PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Oral phenotype and scoring of vascular Ehlers-Danlos Syndrome: a case control study
AUTHORS	Ferre FC, Frank M, Gogly B, Golmard L, Naveau A, Cherifi H, Emmerich J, Gautier F, Berdal A, Jeunemaitre X and Fournier BPJ

VERSION 1 - REVIEW

REVIEWER	Fransiska Malfait and Anne De Paepe Center for Medical Genetics Ghent University Hospital De Pintelaan 185 B-9000 Ghent Belgium No competing interests
REVIEW RETURNED	21/12/2011

THE STUDY	The supplemental documents do not contain information that should be better reported in the manuscript.
GENERAL COMMENTS	This paper reports on a prospective case-control study of oral features of 17 molecularly proven vascular Ehlers-Danlos syndrome (vEDS) patients. Gingival recession, which is a minor diagnostic criterion for vEDS, according to the Villefranche Nosology, was present in a lower percentage of vEDS patients than in controls. On the other hand, patients were shown to have gingival thinness and fragility, a higher prevalence of temporomandibular disorders, increased dental root length and modified dental pulp shape. Based on these findings the authors conclude that gingival recession may be an inappropriate diagnostic criterion for vEDS. They propose an oral score, based on increased root length, modified pulp shape and TMJ arthalgia/arthrosis, which may be of use in the clinical diagnosis of vEDS. This is the first specific report of oral involvement in patients with molecularly proven vEDS. These findings and the diagnostic value of the oral score need to be confirmed in a larger patient cohort. These findings are nevertheless valuable, as they contribute to the in-depth characterization of the phenotypic spectrum of this disorder, and help us to gain insight in the pathophysiology, and to guide management of this rare condition. The development of an oral score probably has only limtied diagnostic value for the clinician. It may contribute, as a minor diagnostic criterion, to the suspicion of vEDS, but when this diagnosis is suspected, confirmation by molecular analysis of the COL3A1 gene remains mandatory. Questions:

	1/ page 11, TMJ status: Patients with vEDS suffer from small joint hypermobility. Did the investigators test for TMJ hyperlaxity ? Could TMJ hyperlaxity contribute to the TMJ arthralgia/arthritis? 2/ page 14, line 54: did the investigators find absent frenulas in the control population? Suggestions: page 5, line 45: (with proven COL3A1 mutations) instead of (with sequenced COL3A1 mutations) page 10, line 41: Periodontitis was present in 23.5 % (n=4) of vEDS patients , compared to 45 % (n= 21) of controls. page 10, line 57: These characteristics were associated with an increase in... page 11, line 16: ... pain, , whereas it was reported in only 3 (6.5%) controls. page 11, line 31: ... as expected in the general population: what do the authors mean exactly by this? Please rephrase, as the sentence is not clear. page 12, line 2: Mandibular dental root length was significantly increased in a large proportion of vEDS patients (please indicate exact number, as this number is also indicated for the controls)... page 13, line 25: Oral mucosa and gingival thinness has to be considered... Figure legends: Figure 2: line 14: "Both gingival and oral mucosa appear very thin" Typing errors: page 3, line 39: periodontitis page 3, line 39: conversely page 5, line 32: ...even in early adulthood... page 10, line 12: of the 21 remaining patients... page 13, line 7: involvement in patients with ..

REVIEWER	Atsushi WATANABE Associate Professor Nippon Meddical School, JAPAN
REVIEW RETURNED	24/12/2011

THE STUDY	1) The references need considerable revision. At present, they do not conform to the journal guidelines. For example, the authors wrote article title in italic and journal abbreviation not italic. And authors wrote not only numbering of volume but also issue. Some references rack information, especially in ref no2 and no3. 2) I cannot evaluate supplemental data in page26 and page27 because of no legend.
RESULTS & CONCLUSIONS	1) The authors mentioned the prevalence of gingival recession was low among patients with vEDS but did not write data to be evaluated. They measured recessions of the gingival margin at six different sites per tooth. It is important to write the measured data. 2) The authors mentioned vEDS patients showed increased gingival thinness and increased root length. Increased gingival thinness and increased root length seems to be related with gingival recession. The authors should discuss the reason why increased gingival thinness increased root length not influences gingival recession because the readers are not familiar with oral phenotype. 3) The authors made new scoring of oral phenotype. The oral score was high in most of vEDS patents. But they checked only in vEDS

