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# **Trivalent influenza vaccine and febrile adverse events in Australia, 2010: Clinical features and potential mechanisms**

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## **Abstract**

### **Introduction**

Increased numbers of children presenting with febrile adverse events following trivalent influenza vaccine (TIV) were noted in Australia in 2010. We describe the epidemiology and

clinical features of the adverse events and explore the biological basis for the adverse events using an in vitro model.

## **Materials and Methods**

Children presenting to a tertiary paediatric hospital in 2010 with adverse events within 72 h of TIV were retrospectively reviewed. Demographics, clinical features, physiological variables and outcomes were examined. Plasma cytokine and chemokine levels were examined in a subgroup of children with vaccine-related febrile convulsions. Peripheral blood mononuclear cells of age-matched children were stimulated with different TIV preparations. Inflammatory cytokine and chemokine analysis was performed on cultured supernatants.

## **Results**

Vaccine-related febrile adverse events were identified in 190 children. Most occurred in healthy children (median age: 1.5 years) within 12 h of vaccination. Twenty-eight (14.7%) required hospital admission. High temperature  $\geq 39.0$  °C (101/190; 53%), vomiting (120/190; 63%) and convulsions (38/190; 20%) were common. All children presenting had received Fluvax<sup>®</sup> or Fluvax Junior<sup>®</sup>.

In the in vitro model, IFN- $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, IP-10 and MIP-1 $\alpha$  levels were significantly higher when measured at 6 and 24 h in cultures stimulated with Fluvax<sup>®</sup> compared with alternative 2010 TIV preparations.

## **Conclusions**

Numerous febrile adverse events (including febrile seizures) were observed following Fluvax<sup>®</sup> or Fluvax Junior<sup>®</sup> in 2010. Clear differences in cytokine production were observed when peripheral blood mononuclear cells were stimulated with Fluvax<sup>®</sup> compared with

alternate TIV preparations. Increased awareness of these potential adverse events is required to ensure earlier detection and prevention in the future.

## **Abbreviations**

TIV, trivalent influenza vaccine; WA, Western Australia; PBMCs, peripheral blood mononuclear cells; ACIR, Australian Childhood Immunisation Register; LPS, lipopolysaccharide; SEB, staphylococcal enterotoxin B; PMH, Princess Margaret Hospital; IQ, interquartile

## **Keywords**

Influenza vaccine; Immunization; Febrile convulsion; Vaccine associated adverse events

## **Introduction**

The increased risk of severe influenza in preschool children<sup>1</sup> has prompted the use of seasonal influenza vaccines in this age group. Routine influenza vaccination of healthy young children using trivalent influenza vaccine (TIV) is recommended in many countries including the United States and Canada.<sup>2 and 3</sup> Large case series have shown this to be well tolerated and rarely associated with serious adverse events including seizures.<sup>4 and 5</sup> Within Australia, Western Australia (WA) is the only state recommending routine immunization of children aged 6 months to 5 years with TIV. Established in 2008, this program has been associated with decreased severity of illness and hospitalizations.<sup>6</sup>

Vaccines from three manufacturers were used in the WA vaccination program in 2010. All vaccines contain the three haemagglutinin antigens recommended by the World Health Organization for the 2010 southern hemisphere influenza season (A/California/7/2009 (H1N1)-like virus, A/Perth/16/2009 (H3N2)-like virus and B/Brisbane/60/2008-like virus).<sup>7</sup> Fluvax<sup>®</sup> (CSL Biotherapies, Parkville, Australia), Influvac<sup>®</sup>, (Solvay SA, Brussels, Belgium) and Vaxigrip<sup>®</sup>, (Sanofi Pasteur, Lyon, France) contain 15 µg of haemagglutinin from each of the three vaccine strains per dose. Children 6 months to 3 years are administered a half dose (0.25 ml) and children three and older are administered an adult dose (0.5 ml). In addition, Fluvax Junior<sup>®</sup> is distributed for children 6 months to 3 years and contain 7.5 µg of haemagglutinin from each of the three vaccine strains per dose. Despite similarities in their composition, differences exist in their manufacturing processes.

