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Coding Microsatellite Frameshift Mutations Accumulate in Atherosclerotic Carotid Artery Lesions: Evaluation of 26 Cases and Literature Review

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Somatic DNA alterations are known to occur in atherosclerotic carotid artery lesions; however, their significance is unknown. The accumulation of microsatellite mutations in coding DNA regions may reflect a deficiency of the DNA mismatch repair (MMR) system. Alternatively, accumulation of these coding microsatellite mutations may indicate that they contribute to the pathology. To discriminate between these two possibilities, we compared the mutation frequencies in coding microsatellites (likely functionally relevant) with those in noncoding microsatellites (likely neutral). Genomic DNA was isolated from carotid endarterectomy (CEA) specimens of 26 patients undergoing carotid surgery and from 15 nonatherosclerotic control arteries. Samples were analyzed by DNA fragment analysis for instability at three noncoding (BAT25, BAT26, CAT25) and five coding (AIM2, ACVR2, BAX, CASP5, TGFBR2) microsatellite loci, with proven validity for detection of microsatellite instability in neoplasms. We found an increased frequency of coding microsatellite mutations in CEA specimens compared with control specimens (34.6 versus 0%; $p = 0.0013$). Five CEA specimens exhibited more than one frameshift mutation, and ACVR2 and CASP5 were affected most frequently (5/26 and 6/26). Moreover, the rate of coding microsatellite alterations (15/130) differed significantly from that of noncoding alterations (0/78) in CEA specimens ($p = 0.0013$). In control arteries, no microsatellite alterations were observed, neither in coding nor in noncoding microsatellite loci. In conclusion, the specific accumulation of coding mutations suggests that these mutations play a role in the pathogenesis of atherosclerotic carotid lesions, since the absence of mutations in noncoding microsatellites argues against general microsatellite instability, reflecting MMR deficiency.

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INTRODUCTION

Atherosclerosis is characterized by intimal lesions (atheromas or atheromatous plaque, consisting of a lipid core, inflammatory cells and a fibrous cap) particularly affecting medium-sized muscular arteries. Hypertension, hyperlipidemia, cigarette smoking and hyperglycemia are major risk factors triggering vascular damage. The contemporary model views atherosclerosis as a chronic inflammatory response to this damage, which involves macrophage and leukocyte adhesion to the endothelium, thrombosis, enhanced smooth muscle cell proliferation and excessive extracellular matrix production (1). These processes are regulated by complex molecular mechanisms that remain mainly obscure. Recent molecular studies of atherosclerotic lesions have revealed that genetic alterations are a common phenomenon in atherosclerosis. The observed alterations encompass large chromosomal alterations such as loss of heterozygosity, as well as small alterations (most notably nucleotide insertions and deletions in repetitive DNA regions, known as microsatellites or tandem repeats) (2,3). However, it remains to be demonstrated whether these alterations are cause or consequence of atherosclerosis.

About 3% of the human genome consists of such short repetitive nucleotide sequences of different length (mononucleotides, dinucleotides, trinucleotides, and up to hexanucleotides) (4). They are located in both noncoding and coding DNA regions and are highly susceptible

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to nucleotide insertion/deletion caused by DNA polymerase slippage during replication. If not repaired properly by the DNA mismatch repair (MMR) system, the coding insertions/deletions may result in frameshift mutations and thus in altered protein sequences that might affect the function and abundance of the encoded protein (examples are shown in Supplementary Figure S1). In contrast, noncoding insertions/deletions are considered to have a lower impact on cell function or even remain neutral.

Because of their high abundance, different variability and relatively simple detectability, microsatellites have found a widespread use as genetic markers for different disorders (5). For example, trinucleotide microsatellite extensions, occurring in the germline, account for several neurological disorders (6). In contrast, high-level somatic instability of mono- and dinucleotide repeats is a molecular feature of tumors associated with defects in the DNA MMR system. The background mutation frequencies in MMR-deficient cells have been well characterized (7). The current understanding implies that somatic mutations in coding microsatellites of certain genes critically affect regulation of cell growth and promote the selective outgrowth of cell clones (8).

