd2 [37].

Function Assays

Cofactor activity of the complement regulators factor H and FHL-1 bound to Gpd2 was assayed as described elsewhere.
Flow Cytometry and Confocal Microscopy

C. albicans yeast was incubated with polyclonal anti-Gpd2 preimmune serum (1:200) or buffer for 30 minutes on ice, followed by incubation with Alexa Fluor 488–labeled goat anti-rabbit serum (Molecular Probes; 1:200) for 30 minutes on ice. After washing, the cell pellet was analyzed by flow cytometry (LSRII, BD Bioscience). For confocal microscopy, C. albicans yeast and hyphae were incubated with polyclonal rabbit anti-Gpd2 serum (1:400) or buffer for 20 minutes on ice, after which Alexa Fluor 488–labeled goat anti-rabbit serum (1:200) and calcofluor white (10 µg/mL) were added. Samples were examined by confocal microscopy (LSM 510, Zeiss, Jena). To determine whether recombinant Gpd2 binds back to the surface of C. albicans, yeast cells or hyphae were incubated with Gpd2 (10 µg/10⁷) or buffer (negative control) at 37°C for 1 hour. Bound Gpd2 was detected by a Penta-His antibody (1:200) and visualized by microscopy.

To analyze binding of Gpd2 to human cells, Gpd2 was incubated with HUVEC, HaCaT, and U937 cells for 30 minutes at 37°C. Bound Gpd2 was detected by a Penta-His antibody (1:200) and visualized by microscopy.

To confirm factor H binding, the fungal Gpd2 was cloned and recombinantly expressed. The corresponding His-tagged protein was purified from the culture medium by nickel affinity chromatography. Yeast culture supernatant, flow through, wash, and eluate fractions were collected, separated by SDS-PAGE, and analyzed either by silver staining or by Western blotting. Recombinant Gpd2 was identified in the eluate fraction as 4 bands with mobilities of 54, 52, 45, and 43 kDa. The 54/52 doublet identified by silver staining most likely represented 2 differently glycosylated forms of the intact recombinant protein. The doublet of 45 kDa and 43 kDa likely represented degradation fragments that included the C-terminal His tag (Supplementary Figure 1A). Western blotting analyses with a mouse Penta-His antibody identified the upper doublet of 54/52 in the culture supernatant and all 4 bands in the eluate fraction (Figure 1B).

Localization of Gpd2 at the C. albicans Surface

Gpd2 localization at the surface of intact C. albicans cells was analyzed by flow cytometry, using Gpd2 antiserum. The specificity of anti-Gpd2 serum was proved using the cell wall and cytoplasmic fractions of C. albicans (Supplementary Figure 1). Gpd2 (green) was detected at the surface of intact yeast cells (Figure 2A). In addition, Gpd2 was detected at the surface of both yeast cells and C. albicans hyphae by confocal microscopy, which was colocalized with calcofluor white (blue) for cell wall staining (Figure 2B).

The fungal factor H–binding protein CRASP 2/Pra1 is both a surface and secreted protein. CRASP 2/Pra1 binds back to the surface of hyphae [27]. We therefore asked whether Gpd2, gel electrophoresis (SDS-PAGE), and a 52 kDa band was identified by mass spectrometry as Candida Gpd2.
similar to CRASP 2/Pra1, binds to the fungal surface. To this end, Gpd2 was incubated with immobilized Candida yeast and hyphae, and bound Gpd2 was determined with a Penta-His antibody that identifies specifically the recombinant but not the native Gpd2. Recombinant Gpd2 bound to the surface of hyphae but not to yeast cells (Figure 2C). Gpd2 binding was dose dependent up to 0.5 µg and was saturated at higher concentrations (Figure 2C). Binding of Gpd2 to C. albicans was confirmed by microscopy. Recombinant Gpd2 bound to Candida hyphae (Figure 2D) but not to yeast cells (data not shown). Binding was prominent at the tip of some hyphae and occurred in a patterned manner. Thus, Gpd2 was found to be expressed at the surface of both Candida yeast cells and hyphae. Recombinant Gpd2 binds to the surface of Candida hyphae.