These vaccines were made available to vaccine providers from the 8th March 2010. Safety concerns were raised in Australia after an apparent increase in adverse events, particularly in young children. Increased numbers of children presenting with severe febrile reactions post-TIV were noted in April 2010, a number of whom developed febrile convulsions. Investigation of these reports resulted in suspension of the WA preschool influenza vaccination program on the 22 April 2010 and the national influenza immunization program for children less than 5 years of age the following day.<sup>8</sup>

Following suspension of the program, the rates of febrile convulsions in WA children < 5 years following Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> were calculated to be 4.4 convulsions per 1000 doses administered (>14,000 doses administered; Armstrong et al.,<sup>23</sup> Manuscript in review, BMJOnline). No febrile convulsions were observed following administration of

Influvac<sup>®</sup> and Vaxigrip<sup>®</sup> (<5000 doses administered). The rate of febrile convulsions following Fluvax<sup>®</sup>/Fluvax Junior<sup>®</sup> was significantly higher in 2010 than that observed with all TIV preparations in 2009 (0.3 febrile convulsions per 1000 doses administered). Likewise, the rate of adverse events in 2010 following Fluvax<sup>®</sup>/Fluvax Junior<sup>®</sup> were significantly higher compared with the rate of adverse events for all other TIVs.<sup>8</sup>

To date, despite extensive analyses, the biological basis for the excess cases of febrile adverse events after the administration of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> remains unclear.<sup>8</sup> This report explores the biological basis for the reactions by describing the clinical features of children with convulsive and non-convulsive febrile adverse events and examining inflammatory cytokine profiles in a group of children with adverse events. Based on these data, we hypothesise that excessive pyrogenic cytokine production contributed to the adverse events. We explored this hypothesis using an in vitro stimulation model in which peripheral blood mononuclear cells (PBMCs) from young children (12–36 months of age) were challenged with different TIV preparations and cytokine responses assessed.

## **Materials and Methods**

### **Clinical data**

Princess Margaret Hospital is the only tertiary paediatric hospital in the state of WA (population 2.26 million) with more than 65,000 emergency paediatric presentations per year. Admission or discharge diagnoses are available through the WA Emergency Department Information System. Children presenting from the 8th March 2010 to 25th April 2010 with possible immunization-related adverse events were identified retrospectively using ICD-10

diagnostic codes: R56.0 (febrile convulsion), T88.8 (other vaccination complication), R50.9 (febrile illness of unknown origin), B34.9 (viral illness), G40.3 (generalized idiopathic epilepsy and epileptic syndromes), T80.5 (anaphylactic shock due to immunization) and T80.6 (vaccination complicated by allergic reaction).<sup>9</sup>

A vaccine-related febrile adverse reaction was defined as any child presenting within 72 h of TIV with a documented fever ( $>37.5$  °C as recorded by health-care providers or parents) where an alternative source of fever was not identified clinically and/or microbiologically. Febrile convulsions were identified using published definitions.<sup>10</sup> All adverse events were reviewed by one author (CCB) to ensure consistency of data collection.

Medical records of all children identified were reviewed. Receipt of TIV in the previous 72 h was confirmed by examining the Australian Childhood Immunization Register (ACIR) and case notes. Demographics, symptoms, physiological parameters, results of any investigations performed, treatment and outcome data were recorded. Where clinical data and immunization status were uncertain, parents and/or immunization providers were contacted.

### **Immunological experiments**

Clinical samples were routinely stored by our pathology service at 4–8 °C for 7 days following collection. Plasma samples were collected into tubes containing lithium heparin (BD Vacutainer<sup>®</sup>, Becton Dickinson, Franklin Lakes, NJ) and centrifuged as per manufactures instructions. Following suspension of the program, any remaining plasma

samples from children sustaining vaccine-related febrile adverse events were identified and stored at  $-20^{\circ}\text{C}$ .

IL-6, IL-10, IP-10, MIP1 $\alpha$ , TNF $\alpha$  and IFN $\gamma$  levels in samples were determined using an in-house multiplex bead-based assay. In brief, primary antibodies (IL-6, IL-10, IP-10, MIP1 $\alpha$ , and IFN $\gamma$ : Becton Dickinson; TNF $\alpha$ : BioScientific Pty Ltd, Australia) were covalently conjugated to carboxylated microspheres (Bio-Rad Laboratories Inc, Hercules, CA). Samples were diluted in PBS/0.05% Tween/2% FCS. Microspheres and samples were incubated at room temperature on an orbital shaker. After 30 min, biotinylated secondary antibodies were added for another 30 min. After washing (PBS, 1% BSA, 0.05% Tween, 0.001% Sodium azide), streptavidin-PE conjugate (Becton Dickenson) was added for 15 min. Samples were washed again and fluorescence in each specific bead region was measured on the BioPlex<sup>®</sup> 200 System (Bio-Rad). Data was acquired electronically in real-time and analysed using BioPlex Manager 5.0 software. Data in pg/ml was generated from a 5-PL standard curve of median fluorescent intensity against a standard curve of recombinant cytokines. IL-1 $\beta$ , IL-8 and IFN $\alpha$  levels were measured using a commercially available ELISA Kit (Bender MedSystems, eBioscience, Inc, San Diego, CA) according to the manufacturer's instructions.