	patients. From these data, the scoring could not be evaluated to be specific in vEDS. The authors should measure the score in control group and other connective tissue diseases (for example; Loeys-Dietz syndrome) and write in the manuscript.
GENERAL COMMENTS	This manuscript by Ferre FC et al. describes oral phenotype of vascular Ehlers-Danlos syndrome (vEDS) from a case control study and makes and scoring of oral phenotype. Oral phenotype is new finding in vEDS. But, the readers in BMJ open may be not familiar with oral phenotype. This manuscript is not suitable for proper reviewing in the journal because the information is not enough.

VERSION 1 – AUTHOR RESPONSE

This paper reports on a prospective case-control study of oral features of 17 molecularly proven vascular Ehlers-Danlos syndrome (vEDS) patients.
Gingival recession, which is a minor diagnostic criterion for vEDS, according to the Villefranche Nosology, was present in a lower percentage of vEDS patients than in controls. On the other hand, patients were shown to have gingival thinness and fragility, a higher prevalence of temporomandibular disorders, increased dental root length and modified dental pulp shape. Based on these findings the authors conclude that gingival recession may be an inappropriate diagnostic criterion for vEDS. They propose an oral score, based on increased root length, modified pulp shape and TMJ arthalgia/arthrosis, which may be of use in the clinical diagnosis of vEDS.

This is the first specific report of oral involvement in patients with molecularly proven vEDS. These findings and the diagnostic value of the oral score need to be confirmed in a larger patient cohort. These findings are nevertheless valuable, as they contribute to the in-depth characterization of the phenotypic spectrum of this disorder, and help us to gain insight in the pathophysiology, and to guide management of this rare condition. The development of an oral score probably has only limtied diagnostic value for the clinician. It may contribute, as a minor diagnostic criterion, to the suspicion of vEDS, but when this diagnosis is suspected, confirmation by molecular analysis of the COL3A1 gene remains mandatory.

We thank the reviewers for their interest concerning this study. We agree that COL3A1 molecular analysis is the gold-standard for vEDS diagnosis. It is obvious that further investigations are needed to use the oral score as a major criterion (that what we suggest in the discussion). We have changed the term of major by minor in the discussion.

Questions:
1/ page 11, TMJ status: Patients with vEDS suffer from small joint hypermobility. Did the investigators test for TMJ hyperlaxity ? Could TMJ hyperlaxity contribute to the TMJ arthralgia/arthritis?

TMJ hyperlaxity is linked to an excessive mouth opening. Normal mouth opening in the general population is considered to be around 30 to 40mm. In patients with vEDS, mouth opening was frequently reduced, as a consequence we believe of a neuromuscular pain relieving reflex (shunning reflex). Yet due to the limited population sample size and the absence of teenagers or very young adults, we cannot exclude that the observed TMJ discorders were at least partly induced by joint hyperlaxity. In our clinical experience though, young vEDS patients seldomly report TMJ laxity, even less instability or subluxations. However this specific issue has not been systematically addressed in our cohort of patients and relatives.

2/ page 14, line 54: did the investigators find absent frenulas in the control population?

We did not observe any missing or hypotrophic frenulas in control subjects. Conversely approx. one third of patients with vEDS had either one absent or hypotrophic frenula. Yet characterization of abnormal frenulas lack standardization and lead to contradictory findings in Ehlers-Danlos syndromes, as extensively discussed elsewhere (Celletti, C. and al. Am J Med Genet A. 2011 Dec;155A(12):3157-9). Therefore, in order not to fuel further controversy, we reported this finding only in the discussion (page 14, 2 last lines).

Suggestions:
page 5, line 45: (with proven COL3A1 mutations) instead of (with sequenced COL3A1 mutations)
page 10, line 41: Periodontitis was present in 23.5 % (n=4) of vEDS patients , compared to 45 % (n= 21) of controls.
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page 12, line 2: Mandibular dental root length was significantly increased in a large proportion of vEDS patients (please indicate exact number, as this number is also indicated for the controls)...
page 13, line 25: Oral mucosa and gingival thinness has to be considered...
Figure legends: Figure 2: line 14: "Both gingival and oral mucosa appear very thin"

We thank the reviewers for these suggestions. Changes were made accordingly in the manuscript.

