## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | CASE-FATALITY RATE AND ASSOCIATED FACTORS IN 22Q11 MICRODELETION SYNDROME PATIENTS: A RETROSPECTIVE COHORT STUDY |
| **AUTHORS** | Repetto, Gabriela; Guzman, Maria Luisa; Delgado, Iris; Loyola, Hugo; Palomares, Mirta; Lay-Son, Guillermo; Vial, Cecilia; Benavides, Felipe; Espinoza, Karena; Alvarez, Patricia |

## VERSION 1 - REVIEW

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| **REVIEWER** | Bernice Morrow<br>Albert Einstein College of Medicine, Bronx, New York, USA |
| **REVIEW RETURNED** | 29-Apr-2014 |

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| **GENERAL COMMENTS** | Major revisions (basically they need to provide details, none were present) or rejection. Also, a native English speaker needs to edit it.<br><br>Summary: The authors were able to collect a 15 years worth of information on a large patient population with the 22q11 Deletion Syndrome. They concluded that cardiac defects, hypocalcemia and airway malacia contributed to the large number of deaths in the population. However, there are some key issues and questions that arise with this paper that would really help strengthen it and are necessary to make this appropriate for publication.<br><br>Major criticisms:<br>Methods: More clarification would be helpful in order to understand this 15 year cohort.<br>Was every patient identified with the deletion consented at diagnosis and then followed for the entire duration?<br>If not, are we sure that deaths that occur in those lost to follow-up? (Biases need to be addressed)<br><br>Discussion:<br>Regarding the premise of increased deaths with CHD due to the deletion (paragraph 3). As the overwhelming majority of deaths are prior to 2 years of age, at what point did these deaths occur - at birth? prior to surgery? intraoperative? immediately postoperative? recovery? maintenance? It is very important to clarify the situation so conclusions can be made. I would recommend a complete review of these charts |

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| | to<br>better understand the situation surrounding the deaths, and perhaps a<br>better<br>comparison of those with the mutation who survived before making conclusions<br>about management. (For example - post operative calcium management<br>does not play a role if the infant never was in surgery).<br>Issues with controls (page 6, line 15) The<br>controls for the cardiac component are from the general Chilean population.<br>They are unmatched and therefore, many other factors could potentially<br>play a role. Additionally, we do not know from the manuscript when the<br>deaths occured over the 15 year span - it is certainly possible that improvements have been made and thus lowering the fatality rate therefore<br>making it an unfair assumption that a 22q over 15 years would have the same<br>rate as the population in 2010.<br><br>Minor criticisms:<br>- A thorough editing is necessary, I would<br>recommend one by a native English speaker as there are phrases that do not<br>translate appropriately.<br>- If at a point you discus male prevalence,<br>female should be stated as well (ie - a male:female ratio). |

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| **REVIEWER** | Erwin Oechslin<br>University Health Network<br>Toronto, Canada |
| **REVIEW RETURNED** | 12-Jun-2014 |

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| **GENERAL COMMENTS** | This is an interesting study reporting the Chilean experience in patients with microdeletion 22q11.2 syndrome. Comparison of survival to the general Chilean population is interesting. This paper would even be stronger if the author could compare survival of microdeletion 22q11.2 patients and CHD with survival of a matched group of CHD patients without microdeletion 22q11.2 syndrome.<br><br>Repetto GM, et al. report the impact of 22q11.2 microdeletion syndrome and its clinical manifestation on survival in a cohort of 419 Chilean patients diagnosed with this syndrome between 1998 and 2013. Mortality was 14%, most of them during the first two years of life. Mortality was associated with congenital heart defect, hypocalcemia and airway malacia. The patient population was recruited from 6 clinical cytogentic laboratories in Chile.<br><br>Comments:<br><br>1) Comparison of survival to the general Chilean population is interesting. This paper would even be stronger if the authors could compare survival of microdeletion 22q11.2 patients and CHD with survival of a matched group of CHD patients without microdeletion 22q11.2 syndrome. |