Recombinant Gpd2 Binds Factor H and FHL-1

To confirm that candida Gpd2 is a factor H–binding protein, binding of different amounts of factor H or FHL-1 to immobilized Gpd2 was analyzed by enzyme-linked immunosorbent assay (ELISA). Gpd2 bound both regulators in a dose-dependent manner (Figure 3A and 3B). This interaction was confirmed in a reverse setting by assaying Gpd2 binding to immobilized factor H and FHL-1 (data not shown). Binding was further confirmed by ligand blotting. Factor H was separated by SDS-PAGE and blotted onto a membrane, and then the membrane was incubated with recombinant Gpd2. Bound Gpd2 was detected with a Penta-His antibody (Figure 3C).

To characterize effect of ionic strength on the Gpd2–factor H interaction, we measured the influence of NaCl on the
binding of factor H to immobilized Gpd2. At the physiological level of 150 mM, NaCl reduced factor H–Gpd2 interaction by approximately 50%, but binding was still present. However, at the highest concentration (600 mM), NaCl blocked factor H binding to Gpd2 (Figure 3D). Thus, the Gpd2–factor H interaction is ionic and is affected by NaCl.

To localize the domains of factor H and FHL-1 that contact candida Gpd2, binding of factor H/FHL-1 deletion mutants to immobilized Gpd2 was analyzed. FHL-1/SCRs 1–7 bound to Gpd2 and constructs SCRs 1–6, SCRs 1–5, and SCRs 1–4 bound with weaker intensity or did not bind (Figure 3E). Factor H deletion mutants that include the middle region (ie, SCRs 8–11 and SCRs 11–15) and also the C-terminal domains (SCRs 19–20) did not bind to Gpd2 (Figure 3E). Thus, both factor H and FHL-1 have 1 binding region that contacts candida Gpd2, and this region is located primarily in SCR 7 (Figure 3F).

**Factor H and FHL-1 Bound to Candida Gpd2 Display Complement Regulatory Activity**

To define whether the 2 complement regulators bound to Gpd2 maintain their regulatory function, factor H and FHL-1 were attached to immobilized Gpd2, and C3b and factor I were added. After incubation, degradation of C3b was evaluated. Both factor H and FHL-1, when bound to candida Gpd2, displayed cofactor activity, as revealed by the appearance of C3b cleavage fragments α′68, α′46, and α′43 (Figure 4). When BSA was used or when factor I was absent, C3b remained intact, and no cleavage occurred (Figure 4). Thus,
factor H and FHL-1 maintained complement regulatory activity when bound to Candida Gpd2.

**Recombinant Gpd2 Binds Plasminogen**

As several microbial factor H–binding proteins also bind plasminogen [25, 30, 39], we asked whether Candida Gpd2 also binds plasminogen. To this end, binding of plasminogen to Gpd2 was tested. Plasminogen bound to immobilized Gpd2, and binding was dose dependent (Figure 5A). This interaction was confirmed with dot blotting (data not shown). Also, plasminogen derived from human serum bound to immobilized Gpd2, as shown by ELISA (Figure 5B) and slot blotting (data not shown).

To analyze whether lysine residues are relevant to the Gpd2-plasminogen interaction, we tested whether the lysine analogue aminocaproic acid (ɛACA) affects Gpd2-plasminogen interaction. ɛACA inhibited the interaction in a dose-dependent manner. ɛACA at a concentration of 0.2 mM inhibited binding by 60% and at a concentration of 1 mM blocked the binding completely (Figure 5A). In addition, the effect of ionic strength on the plasminogen–Gpd2 interaction was tested. NaCl, used at a physiological level (150 mM) and at 600 mM, reduced plasminogen binding by 25% and by 39%, respectively (Figure 5D). Thus, Gpd2 binds plasminogen, and the Gpd2-plasminogen interaction is dependent on lysine residues and is affected by ionic strength.

**Plasminogen Bound to Gpd2 Is Functionally Active**

To assay whether plasminogen bound to Gpd2 is accessible for activators and can be converted to active plasmin, plasminogen was bound to immobilized Gpd2 and treated with the activator urokinase plasminogen activator, and the proteolytic activity of plasmin was assayed. In this setting, plasmin was generated and cleaved the synthetic chromogenic substrate in a time- and dose-dependent manner (Figure 6A). In addition, bound Gpd2 and activated plasmin also cleaved the physiological substrate fibrinogen. Fibrinogen degradation was time dependent, and after 4 hours fibrinogen was completely degraded (Figure 6B). Thus, plasminogen bound to Gpd2 was accessible by the activator urokinase plasminogen activator, and proteolytically active plasmin was generated that cleaved both synthetic and native substrates.

