PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Severe febrile reactions to a 2010 trivalent inactivated influenza vaccine in young children in Western Australia
AUTHORS	Armstrong, Paul; Dowse, Gary; Effler, Paul; Carcione, Dale; Blyth, Chris; Richmond, Peter; Geelhoed, Gary; Mascaro, Filomena; Scully, Megan; Weeramanthri, Tarun

VERSION 1 - REVIEW

REVIEWER	***Kathryn Edwards*** Vanderbilt University, Dept of Pediatrics
REVIEW RETURNED	23-Nov-2010

GENERAL COMMENTS	General comments: In this manuscript the authors describe a clustering of febrile reactions in Western Australia, many associated with seizures, in young children receiving the 2010 trivalent inactivated influenza vaccine (TIV) produced by one manufacturer. This clustering led to the cessation of the universal influenza vaccination program in Western Australia and has great relevance to the acceptance of influenza vaccines for the rest of the world. The manuscript is clearly presented, well analyzed, but still leaves questions regarding the pathogenesis of these reactions. Until it is understood what caused the reactions, it may be difficult to prevent them. Specific comments: The title clearly reflects the study focus. The Abstract highlights the study goals and presents the salient results. The conclusion needs to stress that the pathogenesis of these reactions must be determined to prevent them from happening in the future. The Introduction succinctly presents the needed background to assess the existing burden of febrile seizures associated with vaccine in earlier surveillance studies. It should also describe the ongoing vaccine safety programs that exist within Western Australia. Several additional points should be made in the Methods and will be outlined below. 1. What studies were conducted to characterize the vaccines? Did all the lots meet licensing requirements? Were studies done to see if the vaccines were split appropriately? Whole virus vaccine use in children in the 1970s and 80s were also associated with febrile seizures. Could this be explaining the etiology of the seizures? In the Results, 1. the authors should approximate what percentage of the vaccinated population were included in the assessment of the rate of

	febrile reactions and febrile seizures in the Perth hospitals after 2010 TIV vaccination. 2. the interpretation of the increased rate of fever associated with the Junior vaccine brand is complex and needs to include whether this is because younger children are receiving the Junior vaccine more commonly, or whether there are inherent differences between the Junior and the regular CSL vaccine. 4. Does the historical comparison of febrile seizures, include only those 9 Perth hospitals? Please clarify. The Discussion has a number of points that need to be addressed. 1. The authors state that this is the first vaccination program to be suspended of any type in Australia. Was the Wyeth rotavirus vaccination program suspended in Australia? 2. The higher rates of febrile reactions with the Junior brand of vaccine needs to be acknowledged and potential reasons discussed. 3. The statement that "this is unlikely to represent a new, distinct clinical syndrome resulting from a neurotoxic effect of the vaccine? is interesting but what studies have been conducted to date on the vaccine to ensure that is not the case. Again as mentioned above, earlier studies of whole influenza virus administered to children yielded similar patterns of febrile seizures. Do the CSL vaccines associated with fever, have high concentrations of whole influenza virus? 4. Given the fact that the other vaccines are not associated with these febrile reactions, would a reasonable conclusion be that the other vaccines should be used preferentially over the CSL vaccines until the etiology of the problem is identified? This should be acknowledged in the conclusions. Why was the entire program interrupted when only one vaccine type was associated with the reactions? 5. Were these febrile reactions restricted to only children? Were children that were multiply vaccinated actually at more or less risk for febrile reactions? 6. the comment that the vaccine was not "neurotoxic" needs clarification or should be removed. What were the criteria used for that definition? 7. What was the f/u of the kids with seizures – did any require admission? Were there any complex seizures or were there other neurologic manifestations? Were there any identified sequelae?

REVIEWER	***Janet A. Englund, MD*** Professor of Pediatrics University of Washington/Seattle CHildren's Hospital Potential Conflicts: I am a member of the influenza working group of the ACIP (US CDC). Also, I receive research support from Novartis and medimmune for non-influenza-related research projects, as well as support form Adamas, Inc. and ADMA for antiviral research projects.
REVIEW RETURNED	02-Dec-2010

GENERAL COMMENTS	General: Well written descriptive study of epidemiological investigation of febrile seizures associated with influenza vaccine in Western Australia. The authors have utilized several different types of

	analyses and data sources to investigate the relationship of 2010 TIV influenza vaccine and seizures, an effort enhanced by the central reporting of this geographic area and a retrospective studies performed both on ED data and a retrospective cohort sample. This information needs to be available to agencies and health authorities advocating influenza vaccine for children, and the authors have done a commendable job in presenting the data clearly and cohesively. Specific comments: Abstract: Clearly written, well presented overall. However, in the Interpretation section, next to last sentence: it is not clear why these authors comment on continuing vaccination programs in other countries (first sentence of “interpretations”) since there is no new or direct data presented in this paper; this sentence should be deleted. Introduction: Well presented. Another sentence documenting the lack of fevers associated with previous studies of flu vaccine in children would be useful in the 2nd paragraphs. The lack of significant increased rates of fevers, as well as the absence of seizures, in clinical studies supports the passive data of VAERS and the population-based data of VAERS. It is plausible that the seizures could be related to fevers in these children, and flu vaccines in the US have not had any increased rates of even low grade fevers in recent studies in young infants as well as pre-school children. It is also unfortunate that no data on the rates of fever in the CSL vaccine in other years or previous studies is available. Methods: Would like to clarify that no live-attenuated influenza virus vaccine was utilized in WA. Clear description of ascertainment of rates of febrile convulsions; only item missing was a ‘backward” check in terms of assessing known cases reported directly to the authorities and verifiying the children were indeed coded accurately with the correct ICD10 code. Page 9- rates adjusted by a factor of 1.4: can the authors provide estimates later if the factor is not adjusted upwards? Page 12- Statistical analysis: Appears reasonable. It is not clear how the fever was captured for all patients for the multivariate model, as there is not mention of capturing fever data in patients in this study. If this analysis is utilized, there should be mention in the methods how the temperature data was obtained (by history? Emergency room data? Parental report?) Results: As listed above, report of known temperatures based on measurement in the ED would be useful to report here. What percent of children with “febrile convulsions” had a temperature determined (in an attempt to make sure these were “febrile convulsions”). Figure 1 is very helpful; one comment would be to have the bars with more differential in color so that the different groups could be distinguished in a black and white format. Also, the investigation of diarrhea illness such as rotavirus or norovirus epidemiology during the period that is added in the discussion on page 32 needs to be presented in the methods and results section and not just added into the discussion. Page 15- historical comparison: can the authors demonstrate that the increased rates of reporting of febrile convulsions in 2010 was not related to increased publicity, ie, can the authors show when the

