

Genetic analysis of Factor XII and bradykinin catabolic enzymes in a family with estrogen-dependent inherited angioedema

Qing Ling Duan, PhD,^{a,b} Karen Binkley, MD, FRCPC,^c and Guy A. Rouleau, MD, PhD, FRCPC, OQ^a Montreal, Quebec, and Toronto, Ontario, Canada

Background: Recent studies reported a gain-of-function mutation in the gene encoding coagulation Factor XII (*F12*) among 5 German and French families with estrogen-associated angioedema who share a common ancestor. The role of this factor, additional pathways that might contribute to increased bradykinin levels, or both remain to be determined in other families with estrogen-dependent or estrogen-associated inherited angioedema.

Objective: The purpose of this study was to determine whether mutations in *F12* and polymorphisms in the genes encoding aminopeptidase P (APP) and angiotensin I-converting enzyme (ACE), which have been associated with increased bradykinin levels, contribute to estrogen-dependent inherited angioedema in a large family of Italian origin.

Methods: We screened the coding regions of *F12* and the gene encoding membrane-bound APP (*XPNPEP2*), for genetic variants in the 3 affected female subjects. In addition, we genotyped this family for the insertion/deletion polymorphism in the *ACE* gene, which accounts for variable ACE levels.

Results: The 3 affected female subjects all have the threonine-to-lysine (Thr328Lys) mutation, which is associated with higher Factor XII activity. In addition, they have at least one A allele of rs3788853 at the *XPNPEP2* locus, which is associated with lower APP activity, and at least one I allele in *ACE*, which is associated with reduced ACE activity.

Conclusion: A missense mutation in *F12* is present in the 3 affected female subjects of this family with estrogen-dependent inherited angioedema. In addition, these affected females have polymorphisms associated with lower levels of both APP and ACE, the major enzymes responsible for bradykinin degradation. Thus, our study suggests that multiple genes

might contribute to estrogen-dependent or estrogen-associated inherited angioedema and explain some of the observed heterogeneity. (J Allergy Clin Immunol 115:1111-1116, 2005.)

Key words: Estrogen-dependent inherited angioedema, bradykinin, coagulation Factor XII, angiotensin I-converting enzyme, aminopeptidase P

Classic forms of hereditary angioedema (HAE; types I and II) are autosomal-dominant disorders resulting from decreased levels or function of the inhibitor for the first component of the complement pathway (C1-INH).^{1,2} These manifest as localized swelling of parts of the face, upper respiratory tract, gastrointestinal tract, genitalia, hands, and/or feet (Online Mendelian Inheritance of Man [OMIM] 106100; <http://www.ncbi.nlm.nih.gov/omim/>).³ Inadequate C1-INH fails to restrict the activity of coagulation Factor XII (Hageman factor) and kallikrein, leading to increased production of bradykinin, a potent vasodilator and an important mediator of angioedema.^{2,4} Additional forms of HAE and sporadic angioedema, which are clinically indistinguishable from classic HAE, have also been reported among individuals with normal C1-INH concentrations and function. These include cases of unknown cause (idiopathic angioedema),^{5,6} drug-induced angioedema (eg, angiotensin I-converting enzyme [ACE] inhibitors⁷ and plasminogen activators⁸) and angioedema that affects only women.^{9,10} The latter forms are known as estrogen-dependent (or estrogen-associated) inherited angioedema (EDIA; or HAE type III [OMIM 610618]) because they depend on or are worsened by increased levels of endogenous (eg, pregnancy) or exogenous estrogen (eg, oral contraceptives or hormone replacement therapy).

Recent studies identified a gain-of-function mutation in the gene encoding factor XII (*F12*) among female subjects with estrogen-associated angioedema originating from Germany and France who shared a common ancestor.^{11,12} This Thr328Lys substitution in exon 9 of *F12* was associated with increased Factor XII activity but not protein levels.¹² Earlier studies reported that an estrogen response element in the promoter region of the gene contributes to increased gene expression during increased estrogen states.^{13,14} Taken together, women with increased estrogen levels and a gain-of-function mutation in *F12* have increased levels of a highly active Factor XII. It is hypothesized that increased activity and expression of Factor XII contributes to higher bradykinin production (Fig 1), which increases vascular permeability and risk of angioedema.^{15,16} Thus, similar to classic forms of HAE, estrogen-associated inherited angioedema might result from increased levels of bradykinin.