No baseline values for cytokine levels in plasma from healthy children have been reported for this age group. Cytokine levels from clinical samples were therefore compared with baseline 'normal' values established in plasma from healthy preschool children participating in a study investigating ear disease.



## **Potential mechanisms for the reaction were explored using an in vitro model**

PBMCs ( $1.25 \times 10^5$ ) from 22 donors aged <36 months were cultured in 125  $\mu$ l of RPMI 1640 Medium + l-Glutamine (Invitrogen, Victoria, Australia) with 10% foetal calf serum (SAFC-Biosciences, Victoria, Australia) and stimulated with either 12.5  $\mu$ l of neat Fluvax<sup>®</sup> 2010 (CSL Biotherapies, Parkville, Australia), Influvac<sup>®</sup> (Solvay SA, Brussels, Belgium), Vaxigrip<sup>®</sup> (Sanofi Pasteur, Lyon, France), lipopolysaccharide (LPS; 1 ng/ml) or *Staphylococcal* enterotoxin B (SEB; 1  $\mu$ g/ml) and incubated at 37 °C/5% CO<sub>2</sub>. Culture supernatants were collected after 6 or 24 h and stored at -20 °C until cytokine analysis was performed (as above). Responses to further dilutions (up to 1:1250) of Fluvax<sup>®</sup> 2009 and 2010 were also examined.

## **Statistics**

Data were analysed using SPSS version 16.0.0 (SPSS Inc., Chicago, IL). Categorical variables were compared with  $\chi^2$  using  $2 \times 2$  contingency tables. Continuous variables were compared using Student's *t*-test or paired *t* test with logarithmic transformation where appropriate.

## **Ethics**

Ethics approval for chart audit was obtained as part of the public health investigation (GEKO:2204-04/10). Following review by the Ethics Committee of Princess Margaret Hospital (PMH) for Children, Perth, WA, consent was obtained from parents to perform cytokine studies on stored samples. PBMC collection was approved by PMH Ethics Committee as part of a study of immunity in ear disease (1205/EP).

## Results

A total of 745 children were identified by the initial ICD-10 code search. Of these, 205 had received TIV within 72 h. Children aged >5 years ( $n = 12$ ) or children in whom an alternative source of fever was identified ( $n = 3$ ) were excluded leaving 190 children presenting to Princess Margaret Hospital with febrile adverse events attributable to TIV. This was significantly greater than in previous years (see online supplement; figure A) and included 38 children with febrile convulsions (20.0%). All children presenting with vaccine-related febrile adverse events had received Fluvax<sup>®</sup> or Fluvax Junior<sup>®</sup>.

Adverse events were most frequently observed in children  $\leq 2$  years of age (Table 1). Most children had no pre-morbid conditions and 69.1% received their first influenza vaccination. TIV was administered without other vaccines in 85% of cases. High temperature ( $>39.0$  °C) and vomiting were most frequently observed. More than 96% of reactions occurred within 12 h of immunization: median time from immunization to symptom onset was 6.25 h (interquartile [IQ] range: 5.25–7.5 h); median time from immunization to emergency presentation was 9.95 h (IQ range: 7.8–12.0 h).

Twenty-eight children (14.7%) required hospital admission including eight who developed hemodynamic instability requiring fluid bolus resuscitation. Thirty-five of 38 children with seizures (92.1%) had tonic–clonic seizures. The median length of seizure was 2.5 min (range: <1–103 min; IQ range: 1.25–6 min). Nine children had recurrent seizure during the febrile episode, including two children with known seizure disorders and a further child with recurrent febrile seizures. Two subjects, both infants, had status epilepticus requiring

intensive care admission: one of these two children sustained an irreversible neurological injury with Cranial CT and MRI scans demonstrating changes consistent with hypoxic-ischaemic encephalopathy.