	medical and general community were notified of this increased risk in comparison to the reporting of the seizures (as seen in Figure 1?) Discussion: Again, main points of paper are clearly presented. Page 18, line 32- the point of no evidence of increased rates of childhood infections is important. As stated earlier, this should be presented in methods and results. Can the authors specifically document that no excess of rotavirus (or norovirus) cases were seen in WA during this time period, as rotavirus would be a likely etiology (and easily identified). Page 18- It is fine in the discussion to discuss implications for northern hemisphere countries- I just don't think it belongs in the abstract. Conclusion on page 19 is appropriate Table 2, 3 very helpful.

REVIEWER	***Dr P Mangtani*** Clinical Senior Lecturer in Epidemiology Lodnon School of Hygiene and Trop Medicine UK I have no completing interests
REVIEW RETURNED	22-Dec-2010

GENERAL COMMENTS	The paper provides more detail of an investigation of reports of fever and febrile convulsions associated with one of the formulations of the 2010 trivalent influenza vaccine in Australia and as such is of interest. The investigation and responses to the signal is described in a recent JAMA paper (JAMA. 2010;304(21):2353-2355) and in a report on the Government of Australia website (Therapeutic Goods Administration. Investigation into febrile reactions in young children following 2010 seasonal trivalent influenza vaccination. Woden, Australia: Therapeutic Goods Administration, Department of Health and Ageing; 2010. Available at http://www.tga.gov.au/alerts/medicines/fluvaccine-report100702.htm). The report also notes there was no signal of fever and febrile convulsions seen for the same manufacturer's monovalent pandemic H1N1 vaccine. Thus there are several missing pieces of information. To help put the investigation and findings into its correct context the authors should: refer to the above publications; include the fact that no signal was seen for monovalent pandemic vaccine; discuss the other investigations that were carried out as well as what the final decisions were that were taken. More minor issues are to help make the text a bit clearer. In the introduction the several different epidemiological studies conducted could be stated more clearly. Eg the fact that passive surveillance data cannot be used to make any causal association but can only suggest associations is worth noting. The inherent biases in passive surveillance data due to reporting bias because of a recent vaccination, stimulated reporting, lack of a comparison group and denominator as well as lack of ability to control for confounding means a formal examination using an analytical study is required. As is shown in this paper such studies can provide stronger evidence. The supporting results from investigations in other countries who also used the vaccine or similar vaccine should

	be added. Finally it would be good to know what are the most plausible hypotheses currently being suggested and when investigations will be finished. The definitions of both the intervention (eg one or two dose vaccine) and outcome should be given. It does not appear possible given the limited clinical information available to have used the more standard definition from the Brighton collaboration. Minor additional comments I hope will be helpful to make the findings more understandable to an international audience and improve presentation of results and discussion. Introduction page 6 line 12: it would be helpful to give rates and state how much higher the rates of severe disease are compared to a suitable baseline group or compared to outside the flu season. Line 30: It would be useful to assess the literature based on robustness of the methods used to examine safety ie separating out passive surveillance findings from database analytic studies. A sentence or two about the limitations of passive surveillance would be useful (see above). The authors mention Australia's reporting of adverse events includes the public reporting directly to the national passive reporting system. How long this public contribution has been collected would help in the interpretation of the time trends in AEFIs presented. Line 20 The recommendations in Australia for dose size, number of doses and timing between doses of TIV by age group should be given. Page 7 line 31: A summary of the methods used here or early in the methods section and the time line would make the paper clearer eg a descriptive analysis of the temporal clustering of febrile convulsions was conducted in ? April 2010 followed by.. Page 7 Methods The paper states that previous seasonal flu vaccine exposure / monovalent H1N1 pandemic flu vaccine exposure was not associated. See below regarding the analysis – if two doses are recommended, did the analysis take this into account i.e. did most febrile seizures occur following first or second dose? Probably most febrile convulsions occurred soon after first dose but this should be stated or analysed. Page 9 line 47 to 51: A very small point: linking of individual records of events to records holding individual records on formulation of vaccine given rather than the term "cross-matching " might be a more specific description of what was done. Could the percentage that were successfully linked and what variables were used for linking also be given?. Page 10 line 8 : The case definition for febrile seizure used and timeframe (eg under 72 hours post vaccination) should be mentioned. How the Brighton collaboration case definition for generalised seizure was used to provide "established diagnostic criteria" is unclear. The Brighton definitions have levels 1, 2,3 for diagnostic certainty (i.e. from "witnessed tonic-clonic activity" to only a "history of unconsciousness"). It does seem unlikely there were enough data to use these Brighton levels in this study. It might be the classification was made more simply based on physician (or parent) report of a febrile seizure. Page 10 line 17: Could details of who the vaccine providers were be added and what the response rate was? Some justification of the inflation factor of 1.4 used or some sort of sensitivity analysis is required. Page 10 line 40: What was the definition of "other febrile reactions"?