From ^aCentre for Excellence in Neuromics, University of Montreal, and the Centre Hospitalier de l'Université de Montreal and Ste-Justine Hospital, Montreal; ^bthe Department of Human Genetics, McGill University, Montreal; and ^cthe Division of Clinical Immunology and Allergy, Department of Medicine, University of Toronto.

Supported by the Canadian Institute of Health Research (G.A.R.) and the Allergy, Asthma and Immunology Society of Ontario (K.B.). Q.L.D. was supported by the Heart and Stroke Foundation of Canada.

Disclosure of potential conflict of interest: K. Binkley has received research support from the Research Institute for Fragrance Materials. The rest of the authors have declared that they have no conflict of interest.

Received for publication May 30, 2008; revised December 5, 2008; accepted for publication December 11, 2008.

Reprint requests: Guy A. Rouleau, MD, PhD, J. A. de Séve Pavilion, Room Y-3633, 1560 Sherbrooke St E, Montreal, Quebec, Canada, H2L 4M1. E-mail: guy.rouleau@umontreal.ca.

0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2008.12.010

Abbreviations used

ACE:	Angiotensin I-converting enzyme
APP:	Aminopeptidase P
C1-INH:	Inhibitor of the first component of the complement pathway
des-Arg9-BK:	des-Arginine9-BK
EDIA:	Estrogen-dependent inherited angioedema
<i>F12</i> :	Gene encoding Factor XII
HAE:	Hereditary angioedema
I/D:	Insertion/deletion
UTR:	Untranslated region
<i>XPNEPEP2</i> :	Gene encoding aminopeptidase P

In this study we investigated the *F12* locus in a family of Italian origin with a previously well-characterized, strictly estrogen-dependent angioedema phenotype.¹⁰ Binkley and Davis¹⁰ had confirmed the absence of mutations in the coding region and 5' untranslated region (UTR) of the gene encoding C1-INH (*SERPING1*), as well as the 5'UTR of *F12* among the affected female subjects of this family. In addition, we extend our investigation to examine the potential contribution of bradykinin degradation pathways to the EDIA phenotype in this family. As shown in Fig 1, increased bradykinin levels might result from increased production (eg, higher Factor XII activity) or decreased degradation because of deficiencies of several catabolic enzymes. For example, ACE and aminopeptidase P (APP) are the primary and secondary enzymes responsible for the degradation of bradykinin,¹⁷ with additional enzymes representing minor pathways (Fig 1). Furthermore, ACE and APP also represent the major pathways for the degradation of the active metabolite of bradykinin known as des-Arginine9-BK (des-Arg9-BK).^{18,19} Genetic variants in the gene encoding APP (*XPNEPEP2*), resulting in reduced enzyme activity,²⁰ higher bradykinin and des-Arg9-BK levels,²¹ have been associated with angioedema induced by ACE inhibitors.²⁰ Also, this study investigates the potential role of an insertion/deletion (I/D) polymorphism in the *ACE* gene, which accounts for 50% of the variability in human serum ACE levels.²² Previous studies have demonstrated that the I allele is associated with lower expression of *ACE* mRNA²³ and decreased degradation of bradykinin.²⁴