Typing errors:
page 3, line 39: periodontitis
page 3, line 39: conversely
page 5, line 32: ...even in early adulthood...
page 10, line 12: of the 21 remaining patients...
page 13, line 7: involvement in patients with ..

We thank the reviewers for their careful review of the manuscript. All typing errors were corrected as suggested.

Reviewer: Atsushi WATANABE
Associate Professor
Nippon Medical School, JAPAN

1) The references need considerable revision. At present, they do not conform to the journal guidelines. For example, the authors wrote article title in italic and journal abbreviation not italic. And authors wrote not only numbering of volume but also issue. Some references rack information, especially in ref no2 and no3.

We agree with the reviewer. All references have been checked for inconsistencies with the journal's author guidelines and corrected accordingly.

2) I cannot evaluate supplemental data in page26 and page27 because of no legend.

We agree with the reviewer's remark. An appropriate legend has been added.

1) The authors mentioned the prevalence of gingival recession was low among patients with vEDS but did not write data to be evaluated. They measured recessions of the gingival margin at six different sites per tooth. It is important to write the measured data.

We agree with the reviewer. However, the detailed report of these measurements was withheld of the manuscript in order to increase the general “understandability” and readability of our work for physicians not familiar with dental surgery.
Data on measurements that were performed on patients were as follows:
Four classes of gingival recession have been distinguished according to international guidelines (Miller et al. Int J Periodontics Restorative Dent 1985;5(2):9-13): Class I recession was defined by marginal tissue recession that does not extend to the mucogingival junction (see also last figure). Class II recession was defined by marginal tissue recession that extends to or beyond the mucogingival junction, with no periodontal attachment loss (bone or soft tissue) in the interdental area. Class III recession was defined by marginal tissue recession that extends to or beyond the mucogingival junction, with periodontal attachment loss in the interdental area or malpositioning of teeth. Class IV recession was defined by marginal tissue recession that extends to or beyond the mucogingival junction, with severe bone or soft-tissue loss in the interdental area and/or severe malpositioning of teeth. For each tooth, 6 measurements have been performed. Recession was considered when superior to 1 mm and the highest measurement of each tooth was recorded. Findings were as follows: 41.2% of the vEDS patients had at least one recession. We observed 6 patients with class I, 2 patients with class II (11, 8%), 3 patients with class III (17,6%) and 1 patient with class IV recession (5,8%). For 10 patients, no recession has been observed. It was clinically obvious to us that vEDS patients did not show an increased prevalence in gingival recession when compared to controls and patients in our general practice. Consequently, in the control group, we identified 62% of patients with at least one recession, which is consistent with previous reports.
It is important to note that gingival recession has been implemented as a minor diagnostic criterion for vascular Ehlers-Danlos syndrome without published clinical proof to our best knowledge and without any reassessment since the publication of the diagnostic criteria. The objective of this study was not to focus on gingival recession because we have not observed an increasesd prevalence of recession in vEDS patients. Besides, the large variety of recessions would even call for a specific study which is far out the scope of this work.
patient Recessions (number class)
Patient 1 2 class I, 2 class III
patient 2 no
Patient 3 no
Patient 4 2 class II
Patient 5 no
Patient 6 2 class I, 1class II
Patient 7 no
Patient 8 no
Patient 9 17 class1
Patient 10 no
Patient 11 3 class I, 2 class III
Patient 12 no
Patient 13 no
Patient 14 no
Patient 15 3 class I
Patient 16 no
Patient 17 3 class I, 3 class III, 1 class IV

2) The authors mentioned vEDS patients showed increased gingival thinness and increased root length. Increased gingival thinness and increased root length seems to be related with gingival recession. The authors should discuss the reason why increased gingival thinness increased root length not influences gingival recession because the readers are not familiar with oral phenotype.