So far, it has been unknown whether the observed microsatellite alterations in atherosclerotic plaques result from a deficiency of specific DNA repair mechanisms such as DNA MMR, in analogy to cancers with microsatellite instability (9), or rather reflect selection of random mutation events that affect microsatellites with impact on vascular cell function. To analyze this question, we compared the mutation frequency of functionally neutral, noncoding microsatellites with the mutation frequency of coding microsatellites with potential functional relevance. We hypothesized that a lesion-promoting functional significance of a certain microsatellite mutation would be reflected by an accelerated outgrowth of the affected cell, which should result in a higher likelihood of the mutation to be detectable in the resulting lesion. Accordingly, we hypothesized that an increased frequency of a certain microsatellite mutation may suggest functional relevance during plaque development, according to models that have convincingly demonstrated associations in cancers with MMR deficiency (10–14).

We therefore examined five well-characterized coding microsatellites and three noncoding microsatellites in 26 carotid lesions and 15 apparently healthy arteries. The noncoding microsatellite markers (BAT25, BAT26, CAT25) were selected because of their high sensitivity for the detection of MMR deficiency (9,15). The coding microsatellites (activin receptor 2 [ACVR2], exon 10, A8; absent in melanoma 2 [AIM2], A10; BCL2-associated X protein [BAX], G8; caspase 5 [CASP5], A10; and transforming growth factor, β receptor 2 [TGFBR2], A10) were selected on the basis of the following criteria: minimum length of eight nucleotides, well characterized from studies on MMR deficiency, potential functional significance of the respective gene products as predicted from the analogy to cancer (8,13,16) (www.seltarbase.org) or a known involvement in vascular pathology (17–21). These analyses were complemented by a comprehensive literature search on atherosclerotic lesions and MMR deficiency.

MATERIALS AND METHODS

Tissue Samples and Processing

Atherosclerotic carotid artery specimens derived from consecutive patients undergoing carotid endarterectomy (CEA) between 2007 and 2008 were obtained from the Vascular Biobank Heidelberg. Indications for CEA were high-grade internal carotid artery stenosis (as determined by ultrasound) for asymptomatic patients and transient ischemic attack, amaurosis fugax or ischemic stroke for symptomatic patients, as described previously (22). Clinical data, including medication, bloodwork and risk factors for atherosclerosis, were recorded for all patients. All patients gave written informed consent. Control tissue from medium-sized visceral arteries and renal arteries was anonymously derived from the tissue bank of the National Centre for Tumor Disease Heidelberg (23). Tissue collection and further processing were carried out according to ethical guidelines and approved by the University of Heidelberg Medical Ethics Committee (S-301/2013 and 207/2005). For further processing, all specimens were rinsed in phosphate-buffered saline to remove surface blood and fixed in 5% formalin.

Tissue Staining and Immunohistochemistry

Tissues were formalin-fixed and embedded in paraffin according to standard procedures for conventional histology. For the present study, 26 consecutive CEA specimens and 15 apparently healthy arteries were selected from the tissue collections by visual inspection of the paraffin blocks for tissue quality (well defined lesion or healthy artery with vital tissue). For immunohistochemical detection of protein expression and microdissection, serial 2-μm transversal sections were prepared from each paraffin-embedded tissue specimen. After deparaffinizing and rehydration, sections were either stained with hematoxylin and eosin (AppliChem) or pretreated according to individually optimized protocols for immunohistochemistry as described previously (22). For detection of vascular smooth muscle cells, sections were incubated overnight with anti–smooth muscle actin (α-SMA; clone 1A4; dilution 1:1,000; Dako). The Dako real detection system, containing biotinylated secondary antibody, streptavidin-horseradish peroxidase (HRP) and HRP substrate, was applied for further treatment, following the manufacturer's recommendation.