METHODS

DNA was extracted from blood samples of 29 members of a family of Italian origin with multiple cases of EDIA, which was previously described by Binkley and Davis.¹⁰ Fig 2 shows a modified pedigree comparable with the earlier report, with several additional members who did not participate in the previous study (eg, spouses and children). Blood samples for the current investigation were collected in October 2007 from participants who now reside in Canada and represent a subset of the original pedigree. For example, only 3 affected female subjects (II:2, II:7, and II:8) were available for our study, whereas 3 of their affected relatives (II:4, III:4, and III:9) reside in Italy and were not available to participate in this study. The phenotypic details for this family can be found in the earlier report by Binkley and Davis.¹⁰ In brief, all affected female subjects of this family experienced angioedema symptoms (without urticaria) throughout all their pregnancies (with multiple episodes), as well as during estrogen replacement therapy, but not at any time in between. Their angioedema symptoms included swelling of the face and extremities, airway obstruction, and abdominal pain and vomiting, which are similar to HAE types I and II. All participants provided informed consent, and all protocols were approved by the Ethics Committees at the Centre Hospitalier de l'Université de Montreal and Sunnybrook Health Sciences Centre, Toronto, Canada.

The UTRs and exons of *F12* and *XPNEPEP2* were sequenced for 3 affected female subjects (II:2, II:7, and II:8) and 2 unrelated male control subjects (spouses of II:7 and II:8). These coding regions were amplified by using intronic primers in standard PCRs and sequenced at the Genome Québec Innovation Centre in Montreal, Canada. Sequence traces were viewed and analyzed with the program Seqman (DNASTAR package) to identify genetic variants. Furthermore, all family members ($n = 29$) were screened for exon 9 of *F12* and genotyped for rs3788853 in *XPNEPEP2*, as well as the *ACE* I/D polymorphism. Exon 9 of the *F12* gene was amplified by using the primers 5'-ACACCAAGGCAAGCTGCTAT-3' and 5'-GCTGGCCCGAATCTAGCTC-3', with an optimal annealing temperature of 55°C. The rs3788853 polymorphism located 5' of *XPNEPEP2* was genotyped by using allele-specific PCR with 1 common forward primer, 5'-AACCTCCCCACGTTGAATCA-3, and a choice of 2 allele-specific reverse primers, 5'-GCACTGCTGAAATAGCAGTTGTTAG-3' and 5'-GCACTGCTGAAATAGCAGTTGTTAT-3', in parallel PCRs with an annealing temperatures of 63°C, followed by gel electrophoresis with 1.5% agarose gel for genotype calling.²⁰ Duan et al²⁰ had previously determined the high efficacy and reproducibility of this allele-specific genotyping method through comparisons with sequencing results in more than 300 DNA samples. The I/D polymorphism in *ACE* was genotyped by using the primers 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGC CATCACATTCGTGAT-3', with an optimal annealing temperature of 58°C. Genotypes for this I/D polymorphism were determined by using agarose gel electrophoresis according to a standard PCR because the D allele was 287 bp shorter than the I allele (490 bp).

RESULTS

Fig 2 shows that all 3 affected female subjects with a history of EDIA (II:2, II:7, and II:8) are heterozygous for Thr328Lys (c. 1032 C>A), a gain-of-function mutation in exon 9 of the *F12* gene that has been previously described.^{11,12} In addition, this family has 5 male carriers (III:5, III:19, IV:3, IV:5, and IV:7) and 1 female heterozygote (IV:6) with undetermined phenotype. At age 20 years, this female carrier has not demonstrated symptoms of EDIA, which is in keeping with the fact she has never been pregnant or received exogenous estrogen (oral contraceptives or estrogen replacement).

In addition to the gain-of-function mutation in *F12*, all 3 affected female subjects have at least 1 copy of the A allele at the SNP rs3788853, located 5' of *XPNEPEP2*, which codes for membrane-bound APP. This allele has been associated with decreased APP activity, decreased bradykinin and des-Arg9-BK degradation, and angioedema induced by ACE inhibitors.^{20,21} Ten other blood relatives within this family also carry this polymorphism but are unaffected, including the female of undetermined phenotype (IV:6) with the Thr328Lys mutation in *F12*. Screening of the coding regions of *XPNEPEP2* yielded no mutations in the affected members of this family.

Finally, the 3 affected female subjects have at least 1 copy of the inserted allele in intron 16 of the *ACE* gene (Fig 2), which is associated with lower levels of ACE.^{22,23} Based on previous studies, one can infer that the individuals with the I/I or I/D genotypes have significantly lower ACE levels compared with individuals with the D/D genotype. Three male relatives of these affected female subjects also have the I/D genotype, and 8 relatives have the I/I genotype (5 male and 3 female subjects). The female carrier with undetermined phenotype (IV:6) with the *F12* mutation and the C/A genotype at rs3788853 in the *XPNEPEP2* locus also has the I/I genotype in *ACE*, which is associated with low levels of ACE.

