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ORIGINAL ARTICLE

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DNA polymorphism and mutations in *CPN1*, including the genomic basis of carboxypeptidase N deficiency

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Abstract Carboxypeptidase N (EC 3.4.17.3) regulates the activity of peptides such as kinins and anaphylatoxins. Although deficiency of carboxypeptidase N (MIM 212070) produces a severe allergic syndrome, no human mutations have ever been described. Therefore, using archival genomic DNA from a subject with documented carboxypeptidase N deficiency, we sequenced *CPN1* (MIM 603103), which encodes the catalytic subunit of carboxypeptidase N. In the genomic DNA of the proband, we discovered three *CPN1* variants: (1) 385fsInsG, a frameshift mutation in exon 1 due to a single G insertion at nucleotide 385; (2) 746G>A single-nucleotide polymorphism (SNP), a missense mutation in exon 3 that predicted substitution of aspartic acid for the wild-type conserved glycine at amino acid 178 (G178D); and (3) IVS1 +6C>T, an SNP in intron 1. Among 128 normal Caucasians, the 385fsInsG mutation was absent and the G178D mutation had a frequency of 0.0078, suggesting that these were rare molecular events that likely contributed to the carboxypeptidase N deficiency phenotype. The frequency of the IVS1 +6C>T polymorphism was 0.051. The reagents described here provide tools for further study of association with clinical and biochemical phenotypes related to allergy and immunity.

Key words Nucleotides · Allergy · Genomic DNA · Sequencing · Monogenic disease

Introduction

Carboxypeptidase N (also called arginine carboxypeptidase, EC 3.4.17.3) is a member of the B-type carboxypeptidase group and cleaves basic amino acid residues from the carboxyl terminus of many proteins (Riley et al. 1998). As a serum alphaglobulin metalloenzyme, it inactivates complement components C3a, C4a, and C5a, in addition to bradykinin, kallidin, and fibrinopeptides. Its central role in regulating the biologic activity of peptides such as kinins and anaphylatoxins led to such alternate designations as kininase-1 and anaphylatoxin inactivator (Mathews 1986). Purification of carboxypeptidase N (Skidgel et al. 1988; Gebhard et al. 1989) permitted partial peptide sequencing of the catalytic subunit, resulting in the cloning of *CPN1* (MIM 603103), which encodes the 458-amino acid catalytic subunit (Gebhard et al. 1989). *CPN1* has been mapped to chromosome 10 (Riley et al. 1998).

The only reported family with carboxypeptidase N deficiency (MIM 212070) included a 65-year male Caucasian proband with an 11-year history of angioedema that occurred about once weekly (Mathews et al. 1980). The attacks lasted ~24 h and most often involved the face and tongue, but sometimes involved large regions of pruritic red swellings on the trunk or limbs. Repeated spectrophotometric assays of serum for carboxypeptidase N activity using protamine as a substrate showed that the proband and his sister each had ~20% normal activity (Mathews et al. 1980). Several family members were clinically affected with some combination of angioedema or chronic urticaria, as well as hay fever or asthma, and had slightly depressed serum carboxypeptidase N, suggesting autosomal recessive inheritance (Mathews et al. 1980). To determine the molecular genetic basis of this condition, we sequenced *CPN1* from genomic DNA extracted from cells of the index patient with carboxypeptidase N deficiency.

Subjects and methods

Study subjects

DNA was obtained from the Coriell Cell Repositories (Camden, NJ, USA). Subject GM09276 was the proband from the index kindred with carboxypeptidase N deficiency (Mathews et al. 1980). DNA from five normal Caucasian

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Table 1. Amplification primers for *CPN1*

Exon	Primer sequence (5′ to 3′)	Product size (bp)
1	F- GGT TGT TTA AGG AGG TGG GG	340
	R- ACT GGA TTT GCA AGG GAA AG	
2	F- GGC ATC AAT GAC ACT TCC AA	336
	R- ACA GTG GGA CAG AGA GGG AA	
3	F- AGT ATT CAA TCT GAA ACC TTC ATT TTT	280
	R- AGA TGG CTT AGC AGT CTT TCT G	
4	F- TTT GTT TGG TAG CCC CAG TT	326
	R- CGC ACT GAA AAG GGT TCT GT	
5	F- GGA GCA TTG CCA TTT GAG TT	255
	R- TTT CCC TGA GAA AAC TGG GA	
6	F- TGG GAA CCT GGT TAT GGA AA	263
	R- AAC CAG TGA AAC ACC TTG CC	
7	F- TGA GAC TCT AAA ACA CTG TAC AAA TGG	228
	R- TAC AGT GCA AAG TCA AGC TC	
8	F- CCT AGC TTA TTT GGG ACA GAT TTT	212
	R- TAC AGT GCA AAG TCA AGC TC	
9	F- ATG GCA AAA GAT GGT CCA AA	287
	R- TCC CAG ATA ATA AAA TAG AAA GAA TGC	

F, Forward primer; R, reverse primer

control subjects was also sequenced. In addition, samples from 128 clinically normal Caucasian subjects were screened to determine allele frequencies. The University of Western Ontario Ethics Review Panel approved the study.