To exclude vaccine-related neurological or metabolic toxicities as a potential explanation for the convulsions observed, vaccine-related ( $n = 38$ ) and non-vaccine-related febrile convulsions ( $n = 39$ ) were compared during the same period. No significant differences were observed in mean age (1.5 vs. 1.8 years), mean peak temperature (38.8 °C vs. 38.4 °C), median length of seizure (2.75 vs. 2 min), requirement for anti-convulsant therapy (10.5% vs. 5.1%) and intensive care admission (5.3% vs. 2.6%). Respiratory symptoms were less frequent in vaccine-related febrile convulsions (cough: 7.9% vs. 38.5%,  $p < 0.007$  and rhinorrhoea: 15.8% vs. 46.2%,  $p < 0.003$ ) yet vomiting was more frequent (55.3% vs. 30.8%,  $p < 0.03$ ).

Plasma samples from six subjects with vaccine-related febrile convulsions were available upon suspension of the program (supplemental table A). All plasma samples were collected within 27 h of immunization; four samples were taken within 60 min of febrile convulsion. None of the children had had previous febrile convulsions. One child had a seizure disorder and five of six subjects had no predisposing medical conditions. Only one child received other vaccines at the time of TIV. When measured, IP-10 (CXCL10) and macrophage inflammatory protein (MIP) 1 $\alpha$  were significantly higher in plasma from subjects compared with normal values ( $p < 0.0001$  and  $p = 0.02$ , respectively). A trend towards higher plasma levels of the pyrogenic cytokines IFN $\alpha$ , IL-1 $\beta$  and IL-6 and regulatory cytokine IL-10 in

subjects was observed (Fig. 1). No differences were observed in TNF $\alpha$ , IL-8 and IFN $\gamma$  levels (data not shown).

In the in vitro vaccine PBMC stimulation model, IFN- $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, IP-10 and MIP-1 $\alpha$  levels were all significantly higher in cultures stimulated for 6 h with Fluvax<sup>®</sup> compared to those stimulated with Influvac<sup>®</sup> or Vaxigrip<sup>®</sup> (Fig. 2), with the levels further increased after 24 h (data not shown). A partial response to Influvac<sup>®</sup> was observed for IL-6 and MIP-1 $\alpha$  in some cultures ( $p = 0.051$  and  $0.0025$  compared to Vaxigrip), yet levels were half, to one-tenth that seen with Fluvax<sup>®</sup> in the same cultures at 6 h. TNF- $\alpha$  production was detected after Fluvax<sup>®</sup> stimulation in only in 14 of 22 cultures with relatively low levels that did not increase significantly from 6 to 24 h (mean: 44 pg/ml and 56 pg/ml respectively). IFN- $\gamma$  was low/undetectable after 6 or 24 h vaccine stimulation but was significantly induced by SEB (data not shown).

## **Discussion**

Safety of TIV has been demonstrated following immunization of preschool children in numerous populations.<sup>4, 11 and 12</sup> Investigations following suspension of the Australian 2010 TIV program demonstrate an increased number of vaccine-related febrile convulsions and non-convulsive vaccine-related febrile adverse events in children <5 years, significantly more than previously experienced.<sup>8</sup> This was associated with the administration of the 2010 formulation of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup>. This is the first report to describe the clinical characteristics of the convulsive and non-convulsive adverse events and explore biological mechanisms for the observed events.

The clinical features of the adverse events were extremely consistent in all children, suggesting a common aetiology. A rapid onset of fever and vomiting were seen in most children. This would appear to be related to the specific preparation (2010 Fluvax<sup>®</sup>/Fluvax Junior<sup>®</sup> vaccines), rather than the vaccine components used, given the lack of severe febrile events associated with Influvac<sup>®</sup> and Vaxigrip<sup>®</sup> vaccines in Australia and New Zealand.<sup>8 and 13</sup> No significant differences in febrile events were observed between batches of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> vaccines,<sup>8</sup> suggesting that any changes linked to adverse events were upstream of packaging and distribution. The clinical features of febrile convulsions following Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> were similar to non-vaccine-related febrile convulsions. This, and the rapid recovery observed after onset, supports the theory that convulsions were secondary to high fever in predisposed children rather than any vaccine-related neurological or metabolic toxicities.

