Author's response to reviews

**Title:** Identification of the first intragenic deletion of PITX2 gene causing an Axenfeld-Rieger-Syndrome: Case report

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**Author’s response to reviews:** see over
Fistly, we thank the reviewers for their extremely helpful comments and suggestions. They are experts in the field and we have followed strictly their advices and deepened our analysis of our observations. We send in this letter with our warm thanks to the Editor in Chief and to the Reviewers a list of our answers point by point to the suggestions of the reviewers. We also mention clearly the changes introduced in our manuscript. We believe that these changes might certainly broaden the interest of our manuscript beyond the community of physicians and geneticists involved in the management of ARS affected patients.

To the Attention of Pr Tord Hjalt

We have included the suggestion that some ARS pedigrees might warrant re-examination

1) Figure 5 and its Legend clarify the point raised by the reviewer. Only two bands are visible on the same gel. This experiment has been performed in triplicate separately by two distinct persons GH and VV. The reviewer was right: Artefactual bands were visible in the first gels: thus the experiment has been performed more adequately.

2) We have removed any overstatement about quantitative genomic PCR as well as about novel TAQ Polymerase allowing long range PCR amplifications of large fragments and stated clearly that we have detected a medium size intragenic deletion.

3) We have included the list of the primers used in our study for mutation detection and the length of the exons. We have studied the entire length of each exon (Figure 6 has been added) and part of the intronic sequences: it is specified in the manuscript.

4) The list of the primers used for the RT-PCR of the region concerned by the deletion are clearly indicated as well as their position in the manuscript. Several isoforms can be detected with one set of primers. This set of primer can detect a region common to all PITX2 isoforms. Everything about the experiments is clarified in the text of the Manuscript and in the legends of the figures. Additional figures have been introduced in order to allow the reader to follow more clearly and more easily what has been done.

For the minor points:
We have added arrows and a diagram explaining the structure of the gene and where is located the deletion exactly. We have indicated where the homeobox and the homeodomain are located. We have changed the wording of several sentences

1) we have placed the previous reference 18 at a much well appropriate position;
2) We have clarified what was detected and was undetectable: It is the aberrant form which is impossible to detect. It cannot be detected.
3) We have been unable to get DNA from the previous generation. The Grand parents did not want to participate to the molecular genetics analysis.

ADDITIONNALLY, We have deepened our discussion of the involvement of PITX2, FOXC1 both in development and oncogenesis: This seems us of outstanding importance for all the readers and emphasize the importance of this case report without making any overstatements.

To the Attention of Pr Darryl NISHIMURA

1) Figure 6 has been added and explains very clearly where the deletion is located and what are the domains involved. A visual presentation of the extent of the deletion within the genomic structure of the PITX2 gene is shown in Figure 6.
2) The matter of haploinsufficiency and gene dosage both for PITX2 and FOXC1 thoroughly discussed in relation with our observations and results. The purpose of the RT-PCR experiment is clearly explained in the manuscript. We have taken into accounts all the very important points raised by the reviewer. We clarified the fact that on other presently unavailable tissues, the results might be different for the expression of the mutant allele. The potential consequences of such expression is thoroughly discussed. We think that we have answered all the points raised very rightfully insightfully by Pr Darryl NISHIMURA as well as by Pr Tord Hjalt

Minor Points:

2) Labels and arrows have been added to show what we wanted to present. The arrow on a critical MRI image is indeed of outstanding importance
3) We answered very clearly to this requirement of clarity. The Primers are listed and incorporated
4) The reference has been added and the broader involvement of PITX2 and FOXC1 in oncogenesis has been added to enrich the discussion and to widen the interest of our manuscript for a broader readership
5) We met this requirement also.

We sincerely and strongly hope that the Reviewers and The Editor In Chief will find that the Manuscript is now meeting the requirement of clarity and quality for a publication in BMC Medical Genetics

With again our warm thanks and our kindest regards

Marc Abitbol MD.PhD