DISCUSSION

In this report we identify a gain-of-function mutation in *F12* among 3 affected patients with EDIA in a previously

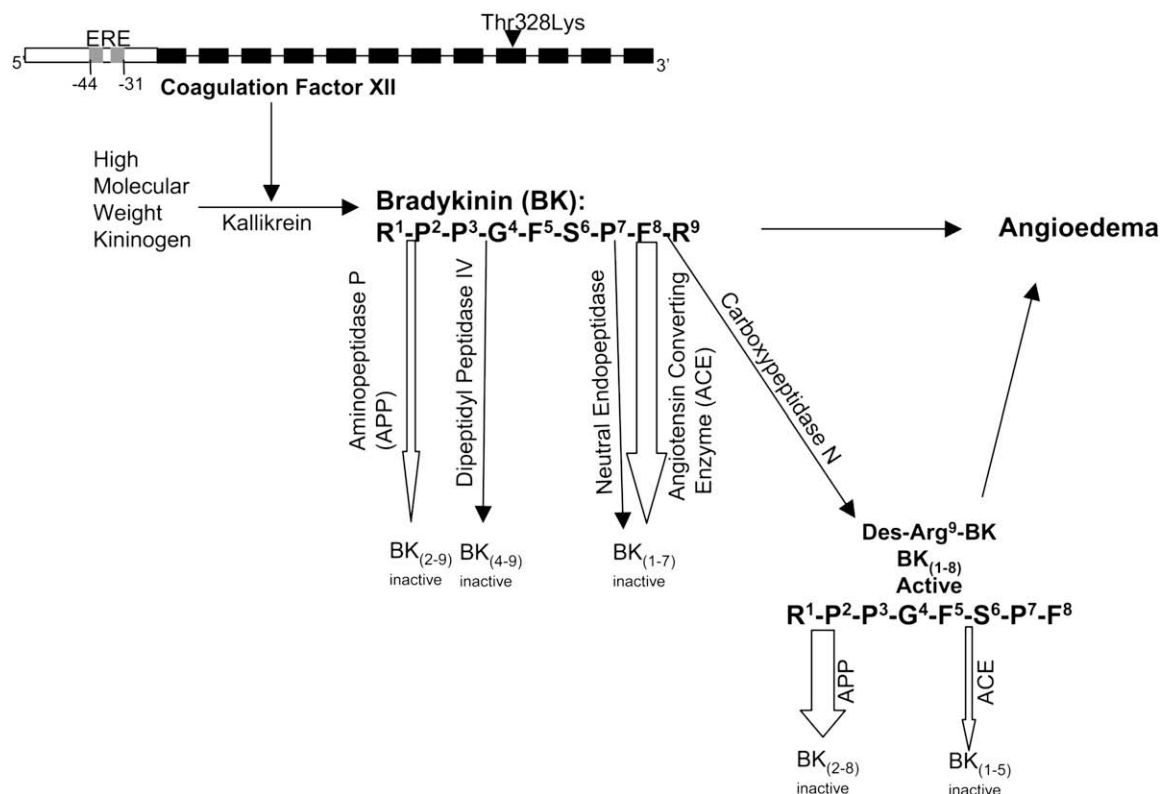


FIG 1. Proposed mechanisms for bradykinin production and degradation in EDIA. The Thr328Lys mutation in exon 9 of *F12* increases activity of the Factor XII protein. High estrogen levels acting through the estrogen response element (ERE) in the promoter region (located between nucleotide positions -44 to -31) increase expression of the overactive Factor XII, leading to increased bradykinin production from high-molecular-weight kininogen through activated kallikrein. This nonapeptide (R¹-P²-P³-G⁴-F⁵-S⁶-P⁷-F⁸-R⁹ [Arginine¹-Proline²-Proline³-Glycine⁴-Phenylalanine⁵-Serine⁶-Proline⁷-Phenylalanine⁸-Arginine⁹]) is an important mediator of angioedema but is normally short lived because it is cleaved by several enzymes. ACE and APP represent the primary and secondary degradation pathways of bradykinin, with reversed importance in the degradation of Des-Arg⁹-BK (R¹-P²-P³-G⁴-F⁵-S⁶-P⁷-F⁸), an active metabolite of bradykinin, which might also contribute to angioedema. Other known enzymes involved in the degradation of bradykinin include carboxy peptidase N, dipeptidyl peptidase IV, and neutral endopeptidase, resulting in inactive metabolites. The relative contribution of each degradation pathway is represented by the width of the arrows.

well-characterized family.¹⁰ High estrogen levels, acting through an estrogen response element in the promoter region of *F12*, increase expression of the mutant Factor XII with a gain of function, which is thought to contribute to increased bradykinin levels and angioedema. Although this mutation has been documented in families from Germany and France,^{11,12} we are the first to report this mutation in a family of Italian origin, demonstrating that *F12* determines the risk for EDIA in other European populations. It remains to be determined whether this Italian family