	It should be made clearer that only the passive reporting system was used for other febrile reactions but for febrile convulsions emergency dept data (EDIS) were additional. It would be useful to also look at the pattern of other febrile reactions in the earlier years. Line 59: how was the commencement of the paediatric TIV programme defined? It would be helpful to understand why the date of onset varied each year. The results are interesting but more details would be helpful. 3 Page 13 line 21: for clarity please add again after "...or vaccine provider" "to the National Drug Adverse Event Reporting System" Line 30: how was the information given in table 1 obtained? It would for instance be of interest to know how history of a previous AEFI was obtained and how accurate it was. Line 19: please could you reiterate the diagnostic criteria here. Line 26: It would be helpful to add what the 62 were out of. Is this out of 63? Page 14 & Table 3: On issue of temporal association - comparison was made with the temporality of febrile convulsions with TIV 2008 & 2009. It would be more helpful in this table to add the strength of the temporal relationship with febrile seizures is only associated with monovalent H1N1 pandemic vaccine in 2009, the H1N1 is an antigen of the current TIV product. Page 15: line 4: the sentence could do with indicating that the results are in table 2. Line 21: the statement could do with noting that this is from ED data in addition not just the ICD coding but who does the coding, how valid are they and what lag period if any there is between admission and obtaining data on reasons for attendance at an ED.. A strong temporal relationship is seen. An explanation is required in the introduction of the need to rule out co-incidence and other possible causes and that to assess if there might be a causal relationship requires a formal analytic study. In this case the authors were successfully able to construct an historical cohort study at short notice. A multivariate model is mentioned but a few more details on how it was constructed is useful. Were there apriori confounders which were always kept in (eg was prior receipt of other vaccines before the 2010 season)? What was the criteria for retaining other factors in the model? What was in the final model, what was the OR and 95 % for younger age and was this independent of vaccine formulation? The discussion would benefit from examining the importance or not of reporting levels being different to previous years because of additional concerns over the inclusion of the pandemic H1N1 strain in the TIV vaccines. Could the rates of reporting for the different vaccine formulations be affected by the different indications and time when they were available different? Of course the main indication, age, was controlled for in the final multivariate analysis. A key issue that is currently absent is the experience of other countries with the same or similar vaccines (see above). If the same product was used elsewhere it would likely to have been checked if a concern existed. Childhood seasonal influenza vaccine is more common in the US and has a large population. The JAMA paper suggests no associations with febrile convulsions were seen. Is there anything more since from the US or other country investigations,. Did quality testing of the vaccine incl pyrogenic testing find anything? 4 Page 17 line 14: I think that not all the points listed are conclusions. The first 3 seem to be findings. Removing the phrase "and allows four important conclusions" and the word "fourth" on page 18 line 11

	would be all that is needed however. Line 37 it is slightly confusing mentioning findings in Perth and then Western Australia. What proportion of data come from Perth's EDs compared to the Western Australia databases used in this analysis? Line 40 mentions the % receiving TIV in previous years. It would appear therefore to be possible and clearer to present rates for the previous years to compare with the year of interest 2010. Page 19 line 15 delete "however" Line 18 replace "mean" with "suggest" Line 20 replace "especially" with " likely to be" Line 20 sentence starting "Also" could perhaps be cut, there does not seem to be any ranges of figures presented or used. Title of table: should more clearly state the number of children in the table. Eg. Table 1: Demographic and clinical details of 63 children aged 6 m

VERSION 1 – AUTHOR RESPONSE

RESPONSE FROM AUTHORS TO REVIEWERS COMMENTS

For clarity. the comments from the reviewers, and the responses to them from the authors, have been combined under the headings of 'General','Abstract, 'Introduction', 'Methods', 'Results', and 'Discussion'.

KEY:
Reviewer KE=Kathryn Edwards, Vanderbilt University, Dept of Pediatrics
Reviewer JE=Janet A. Englund, University of Washington/Seattle CHildren's Hospital
Reviewer PM=Punam Mangtani, London School of Hygiene & Tropical Medicine, Dept of Infectious Disease Epidemiology

GENERAL
Comment: Reference more recent publications on the issue – TGA summary on their website and the JAMA paper.
- include the fact that no signal was seen for monovalent pandemic vaccine (PM)
•Response from authors: the rate of febrile convulsions (FCs) associated with the monovalent pandemic vaccine produced by CSL Biotherapies, Panvax, was found by TGA to be between 0.08/1000 and 0.17/1000 (the time between vaccination and FC for inclusion in this analysis was not stated) http://www.tga.gov.au/alerts/medicines/atagi-tga-report.htm. This is considerably less than the 3.3 febrile convulsions per 1000 vaccine doses found in our study to be associated with Fluvax or Fluvax Jr (febrile convulsion within 72 hours of vaccination), but higher than the figure found in the Vaccine Safety Datalink (VSD) program of 0.014/1000 (FC within 72 hours - ref. 3 in our paper), or the VSD data cited in the TGA paper cited by the reviewer http://www.tga.gov.au/alerts/medicines/vaccine-overview.htm (FC with 24 hours). The authors feel that this information need not be include in the revised manuscript owing the different methodologies used in these studies, which makes direct comparison problematic, a need to keep within the word limit, and the questionable relevance to our paper.

-discuss the other investigations that were carried out as well as what the final decisions were that were taken (PM).
• The TGA summary concluded that the rate of FCs in WA associated with CSL vaccines was 7/1000 in Western Australia and 5/1000 in other states, although it is unclear in the report precisely what numerators and denominators were used to derive these figures. Reference to the non-WA states is made in the Discussion section of the revised manuscript.

The supporting results from investigations in other countries who also used the vaccine or similar vaccine should be added (PM).
• The only other southern hemisphere country to use CSL Biotherapies TIV in 2010 was New

Zealand, which also noted an association with febrile convulsions. This is referenced in the revised manuscript.

It would be good to know what are the most plausible hypotheses currently being suggested and when investigations will be finished (PM).
• Description included in the revised manuscript

ABSTRACT
The conclusion needs to stress that the pathogenesis of these reactions must be determined to prevent them from happening in the future (KE).
• Words to this effect have been included in the Abstract of the revised manuscript.