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| | 2) How was airway malacia diagnosed? When was hypocalcemia diagnosed? Was hypocalcemia diagnosed at the time of diagnosis of microdeletion 22q11.2 or does was hypocalcemia diagnosed before death.<br><br>3) Are there any information about thyroid function?<br><br>4) How many patients underwent repair of their congenial heart defects? Or did they die before repair of their congenital heart defects?<br><br>5) Table 1: This table should also include information about age, gender of the deceased and alive patients.<br><br>6) Univariate and multivariate predictors/OR should be summarized in table.<br><br>7) How many patients presented with a spontaneous mutations and how many patients have a family history of microdeletion 22q11.2 syndrome?<br><br>8) the authors report the mode of death of patients older than 2 years of age. What was the mode of death of the entire population? They report that the presence of a CHD was the direct cause of death in 63% of deceased patients, i.e. did patients die before surgical repair of the congenital heart defect or was the congenital heart defect nor repaired because of microdeletion 22q11.2 syndrome? |

**VERSION 1 – AUTHOR RESPONSE**

REVIEWER 1

"Summary: The authors were able to collect a 15 years worth
of information on a large patient population with the 22q11 Deletion
Syndrome. They concluded that cardiac defects, hypocalcemia and airway
malacia contributed to the large number of
deaths in the population. However, there are some key issues and
questions that arise with this paper that would really help strengthen it
and
are necessary to make this appropriate for publication.

Major criticisms:
Methods: More clarification would be helpful in
order to understand this 15 year cohort.
Was every patient identified with the deletion consented at diagnosis
and then followed for the entire duration?
If not, are we sure that deaths that occur in those lost to
follow-up? (Biases need to be addressed)"
R: A more detailed description of inclusion and follow up was included in the Methods section. In particular, we have described the fact that the authors reviewed alive/deceased status in the National Civil Registry (www.registrocivil.cl) at the end of the study period in December 2013, to avoid biases due to incomplete follow-up.

"Discussion:

Regarding the premise of increased deaths with
CHD due to the deletion (paragraph 3). As the overwhelming majority of
deaths are prior to 2 years of age, at what point did these deaths occur -
at
birth? prior to surgery? intraoperative? immediately postoperative?
recovery?
maintenance? It is very important to clarify the situation so conclusions
can be made. I would recommend a complete review of these charts to
better understand the situation surrounding the deaths, and perhaps a
better
comparison of those with the mutation who survived before making
conclusions
about management. (For example - post operative calcium management
does not play a role if the infant never was in surgery)."
R: We included information regarding the timing of death related to surgery in the Results section, when the information was available.

"Issues with controls ¬ (page 6, line 15) The
controls for the cardiac component are from the general Chilean population.
They are unmatched and therefore, many other factors could potentially
play a role".
R: We agree with both reviewers that a matched case-control study would be useful in discerning factors associated with fatality in this syndrome, but this was not part of the study design (aimed at identifying factors associated with variable expressivity among individuals with the deletion) and including non-deleted controls would require another, different protocol.

"Additionally, we do not know from the manuscript when the
deaths occured over the 15 year span - it is certainly possible that
improvements have been made and thus lowering the fatality rate therefore
making it an unfair assumption that a 22q over 15 years would have the same
rate as the population in 2010"
R: We also agree that this is relevant information, and we have added yearly case fatality rates for the 22q11 patients as well as for national statistics in Table 3. The data show little change in survival rates for 22q11DS patients with congenital heart disease in the 10- year period.

"Minor criticisms:
- A thorough editing is necessary, I would
recommend one by a native English speaker as there are phrases that do not
translate appropriately"
R: The manuscript has been re-reviewed and re-edited by a native English speaker
" If at a point you discus male prevalence,
female should be stated as well (ie - a male:female ratio)"
R. These figures have been added to the text and Table 2.

Reviewer: 2
"This is an interesting study reporting the Chilean experience in patients with microdeletion 22q11.2 syndrome. Comparison of survival to the general Chilean population is interesting. This paper would even be stronger if the author could compare survival of microdeletion 22q11.2 patients and CHD with survival of a matched group of CHD patients without microdeletion 22q11.2 syndrome.

