Micro opioid receptor A118G polymorphism and post-operative pain: Opioids' effects on heterozygous patients

Article in International journal of immunopathology and pharmacology · December 2011

9 authors, including:

Antonella De Capraris
Università degli studi di Foggia

Gilda Cinnella
Università degli studi di Foggia

Loreto Gesualdo
Università degli Studi di Bari Aldo Moro

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MICRO OPIOID RECEPTOR A118G POLYMORPHISM AND POST-OPERATIVE PAIN:
OPIOIDS' EFFECTS ON HETEROZIGOUS PATIENTS

A. DE CAPRARIS1, G. CINNELLA1, A. MAROLLA1, P. SALATTO1, S. DA LIMA1,
P. VETUSCHI1, L. CONSOLETTI1, L. GESUALDO2 and M. DAMBROSIO2

1Unit of Anesthesia and Intensive Care, University Hospital of Foggia, Foggia; 2Department of
Surgical Science, Unit of Anesthesia and Intensive Care, University Hospital of Foggia, Foggia;
3Department of Surgical Sciences, University Hospital of Foggia, Foggia; 4Department of
Biomedical Sciences, Unit of Nephrology, University Hospital of Foggia, Foggia, Italy

Received February 4, 2011 – Accepted September 5, 2011

The Single-Nucleotide-Polymorphism (SNP) 118A>G in the μ-1 Opioid Receptor gene (OPRM1) is
associated with a decrease in the analgesic effects of opioids. The aim of this study is to assess whether
118A>G polymorphism could influence the analgesic response to opioid-based postoperative pain (POP)
treatment. The study consisted of two parts: section α, observational, included 199 subjects undergoing
scheduled surgical procedures with pain management standardized on surgery invasiveness and on
expected level of postoperative pain; section β, randomized, included 41 women undergoing scheduled
caesarean delivery with continuous intra-operative epidural anesthesia and post-operative analgesia
(CEA). In both sections, POP was measured over 48 h (T6h-T24h-T48h) by the visual analogue scale
(VAS). In section β we also tested the responsiveness of hypothalamic-pituitary-adrenal axis (HPA)
expressed by cortisol levels. In section α, with cluster analysis, subjects were analyzed according to
their genotype: a group (1#) of 34 patients reporting VAS score ≥3 at every time lapse was identified and
included only A118G carriers, while wild-type (118A - absence of 118A>G polymorphism) patients
were unevenly distributed between those with cluster #2 (VAS score <3 at every study steps) and those
with cluster #3 (VAS score progressively reducing from T6h). In section β, A118G carriers receiving
epidural sufentanil had the lowest VAS scores at T24h; also in these patients, cortisol levels remained
more stable, with a mild decrease at T6h. This study shows that the OPRM1 118A>G polymorphism
affects postoperative pain response in heterozygous patients: they have a different postoperative pain
response than patients with wild-type genes, which may affect the efficacy of the analgesic therapy.

Opioids play an important role in the3 clinical management of pain. The μ-opioid receptor, encoded by
the gene of human opioid receptor μ-1 (OPRM1), is the primary site of action for the most commonly
used opioids (1). In literature a wide interindividual variation in sensitivity to opioids has been demonstrated,
suggesting potential variability in the gene (OPRM1) and in the μ opioid receptor protein (MOR). Several studies
tried to relate OPRM1 genotyping in the context of pain therapy, to identify patient populations that will benefit
from a pharmacogenetically-guided pain therapy (2).

Single-nucleotide polymorphisms (SNPs) are

Key words: postoperative pain; μ opioid receptor; 118A>G polymorphism, cortisol

0394-6320 (2011)
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Mailing address: Dr. Antonella De Capraris,
Istituto di Anestesia e Rianimazione Universitaria,
Ospedali Riuniti, Università degli Studi di Foggia,
Viale L. Pinto, 1
71100 Foggia, Italy
Tel/Fax ++39 0881 723287
email: antonelladecapraris@yahoo.it
naturally occurring variants in the structure of genes, and SNPs of OPRM1 could alter opioid binding and signal transduction in the resulting receptor, thus having a significant influence on clinical effectiveness and side effects of these drugs (3).