However, in the Interpretation section, it is not clear why these authors comment on continuing vaccination programs in other countries (first sentence of “interpretations”) since there is no new or direct data presented in this paper; this sentence should be deleted (JE).
• Wording in the Abstract of the revised manuscript has been altered to reflect this.

INTRODUCTION
Should also describe the ongoing vaccine safety programs that exist within Western Australia (KE).
• The wording describing the systems for monitoring adverse events following immunisation (AEFI) in Australia have been modified and are now included in the Introduction.

Another sentence documenting the lack of fevers associated with previous studies of flu vaccine in children would be useful in the 2nd paragraphs. The lack of significant increased rates of fevers, as well as the absence of seizures, in clinical studies supports the passive data of VAERS and the population-based data of VAERS. It is plausible that the seizures could be related to fevers in these children, and flu vaccines in the US have not had any increased rates of even low grade fevers in recent studies in young infants as well as pre-school children. It is also unfortunate that no data on the rates of fever in the CSL vaccine in other years or previous studies is available (JE).
• Reference to fever as a side effect of TIV in children has now been included in the Introduction of the revised manuscript. In fact, a paper does exist that describes the rate of fever in CSL vaccines (22 to 40%, depending if was first, second or 3rd dose of TIV being given).

The several different epidemiological studies conducted could be stated more clearly. eg the limitations of passive surveillance data means a formal examination using an analytical study is required (PM).
• This notion has been included in the revised manuscript.

page 6 line 12: it would be helpful to give rates and state how much higher the rates of severe disease are compared to a suitable baseline group or compared to outside the flu season (PM).
• Reliable figures comparing severity of influenza in children compared to other groups are lacking. A qualitative statement has been added in the Introduction stating that hospitalisation for influenza in children less than two years is comparable to rates in other groups considered to be at higher risk for complications for influenza, including the elderly.

P6 Line 30: It would be useful to assess the literature based on robustness of the methods used to examine safety ie separating out passive surveillance findings from database analytic studies. (PM)
• The difference in robustness of data derived from passive reporting systems versus population-based studies has been made clearer in the introduction, including a statement of the limitations of data from passive reporting systems.

A sentence or two about the limitations of passive surveillance would be useful. (PM).
• Included in revised manuscript.

The authors mention Australia's reporting of adverse events includes the public reporting directly to the national passive reporting system. How long this public contribution has been collected would help in the interpretation of the time trends in AEFIs presented. (PM).
• Public reporting of AEFI has been long-standing. The term “established” has been included in the revised manuscript to describe the reporting system that accepts reports from the public and a

reference to this system has been added.

P6 line 20: The recommendations in Australia for dose size, number of doses and timing between doses of TIV by age group should be given (PM).
• Included in revised manuscript.

Page 7 line 31: A summary of the methods used here or early in the methods section and the time line would make the paper clearer eg a descriptive analysis of the temporal clustering of febrile convulsions was conducted in ?April 2010 followed by (PM).
• In the final paragraph of the Introduction, reference is now made to the number of analytical studies described in the manuscript to determine the association between TIV and adverse events in children.

METHODS
What studies were conducted to characterize the vaccines (KE)? Did all the lots meet licensing requirements (KE)? Were studies done to see if the vaccines were split appropriately (KE)? Whole virus vaccine use in children in the 1970s and 80s were also associated with febrile seizures. Could this be explaining the etiology of the seizures (KE)?
• Ours was an epidemiological study to determine the association between TIV and adverse events in children, and was not focussed on determining the cause of the reactions, hence, we have not included this in the Methods or Results. We do, however, now make reference to the scientific studies undertaken by others to determine the biological cause.

Would like to clarify that no live-attenuated influenza virus vaccine was utilized in WA (JE).
• Live-attenuated influenza virus vaccines are not licensed for use in Australia.

Clear description of ascertainment of rates of febrile convulsions; only item missing was a 'backward' check in terms of assessing known cases reported directly to the authorities and verifiying the children were indeed coded accurately with the correct ICD10 code (JE).
• Of the 25 cases directly to the authorities, 22 went to EDs of which 18 were EDIS-enrolled hospitals. None of the 18 were coded as R56.0 that we are aware of, but what codes were used are unknown.
• The methods used to ascertain febrile convulsions were the same for the three years for which data was collected. Therefore, we do not feel that there is a risk of introducing a significant a bias and we have not included this analysis in the paper.

Page 7 -The paper states that previous seasonal flu vaccine exposure / monovalent H1N1 pandemic flu vaccine exposure was not associated. See below regarding the analysis - if two doses are recommended, did the analysis take this into account i.e. did most febrile seizures occur following first or second dose? Probably most febrile convulsions occurred soon after first dose but this should be stated or analysed (PM).

• First dose of TIV given in 2010 - 57 (90%)
• Second dose of TIV given in 2010 - 0 (0%)
• Dose number unknown - 6 (10%)
• Total - 63

• Data collection methods for vaccination history has now been included in the Methods section and the outcomes in the Results section.

Page 9- rates adjusted by a factor of 1.4: can the authors provide estimates later if the factor is not adjusted upwards (JE)?
• If the vaccine dose denominators are not adjusted upwards by the factor of 1.4, then the rates will be 40% higher than as presented in Table 2. However, this would be an over-estimate of the real rates. The 40% correction is based on cross-checking of the number of returned survey forms from immunization providers (of TIV used in children in 2010), versus the expected number of immunization providers who provide influenza vaccines to children. The latter was determined based on vaccine ordering records. The comparison provided the estimate that 40% of providers of childhood TIV did not return survey forms on the number of TIV doses (by formulation) given. It is believed that the providers who did not return data were likely to use fewer doses than those who did

return data. Hence using the 40% correction across all vaccine formulations and batches is likely to "provide the upper limit of the number of doses that might really have been given" (as stated in the Methods), and a relatively conservative estimate of the rate of adverse events.