shares a common ancestor with the German and French families previously described. Our study demonstrates that genetic screening of *F12* in additional families with EDIA is warranted, regardless of their country of origin. Additional studies are necessary to determine the role of this gene in determining EDIA risk in non-European populations.

This study is the first investigation to examine the role of bradykinin catabolic pathways in EDIA. ACE and APP represent the primary and secondary degradation pathways of bradykinin, respectively, with reversed importance in the degradation of des-Arg⁹-BK, an active metabolite of bradykinin (Fig 1). Reduced levels or activities of ACE, APP, or both would result in

reduced degradation and increased levels of bradykinin and des-Arg⁹-BK and therefore greater risk of angioedema.¹³ All 3 affected female subjects studied in this family have at least 1 copy of the A allele of rs3788853 at the *XPNPEP2* locus, which has been associated with low APP activity and increased risk for angioedema during ACE inhibitor therapy.²⁰ This minor allele (frequency of 27% in the white population [CEU] of the Hapmap project) is also present in 10 unaffected relatives and 2 unaffected individuals married into the family. This supports the previous hypothesis that individuals with reduced APP activity are asymptomatic unless a biologically relevant molecule, such as ACE, is also deficient or inhibited.²⁰ Genotyping of the ACE I/D polymorphism revealed that all 3 affected female subjects studied also had at least 1 copy of the I allele, which is associated with reduced ACE expression. This allele is also common in the general population, with reported genotype frequencies of 25.6% (I/I), 48.3% (I/D), and 26.1% (D/D).²⁵ This polymorphism has been associated with reduced gene expression²³ and higher bradykinin levels.²⁴

The effect of estrogen on the levels, activities, or both of bradykinin catabolic enzymes might have particular importance