Screening the *CPN1* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, a primer set was developed using GenBank accession numbers NT_030059 and NM_001308. Primer sequences are shown in Table 1. Amplification conditions for all exons were 94°C for 5 min, followed by 30 cycles comprising 30 s each at 94°C, 60°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of *CPN1* variants

The *CPN1* exon 1 385fsInsG frameshift mutation was genotyped using mismatch primers F- TCT ACA GCA TTG GGC GCA GCG TGG AG**T** **C**G and R- ACT GGA TTT GCA AGG GAA AG. The mismatched nucleotides underlined and in bold introduced a recognition site for *Taq*I. Amplification conditions were as described earlier, except for annealing at 58°C. After digesting the 141-bp product with *Taq*I, the wild-type allele gave 114- and 27-bp fragments, whereas the allele encoding the frameshift mutation gave a single 141-bp fragment. All fragments were resolved in 2% agarose gels.

The *CPN1* G178D (746G>A) mutation was amplified using the same conditions specified for amplification of exon 3 for the sequencing experiments. After digesting the 280-bp product with *Mnl*I, the wild-type 746G allele gave 161- and 119-bp fragments, whereas the 746A allele encoding the missense mutation gave a single 280-bp fragment. All fragments were resolved in 2% agarose gels.

Finally, the *CPN1* intron 1 (IVS1) +6T>C single-nucleotide polymorphism (SNP) was genotyped using mismatched primers F- CCC TGG AAT CCA CGA GCC CTG **G**AA **T**T and R- ACT GGA TTT GCA AGG GAA AG. The mismatched nucleotides underlined and in bold introduced a recognition site for *Eco*RI. Amplification conditions were as described earlier, except for annealing at 58°C. After digesting the 80-bp product with *Taq*I, the wild-type IVS1 +6T allele gave a single 80-bp fragment, whereas the IVS1 +6C allele gave 58- and 22-bp fragments. All fragments were resolved in 3% agarose gels.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Chi-square analysis tested differences in proportions and the deviation of genotype frequencies from Hardy-Weinberg predictions, with the nominal $P < 0.05$. Allele frequencies of less common alleles in 128 normal control subjects, with 95% confidence intervals (CI), were determined using the modified Wald method in SAS.

Results

Identification of genomic variants

Genomic DNA sequencing experiments uncovered three variants. Two of these were found in the cell line from the affected subject namely, 385fsInsG, a frameshift mutation in exon 1 due to a single G insertion at nucleotide 385 (codon R58), which predicted an abnormal and prematurely truncated protein of 164 amino acids, and a missense 746G>A SNP in exon 3, which predicted substitution of aspartic acid for the normal glycine at amino acid 178 (G178D). Heterozygosity for the *CPN1* intron 1 (IVS1) +6T>C SNP was observed both in the affected subject and in one normal subject.

Variant frequencies in normal samples

Among 256 normal Caucasian alleles, the genotype frequencies for all three variants did not deviate from predictions of the Hardy-Weinberg equation. The 385fsInsG frameshift mutation was absent from the samples of normal subjects, meaning that the 95% CI for true allele frequency ranged from 0.0000 to 0.0116. Among 256 normal Caucasian alleles, the 746G>A mutation had a frequency of 0.0078 (95% CI 0.0003 to 0.030). Among 256 normal Caucasian alleles, the IVS1 +6C allele frequency was 0.051 (95% CI 0.029 to 0.086).

Discussion

We report (1) primer sets to amplify coding regions of the *CPN1* gene, and (2) use of these primers to sequence genomic DNA. This has resulted in identification of three *CPN1* variants in the genomic DNA from the cell line of a subject with carboxypeptidase N deficiency. The affected subject was heterozygous for each variant. One of these variants, 385fsInsG, was a frameshift mutation that would be expected to have a profound effect on protein structure, with little or no active enzyme produced. Also, the frameshift mutation was a rare molecular event, because it was absent from the 128 normal Caucasian genomes, supporting the idea that it contributed to the low carboxypeptidase activity in the affected subject. The second variant, 746G>A, altered the coding sequence, G178D, but was also seen in the heterozygous state in normal control subjects, albeit rarely (<1%). G178 of carboxypeptidase has been conserved in species as diverse as cow, rat, and mouse, and is also conserved among most members of the human carboxypeptidase family. Because the affected subject had ~20% of normal carboxypeptidase N activity (Mathews et al. 1980), it is possible that the G178D form of the enzyme had some residual activity. Finally, the IVS1 +6C>T SNP did not affect the coding sequence and was relatively common among normal controls, suggesting that it did not contribute to the carboxypeptidase N deficient phenotype.

No other families have been reported with carboxypeptidase N deficiency. The index family was ascertained by screening of sera from 172 subjects with chronic urticaria and/or angioedema using an uncommon spectrophotometric assay (Mathews et al. 1980). The maximum frequency of homozygosity for the severe deficiency imparted by the 385fsInsG frameshift mutation would be ~1 in 30000 individuals, assuming the upper end of our 95% CI for allele frequency. In contrast, the maximum frequency of homozygosity for the putative milder deficiency imparted by the 746G>A (G178D) SNP would be ~1 in 4000 individuals, assuming the upper end of the 95% CI for allele frequency. The findings suggest that carboxypeptidase N deficiency might be a rare molecular cause of urticaria or related conditions. The reagents described here should improve the ability to molecularly diagnose carboxypeptidase N deficiency because they will facilitate screening either for the reported variants or for new genomic DNA variants. The IVS1 +6C>T SNP might also find application in genetic association studies with allergy phenotypes.

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