Page 9 line 47 to 51: A very small point: linking of individual records of events to records holding individual records on formulation of vaccine given rather than the term "cross-matching" might be a more specific description of what was done. Could the percentage that were successfully linked and what variables were used for linking also be given? (PM)
• The term "cross-matched" has been changed to "cross-checked" in the manuscript (note: "cross-matched" was also used on page 11 of 30, line3).
• The variables used to cross-check ACIR were: Surname, first name and DOB.
• 98 out of 99 children who presented to Perth EDIS hospitals and were coded as R56.0 'febrile convulsions' in the study period of 2010 were listed on ACIR.
• Of the 63 cases of febrile convulsions we found to be associated with 2010 TIV in our study, all were listed on ACIR, but only 41 (65%) of the ACIR records had influenza vaccination information included (the information for these 41 cases was corroborated by their primary care giver and/or immunisation provider). The remaining 22 febrile convulsion cases (35%) had their influenza vaccination status ascertained through interviews with their primary care giver and/or immunisation provider (now stated in manuscript).

Page 10 line 8: The case definition for febrile seizure used and timeframe (eg under 72 hours post vaccination) should be mentioned. How the Brighton collaboration case definition for generalised seizure was used to provide "established diagnostic criteria" is unclear. The Brighton definitions have levels 1, 2,3 for diagnostic certainty (i.e. from "witnessed tonic-clonic activity" to only a "history of unconsciousness"). It does seem unlikely there were enough data to use these Brighton levels in this study. It might be the classification was made more simply based on physician (or parent) report of a febrile seizure. (PM)
• All R56.0 coded febrile convulsions and any additional febrile convulsions identified through passive reporting were reviewed by one author (CCB) to ensure diagnostic accuracy. A febrile convulsion was defined as any generalised convulsive seizure (level 1,2 or 3 of the Brighton collaboration case definitions) that occurred in a febrile child (temperature on presentation to a health practitioner > 37.5degC). A vaccine associated febrile convulsion was defined as any febrile convulsion that occurred within 72 hours of either the first or subsequent dose of TIV for whom an alternative cause of fever could not be identified either clinically or microbiologically. This information included in revised manuscript.

Page 10 line 17: Could details of who the vaccine providers were be added and what the response rate was? (PM)
• Vaccination providers in WA are predominantly general medical practitioners (~65%) and government health services (~35%)). These have been mentioned in the revised manuscript.

Page 10 line 30: Some justification of the inflation factor of 1.4 used or some sort of sensitivity analysis is required. (PM)
• See response to JE, above

Page 10 line 40: What was the definition of "other febrile reactions"? (PM)
• "Other febrile reactions" included all non-convulsive febrile episodes derived from reports by parents during standardised interviews or recorded during healthcare attendances.

Page 10 line 40: It should be made clearer that only the passive reporting system was used for other febrile reactions but for febrile convulsions emergency dept data (EDIS) were additional. (PM)
• This has been made clearer in the revised manuscript.
It would be useful to also look at the pattern of other febrile reactions in the earlier years. (PM)
• We did not collect detailed data from cases from earlier years that would allow comparison.

Page 10 line 59: how was the commencement of the paediatric TIV programme defined? It would be helpful to understand why the date of onset varied each year. (PM)
• The commencement of the program varied from year-to-year according to the first availability of vaccine.

Page 12- Statistical analysis: Appears reasonable. It is not clear how the fever was captured for all patients for the multivariate model, as there is not mention of capturing fever data in patients in this study. If this analysis is utilized, there should be mention in the methods how the temperature data was obtained (by history? Emergency room data? Parental report?) (JE)
• The multivariate model was used to analyse the data from the retrospective cohort study. This data was collected by standardized interviews with parents of study subjects and included questions around fever, either self-reported or recorded. This point has been added to the revised manuscript.

RESULTS
The authors should approximate what percentage of the vaccinated population were included in the assessment of the rate of febrile reactions and febrile seizures in the Perth hospitals after 2010 TIV vaccination (KE).
• In 2008 and 2009, 83% and 86%, respectively, of WA children vaccinated with TIV resided in the catchment of the EDIS-enrolled hospitals. The percentage in 2010 is thought to be similar (now stated in manuscript).

The interpretation of the increased rate of fever associated with the Junior vaccine brand is complex and needs to include whether this is because younger children are receiving the Junior vaccine more commonly, or whether there are inherent differences between the Junior and the regular CSL vaccine (KE).
• The fact that the difference in overall rates (ages <5yrs) of febrile convulsions between Fluvax and Fluvax Jr cannot be explained solely by age differences is shown in the results through the age-stratification. Some additional discussion on this observation is now included in the Discussion section of the revised manuscript.

Does the historical comparison of febrile seizures, include only those 9 Perth hospitals? Please clarify (KE).
• Yes. This has now been made clearer in the manuscript.

As listed above, report of known temperatures based on measurement in the ED would be useful to report here. What percent of children with “febrile convulsions” had a temperature determined (in an attempt to make sure these were “febrile convulsions”) (JE).
• All 63 cases reported fever, and 58/63 (92%) had a recorded temperature, either by the parent at home, on presentation to ED or in ambulance (see table below). This information now included in Table 1 in the manuscript.

<38 deg C -1
38-≤39 - 19
39-≤40 - 25
40-≤41 - 8
41-≤42 - 3
≥42 - 2
Unknown - 5

Figure 1 change the bars to have more differential in color so that the different groups could be distinguished in a black and white format (JE).
• A black and white version of Figure 1 has been prepared (attached).

The investigation of diarrhea illness such as rotavirus or norovirus epidemiology during the period that is added in the discussion on page 32 needs to be presented in the methods and results section and not just added into the discussion (JE).
• This has now been included in the Methods and Results sections of the revised manuscript.