One of the most widespread SNP of the OPRM1 is 118A>G, an exchange of the nucleotide adenine (A) with guanine (G) at position 118. In the Caucasian population, the OPRM1 118A>G SNP has a prevalence of 10%-15% (4), which increases to 16.7% for the populations tracked by the HapMap Project (www.hapmap.org), and its effects are already present in heterozygous carriers (A118G) (5). This prevalence suggests that this SNP could contribute to the known heterogeneity in pain perception, pain threshold, and opioid responsiveness.

Previous in vitro studies demonstrated altered function and binding in the variant A118G MOR: an affinity three times higher for β-endorphins (3), reduced expression levels (6-7), and alteration in intracellular signal transduction (8-9). In vivo studies have demonstrated that OPRM1 118A>G polymorphism is associated with a decrease in the analgesic effects of opioids. In individuals carrying the G allele, clinical research verified an altered opioid effectiveness, a decreased analgesic effect of morphine and its active metabolite M6G (morphine-6-glucuronide), as well as a decreased incidence of opioid side effects (2). The relevance of the 118A>G OPRM1 polymorphism has also been studied in relation to responsiveness of the hypothalamic-pituitary-adrenal axis, showing a reduced cortisol response in A118G heterozygous individuals (10-12). Our study enrolled patients with acute postoperative pain, undergoing standardized plans of pain therapy limited to 48 h, receiving the same dose of opioids. Such cohort of patients represents the ideal population to investigate a possible “gene variation-pain response” correlation, as suggested recently by Ikeda (13).

The aim of this study is to assess the hypothesis that the 118A>G polymorphism could influence the analgesic response to an opioid based postoperative pain (POP) therapy.

**MATERIALS AND METHODS**

**Patients**

The study protocol was approved by the local Ethics Committee. Subjects entering the study were required to understand the study procedures and sign the Ethics Committee-approved written informed consent. The protocol consisted of two parts: section α, observational and section β, randomized. In section α, we recruited prospectively all patients undergoing scheduled surgical procedures (major abdominal surgery, gynaecological and obstetrical surgery, urological and thoracisurgery) in our University Hospital during an 18-month period. Inclusion criteria were: age >18 years and Caucasian race. Exclusion criteria: known hyper-sensibility towards the drugs used, pre-operative chronic pain treatment, and hepatic diseases.

Standardized anesthesiology procedures (general, combined general-epidural, spinal or epidural) were performed according to the type of surgery.

Evaluation of post-operative pain was performed using a visual analogue scale (VAS) at T6h, T24h and T48h. In our institution, all surgical procedures are divided into three levels of expected post-operative pain (low, moderate, and severe) based on invasiveness: we enrolled only patients undergoing moderate and severe pain. Treatment of POP was standardized using plans appropriate to the pain level expected. Protocols of post-operative pain therapy were formulated by our institution, based on guidelines from the Italian National Society of Anaesthesia, Analgesia and Intensive Care (SIAARTI) (14) and on literature recommendations.

Plans for moderate pain (in case of caesarean section, laparoscopic hysterectomy, vaginal hysterectomy, gynaecological laparoscopy, thyroidectomy, mastectomy, laparoscopic cholecystectomy): continuous intravenous administration (IV) with tramadol (150-200mg/die) plus ketorolac (90mg/die) over 48 h by means of elastomeric pump, flow-rate 2ml/h, with ranitidine (150mg/die) for gastric protection and omedetason (4mg/die) to prevent PONV (Post-Operative Nausea and Vomiting); or Continuous Epidural Analgesia (CEA) with ropivacaine 2mg/ml or levobuvicaincaine 2.5mg/ml by means of an elastomeric pump, flow-rate 5ml/h, over 48 h.

Plans for SEVERE pain (in case of gastric, bowel or hepatic resection, lung resection, cystectomy, prostatectomy, nephrectomy, suprarenal surgery): continuous IV with tramadol (300-400mg/die) plus ketorolac (90mg/die) or continuous IV with morphine (10mg/die) for 48 h by means of an elastomeric pump, flow-rate 2ml/h, with ranitidine (150mg/die) for gastric protection and omedetason (4mg/die) to prevent PONV, or CEA with ropivacaine 2mg/ml or levobuvicaincaine 2.5mg/ml plus Fentanyl 100mcg/die or Sufentanil 25mcg/die, by means of an elastomeric pump, flow-rate 5ml/h, during 48 h.