**Gpd2 Binds Human Endothelial and Epithelial Cells**

Candida Gpd2 is expressed at the surface of yeast cells and hyphae. To assay whether this fungal virulence protein binds to human cells, Gpd2 was incubated with HUVEC, HaCaT, and U937 cells, and bound Gpd2 was detected by flow cytometry. Gpd2 bound both HUVEC and HaCaT cells (Figure 7A and B) but not U937 cells (Figure 7C).

Gpd2 binding to HUVEC and HaCaT was further confirmed by confocal microscopy. Gpd2 bound to both HUVEC and HaCaT cell surfaces, as revealed by green fluorescence (Figure 7D...
Figure 5. Candida glycerol-3-phosphate dehydrogenase 2 (Gpd2) binds plasminogen. A, Binding of purified plasminogen to Gpd2 was analyzed by enzyme-linked immunosorbent assay. Gpd2 were immobilized at the indicated amounts, purified plasminogen was added, and plasminogen binding was assayed with a polyclonal goat plasminogen antiserum. Purified plasminogen bound to Gpd2, and binding was dose dependent. B, Plasminogen derived from normal human serum bound to Gpd2. Gpd2 was immobilized, human serum was added, and, after washing, bound plasminogen was identified with a polyclonal goat plasminogen antiserum. C, The lysine analog aminocaproic acid (εACA) affects plasminogen-Gpd2 interaction. Plasminogen was incubated with immobilized Gpd2 in the presence of εACA. Bound plasminogen was detected by a polyclonal goat plasminogen antiserum. εACA inhibited plasminogen binding to Gpd2, and the effect was dose dependent. D, NaCl affects the plasminogen-Gpd2 interaction. The effect of NaCl, used at increasing concentrations, on plasminogen binding to immobilized Gpd2 was assayed. In the absence of NaCl, plasminogen bound strongly to immobilized Gpd2, and in the presence of NaCl binding was slightly decreased. Data represent mean values ± SD of 3 independent experiments.

Figure 6. Plasminogen bound to glycerol-3-phosphate dehydrogenase 2 (Gpd2) is functionally active. A, Plasminogen was bound to Gpd2 which was immobilized at the indicated amounts. After extensive washing, the activator urokinase plasminogen activator (uPA) together with the chromogenic substrate S-2251 was added, and at the indicated time points the absorbance was measured. Proteolytically active plasmin was generated. The S-2251 degradation was both dose and time dependent. B, Degradation of fibrinogen. Plasminogen was bound to immobilized Gpd2. After extensive washing, the activator uPA and the substrate fibrinogen were added. At 0, 1, 2, and 4 hours (lanes 4–7), the reaction was stopped by adding reducing buffer. Then samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and degradation of fibrinogen was analyzed by Western blotting, using polyclonal rabbit fibrinogen antiserum. Fibrinogen degradation was time dependent (lanes 4–7). After 4 hours, fibrinogen was completely degraded. In comparison, after 4 hours of incubation, wells immobilized with BbCRASP-1 also showed completed degradation of fibrinogen (lane 2) but not bovine serum albumin (line 3). Equal amount of fibrinogen was loaded in line 1. One representative of 3 independent experiments is shown.
and E). In addition, bound Gpd2 was also detected in the extracellular space (Figure 7D), suggesting that Gpd2 also bound to extracellular matrix components that are released by human endothelial cells.

DISCUSSION

Here, we identified Gpd2 as a novel immune evasion protein of the human pathogenic fungus C. albicans. C. albicans Gpd was initially identified as a nicotinamide adenine dinucleotide-dependent enzyme that functions in glycerol accumulation. Two isoforms, Gpd1 and Gpd2, exist and are encoded by the homologous GPD1 and GPD2 genes, respectively [40–42]. Gpd1, which consists of 403 amino acids, has a predicted molecular mass of 44 kDa, and Gpd2, which contains 371 amino acids, has a predicted molecular mass of 41 kDa.