Page 15- historical comparison: can the authors demonstrate that the increased rates of reporting of febrile convulsions in 2010 was not related to increased publicity, ie, can the authors show when the medical and general community were notified of this increased risk in comparison to the reporting of the seizures (as seen in Figure 1?) (JE)
• There was no communication to the public or to clinicians prior to the suspension of the program.

This point has been included in the Discussion section of the revised manuscript.

Page 13 line 21: for clarity please add again after "...or vaccine provider" "to the National Drug Adverse Event Reporting System" (PM)
• These words have been added.

P13 Line 30: how was the information given in table 1 obtained? It would for instance be of interest to know how history of a previous AEFI was obtained and how accurate it was. (PM)
• The information shown in table 1 was derived from a number of sources: case record review of all of those who attended hospital, and parent and vaccination provider interview for those who did not attend hospital.

P13 Line 19: please could you reiterate the diagnostic criteria here. (PM)
• Using the same definitions as described above (i.e. response to reviewer's comment to page 10 line 8), an additional 25 TIV-associated febrile convulsions, not coded as R56.0 were identified. These were identified prospectively through formal vaccine adverse events reports and retrospectively by ED clinicians, primary caregivers and vaccine providers following suspension of the program.
• The text in the Methods has been modified previously and is not required again.

P13 Line 26: It would be helpful to add what the 62 were out of) (PM)
• It is out of 63 and this has been clarified.

Page 14 & Table 3: On issue of temporal association - comparison was made with the temporality of febrile convulsions with TIV 2008 & 2009. It would be more helpful in this table to add the strength of the temporal relationship with febrile seizures is only associated with monovalent H1Nl pandemic vaccine in 2009, the H1Nl is an antigen of the current TIV product. (PM)
• It is not clear why the reviewer is referring to an association with monovalent H1Nl pandemic vaccine. No data were collected on H1Nl pandemic vaccine for this study.

Page 15: line 4: the sentence could do with indicating that the results are in table 2. (PM)
• Page 15, line 4 does not refer to results in table 2.

P15 Line 21: the statement could do with noting that this is from ED data in addition not just the ICD coding but who does the coding, how valid are they and what lag period if any there is between admission and obtaining data on reasons for attendance at an ED. (PM)
• The data from EDIS-enrolled hospitals is derived in near-real-time from diagnoses by ED doctors based on ICD-10 codes as the patient is discharged from the ED. There is no reason to suspect that there would be a systematic difference in this coding over the 3 years of data collection.

A strong temporal relationship is seen. An explanation is required in the introduction of the need to rule out co-incidence and other possible causes and that to assess if there might be a causal relationship requires a formal analytic study. In this case the authors were successfully able to construct an historical cohort study at short notice. (PM)
• Reference to the need for formal analytical studies has been included in the Introduction of the revise manuscript.

A multivariate model is mentioned but a few more details on how it was constructed is useful. Were there a priori confounders which were always kept in (eg was prior receipt of other vaccines before the 2010 season)? What was the criteria for retaining other factors in the model? What was in the final model, what was the OR and 95 % for younger age and was this independent of vaccine formulation? (PM)
• The model was created to see if vaccine brand was still associated with ‘fever’ or ‘significant febrile adverse event’ (defined as measured fever > 38.90 and/or rigors and/or convulsions) when simultaneously controlling for three potential a priori confounders: age, prior TIV, prior PANVAX (monovalent pandemic vaccine).
• Model set-up: Clinical outcome (Fever or Sig AEFI) as a yes/no = (age in days) + (Prior Seasonal TIV) + (Prior PANVAX) + (Vaccine Brand)
• In all the models, receiving CSL brand TIV in 2010 (any CSL vaccine, or just Fluvax, or just Fluvax Junior) remains significantly associated with reporting ‘fever’ or ‘significant febrile adverse event’.

DISCUSSION
The authors state that this is the first vaccination program to be suspended of any type in Australia. Was the Wyeth rotavirus vaccination program suspended in Australia (KE)?
• Wyeth rotavirus vaccine, Rotashield, was never registered for use in Australia.

The higher rates of febrile reactions with the Junior brand of vaccine needs to be acknowledged and potential reasons discussed (KE).
• Some additional discussion on this observation is now included in the Discussion section of the revised manuscript.

The statement that “this is unlikely to represent a new, distinct clinical syndrome resulting from a neurotoxic effect of the vaccine” is interesting but what studies have been conducted to date on the vaccine to ensure that is not the case. Needs clarification or should be removed. What were the criteria used for that definition? (KE)
• We were using the term “neurotoxic” in a generic sense to describe the possible scenario of the vaccine causing convulsions due to some direct effect on the brain. Our study showed that this was not the case and that the likely pathogenesis is the same as that for usual febrile convulsions. To prevent ambiguity, we have deleted the term “neurotoxic” from the revised manuscript.

Earlier studies of whole influenza virus administered to children yielded similar patterns of febrile seizures. Do the CSL vaccines associated with fever, have high concentrations of whole influenza virus? (KE)
• The laboratory investigation of Fluvax® and Fluvax Junior® undertaken by the Therapeutic Goods Administration, the Australian regulator of prescription medicines including vaccines, has not shown any abnormal presence of whole virus particles. This point has been added to the revised manuscript.

Given the fact that the other vaccines are not associated with these febrile reactions, would a reasonable conclusion be that the other vaccines should be used preferentially over the CSL vaccines until the etiology of the problem is identified? This should be acknowledged in the conclusions (KE).
• This conclusion was included in final paragraph of the Discussion section in the original manuscript. This advice has been strengthened in the revised manuscript.

Why was the entire program interrupted when only one vaccine type was associated with the reactions (KE)?
• At the time of the suspension of the use of TIV in children under 5 years on 23 April 2010, it was not known that only one vaccine type was implicated and, therefore, all TIV formulations were included in the suspension. On 30 July 2010, after a detailed examination of the epidemiological evidence concluded that CSL-branded alone were responsible for the reactions, the suspension was lifted for non-CSL-branded vaccines.
Were these febrile reactions restricted to only children? Were children that were multiply vaccinated actually at more or less risk for febrile reactions (KE)?
• Our study was confined to determining adverse reactions in children under the age of 5 and we are unable to comment on adverse reactions in other age groups.
• There were too few children identified in our study who were multiply vaccinated to allow as association to be demonstrated.