In section β, we recruited healthy women undergoing
scheduled caesarean delivery (not in active labour), homogeneous in demographic characteristics and type of surgery, with continuous epidural intra-operative anaesthesia and post-operative analgesia (CEA). Women were randomized using a computer-generated table into 2 groups: group SUF+ recruited to receive epidural anaesthesia with levobupivacaine 0.6% + sufentanil 10 mcg and epidural postoperative analgesia with levobupivacaine 0.25% + sufentanil 0.75 mcg/ml; and group NO SUF that received epidural anaesthesia with levobupivacaine 0.6% and epidural postoperative analgesia with levobupivacaine 0.25%. Post-operative pain therapy was performed by means of an elastomeric pump, flow-rate 5 ml/h, over 48 h; Post-Operative Pain was assessed using the visual analogue scale (VAS) at T6h, T24h and T48h.

Each patient underwent blood sample collection at T0 (basal – before surgery) for genetic analysis of OPRM1. In section β, we also collected blood samples to dose serum cortisol (chemiluminescent method) at T0 (basal), T45° (45 minutes after the beginning of surgery), and at T6h and T24h from the end of surgical procedures.

Genetic analysis

The physicians and nurses who performed post-operative pain assessments were blind to the ultimately determined genotypes of the study subjects. Similarly, the identity and categorization of the study subjects were and remained unknown to the laboratory research personnel. According to their OPRM1 genotype, patients were divided out into: wild-types (A118A - absence of 118A>G polymorphism); heterozygotes (A118G); and homozygotes (G118G).

DNA collection

Peripheral blood was collected in 3 ml EDTA tubes from patients who gave consent to participate in the study. All blood samples were coded in order to ensure full confidentiality. Blood samples were processed for DNA extraction with a commercial DNA isolation kit (Wizard Genomic Kit, Invitrogen), and stored at -20°C. Quantification and purity of DNA were assessed using spectrophotometric evaluation at 260 nm and 260:280 ratio.

SNP genotyping

For identification of OPRM1 118A>G polymorphism, the exon 1 was amplified by polymerase chain reaction (PCR), by means of specific primer pairs at the boundary of exon 1. The genomic structure and intron boundary sequences were deduced from the Homo sapiens chromosome 10 genomic contig NC_000006.10 and the cDNA sequence; PCR primers were designed with the Primer-3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Amplicons were checked using agarose gel electrophoresis and ethidium bromide and then were directly sequenced using a 3100 Genetic Analyser (Applied Biosystems, Foster City, CA) using both forward and reverse amplification primers and Big Dye terminator sequencer chemistries. The chromatograms were analyzed with Chromas 2.23.

Statistical analysis

Data recorded are reported as mean ± standard deviation (SD) or 95% confidence limits (CL), as appropriate. The allele and genotype frequencies were calculated using gene-counting. For section α, a multivariate regression analysis together with a stepwise model-selection procedure was used to identify variables in statistical relation to pain scores. Cluster analysis was used to categorize patients: first a joining analysis (tree/hierarchical clustering) was performed to evaluate how many “natural” clusters (those that could be labelled in a meaningful manner) were formed by our patients; afterwards the k-mean clustering method was applied on the basis of this number of clusters to assign observations to each cluster. Cluster analysis was conducted using VAS pain-intensity scores recorded at 6 h, 24 h and 48 h, such that the clusters that emerged were distinct in terms of pain at every time-point. The next step was to use mixed-model analysis of variance (ANOVA) for repeated measures, to examine whether clusters were significantly different in terms of genotype, age, sex, and expected level of pain over time. P < 0.05 was the minimal value accepted as statistically significant. Data were analysed in the statistical program STATA-SOFT version 8.0. In the randomized arm (section β), data were analysed with the ANOVA test for repeated measures. The calculation of sample size was based on a power calculation assuming a ratio of 3:1 between the carriers of wild-type OPRM1 and the carriers of the variant allele, to achieve 80% power (type II error at 0.2) to detect a clinically relevant change by 50% or more in VAS scale evaluation, with α = 0.05. The sample size calculated was of 15 patients per group, increased to 20 in order to detect the expected relevant VAS changes.