We agree with the reviewer on the fact that physicians may not be familiar with dental semiology and that the odontologic description should be simplified as much as possible. The whole manuscript has therefore been “adapted” to a broad readership. Residual difficulty may subsist but we believe it to be minor.
We disagree with the reviewer regarding the possibility of a general correlation between dental root length, gingival thinness and gingival recession. As shown on the illustration below (from Chapple, Sex Transm Infect 2000;76:236-243), gingival thinness is assessed by measuring thickness of the gingiva at the base of teeth (transversal measure), whereas gingival recession is a vertical process, and therefore measured vertically. Our findings do not support a correlation between thinness and recession (gingival may be thin without recessing). Furthermore, there is no anatomical and published evidence as to a possible correlation between dental root length and gingival recession: 1) dental roots are located behind the alveolar bone of the mandibula (for example) and therefore physically separated from the gingiva. Furthermore, increased root length should not be interpreted as “longer teeth” meaning higher crown location and therefore teeth that are more prone to gingival recession because they are longer. In fact increased root length is a radiological finding, with roots diving excessively into or behind alveolar bone.

3) The authors made new scoring of oral phenotype. The oral score was high in most of vEDS patents. But they checked only in vEDS patients. From these data, the scoring could not be evaluated to be specific in vEDS. The authors should measure the score in control group and other connective tissue diseases (for example; Loeys-Dietz syndrome) and write in the manuscript.

We agree and disagree with the reviewer’s observations: it is certain that this oral score was not designed or intended to be a substitution to clinical diagnostic criteria, especially major diagnostic criteria as arterial or digestive fragility. Yet it suggests that these signs, measured in a standardized setting may be sufficiently specific to independently predict vascular Ehlers-Danlos syndrome. This is not a validation study and as the reviewer suggests our findings
1) need to be confirmed in a larger setting with appropriate sample size calculation,
2) need comparison to patients with arterial fragility without clinical evidence of vEDS.
3) need to be confronted to other inherited connective tissue diseases, to determine the exact significance of these finding within this population of patients. Off interest would be to determine its specificity within Ehlers-Danlos syndromes, especially the classical and hypermobile types. But this will only be possible and reasonable to be done once these diseases will be genetically characterized. As for Marfan syndrome, the interest would be limited since there may be only little confusion in diagnosis as patients have differing phenotypes. Of more interest to study would be patients with Loeys-Dietz syndromes, which are described to have a phenotype that is at least in part related to vEDS.

This manuscript by Ferre FC et al. describes oral phenotype of vascular Ehlers-Danlos syndrome (vEDS) from a case control study and makes and scoring of oral phenotype.
Oral phenotype is new finding in vEDS. But, the readers in BMJ open may be not familiar with oral

phenotype. This manuscript is not suitable for proper reviewing in the journal because the information is not enough.

VERSION 2 – REVIEW

REVIEWER	Atsushi WATANABE Associate Professor Nippon Medical School, JAPAN No competing interests
REVIEW RETURNED	21/02/2012

RESULTS & CONCLUSIONS	The scoring system the authors established is important in this paper. I previously suggested comparing the number of scores in between vEDS and controls at least because the scoring could not be evaluated to be specific in vEDS from paper's data. I believe it is a minimal requirement for publication to compare the number of scores in between vEDS and controls they used, but the authors did not comply with this request. My request might be not needed for a larger setting.

VERSION 2 – AUTHOR RESPONSE

The scoring system the authors established is important in this paper. I previously suggested comparing the number of scores in between vEDS and controls at least because the scoring could not be evaluated to be specific in vEDS from paper's data.
I believe it is a minimal requirement for publication to compare the number of scores in between vEDS and controls they used, but the authors did not comply with this request. My request might be not needed for a larger setting.

We apologize the reviewer for the incomplete answer. The supplemental data show the number of false/true negative scoring counted in the control group. The oral scores of control patients are shown below:

Oral score Number in control group (%)-n=46
0 28 (60,8)
1 17 (37)
2 1 (2,2)
3 0
4 0

For the record, in vEDS patients, the oral score repartition was:

Oral score Number in vEDS group (%)-n=16
0 1 (6,25)
1 1(6,25)
2 5 (31,25)
3 4 (25)
4 5 (31,25)