Microdissection and Extraction of Genomic DNA

For selection of vital areas within the atherosclerotic CEA specimen and controls, tissue sections were screened for expression of smooth muscle actin and absence of necrosis or hemorrhage (Figure 1A).

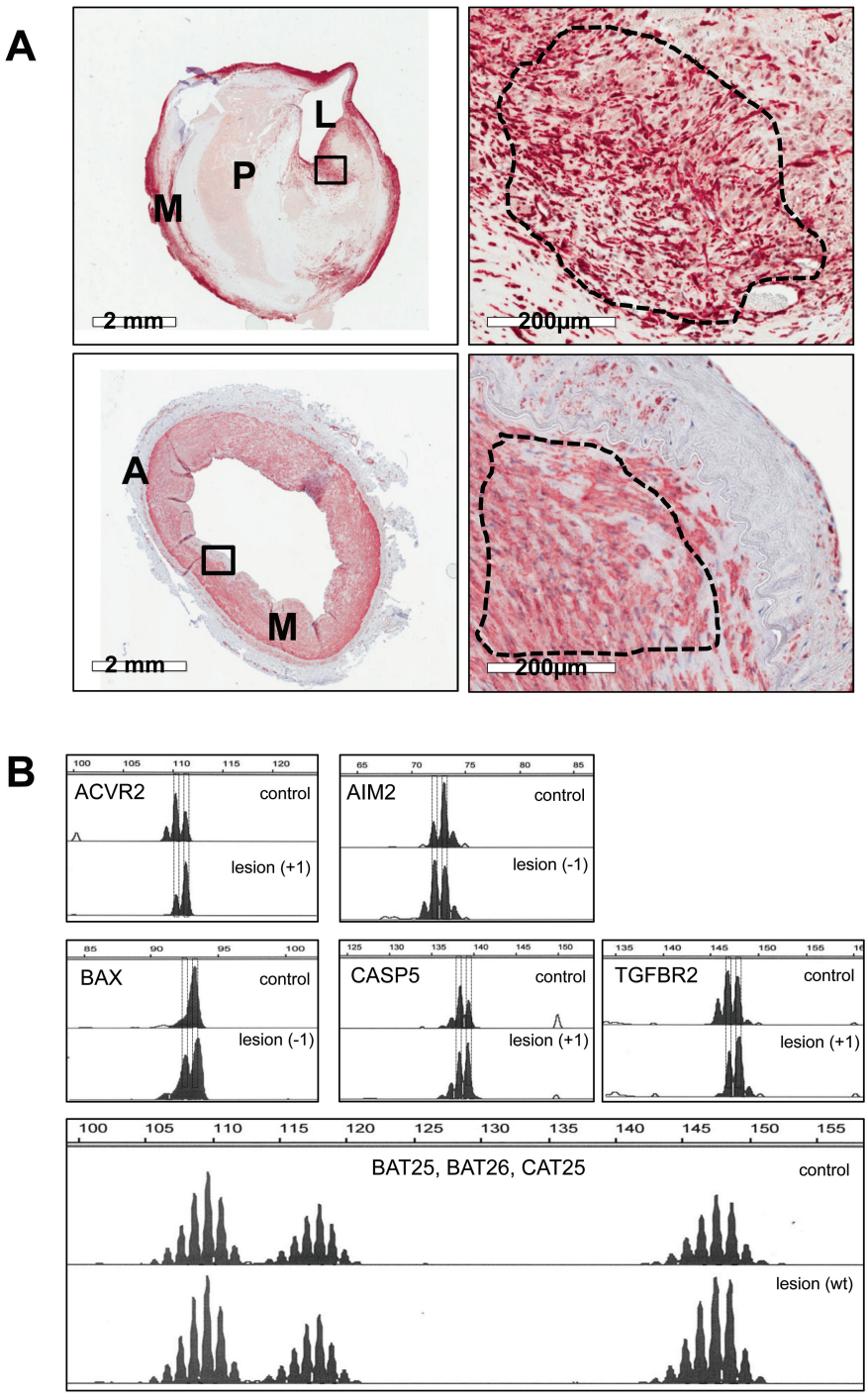

Figure 1. (A) Representative micrographs of one atherosclerotic (upper panels) and one apparently healthy (lower panels) carotid artery. Transversal sections were immunohistochemically stained for detection of α-SMA (red) as described in materials and methods. Original magnification 10× (right panels) and 200× (left panels). The dashed line designates vital areas with cells stained positive for α-SMA that were selected for microdissection. A, tunica adventitia; M, tunica media; L, lumen; P, plaque. (B) Electropherograms of the amplified DNA fragments derived from CEA specimens (lesion) and controls, exhibiting microsatellite instability at different loci. Representative examples of the coding microsatellite markers ACVR2, AIM2, BAX, CASP5 and TGFBR2 are shown with normal (upper panels) and mutated (–1, + 1 deletion; lower panels) peak patterns. No shifts were detected in noncoding microsatellite markers; therefore, only wild-type (wt) patterns are shown for the BAT25, BAT26 and CAT25 markers. Regional microdissection was performed to extract genomic DNA for fragment-length analysis in control (nonatherosclerotic arteries) and atherosclerotic carotid samples.

Table 1. Microsatellite markers and primers used for DNA fragment-length analysis.

Marker	Location and accession number	Repeat	Sense	Antisense
Coding				
ACVR2	M93415	A8	GTT GCC ATT TGA GGA GGA AA	CAG CAT GTT TCT GCC AAT AAT C
AIM2	AF024714	A10	TTC TCC ATC CAG GTT ATT AAG GC	TTA GAC CAG TTG GCT TGA ATT G
BAX	L22473	G8	ATC CAG GAT CGA GCA GGG CG	ACT CGC TCA GCT TCT TGG TG
CASP5	U28015	A10	CAG AGT TAT GTC TTA GGT GAA GG	ACC ATG AAG AAC ATC TTT GCC CAG