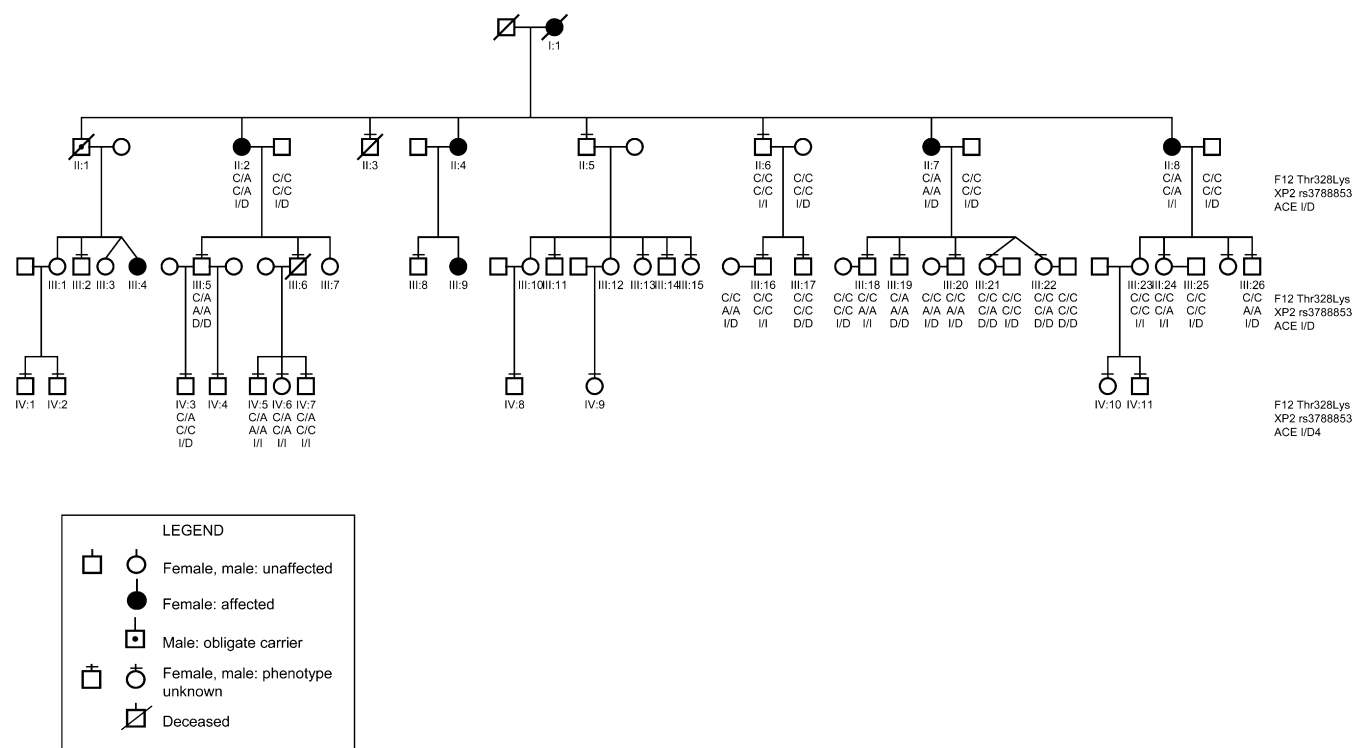


FIG 2. Segregation of the Thr328Lys mutation in *F12*, rs3788853 in *XPNPE2* (abbreviated *XP2*), and the *ACE* I/D polymorphism. Affected female subjects (solid circles) are heterozygous for Thr328Lys and carry at least 1 copy of the A allele of rs3788853 and at least 1 copy of the I allele in *ACE*. Individual IV:6 is a young female subject who is heterozygous for Thr328Lys and rs3788853 and homozygous for the I allele in *ACE*; however, her phenotype is unknown because she has not experienced high estrogen states. There are 5 male carriers of the Thr328Lys mutation, 11 individuals with at least 1 copy of the A allele of rs3788853, and 11 relatives with 1 or more copies of the I allele in *ACE*. Note that rs3788853 is located on the X chromosome, and thus male subjects are hemizygotes.

in the generation of the estrogen-dependent nature of the phenotype in this family. Previous studies have demonstrated that estrogen suppresses ACE expression²⁶ and increases bradykinin concentrations^{27,28} independent of the *ACE* I/D genotype.²⁹ Although the effect of estrogen on APP activity is unknown, a recent report suggests that androgens increase APP levels.³⁰ Because androgens and estrogens often have antagonistic effects, it is not unreasonable to speculate that estrogen might reduce APP activity. In healthy individuals high estrogen states would facilitate high bradykinin levels through both increased production (increased Factor XII level) and reduced degradation (reduced ACE and possibly APP levels). In affected members of this family, the normal effects of estrogen on bradykinin levels would be accentuated by the presence of the mutant Factor XII with a gain of function and the polymorphisms associated with pre-existing low activities of ACE and APP.