What was the f/u of the kids with seizures – did any require admission? Were there any complex seizures or were there other neurologic manifestations? Were there any identified sequelae (KE)?
• 28/63 (44%) were admitted to hospital for at least one night (mean length of stay=3 days; range 1 to 50 days). Two children were admitted to the intensive care unit with status epilepticus, one of whom suffered significant neurological sequelae (hypoxic ischaemic encephalopathy) (information now added to manuscript).

Page 17 line 14: I think that not all the points listed are conclusions. The first 3 seem to be findings. Removing the phrase "and allows four important conclusions" and the word "fourth" on page 18 line 11 would be all that is needed however. (PM)
• The word “conclusions” has been replaced with “observations”.

P17 Line 37 it is slightly confusing mentioning findings in Perth and then Western Australia. What proportion of data come from Perth's EDs compared to the Western Australia databases used in this analysis? (PM)
• The text has been altered in the revised manuscript to confine the point in this paragraph to the Perth metropolitan area.

P17 Line 40 mentions the % receiving TIV in previous years. It would appear therefore to be possible and clearer to present rates for the previous years to compare with the year of interest 2010. (PM)
• It was not possible to calculate rates reliably for the previous years for the data presented in Table 3. While information on the number of doses reported are available for 2008 and 2009, which allows calculation of coverage, these are likely under-estimates, as there is no corresponding estimate of the proportion of providers who did not report vaccination in these years. The latter information was available for the 2010 survey of providers, and hence the upwards correction of the vaccine dose data for 2010. Hence, given the non-comparability of the denominator data for the different years, odds ratios have been calculated for the data in Table 3.
Page 18, line 32- the point of no evidence of increased rates of childhood infections is important. As stated earlier, this should be presented in methods and results. Can the authors specifically document that no excess of rotavirus (or norovirus) cases were seen in WA during this time period, as rotavirus would be a likely etiology (and easily identified) (JE).
• This point is now included in the Methods and Results section of the revised manuscript.

Page 18- It is fine in the discussion to discuss implications for northern hemisphere countries- I just don't think it belongs in the abstract. Conclusion on page 19 is appropriate (JE)
• We feel that the implications of our study for childhood influenza vaccination programs in other countries is very important and would prefer that it remains in the Abstract. The words have been modified, however, so as not to specify the programs of any particular hemisphere.

The discussion would benefit from examining the importance or not of reporting levels being different to previous years because of additional concerns over the inclusion of the pandemic H1NI strain in the TIV vaccines. (PM)
• It is possible that vaccination providers would have been more likely to report adverse events in 2010 owing to the publicity around the 2009 pandemic vaccination program. However, this would be unlikely to have resulted in biased results as all TIV formulations used in 2010 included the pH1N1 strain and there is no plausible reason for adverse events related to Fluvax and Fluvax Jr to have been preferentially reported. Also, a heightened awareness by the public would not be expected to alter the propensity of parents to present their child to an ED if they suffered a febrile convulsion. Could the rates of reporting for the different vaccine formulations be affected by the different indications and time when they were available different? Of course the main indication, age, was controlled for in the final multivariate analysis. (PM)
• These are very unlikely to have altered rates of reporting.

A key issue that is currently absent is the experience of other countries with the same or similar vaccines (see above). If the same product was used elsewhere it would likely to have been checked if a concern existed. Childhood seasonal influenza vaccine is more common in the US and has a large population. The JAMA paper suggests no associations with febrile convulsions were seen. Is there anything more since from the US or other country investigations.
• The only other southern hemisphere country to use CSL Biotherapies TIV in 2010 was New Zealand, which also noted an association with febrile convulsions. This is referenced in the revised manuscript. The authors are unaware if any northern hemisphere countries used CSL vaccines in their paediatric influenza programs, and to our knowledge no reports of adverse events have been published.

Did quality testing of the vaccine incl pyrogenic testing find anything? (PM)
• The laboratory investigation of Fluvax® and Fluvax Junior® undertaken by the Therapeutic Goods Administration, the Australian regulator of prescription medicines including vaccines, did include pyrogenic studies, but these were conflicting. In a rabbit model, no difference was found in pyrogenicity of CSL vaccines compared to other vaccines. In a ferret model, however, a significant difference was detected. Reference to these studies has been included in the revised manuscript.

Page 19 line 15 delete "however" (PM)
• The authors would prefer to leave the word “however” in the manuscript.

P19 Line 18 replace "mean" with "suggest" (PM)
• "mean" replaced with "suggest".

P19 Line 20 replace "especially" with" likely to be" (PM)
• "especially" deleted.

P 19 Line 20 sentence starting "Also" could perhaps be cut, there does not seem to be any ranges of figures presented or used. (PM)
• This sentence has been deleted.

Table
Title of table: should more clearly state the number of children in the table. ie. Table 1: Demographic and clinical details of 63 children aged 6 months..." (PM)
• The number “63” has been included in the table title.