RESULTS

Section α

The flow-chart (based on Consort E-Flow-chart) shows the diagram of enrolment and follow-up (Fig. 1). Our final study population was made up of 199 patients. A summary of patients, genotypes, and surgery characteristics is presented in Table I.
The frequency of the 118A>G polymorphism and the genotype distribution among the 199 patients is given in Table II. All patients are Caucasians. The frequency of the 118G allele in our population is 15%.

Only two study subjects were homozygous for the minor allele 118G and they were not included in our statistical analysis. (A male patient belonging to the group “severe” pain received general anaesthesia plus postoperative continuous epidural analgesia with sufentanil, and showed VAS = 1 at every time lapse without rescue dose. A female patient belonging to group “moderate” pain received spinal anaesthesia and postoperative intravenous analgesia with tramadol with two rescue doses, and she had high pain scores: VAS T6h=3, T24h=8, and T48h=10.) Consequently, our study population was made up of A118G heterozygous and randomly selected subjects.

The multivariate regression analysis, together with a stepwise model-selection procedure to identify the variables that explain pain scores, showed that the presence of 118A>G polymorphism could influence VAS values (p<0.01); other variables such as age, sex, type of surgery and type of anaesthesia are not related to pain scores.

The hierarchical clustering analysis identified three clusters based on the identification of pain perception at T6h, T24h and T48h (Fig. 2). These three clusters are populated as follows: 34 patients had uncontrolled severe pain and VAS >5 at every study step (cluster 1); 98 patients had perfectly
Table II. Frequency and distribution of the 118A>G polymorphism into study population; section α and section β (n.199 patients and n.41 patients respectively).

<table>
<thead>
<tr>
<th></th>
<th>Genotypes</th>
<th>n. patients</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Section α</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A118G polymorphism</td>
<td>A/A</td>
<td>139</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>58</td>
<td>0.291</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>2</td>
<td>0.010</td>
</tr>
<tr>
<td>Major allele</td>
<td>A</td>
<td>336</td>
<td>85% *</td>
</tr>
<tr>
<td>Minor allele</td>
<td>G</td>
<td>62</td>
<td>15% *</td>
</tr>
<tr>
<td><strong>Section β</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A118G polymorphism</td>
<td>A/A</td>
<td>31</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>10</td>
<td>0.25</td>
</tr>
<tr>
<td>Major allele</td>
<td>A</td>
<td>72</td>
<td>88% *</td>
</tr>
<tr>
<td>Minor allele</td>
<td>G</td>
<td>10</td>
<td>12% *</td>
</tr>
</tbody>
</table>

* Frequency of occurrence = (detected variant alleles/total of individuals x 100%.

controlled pain and VAS <3 at every study step (cluster 2); and 65 patient had severe pain with VAS ≥5 at 6 h that decreased to values <3 at 24 h and 48 h (cluster 3). The k-mean cluster analysis allowed to identify members of each cluster for the subsequent ANOVA analysis, which confirmed a significant difference in mean VAS score between the three clusters (Fig. 3). Cluster 1 patients had mean VAS scores significantly higher than cluster 2 at every time-point, and compared with cluster 3 patients at T24h and T48h (p<0.0001). Subsequently, cluster members were analyzed according to their genotype: wild-type patients were unevenly distributed between clusters #2 (VAS score <3 at every study steps) and cluster #3 (VAS score progressively reducing from T6h) (Fig. 4, top), while cluster #1 included only A118G carriers (Fig. 4, bottom). This cluster #1 had higher VAS scores (>3) at every time lapse. The difference was significant when compared to clusters #2 (T6h to T48h) and #3 (T24h and T48h) (p<0.0001).