Repetto GM, et al. report the impact of 22q11.2 microdeletion syndrome and its clinical manifestation

on survival in a cohort of 419 Chilean patients diagnosed with this syndrome between 1998 and 2013. Mortality was 14%, most of them during the first two years of life. Mortality was associated with congenital heart defect, hypocalcemia and airway malacia. The patient population was recruited from 6 clinical cytogentic laboratories in Chile.

Comments:

1) Comparison of survival to the general Chilean population is interesting. This paper would even be stronger if the authors could compare survival of microdeletion 22q11.2 patients and CHD with survival of a matched group of CHD patients without microdeletion 22q11.2 syndrome"
We agree with both reviewers that a matched case-control study would be useful in discerning factors associated with fatality in this syndrome, but this was not part of the study design (aimed at identifying factors associated with variable expressivity among individuals with the deletion) and including non-deleted controls would require another design.

2) "How was airway malacia diagnosed?"
R. As stated in the Methods, malacia was diagnosed by laryngoscopy/bronchoscopy, performed only if there was a clinical indication (stridor, recurrent atelectasis, etc).

"When was hypocalcemia diagnosed? Was hypocalcemia diagnosed at the time of diagnosis of microdeletion 22q11.2 or does was hypocalcemia diagnosed before death."
R: This varied widely in the series: before or after deletion diagnosis; before or after surgery; due to symptoms or during yearly screening. For deceased patients with CHD, diagnosis of hypocalcemia occurred pre or post surgery.

3) "Are there any information about thyroid function?"
R. We have collected information on thyroid function, but there is a substantial proportion of missing data, and it was not included in the analysis. This was made explicit in the Results and Discussion section.

4) "How many patients underwent repair of their congenial heart defects? Or did they die before repair of their congenital heart defects?"
R. This is a relevant issue, and we included available information in the Results section.

5) "Table 1: This table should also include information about age, gender of the deceased and alive patients"
R. Information on age was added to the Results, and gender information was clarified in Table 2.

6) "Univariate and multivariate predictors/OR should be summarized in table"
R: Univariate predictors are included in Table 2 (former Table 1). Due to the exploratory nature of the study and the large proportion of missing data in key potential confounders, multivariate analysis was not performed. This has been made explicit as a limitation of the study in the discussion section and considered relevant for future research in the field.

7) "How many patients presented with a spontaneous mutations and how many patients have a family history of microdeletion 22q11.2 syndrome?"
R. This information was included in the manuscript. Due to insurance limitations, deletion testing was performed only in parents with clinical suspicion of the deletion, and not in all parents.

8) “the authors report the mode of death of patients older than 2 years of age. What was the mode of death of the entire population? They report that the presence of a CHD was the direct cause of death in 63% of deceased patients, i.e. did patients die before surgical repair of the congenital heart defect or was the congenital heart defect nor repaired because of microdeletion 22q11.2 syndrome?”
R. Causes of death are described in the manuscript, and we included information on temporal relationships surgery. We rephrased “direct cause of death” to “direct cause(s) of death listed in the Death Certificate” in the Results section.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Erwin Oechslin<br>University Health Network, Ontario, Toronto, Canada |
| **REVIEW RETURNED** | 10-Aug-2014 |

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| **GENERAL COMMENTS** | There are still major issues/questions which have to be addressed by the authors. The manuscript still needs some language editing<br><br>Comments:<br>1) Comment #2 (reviewer#2): ‘ How was airway malacia diagnosed? The authors mention that this definition was clarified in the Method Seciton. I cannot find any pargraphy defining airway malcia.<br><br>2) Result section, 3rd paragraph: “Causes of death documented in the death certificate are listed in table 1……”. The wording of this paragraph is quite complicated and it is difficult to follow the content. In addition, the numbers don’t seem match with the numbers in table 1. I suggest shortening this paragraph. I would refer to table 1 which lists the ‘cause auf death’<br><br>3) Table 1: the authors list cardiac or non-cardiac deaths as cause of death. It would be interesting to know the mode of death (i.e. sudden death, heart failure death, etc).<br><br>4) Results, 5th paragraph: “Information regarding cardiac surgery was available….”. 3 patients died during surgery and 27 died after surgery. This needs some clarification. Did the 3 patients die on the operating table or during the first 24 hours after surgery? When did the 27 patients die after surgery? Were these deaths during 30 days after surgery? Patients with CHD who underwent surgical repair died at a mean of 6 months…… Did they die at the age of 6 months or 6 months after surgery? Six patients without CHD died at a mean of 7 years, ranging from 2.8 months to 32.4 years. The authors should report the MEDIAN age of death and not the mean age of death (the range is very wide).<br><br>5) The authors mention some limitations of the study. But they should more elaborate on the limitations, e.g. timing of measurement of serum calcium.<br><br>6) Table 2: the title of this table should be changed to ‘Univariate Predictors of Mortality’.<br><br>7) The manuscript still needs some language editing. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 2

