Our findings indicate that p.Arg494Gln-C1-INH allows protein synthesis, but impaired cytoplasmic secretion. The same condition seems to apply to the other SERPING1 structural variants that cause recessive forms of HAE. No homozygous p.Arg494Gln has ever been described in homozygosity. Hence, for regulatory reasons, the formation of the complex with C1-inhibitor in vivo is necessary for efficient activation. Activation of the lectin pathway of complement is initiated by mannose-binding lectin (MBL)-associated serine proteases 1 and 2 (MASP-1 and MASP-2). MASP-1 and MASP-2 circulate in the blood aszymogens in complex with pattern recognition molecules (PRMs), such as MBL, other collectins, and ficolins. The third serine protease of the lectin pathway (MASP-3), which is also complexed with PRMs, was shown to be the major physiological activator of pro-factor D (pro-FD) in the blood, linking the alternative and the lectin complement pathways. We have demonstrated earlier that only activated MASP-3 is capable of converting pro-FD to factor D (FDI), and indeed the major form of MASP-3 in the blood is the active form. The activation mechanism of MASP-3, however, remains unclear. In vitro MASP-3 can activate MASP-3, and C1-inhibitor is the major physiological regulator of MASP-3. We hypothesized that if MASP-1 is the physiological activator of MASP-3 then individuals with low C1-inhibitor levels would exhibit altered MASP-3 activation.

The activation state of endogenous MASP-3 was detected by Western blot, whereas in other experiments fluorescently labeled recombinant MASP-3 variants were used. We found that a significant portion of full-length, labeled MASP-3 became "activated" (cleaved) in hirudin-plasma in the matter of hours even when the inactive S664A variant was used. The activation was less efficient, but still occurred, when the N-terminally truncated catalytic fragment was used. On the other hand, we found that the ratio of active MASP-3 to C1-HAE patients was virtually identical with that in healthy individuals, namely 82 ± 3%, versus 81 ± 4%. This indicates that a protease other than MASP-1 is responsible for the activation of MASP-3. To confirm this assumption we monitored the cleavage of labeled MASP-3 in the presence or absence of a MASP-1-specific inhibitor. Again, no difference was observed. In conclusion, our results imply that a protease is present in the blood that converts MASP-3 to the active form. The activation is not autocatalytically induced.

**O17**

**Simultaneous determination of human plasma serine proteases**

Zsófia Janrusics*,1 Erika Kajdácsi1,2 Nőra Veszielő2,4 Vera Makó4, Anna Koncz1, Kinga Viktória Köhalmi1, Laszlo Cervenak1, Péter Gál1, József Dobó5, Steven de Maat1, Coen Maas1, Henriette Farkas3, Péter Gál1, József Dobó5, Steven de Maat1, Coen Maas1, Henriette Farkas3, Péter Gál1, József Dobó5, Steven de Maat1, Coen Maas1, Henriette Farkas3, Péter Gál1, József Dobó5.

1Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary; 2Hungarian Angioedema Reference Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary; 3Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary; 4Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary; 5Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

**Correspondence:** József Dobó (dobo.jozsef@tkk.mta.hu)

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**O16**

**Activation of complement MASP-3 in healthy donors and in patients with C1-inhibitor deficiency**

Gabor Orozczan1, Gabor Pal2, Peter Zavodsky1, Henriette Farkas3, Péter Gál1, József Dobó5,1

1Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary; 2Department of Biochemistry, Eotvos Lorand University, Budapest, Hungary; 3Hungarian Angioedema Reference Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

**Correspondence:** József Dobó (dobo.jozsef@tkk.mta.hu)

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**O15**

**A SERPING1 variant that causes C1-inhibitor deficiency without hereditary angioedema**

Silvia Berra1, Chiara Sufritti1, Andrea Zanchelli1,2, Debora Parolin1, Maddalena A. Wu, Marco Cavalleri3,4, Sonia Caccia4,5

1L. Sacco Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy; 2ASST Fatebenefratelli Sacco, Milan, Italy; 3Istituti Clinici Scientifici Maugeri IRCCS, Milan, Italy; 4Department of Internal Medicine, Semmelweis University, Budapest, Hungary; 5Department of Internal Medicine, Semmelweis University, Budapest, Hungary.

**Correspondence:** Sonia Caccia (sonia.caccia@unimi.it)

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More than 400 mutations (around 25% de novo) in SERPING1 have been described to cause functional C1-inhibitor (C1-INH) deficiency and hereditary angioedema (HAE). Angioedema symptoms and C1-INH deficiency co-segregate within the families with autosomal dominant inheritance. Two promoter and two structural SERPING1 variants escape this rule. These four mutations represent a recessive character. They cause the clinical phenotype of HAE only when present in homozygosity.

Here we describe variant g.22006G→A (p.Arg494Gln) in SERPING1, detected in 12 subjects from four families with C1-INH deficiency and no HAE. The variant is located in a region of the molecule that is critical for regulating SERPIN conformational state.

Index cases in each family were identified due to urticaria symptoms for regulating SERPIN conformational state. They cause the clinical phenotype of HAE only when present in homozygosity.