WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP
Impact Factor 8.63
Volume 15, Issue 7, 1179-1188.
Research Article
ISSN 2277–7105

GENETIC VARIATION IN OLFACTORY RECEPTOR GENES AND ITS IMPACTS ON CILANTRO PREFERENCE IN HUMANS

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Article Received on 03 March 2026,
Article Revised on 24 March 2026,
Article Published on 01 April 2026,

https://doi.org/10.5281/zenodo.19333060

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How to cite this Article: Swetha K.[1], Sona A.[1], Priya Senan V.[1]* (2026). Genetic Variation In Olfactory Receptor Genes And Its Impacts On Cilantro Preference In Humans. World Journal of Pharmaceutical Research, 15(7), 1179–1188.

ABSTRACT

The leaves of the *Coriandrum sativum* plant, commonly referred to as cilantro or coriander, are a popular ingredient in various global cuisines. However, the appeal of cilantro has been a topic of debate for centuries. Genetic variation is a key factor in shaping individual food preferences. In particular, Olfactory Receptor (OR) genes in humans exhibit significant DNA sequence diversity, which likely contributes to differences in how people perceive and respond to certain tastes and smells. Human genome exhibits high sequence variability that it consists of approximately 800 olfactory receptor genes. Some people claim to perceive certain odors significantly different from others in terms of intensity, valence, or detection threshold. In this work, we determine the variation of olfactory receptor genes in human's cilantro preference. A survey was conducted based on the smell of cilantro, and saliva samples were collected from individuals who perceived coriander leaves as pleasant and those who found them unpleasant. The study explains how a heterozygous mutation in the olfactory receptor gene ORA2 can be identified and associated with an individual's perception of coriander as either pleasant or unpleasant. PCR analysis and sequencing were used to detect sequence differences in the samples.

KEYWORDS: Cilantro, olfactory receptor genes, unpleasant, pleasant, ORA2 gene.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is a uniquely fascinating herb or spice, primarily due to the distinctly polarizing chemosensory properties associated with its fresh leaves, especially when used in food (Spence, 2023)**.** The *Coriandrum sativum* plant has been cultivated since at least the 2nd millennium BCE (Zohary and Hopf, 1988). Its fruits, known as coriander seeds, and its leaves, referred to as cilantro or coriander, are key ingredients in a wide range of cuisines. Notably, South Asian cuisines make extensive use of both the leaves and seeds, while Latin American dishes frequently feature the leaves (Bhat *et al*., 2014). The smell of cilantro is frequently described as pungent or soapy. Although not definitively proven, it is believed that the dislike to cilantro is primarily influenced by its odor rather than its taste. The key aromatic compounds in cilantro include various aldehydes, particularly (E)-2-alkenals and n-aldehydes (Knaapila *et al*., 2012; Cadwallader *et al*., 2005). The unsaturated aldehydes (mostly decanal and dodecanal) in cilantro are described as fruity, green, and pungent; the (E)-2-alkenals, (mostly (E)-2-decenal and (E)-2 dodecenal) as soapy, fatty, like cilantro, or pungent (Eyres *et al*., 2005)**.**The prevalence of cilantro (*Coriandrum sativum*) dislike varies significantly across different ethnocultural groups (Satyal and Setzer,2020). The desirability of cilantro has been a topic of debate for centuries, and the reasons behind its differential perception remain unclear. The proportion of people who dislike cilantro varies significantly by ancestry, yet it is still uncertain how much this may be influenced by environmental factors, such as the frequency of exposure (Mauer and El-Sohemy 2012). Food preferences and liking are complex and multidimensional, shaped by the interaction of various factors such as biological, genetic, physiological, psychological, and personality traits, as well as health, sociodemographic, lifestyle, and cultural influences (Monteleone *et al*., 2017). The high frequency of genetic variations within taste and olfactory receptors is unique in the human genome (Hasin-Brumshtein *et al*., 2009; Nei *et al.*, 2008). Therefore, variations in these two perceptions may be considered as a landmark of human evolution (Fujikura, 2014)**.** The genetic variation that influences people's response to coriander leaves has been traced to a mutation in an olfactory receptor gene (McGee, 2010). Human olfaction plays a crucial role in various functions, provides us a deep understanding of both our social and physical environment (McGann, 2017; Stevenson, 2010). In humans, odor perception is primarily mediated by olfactory receptors (ORs) encoded by OR genes, along with a small group of trace amine-associated receptors that also function as olfactory receptors (Malnic *et al*., 2004; Glusman *et al*., 2001; Liberles and Buck 2006; Liberles, 2015; Gainetdinov *et al*., 2018.)**.** The human genetic revolution has played a pivotal role in identifying the genetic factors underlying sensory perception, providing the opportunity to explore the extent to which food preferences are genetically determined (Newcomb *et al*., 2010). Genetic variation plays a significant role in shaping individual differences in food preferences, which in turn affect food choices and ultimately impact health through dietary intake (Dioszegi 2019; Feeney *et al*., 2021; Robino *et al*., 2019; Chamoun *et al*., 2018). It has been noted that variations in smell perception can influence food preferences, and these differences should be considered, particularly when the volatile composition plays a key role in a food product's flavor profile. However, research on the genetic effects on food odor and flavor perception is still limited (Hayes, 2013).