Despite extensive investigations to date, no cause has been identified for the increased number of adverse events with Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup>.<sup>8</sup> No abnormalities in pharmacopoeial parameters (endotoxin; potency) have been identified in samples of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup>. Further testing has failed to identify whole virions, viable virus (cell culture), ribonucleic acid or contamination in Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup>.<sup>8</sup>

Increased pyrogenic cytokines were present in plasma of a small subset of children sustaining febrile adverse events compared with plasma levels in healthy age-matched controls. These findings compelled us to explore whether rapid, cytokine driven, febrile reactions to Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> were responsible for the adverse events observed. Higher

immunostimulatory properties of Fluvax<sup>®</sup> were evident when compared to the other TIV preparations in our in vitro model, even after only 6 h of stimulation.

The cytokine profile in response to Fluvax<sup>®</sup>/Fluvax Junior<sup>®</sup> is noteworthy. Significant type I IFN responses were noted (IP-10; IFN $\alpha$ ), as were the production of other pyrogenic cytokines such as IL-6 and IL-1 $\beta$ . In contrast to the stimulation pattern observed with LPS, significant induction of TNF- $\alpha$  was not observed, providing further evidence that endotoxin contamination was not responsible for the vaccine adverse events. Instead, the Fluvax<sup>®</sup> cytokine profile resembles that observed during in vitro activation with inactivated influenza A virus<sup>14</sup> and in the early/innate response to natural influenza infection, where IFN $\alpha$  and IL-6 levels are closely associated with peak temperature and respiratory symptoms.<sup>15 and 16</sup> This may suggest that the formulation of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> 2010 contains innate immune-activating components that resemble, or are derived from influenza virions. In this regard, we detected significantly higher levels of the chemokine IP-10 (CXCL10) in cases. IP-10 has been implicated in immune response to several viruses, including influenza A<sup>14</sup>, and was recently shown to be a useful biomarker of respiratory infection itself.<sup>17</sup> The chemokine also serves as a useful biomarker of human IFN- $\beta$  responses and strongly correlates with febrile responses in multiple sclerosis patients treated with recombinant IFN- $\beta$ .<sup>18</sup>

Elevated pyrogenic cytokine responses have been previously associated with febrile seizure events. IFN $\alpha$  levels were significantly higher in children with confirmed influenza who developed febrile seizures compared with those without seizures.<sup>16</sup> Seizures have also been

reported in recipients of therapeutic IFN $\alpha$ , particularly children.<sup>19, 20 and 21</sup> This possible mechanism warrants further investigation.

Based on the available data, we postulate that the adverse events observed were related to a rapidly induced (within 6 h) viral-like, pyrogenic cytokine response. The recent findings by Osterlund et al., demonstrating that influenza A/H1N109 is a relatively weak inducer of type I interferons, would indicate that this pyrogenic response is not due to the inclusion of the pandemic strain *per se*.<sup>22</sup> Indeed, we found, perhaps somewhat surprisingly, that the 2009 formulation of Fluvax<sup>®</sup>, was also equally capable of inducing a type I IFN pyrogenic response in vitro. Notably, the 2009 vaccine program was not associated with a significant increase in adverse events. This might suggest that vaccine-induced pyrogenic cytokine responses alone are not sufficient to trigger severe adverse events like those seen in 2010. It should be noted that although our in vitro model tested responses in subjects in the age-group at most-risk of adverse events, it did not specifically include samples from those with a history of vaccine-related febrile responses or seizures. Clearly, the epidemiology data demonstrates that there are individual differences in the risk of vaccine-related adverse events. Testing of in vitro vaccine responses in those subjects most prone to adverse febrile outcomes may reveal threshold effects in pyrogenic cytokine production. Alternatively, vaccine storage for more than a year may lead to protein aggregates thereby enhancing the innate immune response of the 2009 vaccine. Nevertheless these data suggest that the TIV component that led to the innate response relates to the methods of vaccine preparation and has been present in previous Fluvax<sup>®</sup> preparations.

The retrospective nature and difficulty in using diagnostic coding data may have limited our ability to identify all cases requiring hospital presentation. Clinical data were collected from case notes limiting the volume of data collected. As many children with fever and/or febrile convulsions did not undergo diagnostic investigations, it is possible that a proportion of presenting children had undiagnosed infections. The lack of symptoms or signs of an infectious cause suggests that the proportion would be small. Plasma from a small number of children with febrile convulsions were collected as part of clinical care and stored for up to 1 week prior to retrieval. It is possible that cytokine levels degraded during storage and that measured levels may be inappropriately low. Due to this, differences between case and normal values may have been potentially underestimated in our study. It is probable that comparing in vivo cytokine and chemokine levels with healthy age-matched controls may exaggerate any identified differences. These were used as true age-matched controls (i.e. those who have received an alternative vaccine or those who received Fluvax<sup>®</sup>/Fluvax Junior<sup>®</sup> without adverse events) were not available for analysis due to the suspension of the program.