Although specific measurements for Factor XII, APP, and ACE levels were not performed, this study suggests that genetic variants in multiple loci can contribute to angioedema, possibly through their effects on bradykinin levels. The presence or absence of genetic variants at these additional loci might contribute to the observed heterogeneity of clinical phenotypes (ie, estrogen-dependent vs estrogen-associated angioedema)³¹ and even heterogeneity within the classic forms of HAE. Further studies of bradykinin catabolic enzymes in other families with EDIA would help delineate these issues.

This report also demonstrates the application of predictive testing to identify presymptomatic carriers of risk alleles, which will aid in the management of EDIA. Because the affected

members of this family have reported multiple episodes of near-fatal laryngeal edema, the prevention of clinical symptoms in at-risk individuals might be life-saving. For example, a female subject of undetermined phenotype (IV:6) carries 1 risk allele of the Thr328Lys mutation in *F12*, as well as alleles associated with lower APP and ACE levels. This genetic information will allow this individual to avoid symptoms by selecting contraceptive strategies other than estrogen-containing agents, careful observation during pregnancy, and, later in life, alternative strategies to estrogen-containing hormone replacement regimens to manage menopausal symptoms. In addition, we have also identified several male family members who carry the mutation but have never been affected because of the strict estrogen dependence of the phenotype in this family. Identification of such male carriers will allow targeted screening of their offspring so that symptoms can be avoided in subsequent generations.

We thank the family for their participation in this study, Ms Olga Likhodi for sample collection, and Dr Patrick Dion for careful review of this manuscript.

Clinical implications: Increased bradykinin production and decreased bradykinin catabolism might contribute to EDIA.

REFERENCES

1. Cyr M, Eastlund T, Blais C Jr, Rouleau JL, Adam A. Bradykinin metabolism and hypotensive transfusion reactions. *Transfusion* 2001;41:136-50.
2. Davis AE 3rd. The pathogenesis of hereditary angioedema. *Transfus Apheresis Sci* 2003;29:195-203.