Section β

We recruited 41 patients undergoing scheduled caesarean delivery, in continuous intra-postoperative epidural anaesthesia and analgesia. Fig. 5 shows the flowchart diagram of enrolment and follow-up based on CONSORT E-Flow-chart. A summary of patients’ characteristics is presented in Table I, and the frequency of the 118A>G polymorphism is shown in Table II. We found differences among the VAS value in the two groups, with regards to genotype and to the effect of time. Intracroup comparison of VAS over time showed that in both SUF+ and NO SUF groups the mean VAS values decreased from T6h to T48h. Intergroup comparison showed that in group SUF+ A118G carriers had higher VAS values, although not at a significant level, at T6h (2.8±2) when compared to the wild-type (VAS 1.5±1) (Table III).

With regard to the evaluation of serum cortisol,
Table III. Post-Operative Pain Scores (VAS values) and cortisol levels of a randomized group of patients undergoing scheduled caesarean delivery (section 6).

<table>
<thead>
<tr>
<th></th>
<th>SUF+</th>
<th>NO SUF</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS Wild-type</td>
<td>2.8±2</td>
<td>2.2±2</td>
</tr>
<tr>
<td>T6h</td>
<td>2.8±2</td>
<td>2.2±2</td>
</tr>
<tr>
<td>T24h</td>
<td>2.3±2</td>
<td>1.75±1</td>
</tr>
<tr>
<td>T48h</td>
<td>1.8±1</td>
<td>1.75±1</td>
</tr>
<tr>
<td>Cortisol (mcg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0 Wild-type</td>
<td>24±5</td>
<td>26±10</td>
</tr>
<tr>
<td>T45'</td>
<td>29±8</td>
<td>30±10</td>
</tr>
<tr>
<td>T6h</td>
<td>14±4</td>
<td>13±3</td>
</tr>
<tr>
<td>T24h</td>
<td>19±13</td>
<td>29±12</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± standard deviation (*p < 0.05).

Basal levels were similar among groups, but higher than normal according to the typical physiology of pregnancy. In both groups cortisol levels had the same path over time, with a tendency to increase at T45' and to decrease at T6h. In group SUF+ no differences were found between genotypes at every time lapse; wild-type subjects had a stronger decrease of cortisol at T6h compared to T0 (24±6 mcg/dl vs 14±4 mcg/dl with p<0.05, respectively) (Table III). Regarding the NO SUF group, a comparison between genotypes shows lower cortisol levels at T6h of A118G patients vs wild-type (13±3 mcg/dl vs 25±6 mcg/dl - p<0.05, respectively).

DISCUSSION

This study shows that the OPRM1 118A>G polymorphism affects post-operative pain and, as a result, pain intensity differs between genotypes: with the same dose of opioids, given during intra and post-operative periods, heterozygous patients have different analgesic response compared to wild-type patients. Among A118G heterozygous patients, some have higher VAS scores (cluster #1) than their wild-type counterparts at every point in time.

In the literature it is well demonstrated that the presence of 118A>G polymorphism can alter pain scores and the response to opioid analgesics such as morphine (4, 17). In healthy subjects with acute induced pain, those G118G homozygous required more morphine and showed smaller incidence of side effects such as nausea and respiratory depression compared to heterozygotes and wild-type subjects (18). Few studies have investigated the effect of the 118A>G polymorphism on post-operative pain or its interaction with other opioids. Two studies were conducted on an Asian population undergoing scheduled gynaecological and orthopaedic surgery; they received POP therapy with morphine by means of PCA (Patient-Controlled-Analgesia) over 48 h: female G118G homozygotes needed more morphine (33±10mg) in the first postoperative day than A118G heterozygotes (29±8mg) or wild-type (27±10mg) (19, 20). More recently, a study conducted on 138 Japanese patients, undergoing major open abdominal surgery, confirmed that G118G homozygous
patients required more 24 h postoperative analgesics compared with wild-type and heterozygotes (21). On the contrary, another study on opioid-naïve subjects with acute postoperative pain (n=101) undergoing laparoscopic abdominal surgery, found a lack of correlation between the 118A>G polymorphism and clinical response to opioids. In this paper, the length of stay and pain monitoring was limited to 1 h, where probably the effects of high dose of intraoperative opioids are still present (22). In our study postoperative pain was evaluated over 48 h. Despite the appropriate opioid dosage for each protocol of POP treatment based on surgery invasiveness, the standard dosing of opioid is still not enough for a group of A118G heterozygous patients (cluster #1). Further studies are required to better identify the characteristics of these patients, accounting for type of surgery or opioid used.