In the present study, a survey was conducted among individuals who perceive the smell of coriander leaves as either unpleasant or pleasant. Saliva samples were then collected from these individuals based on their perception of the smell. The DNA was isolated and amplified using olfactory receptor gene primers. DNA fragments of olfactory receptor genes, ORA2 gene was sequenced. The selection of the ORA2 gene provides a strategic entry point for elucidating the complex mechanisms of olfactory perception and its implications for human health. Sequence analysis was performed to identify the differences between two samples: individuals who perceive the smell of coriander leaves as unpleasant and those who perceive it as pleasant.

MATERIALS AND METHODS

Sample Collection

Saliva samples were collected from individuals with different perceptions of coriander leaves that is individuals with pleasant or unpleasant smell. Then a survey was conducted in 35 people based on the smell of the coriander that is unpleasant and pleasant. Saliva samples were then collected from two individuals: one who finds the smell of coriander leaves pleasant and the other who finds it unpleasant.

Primer Designing

The primer specific to the ORA2 gene is synthesized. This primer will be used to amplify the gene region associated with olfactory receptors involved in smell perception. Primers were designed for the olfactory receptor gene, ORA2. Forward and reverse primers of ORA2 were designed as follows.

Forward primer – ORA2-F-5’ AGCCTGTGTCTTTGCTAGTC 3’

Reverse primer – ORA2-R-5’ GACAGCCCTGTAGTGTTTGG 3’

DNA Isolation

DNA is extracted from saliva samples of individual who have unpleasant and pleasant smell. This DNA will serve as the source material for further analysis. The isolation of the DNA were carried out using QIAGEN - DNeasy Blood &tissue kit (Germany).

PCR Amplification

The isolated DNA is then amplified using PCR. This technique selectively amplifies the region of the ORA2 gene, making it easier to analyze. Isolated DNA were amplified using the forward and reverse primers. The PCR-amplified DNA fragments are separated using 2% gel electrophoresis based on the size of the DNA fragments. The amplified product was visualized under UV transilluminator and compared the amplified product with DNA ladder.

Sequence Analysis

The selected DNA fragment of the sample from unpleasant taste were then sequenced to determine the exact nucleotide sequence of the ORA2 gene. The sequence obtained is compared to known reference sequences. Differences in the sequence can indicate mutations. The sequencing and analysis of band quality reveals whether the mutation is present in a heterozygous state.

Sequencing was performed using forward and reverse primers in ABI 3730 XL cycle sequencer. Forward and reverse sequence were assembled and contig was generated after trimming the low-quality bases. The sequence was aligned using sequencher and then SNP is determined. Software used was Sequencher 5.4.6, MEGA X.

RESULTS

Sample analysis

The survey was conducted based on the taste preference on the smell of the coriander that is pleasant and unpleasant. 35 individuals were participated in the survey, the results showed that four were felt pleasant and thirty one were felt unpleasant.