We are the first to show that surface-attached Gpd2 functions as a virulence factor that mediates complement evasion by C. albicans, which is different from the metabolic function of Gpd2 in the cytoplasm. Thus, Gpd2, in addition to Tsa1p, Gpm1p, and GAPDH, is a novel moonlighting protein for C. albicans. Candida Tsa1p is implicated both in oxidative stress response and in cell wall biogenesis [43]. Gpm1p acts during glycolysis and the reverse reaction in gluconeogenesis in the cytoplasm and also mediates the immune evasion process at the cell surface [25]. Candida GAPDH functions both at the cell wall surface for fibronectin and laminin binding and also in the cytoplasm as a glycolytic enzyme [44]. However, all of the 4 fungal moonlighting proteins lack a classic signal peptide and are exported from the cells in the absence of a functional endoplasmic reticulum/Golgi system. Therefore, nonconventional transport mechanisms should be considered as directing the exportation of those proteins to the cell wall [45].

Candida Gpd2 is a novel fungal factor H–, FHL-1–, and plasminogen-binding protein. When attached to Gpd2, each of the 3 human regulators of complement activation are functionally active. Thus, during the infection process, C. albicans uses surface Gpd2 to acquire the human complement regulators factor H and FHL-1 on the fungal surface. The attached factor H and FHL-1 display complement regulatory activity,
thereby inhibiting complement activation and generation of toxic and inflammatory complement-activation products. In addition, C. albicans acquires plasminogen at the surface via Gpd2. Surface-bound plasminogen is converted to the active serine protease plasmin and degrades both the synthetic chromogenic and the native substrate, thereby contributing to complement inhibition and degradation of extracellular matrices for tissue invasion.

Factor H and FHL-1 contact Gpd2 mainly with SCR7, which contains the polymorphic residue 402. The human polymorphisms in SCR7 (Y402H) of factor H/FHL-1 might affect the Gpd2-factor H/FHL-1 interaction. Other groups have shown that the polymorphism Y402H markedly reduces factor H binding to malondialdehyde, a common lipid peroxidation product that accumulates in many pathophysiological processes [46]. Y402H polymorphism also causes reduction factor H/FHL-1 binding to C-reactive protein and streptococcal M protein [47]. In addition, the Y402H polymorphism affects factor H surface recruitment by monomeric C-reactive protein to specific patches on the surface of necrotic retinal pigment epithelial cells [48].

Binding of factor H to Gpd2 is based on ionic interaction and is affected by NaCl but not by the lysine analogue (data not shown). However, Gpd2-plasminogen interaction is strongly inhibited by lysine analogue, indicating that Gpd2 binds plasminogen mainly via lysine residues. In addition, the factor H–Gpd2 interaction is strongly affected by ionic strength, but the effect on the plasminogen-Gpd2 interaction is weakly affected by ionic strength. These binding properties indicate that factor H/FHL-1 and plasminogen can bind simultaneously to candida Gpd2 and that these proteins mainly bind to distinct sites within the candida Gpd2, although they also may share some contact sites. However, whether the sequence of amino acids within the Gpd2 protein mediates such binding has not been defined so far.

Gpd2 is a novel fungal factor H–binding surface protein identified in C. albicans that also binds plasminogen. Previously, Candida Gpm1/CRASP1 and Pral/CRASP2 were identified as factor H/FHL-1– and plasminogen-binding proteins. Recently, Hgt1p was identified as third factor H–binding protein. Seven additional Candida plasminogen-binding surface proteins have been identified by a proteome approach [25, 27, 49]. Thus, C. albicans uses an arsenal of surface proteins to acquire human complement regulators and plasminogen for complement evasion and tissue invasion. Knocking out 1 of these proteins from C. albicans is not expected to show a significant reduction in binding to these regulators.

Gpd2 binds to nonphagocytic cells, human endothelial cells, and keratinocytes but not to phagocytic U937 cells. Because Gpd2 also binds factor H, and factor H is attached on the surface of HUVEC and HaCaT cells, factor H might act as a bridge molecule for the Gpd2–host cell interaction. The specific binding to human nonphagocytic cells and the lack of Gpd2 secretion suggest a role for Gpd2 as a surface protein in fungal adhesion and direct contact between the fungus and human endothelial and epithelial cells. In contrast, no binding of surface-expressed Gpd2 to human effector cells can avoid the migration, recognition, and further killing of C. albicans by these immune effector cells.

In summary, candida Gpd2 is a moonlighting protein that acts as a novel virulence factor on the surface and as an enzyme in the cytoplasm. A detailed understanding of the multiple roles of Gpd2 will help define new strategies to interfere with and fight Candida infection.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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### References