At the same time, section β patients were enrolled to remove any possible bias factor stemming from the type of opioid used or the means of delivery. Consequently, our obstetric subjects received epidural Sufentanil or continuous infusion over postoperative 48 h with a standard dose for both genotypes: A118G patients (SUF+) had a different analgesic response to opioids. A previous study by Landau tested CSE (Combined Spinal-Epidural) analgesia with fentanyl on 158 women in labour: medium effective dose (ED$_{50}$) of fentanyl was lower in A118G carriers compared to wild-type subjects (G=17.7µg vs A=26.8µg) (23). Landau showed that A118G subjects had lower epidural opioid ED$_{50}$. 

Fig. 1. Diagram of enrolment and follow-up for section α patients.
Fig. 2. Dendogram for the hierarchical clustering of section α patients' tree (because of the limited resolution of the dendogram, cases were not labeled).

Fig. 3. Results of ANOVA analysis applied on members of clusters 1, 2, and 3 identified by k-mean cluster analysis. Data are expressed as the means ± 95% of Interval of Confidence.
Fig. 4. Results of ANOVA analysis applied on cluster members according to their genotype. Data are expressed as the means ± 95% of Interval of Confidence.

Similarly, our findings suggest that epidural Sufentanil has a strong analgesic effect on A118G heterozygous carriers with a standard dose on T24 h.

Furthermore, in section β patients we tested the effect of Sufentanil on A118G OPRM1 and the consequent cortisol production. In physiological conditions, β-endorphins (and similarly, opioid drugs) can determine tonic inhibition on neurons which release corticotrophin hormones, and consequently can inhibit hypothalamic-pituitary-adrenal axis (HPA) (24) and can decrease plasma concentrations of the adrenocorticotropic hormone (ACTH) and cortisol (25-26). In our study, sufentanil caused a strong decrease of cortisol concentration at T6h in wild-type subjects as expected, but cortisol levels remained constant in A118G carriers. Probably A118G receptors have functional alterations, affecting the interaction with HPA axis.
There are several hypotheses that attempt to explain functional alterations of the 118A>G β-receptor (15-16): considering in vitro studies using a transfected 118A>G β-receptor, these indicate that receptor expression, agonist-induced responses to adenyl cyclase activity, dimension of agonist molecule, and binding affinity of ligands to the 118A>G β-receptor (alteration in N-glycosylation) are likely to be altered by the polymorphism.

This could suggest that we have to consider particular functional variants of pain genes during our daily clinical practice. In two recent studies (27-28) and one meta-analysis (29) it was observed that, despite several positive single studies on the clinical relevance of OPRM1 118A>G polymorphism, it is still not possible to decide to apply a genetically guided pain therapy, for example with larger opioid doses in G allele carriers.

In conclusion, the influence of 118A>G polymorphism on pain scores and opioid therapy is well demonstrated in the literature for homozygous patients, while studies on heterozygous patients are discordant. Postoperative pain is an excellent model of acute pain, to search for the relationship between A118G polymorphism and pain response, in opioid-naïve patients. In our study, wild-type and A118G heterozygous patients received the same opioid dosage during intra and post-operative periods which produced three groups with different analgesic response, in which the cluster with the higher VAS scores over time (till 48 h) was composed only by A118G carriers. Also in section β
patients. A118G heterozygous patients had a different analgesic response. Our study adds new data to the clinical study of OPRM1 A118G polymorphism: heterozygotes have a different postoperative pain response than wild-type subjects, which may affect the efficacy of analgesic therapy.

ACKNOWLEDGEMENTS

This study was supported financially by the University of Foggia, Italy, and in part by a grant from the Italian Government as a National Interest Research Programme (PRIN – 2004).

None of the authors has a financial interest in any of the drugs, devices, or products mentioned in this article, which might pose a conflict of interest.

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