TGFBR2	M85079	A10	CTT TAT TCT GGA AGA TGC TGC	GAA GAA AGT CTC ACC AGG
Noncoding				
BAT25	Intragenic to the c-*kit* protooncogene	T25	TCG CCT CCA AGA ATG TAA GT (HEX)	TAT GGC TCT AAA ATG CTC TGT TC
BAT26	Fifth intron of *hMSH2* gene	A26	TGA CTA CTT TTG ACT TCA GCC (FITC)	AAC CAT TCA ACA TTT TTA ACC C
CAT25	3′ UTR of *Caspase 2* gene; NM_032982	T25	CCT AGA AAC CTT TAT CCC TGC TT (FITC)	GAG CTT GCA GTG AGC TGA GA

FITC, fluorescein isothiocyanate.

Areas of interest were marked by a pathologist and microdissected manually from sequential hematoxylin and eosin–stained sections under the microscope with a microlance needle or by using LASER microdissection (P.A.L.M. MikrolaserTechnologie). The resulting tissue was lysed in ATL buffer (Qiagen), and genomic DNA was extracted by using a DNeasy Blood and Tissue Kit (Qiagen), following the instructions of the manufacturer.

Analysis of Microsatellite Instability (MSI) by DNA Fragment-Length Analysis

Genomic DNA from CEA specimens and control samples were analyzed for alterations in five coding (ACVR2, AIM2, BAX, CASP5, TGFBR2) and three noncoding (BAT25, BAT26, CAT25) mononucleotide markers as previously described (15,16). Briefly, specific primer sets (Table 1) were used for polymerase chain reaction (PCR) amplification of DNA fragments surrounding the microsatellites, and 2 μL of the resulting PCR fragments were mixed with 12 μL formamide and 0.2 μL ROX500 length standard (Applied Biosystems) for analysis on an ABI 3100 genetic analyzer (Applied Biosystems). Size, height and profiles of microsatellite peaks were analyzed with GeneScan 3.7 fragment analysis software (GeneScan, PE Applied Biosystems). The electropherogram peak with the largest area was defined as the "main product." Only patterns showing unequivocally distinct additional peaks or shifts in atherosclerotic tissue DNA in comparison to nonatherosclerotic tissue DNA were recorded and classified as unstable (Figure 1B).