3. Agostoni A, Aygoren-Pursun E, Binkley KE, Blanch A, Bork K, Bouillet L, et al. Hereditary and acquired angioedema: problems and progress: proceedings of the third C1 esterase inhibitor deficiency workshop and beyond. *J Allergy Clin Immunol* 2004;114(suppl):S51-131.
4. Davis AE 3rd. C1 inhibitor and hereditary angioneurotic edema. *Ann Rev Immunol* 1988;6:595-628.
5. Champion RH, Roberts SO, Carpenter RG, Roger JH. Urticaria and angio-oedema. A review of 554 patients. *Br J Dermatol* 1969;81:588-97.
6. Cicardi M, Bergamaschini L, Zingale LC, Gioffre D, Agostoni A. Idiopathic non-histaminergic angioedema. *Am J Med* 1999;106:650-4.
7. Vleeming W, van Amsterdam JG, Stricker BH, de Wildt DJ. ACE inhibitor-induced angioedema. Incidence, prevention and management. *Drug Saf* 1998;18:171-88.
8. Rafii MS, Koenig M, Ziai WC. Orolingual angioedema associated with ACE inhibitor use after rtPA treatment of acute stroke. *Neurology* 2005;65:1906.
9. Bork K, Barnstedt SE, Koch P, Traupe H. Hereditary angioedema with normal C1-inhibitor activity in women. *Lancet* 2000;356:213-7.
10. Binkley KE, Davis A 3rd. Clinical, biochemical, and genetic characterization of a novel estrogen-dependent inherited form of angioedema. *J Allergy Clin Immunol* 2000;106:546-50.
11. Dewald G, Bork K. Missense mutations in the coagulation factor XII (Hageman factor) gene in hereditary angioedema with normal C1 inhibitor. *Biochem Biophys Res Commun* 2006;343:1286-9.
12. Cichon S, Martin L, Hennies HC, Muller F, Van Driessche K, Karpushova A, et al. Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am J Hum Genet* 2006;79:1098-104.
13. Klein-Hitpass L, Tsai SY, Greene GL, Clark JH, Tsai MJ, O'Malley BW. Specific binding of estrogen receptor to the estrogen response element. *Mol Cell Biol* 1989;9:43-9.
14. Farsetti A, Misiti S, Citarella F, Felici A, Andreoli M, Fantoni A, et al. Molecular basis of estrogen regulation of Hageman factor XII gene expression. *Endocrinology* 1995;136:5076-83.
15. Proud D, Kaplan AP. Kinin formation: mechanisms and role in inflammatory disorders. *Annu Rev Immunol* 1988;6:49-83.
16. Nussberger J, Cugno M, Cicardi M. Bradykinin-mediated angioedema. *N Engl J Med* 2002;347:621-2.
17. Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* 1992;44:1-80.
18. Blais C Jr, Rouleau JL, Brown NJ, Lepage Y, Spence D, Munoz C, et al. Serum metabolism of bradykinin and des-Arg9-bradykinin in patients with angiotensin-converting enzyme inhibitor-associated angioedema. *Immunopharmacology* 1999;43:293-302.
19. Blais C Jr, Marc-Aurele J, Simmons WH, Loute G, Thibault P, Skidgel RA, et al. Des-Arg9-bradykinin metabolism in patients who presented hypersensitivity reactions during hemodialysis: role of serum ACE and aminopeptidase P. *Peptides* 1999;20:421-30.
20. Duan QL, Nikpoor B, Dube MP, Molinaro G, Meijer IA, Dion P, et al. A variant in XPNPEP2 is associated with angioedema induced by angiotensin I-converting enzyme inhibitors. *Am J Hum Genet* 2005;77:617-26.
21. Molinaro G, Duan QL, Chagnon M, Moreau ME, Simon P, Clavel P, et al. Kinin-dependent hypersensitivity reactions in hemodialysis: metabolic and genetic factors. *Kidney Int* 2006;70:1823-31.
22. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86:1343-6.
23. Suehiro T, Morita T, Inoue M, Kumon Y, Ikeda Y, Hashimoto K. Increased amount of the angiotensin-converting enzyme (ACE) mRNA originating from the ACE allele with deletion. *Hum Genet* 2004;115:91-6.
24. Murphey LJ, Hachey DL, Oates JA, Morrow JD, Brown NJ. Metabolism of bradykinin in vivo in humans: identification of BK1-5 as a stable plasma peptide metabolite. *J Pharmacol Exp Ther* 2000;294:263-9.
25. Scheer WD, Boudreau DA, Hixson JE, McGill HC, Newman WP 3rd, Tracy RE, et al. ACE insert/delete polymorphism and atherosclerosis. *Atherosclerosis* 2005;178:241-7.
26. Stevenson JC, Oladipo A, Manassiev N, Whitehead MI, Guilford S, Proudler AJ. Randomized trial of effect of transdermal continuous combined hormone replacement therapy on cardiovascular risk markers. *B J Haematol* 2004;124:802-8.
27. Sumino H, Ichikawa S, Kanda T, Sakamaki T, Nakamura T, Sato K, et al. Hormone replacement therapy in postmenopausal women with essential hypertension increases circulating plasma levels of bradykinin. *Am J Hypertens* 1999;12:1044-7.
28. Nogawa N, Sumino H, Ichikawa S, Kumakura H, Takayama Y, Nakamura T, et al. Effect of long-term hormone replacement therapy on angiotensin-converting enzyme activity and bradykinin in postmenopausal women with essential hypertension and normotensive postmenopausal women. *Menopause* 2001;8:210-5.
29. Sumino H, Ichikawa S, Ohyama Y, Nakamura T, Kanda T, Sakamoto H, et al. Effects of hormone replacement therapy on serum angiotensin-converting enzyme activity and plasma bradykinin in postmenopausal women according to angiotensin-converting enzyme-genotype. *Hypertens Res* 2003;26:53-8.
30. Drouet C, Desormeaux A, Robillard J, Ponard D, Bouillet L, Martin L, et al. Metalloproteinase activities in hereditary angioedema: effect of androgen prophylaxis on plasma aminopeptidase P. *J Allergy Clin Immunol* 2008;121:429-33.
31. Binkley KE, Davis AE 3rd. Estrogen-dependent inherited angioedema. *Transfus Apheresis Sci* 2003;29:215-9.