VERSION 2 - REVIEW

REVIEWER	***Punam Mangtani***
REVIEW RETURNED	12-Apr-2011

THE STUDY	I thank the authors for the helpful changes made. I would urge however that reference be made to the Jama paper and the latest version of TGA report. Readers may come across them separately and so they are important to note briefly with the caveats the authors note in their reply to the initial comments, a) to see the results of the current study in context in the introduction b) to allow the readers to appropriately consider those references. Results paragraph 2: Could the authors confirm or check with a statistican that the p value for the comparison of the rate of FC with CSL lab vaccines and other vaccines is done taking into account the 0 in one cell. My understanding is that the package used assumes large numbers but exact methods should be used or adding a half case to all cells and mention this. One can of course point to the CI in table 2 which don’t overlap suggesting a significant effect. More important is the difficulty of comparing the rate in this study with the rate in a different (US) population in a different year and different health services. The key messages should be clear what the comparision rate is and where from and not calculate a 200 fold difference. Major edits The changed title shold use the term ‘associated’ rather than ‘caused ‘ given other evidence from other sources not obtained in these presented studies are needed. The article focus second bullet point indicates as number of analytical studies were conducted. Minor edits Introduction para 1 sentence 3:Could the authors be clear what age ranges are recommended ‘flu vaccinations ie >6 months to ? Could the authors note in the methods section what records were available for the classification of febrile convulsions in the 25 those not identified through the hospital system as they mention in their

	reply to an earlier query on table 1. Some confirmation that these were witnessed convulsions or based on history of convulsions would help compare with future research or surveillance activities on AEFIs. If wish to save words the first sentence in para 3 on page 12 ' A number of data ...over this time period' could be deleted. Could the authors note in the legend for table 3 or elsewhere that no data were collected on febrile convulsions associated with the H1N1 pandemic vaccine.

VERSION 2 – AUTHOR RESPONSE

REVIEWER'S COMMENT:I thank the authors for the helpful changes made.
I would urge however that reference be made to the JAMA paper and the latest version of TGA report. Readers may come across them separately and so they are important to note briefly with the caveats the authors note in their reply to the initial comments, a) to see the results of the current study in context in the introduction b) to allow the readers to appropriately consider those references.
AUTHORS' RESPONSE:The JAMA paper mentioned is a reprint from an MMWR paper (with one table omitted), which is now referenced in the revised manuscript. The TGA report was already referenced in the report (reference 17). With respect to the reviewer's comment (in the first review round) that reference should be made to the lack of safety signal to Panvax found by passive surveillance in Australia cited in the TGA report, the authors contend that this need not be included in our paper as the data presented in the TGA report do not seem to support this notion (eg1. the rate of febrile convulsions in young children to Panvax was found to be between 8 and 17 per 100 000 doses, which is considerably higher than the background rate quoted in the report from the Vaccine Safety Datalink Project of 3 per 100 000; eg2 in the quoted cohort study of children receiving Fluvax, Panvax and Influvac, the rate of fever following Panvax was more than double that for Influvac (but less than half that for Fluvax). However, if the editors feel that reference to the safety signal for Panvax must be included, the following sentence could be added as the final sentence of paragraph 1 in the Discussion: "…In the same report, the rate of febrile convulsions following the monovalent H1N1 pandemic influenza vaccine (Panvax) manufactured by CSL Biotherapies, the same manufacturer as Fluvax, was reported to be between 8 and 17 per 100 000 doses (age group and time of event post TIV not stated)….".

COMMENT:Results paragraph 2: Could the authors confirm or check with a statistican that the p value for the comparison of the rate of FC with CSL lab vaccines and other vaccines is done taking into account the 0 in one cell. My understanding is that the package used assumes large numbers but exact methods should be used or adding a half case to all cells and mention this. One can of course point to the CI in table 2 which don't overlap suggesting a significant effect.
RESPONSE: The manuscript has been revised to include the fact that Fisher's exact test was used if expected cell frequencies were <5 (para 1 of the 'Statistical analysis' section of the Methods). The footnote 2 of Table 2 has also been altered to reflect this.

COMMENT: More important is the difficulty of comparing the rate in this study with the rate in a different (US) population in a different year and different health services. The key messages should be clear what the comparision rate is and where from and not calculate a 200 fold difference.
The manuscript has been revised to now simply state the rate estimated from the comparison study from the US, rather than calculating the fold-difference.

Major edits
COMMENT: The changed title should use the term 'associated' rather than 'caused ' given other evidence from other sources not obtained in these presented studies are needed.

RESPONSE: The authors contend that enough of the Bradford-Hill criteria for causality are fulfilled to establish causality i.e., strength of the association (200 times higher than the accepted rate); consistency of findings (similar findings were found across the various states of Australia and in New Zealand); plausibility (vaccines are known to be immunogenic); specificity; experimental evidence (ceasing Fluvax caused the rate to decrease to normal); coherence; temporal association. However, if the Editor feels strongly that the term 'associated with' is preferable to 'caused by' in the title, the authors will accept the change.

COMMENT: The article focus second bullet point indicates as number of analytical studies were conducted.
RESPONSE: The bullet point has been changed to indicate that three separate analytical studies were conducted (ie. (i) comparison of rates of febrile convulsions in 2010 to those from 2008 and 2009; (ii) comparison of rates of febrile convulsions associated with different TIV formulations; (iii) retrospective cohort study comparing rate of side effects of different TIV formulations)

Minor edits
COMMENT: Introduction para 1 sentence 3:Could the authors be clear what age ranges are recommended 'flu vaccinations ie >6 months to ?
RESPONSE: Manuscript has been revised to indicate that in the US children aged 6 months and over are recommended universal TIV (para 1).

COMMENT: Could the authors note in the methods section what records were available for the classification of febrile convulsions in the 25 those not identified through the hospital system as they mention in their reply to an earlier query on table 1. Some confirmation that these were witnessed convulsions or based on history of convulsions would help compare with future research or surveillance activities on AEFIs.
RESPONSE: A description of the methods used to collect case details of the passively-reported cases has now been included in para 6 of the Methods section.

COMMENT: If wish to save words the first sentence in para 3 on page 12 ' A number of data ...over this time period' could be deleted.
RESPONSE: The authors feel that this sentence provides important information as to how other possible causes of the reactions were investigated and would prefer to keep this in the manuscript. One of the initial reviewers (JE) expressed a particular keenness for including this information in the Methods.

COMMENT: Could the authors note in the legend for table 3 or elsewhere that no data were collected on febrile convulsions associated with the H1N1 pandemic vaccine.
RESPONSE: This information has been added to the footnotes of Table 3.