Comprehensive Literature Search

A comprehensive literature search was performed in PubMed by using the following criteria: search terms position 1: microsatellite instability, mismatch repair deficiency, MSI, MMR deficiency, MMR, TGFBR2; search terms position 2: plaques, plaque, atherosclerosis, arteriosclerosis, vascular lesion, vascular lesions, carotid artery; inclusion criteria for full text analysis: microsatellite instability analysis of at least one repeat, atherosclerotic lesion; exclusion criteria for full-text-analysis: metaanalysis, no-human tissue, no atherosclerotic tissue, no MSI analysis, germ line tissue.

Statistical Analysis

Statistical analysis was performed with IBM SPSS software, version 20. Patient samples and controls were compared by Fisher exact tests. p values <0.05 were considered statistically significant. Detailed information is described in the respective tables.

All supplementary materials are available online at www.molmed.org.

RESULTS

Characteristics of Patients Included in the Experimental Microsatellite Analysis

Clinical data were available from 25 of the 26 patients undergoing carotid endarterectomy due to symptomatic or asymptomatic carotid stenosis. The patients were aged between 54 and 91 years (mean 70 years), the degree of stenosis ranged from 70 to 99% (mean 85%), 20 were male and 6 were female. A total of 22 patients suffered from hyperlipidemia, 20 from hypertension and 9 from diabetes. Five of the 25 patients were former smokers, whereas 20 were nonsmokers (Table 2).

DNA Fragment-Length Analysis of 26 Atherosclerotic Carotid Lesions (CEA Specimens) Using Mononucleotide Markers with a High Sensitivity for Detection of MSI

None of the 26 CEA specimens and none of the control samples revealed any shift within the noncoding microsatellite markers BAT25, BAT26 and CAT25 (Table 3, Supplementary Table S1). In contrast, frameshift mutations were detected in 9 of the 26 CEA specimens, of which 5 were affected in more than one of the coding microsatellites (Table 3, Supplementary Table S1). Frameshift mutations in both ACVR2 and CASP5 were detected in three CEA specimens, whereas one specimen was affected in three of the markers

Table 2. Patient characteristics.

Age (years)	54–91 (mean 70.2)
Level of stenosis (%) (NASCET criteria)	70–99 (mean 84.7)
Sex	
Male	20 (77%)
Female	6 (23%)
Diabetes	
Nondiabetic	16 (61.5%)
Diabetic	9 (34.6%)
Unknown	1 (4.0%)
Hyperlipidemia	
No	3 (11.5%)
Yes	22 (84.6%)
Unknown	1 (4.0%)
Hypertension	
No	5 (19.2%)
Yes	20 (76.9%)
Unknown	1 (4.0%)
Smoker	
No	20 (76.9%)
Former	5 (19.2%)
Unknown	1 (4.0%)
Vascular risk score (1 to 5)	
Mean	2.9
Score 1	1 patient
Score 2	7 patients
Score 3	11 patients
Score 4	5 patients
Score 5	1 patient
Unknown	1 patient
Symptomatic stenosis	
Not symptomatic	14 (53.8%)
Symptomatic	9 (34.6%)
Unknown	1 (4.0%)

NASCET, North American Symptomatic Carotid Endarterectomy Trial.

(ACVR2, CASP5 and TGFBR2). Alterations observed by fragment-length analysis could be confirmed also by Sanger sequencing (data not shown). We cannot deduce from our data whether the observed frameshifts represent heterozygous mutations or rather represent a mix of cells with biallelic mutations and cells with nonaffected gene sequences. Notably, none of the 15 control specimens showed mutations in any of the coding microsatellites.