PCR amplification and gel analysis

The DNA were isolated from the saliva of two individuals, one who fell pleasant and other who feel unpleasant on coriander leaves. The samples were amplified using the designed primers for the olfactory receptor gene, ORA2. The gel electrophoresis showed that there is only single band in individual shows pleasant smell and two bands for unpleasant (Figure 1).

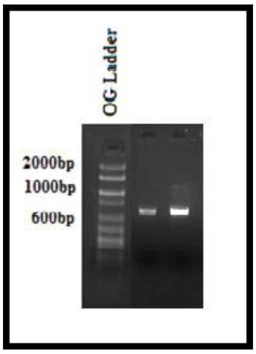

Fig 1: Agarose gel electrophoresis showing PCR amplification of the ORA2 gene.

1st well: unpleasant 2nd well: pleasant

Sequence analysis

Sequence analysis of the unpleasant sample was done. Figure 2 and 3 showing heterozygous mutation. Sequence analysis was shown a region with heterozygous mutation. The homozygous normal condition is CC and homozygous mutated condition is AA. Here we observed two peaks in the graph of unpleasant sample, which represents the heterozygous mutation. C/A heterozygosity in alleles of data confirming the presence two alleles of the gene (Figure 4). With repeated sequencing, band quality sequence was obtained. That may be heterozygous mutation, and obtained double bands (Figure 5). So that we can conclude that there is heterozygous mutation. The heterozygous mutation found in the ORA2 gene is linked to the perception of coriander leaves as unpleasant. This is consistent with research that has shown specific olfactory receptor gene variants can influence how individuals perceive the smell of certain foods, such as coriander, which some people find soapy or unpleasant due to genetic variations.

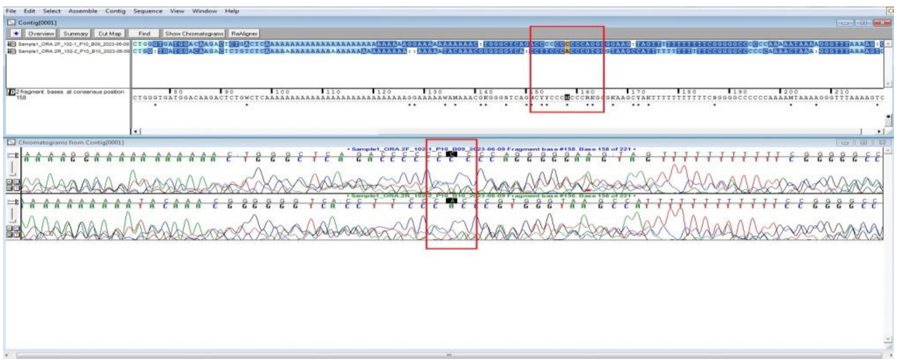

Figure 2: Sequence analysis- The region showing heterozygous mutation.

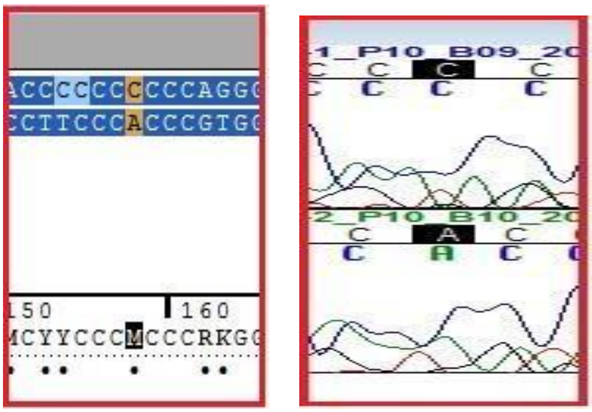

Fig 3: Sequence analysis-The enlarged images showing heterozygous mutation.