"There are still major issues/questions which have to be addressed by the authors. The manuscript still needs some language editing"

Comments:
1) "Comment #2 (reviewer#2): "How was airway malacia diagnosed? The authors mention that this definition was clarified in the Method Seciton. I cannot find any pargraphy defining airway malcia".
R: Malacia was diagnosed using bronchoscopy, as previously mentioned in the Results section. We have added information about the diagnostic criteria that the pulmonologists used in the Methods section

2) "Result section, 3rd paragraph: "Causes of death documented in the death certificate are listed in table 1……..". The wording of this paragraph is quite complicated and it is difficult to follow the content. In addition, the numbers don't seem match with the numbers in table 1. I suggest shortening this paragraph. I would refer to table 1 which lists the 'cause auf death'"
R: The paragraph has been re-worded, and the reference to table 1 was also added. We reviewed the numbers in table 1 and they are correct. For example, 27 patients had cardiac cause as the single immediate cause of death in their certificate, but other 19 had cardiac and another cause listed (infection respiratory failure, etc).

3) "Table 1: the authors list cardiac or non-cardiac deaths as cause of death. It would be interesting to know the mode of death (i.e., sudden death, heart failure death, etc)"
R: There were no sudden cardiac deaths described in the medical records or the death certificates. The information on specific mode of death was unfortunately unavailable for most cases.

4) "Results, 5th paragraph: "Information regarding cardiac surgery was available….". 3 patients died during surgery and 27 died after surgery. This needs some clarification. Did the 3 patients die on the operating table or during the first 24 hours after surgery? When did the 27 patients die after surgery? Were these deaths during 30 days after surgery? Patients with CHD who underwent surgical repair died at a mean of 6 months…… Did they die at the age of 6 months or 6 months after surgery? Six patients without CHD died at a mean of 7 years, ranging from 2.8 months to 32.4 years. The authors should report the MEDIAN age of death and not the mean age of death (the range is very wide)"
R: The 3 patients died during surgery because of ruptured pulmonary artery aneurysms in 2 cases and a cerebrovascular hemorrhage in 1; all 3 in the operating room. The remaining issues have been clarified and/or corrected in the text. Specifically, we added information on median and range of the interval between surgeries and demise.

5) "The authors mention some limitations of the study. But they should more elaborate on the limitations, e.g. timing of measurement of serum calcium"
R. More information on limitations was added to the Discussion section.

6) "Table 2: the title of this table should be changed to 'Univariate Predictors of Mortality'."
R: The title has been changed as requested.

7) "The manuscript still needs some language editing."
R: The manuscript was re-edited by a United States based editing service.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Erwin Oechslin<br>University Health Network, Toronto, Canada |
| **REVIEW RETURNED** | 22-Sep-2014 |

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| **GENERAL COMMENTS** | The authors have addressed the reviewers' suggestion to my satisfaction. There are minor issues which have to be addressed prior to publication; no further review is needed<br><br>I have only minor comments:<br><br>Specific comment:<br>1) Information about specific mode of death was not available in most cases. The authors should add a statement in the method section or limitations section as this is a weakness of the study.<br><br>2) Page 6, result section, 3rd paragraph, 1st sentence: the word 'table' is missing.<br><br>3) Some text editing is still needed, e.g. on page 8, 2nd paragraph, 1st sentence. 'Because the type of CHD in …..severe than those…..'<br><br>4) Table 2: there is a mistyping in line 2: male deceased 26, male alive 172. Sum 198 |