The rate of frameshift mutations in CEA specimens was significantly higher than the total rate of noncoding microsatellites ($p = 0.0013$). When comparing the prevalence of frameshift mutations in CEA specimens with control samples, there was a trend for CASP5 mutation rate to be higher in CEA specimens ($p = 0.07$).

Neither the total nor the individual mutation rates of coding microsatellites in CEA specimen were associated with age or with cardiovascular risk factors (diabetes, hyperlipidemia, hypertension, smoking) or with the level of stenosis or whether the stenosis was symptomatic or asymptomatic (data not shown).

Literature Review: Instability in Coding and Noncoding Microsatellites

A literature search was performed by using the PubMed database to discover previous reports on microsatellite instability in vascular tissue. Seven publications were identified according to our previously defined criteria. Results are summarized in Table 4. Five of the seven studies examined instability in noncoding microsatellites, whereas two studies investigated the instability in coding microsatellites, namely in TGFBR2, and in the *hMSH3* and *hMSH6* genes (24,25). Instability in the TGFBR2 microsatellite was discovered in 3 and 27%, respectively, of vascular tissues in these studies. Only McCaffrey *et al.* (25) included matched nonatherosclerotic control samples derived from the same patients for their analysis.

Alterations in noncoding microsatellite markers were reported to occur in up to 43.8% of atherosclerotic arteries (Table 4). It should be noted, however, that the markers used for analysis in these studies were primarily dinucleotide and tetranucleotide markers that were actually selected for analysis of loss of heterozygosity in the vascular specimen. No study could be identified in the literature reporting on the use of noncoding mononucleotide markers, since they are applied for highly sensitive MSI detection in cancer cells (9,15).

DISCUSSION

The present study of MSI frequencies in CEA specimens from carotid arteries

Table 3. Prevalence of MSI in atherosclerotic carotid artery lesions and control specimen.

	Atherosclerotic CEA specimens			Control specimens			
	Positive	Negative	Missing	Positive	Negative	Missing	Difference (p)
Coding microsatellites							
ACVR2	5 (19.2%)	21 (80.8%)	0	0	15 (100%)	0	0.14
AIM2	1 (3.8%)	25 (96.2%)	0	0	15 (100%)	0	1.00
BAX	1 (3.8%)	25 (96.2%)	0	0	15 (100%)	0	1.00
CASP5	6 (23.1%)	20 (76.9%)	0	0	15 (100%)	0	0.07
TGFBR2	2 (7.9%)	24 (92.3%)	0	0	15 (100%)	0	0.52
*Σ coding microsatellites	15	115	0	0	75	0	0.0013
Noncoding microsatellites							
BAT25	0	26 (100%)	0	0	15 (100%)	0	1.00
BAT26	0	26 (100%)	0	0	15 (100%)	0	1.00
CAT25	0	26 (100%)	0	0	15 (100%)	0	1.00
*Σ noncoding microsatellites	0	78	0	0	45	0	1.00

*In atherosclerotic CEA specimens, the frequency of coding MSI alterations is different from the frequency of noncoding MSI alterations (15/130 versus 0/78; Fisher exact test: $p = 0.0013$).

Table 4. Comprehensive literature search.