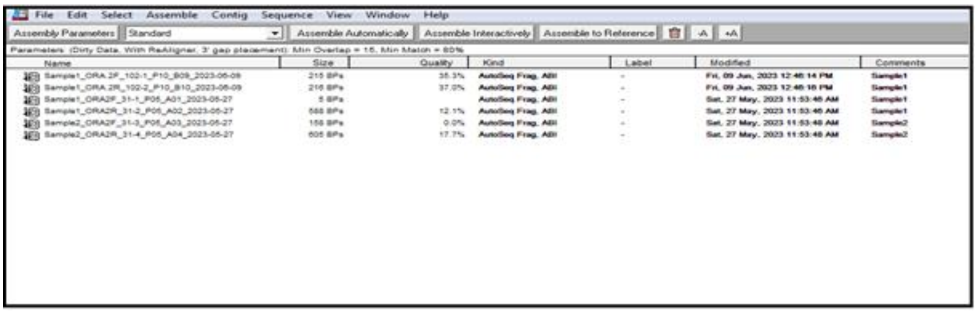

Fig 4: Sequence size and quality.

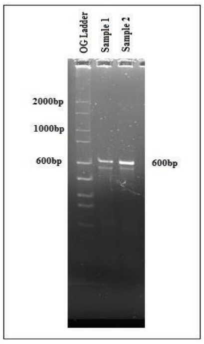

Fig 5: Agarose gel electrophoresis of unpleasant sample.

DISCUSSION

Human genome exhibits high genetic variability that it consists of approximately 800 odorant receptor genes, also human's exhibit variation in the odorant perception. Many love the smell of cilantro while some others claim that it smells foul like a soap or dirt. Studies showed on the olfactory receptor gene in human with preference to cilantro. Among this OR6A2 which has the high binding specificity for several aldehydes which gives the cilantro particular odor (Mauer, 2011). So we have selected the OR6A2 gene for the study. Here we conducted on study of 35 people, how they feel the smell of cilantro. We collected the data of the people who have pleasant smell of coriander leaves, and unpleasant smell of coriander leaves. The saliva is collected from the people who have pleasant and unpleasant smell on coriander leaves. Here we have conducted series of steps which includes DNA isolation, PCR, gel electrophoresis, then sequencing and analysis. Gel electrophoresis revealed multiple bands. Sequencing identified a heterozygous mutation in the unpleasant sample. Other different technique includes microarray testing, exome sequencing, insilco analysis etc. may be used to confirm the heterozygous mutation. There aredifferent types of heterozygous mutations. Here only single base change is observed, it reveals the heterozygous mutation. Finally, we conclude that there is heterozygous mutation in the sample of unpleasant smell. Our results are in consistent with the studies reveals that there is a significant association for cilantro soapy taste in the cilantro preference population (Eriksson *et al*., 2012). The perception of odor is definitely more complex than taste, primarily because olfactory receptor genes form the largest gene family in the genome, with around 400 functional loci. These receptors exhibit a high level of genetic variation compared to other proteins, as approximately 30% of olfactory receptor alleles function differently between individuals (Hasin-Brumshtein *et al*., 2009). So in this study heterozygous mutation is confirmed for unpleasant samples.

CONCLUSION

This study identified genetic variations in olfactory receptor genes in humans, specifically in relation to the perception of cilantro.A survey was conducted with 35 participants to explore the difference in perception of cilantro smell, specifically focusing on the pleasant and unpleasant odors of coriander. Saliva samples were then collected from individuals who found the smell of coriander either pleasant or unpleasant. The primer for the olfactory receptor gene ORA2 was identified and synthesized. Next, DNA was isolated from the saliva samples. The isolated DNA was amplified using polymerase chain reaction (PCR). The amplified DNA fragments were separated through gel electrophoresis. Following electrophoresis, the DNA fragments were sequenced, allowing for the identification of the full sequence and the determination of sequence differences. Through repeated sequencing, a high-quality band sequence was obtained. The sample may contain a heterozygous mutation. To investigate, we ran the sample on a 2.5% gel over a long distance, which resulted in the appearance of double bands. This suggests the presence of a heterozygous mutation in the genomic sequence of the unpleasant-smelling sample.The person who feels unpleasant on coriander leaves because they have heterozygous mutation in their genome. Our study provides a foundation for future research, despite its limitations, and highlights the importance of continued investigation into the genetic basis of olfactory perception.

Conflict of interest

The authors declared that there is no conflict of interest.

ACKNOWLEDGEMENT

We thank the Managing Director, OmicsGen Life Sciences Pvt.Ltd, Ernakulam, Kerala, India for the valuable guidance and support.

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