Reference	Atherosclerotic tissue type	Controls	MS markers	(Non)coding	%MSI
McCaffrey *et al.* (25)	6 carotid arteries, 5 coronary arteries	4 coronary arteries	TGFBR2, hMSH3, hMSH6	3 coding	27%, only in TGFBR2
Clark *et al.* (24)	22 coronary arteries, 9 aorta	No	TGFBR2	1 coding	3% (1/31)
Inafuku *et al.* (36)	29 aorta	No	D1S213, D8S135, D10S197, D10S221, D17S518, D17S799, D17S807, D18S34	8 noncoding	43.8%
Hatzistamou *et al.* (37)	20 aorta, 10 basilar artery	No	D5S111, ANK1, D9S54, D9S103, D17S578, D17S250, D18S34, TPO, D3S1234, D4S194, AMY2B, HRM, CRP, ILIA, MYC, D12S43, D10S109, D7S473	18 noncoding	33% (10/30)
Spandidos *et al.* (38)	20 aorta, 10 basilar artery	No	D13S175, IFNA, TCRD, THRA1, HRM, D9S171, D19S49	7 noncoding	20% (6/30)
Miniati *et al.* (39)	27 cerebral arteries	No	D2S123, D2S177, D2S147, D2S2182, D2S288, D2S2291, D2S138, D2S103, D2S105, D2S115, D2S164, D2S311, D3S1611, D3S1260, D3S1561, D3S1612, D3S647, D3S1478	25 noncoding	7%
Flouris *et al.* (40)	50 aorta	No	D2S119, D2S123, D2S177, D2S147, D2S2182, D2S288, D2S2291, D2S138, D2S103, D2S105, D2S115, D2S164, D2S311, D3S1611, D3S1260, D3S1561, D3S1612, D3S647, D3S1478	19 noncoding	0%

presents evidence for a nonrandom accumulation of frameshift MSI in atherosclerotic lesions. In contrast to previous studies on MSI in vascular lesions, we chose mononucleotide noncoding microsatellite markers for our analysis, because of their high sensitivity for detection of MMR deficiency (9,15). The protein-coding microsatellites used here are well characterized from studies on MSI in a variety of different disorders. They represent repeats of 8 (ACVR2 [A8]; BAX [G8]) to 10 (AIM2 [A10]; CASP5 [A10], TGFBR2 [A10]) mononucleotides with particularly high mutation rates and predicted functional relevance in microsatellite unstable colorectal and endometrial cancers (10,13). Length alterations in these microsatellites have a well-documented impact on protein-coding sequences and are associated with protein function by truncating or altering bigger parts of the amino acid sequence of the translated protein (16,26) (Supplementary Figure S1). Accordingly, these frameshift MSIs are commonly used as markers for tumor classification, disease progression and prognosis after chemotherapy (8).

The functional significance for atherosclerotic lesions of the here-detected frameshift MSI is still elusive. However, the exclusive appearance of MSI in protein-coding microsatellites in our study argues for a selection mechanism (that is, cells harboring mutations in these repeats were selected for further growth within the lesion). This conclusion is based on the following considerations: first, numerous mononucleotide coding and noncoding microsatellites of different length exist in the human genome, and alterations in microsatellites are expected to occur randomly, with frequencies depending on the repeat length and activity of the MMR system (7,27). The noncoding microsatellites used for our analysis thus have a higher chance to be affected by alterations than the short coding microsatellites selected here. Second, because no alterations were detected in the highly sensitive noncoding MMR markers BAT25 (A25 mononucleotide repeat), BAT26 (A26 mononucleotide repeat) and CAT25 (T25 mononucleotide repeat), we conclude that the MMR system is not affected in the atherosclerotic carotid lesions, and randomly occurring insertions or deletions within the microsatellites are effectively repaired before DNA replication starts. Third, detection of accumulated alterations in several protein-coding microsatellites argues for an outgrowth of the affected cells, which escaped from repair by the MMR system and accumulated in the lesion until detection level. This mechanism was originally suggested to drive tumorigenesis of MMR-deficient cancers, where it appears to be the main molecular mechanism by which cancer cells accumulate functional changes with a putative oncogenic effect in this tumor type (28). Monoclonality of atherosclerotic lesions is well established, although the mechanism by which it occurs is yet unclear (29).

Because little is known about the function of proteins encoded by *ACVR2*, *TGFBR2*, *BAX*, *AIM2* and *CASP5* genes during vascular pathogenesis, including

atherosclerosis, we can only speculate about the potential growth advantage of vascular cells affected by protein-coding MSI in these genes.

Our finding of MSI in the TGFBR2 protein-coding microsatellite agrees well with previous studies on coronary and carotid arteries (24,25). The deletion of nucleotides within this repeat results in a frameshift and thus formation of a truncated protein that is associated with loss of its function in the TGF-β signaling pathway and outgrowth of selected cells (14). Thus, inactivation of the *TGFBR2* gene by MSI in vascular cells may likewise affect cell function and contribute to vascular remodeling during pathogenesis. The significant role of TGFBR2 and TGF-β signaling in vascular integrity has been well documented for different cardiovascular disorders, such as atherosclerosis, restenosis and others (30). Advanced atherosclerotic lesions have been shown to express reduced levels of the TGF-β receptor. Consequently, it was suggested, that receptor-negative cells might be allowed to grow in a slow but uncontrolled fashion, while overproducing extracellular matrix components (31). Moreover, germline mutations in the *TGFBR2* gene result in the development of a Marfan type II syndrome, which is characterized by connective tissue disorder and cardiovascular manifestations, such as elastic-fiber calcification, local recruitment of inflammatory cells and increased expression of selected matrix-degrading enzymes within the vasculature (32). It remains to be demonstrated whether the here-observed MSI in the *TGFBR2* gene results in reduced expression levels of the receptor protein and whether it functionally contributes to progression of atherosclerosis in carotid lesions.

In our study, *ACVR2*, encoding the activin type 2B receptor, was particularly often affected by frameshifts in carotid lesions. The ACVR2 pathway parallels the TGF-β signaling pathway and uses the same intracellular mediators. In gastrointestinal neoplasms, *ACVR2* frameshift mutations were shown to accumulate during carcinogenesis, thereby providing evidence for selection of clones having genetic inactivation of the *ACVR2* gene (33). Its role in vascular pathogenesis is completely unknown. However, given the similarities between ACVR2 and TGFBR2 signaling, the total frequency of *ACVR2* and *TGFBR2* frameshifts found in CEA specimens here (27%) strengthens previous research studies, suggesting that this pathway is an important regulator of vascular cell integrity.

BAX, which was affected by frameshift mutation in 1 of 25 CEA specimens in our study, is a member of the mitochondria-induced apoptosis pathway. Its loss or reduced expression in response to mutations has been linked to reduced apoptosis of endothelial cells and intima hyperplasia. Endothelial cells in plexiform lesions of primary pulmonary hypertension have been demonstrated to display microsatellite site mutations and reduced protein expression of TGFBR2 (6 of 19 lesions) and *BAX* (4 of 19 lesions) (34). Whether the BAX frameshift mutations in our study likewise result in reduced *BAX* expression and reduced apoptosis during carotid stenosis requires further analysis.

AIM2 and CASP5 proteins are members of the inflammasome, an intracellular multiprotein complex that forms during cell damage response and results in release of the activated form of proinflammatory interleukin (IL)-1β (35). We have recently shown increased expression and induction of AIM2 and CASP5 in both atherosclerotic lesions and lymphocytic infiltrates of aortic aneurysms (18,19). The functional consequence of MSI in these genes on cell proliferation and apoptosis, however, are yet unknown.

CONCLUSION

This study represents the first systematic analysis of MMR deficiency and MSI in atherosclerotic carotid lesions, which compared the frequencies of well-characterized noncoding with coding mononucleotide MSI markers. Our results indicate that coding MSI is a common event in these lesions, which is not attributed to defects in the MMR system. On the basis of our findings, we conclude that frameshift mutations resulting from MSI in coding microsatellites of vascular cells accumulate during disease progression, which argues for a selection process favoring the outgrowth of cells affected by these mutations. This hypothesis is in line with the clonal outgrowth of vascular cells in atherosclerotic lesions resulting from imbalanced cell proliferation and apoptosis, similarly to neoplastic growth.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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