## **Conclusions**

A significant number of febrile adverse events were observed in preschool children administered Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup> in 2010. The clinical features of these events have not been previously described and pathogenesis not determined. It is likely that a component(s) of the vaccine induced the fever and seizures in young children. These components induced a potent pyrogenic response which was rapid and distinct from contaminants such as LPS. Further research is underway in our laboratory to identify the immunostimulatory components of Fluvax<sup>®</sup> and Fluvax Junior<sup>®</sup>.



This research demonstrates the clear differences between preparations of TIV. It is possible that these are related to differences in the manufacturing process. This investigation highlights the importance of ongoing global safety monitoring for all TIV preparations in all age groups. This needs to be performed annually given the changing formulation of influenza vaccines. Increased awareness amongst manufacturers, immunization providers, family practitioners and emergency physicians of potential adverse events following TIV is required to ensure earlier detection and prevention of significant febrile adverse events in the future.

### **Declaration**

All authors were involved in the investigation, collection and analysis of data. All authors were involved in the drafting and revision of the manuscript.

All authors have approved the final version of the article to be submitted.

### **Conflicts of interest**

CCB, AJC, SPW, NC, LASK, AF and PCR are members of the Vaccine Trials Group, Telethon Institute for Child Health Research. The Vaccine Trials Group has received funding for clinical trials from vaccine providers including CSL Biotherapies and Sanofi Pasteur. PCR also reports previously being a member of a CSL Limited vaccine advisory board and receiving an honorarium. No industry funding was received to perform this public health investigation into TIV related adverse events.

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Table 1. Trivalent influenza vaccine (TIV) related febrile convulsions and non-convulsive vaccine-related febrile adverse events.

	Vaccine related febrile convulsions (n = 38)	Non-convulsive vaccine related febrile adverse events (n = 152)	Total vaccine related febrile adverse events (n = 190)
<i>Demographics and background</i>			
Median age in years (interquartile range)	1.49 (1.0–2.5 years)	1.44 (0.9–2.5 years)	1.46 (1.0–2.5 years)
Male: Sex (%)	25 (65.8%)	92 (60.5%)	117 (62.9%)
Chronic medical condition	9 (23.7%)	24 (15.8%)	33 (17.4%)
Prematurity	5	17	22
Neurological condition*	5 <sup>†</sup>	4 <sup>†</sup>	9 <sup>†</sup>
Chronic respiratory condition	2	10	12
Chronic cardiac condition	2	2	4
<i>Immunization</i>			
Other vaccines given with TIV	9 (23.7%)	20 (13.2%)	29 (15.3%)
First TIV vaccine	17/26 (65.4%)	48/68 (70.6%)	65/94 (69.1%)
<i>Clinical features</i>			
Mean peak temperature (95% CI)	39.1 °C (38.9–39.4)	38.9 °C (38.8–39.1) <sup>‡</sup>	39.0 °C (38.9–39.1)
Peak temperature ≥ 39.0 °C	24 (63.2%)	77 (50.7%)	101 (53.2%)
Vomiting	21 (55.3%)	99 (65.1%)	120 (63.2%)
Other gastrointestinal symptoms	3 (7.9%)	18 (11.8%)	21 (11.1%)
Respiratory symptoms including cough, rhinorrhoea, pharyngitis	9 (23.7%)	44 (28.9%)	53 (27.9%)
Rash	1 (2.6%)	10 (6.6%)	11 (5.8%)
<i>Outcomes</i>			
Admitted to hospital	20 (52.6%) <sup>#</sup>	8 (5.3%) <sup>#</sup>	28 (14.7%) <sup>#</sup>
Overnight admission	4	4	8
ICU admission	2	0	2

\* Not including a past history of febrile seizures (6/37; 16.2% had a past history of febrile seizures).

† Neurological conditions more frequent in those with febrile seizures compared with those without febrile seizures ( $p < 0.02$ ).

‡ Rigors observed in 49 (32.2%) with non-convulsive adverse events.

# Admission more frequent following febrile convulsion compared with those without febrile seizures ( $p < 0.0001$ ).

Fig. 1. Cytokine and chemokine levels in plasma from 6 subjects (clinical data available in supplemental table A) following febrile convulsions compared with plasma from healthy age-matched controls (mean, standard error of the mean and p value where significant).

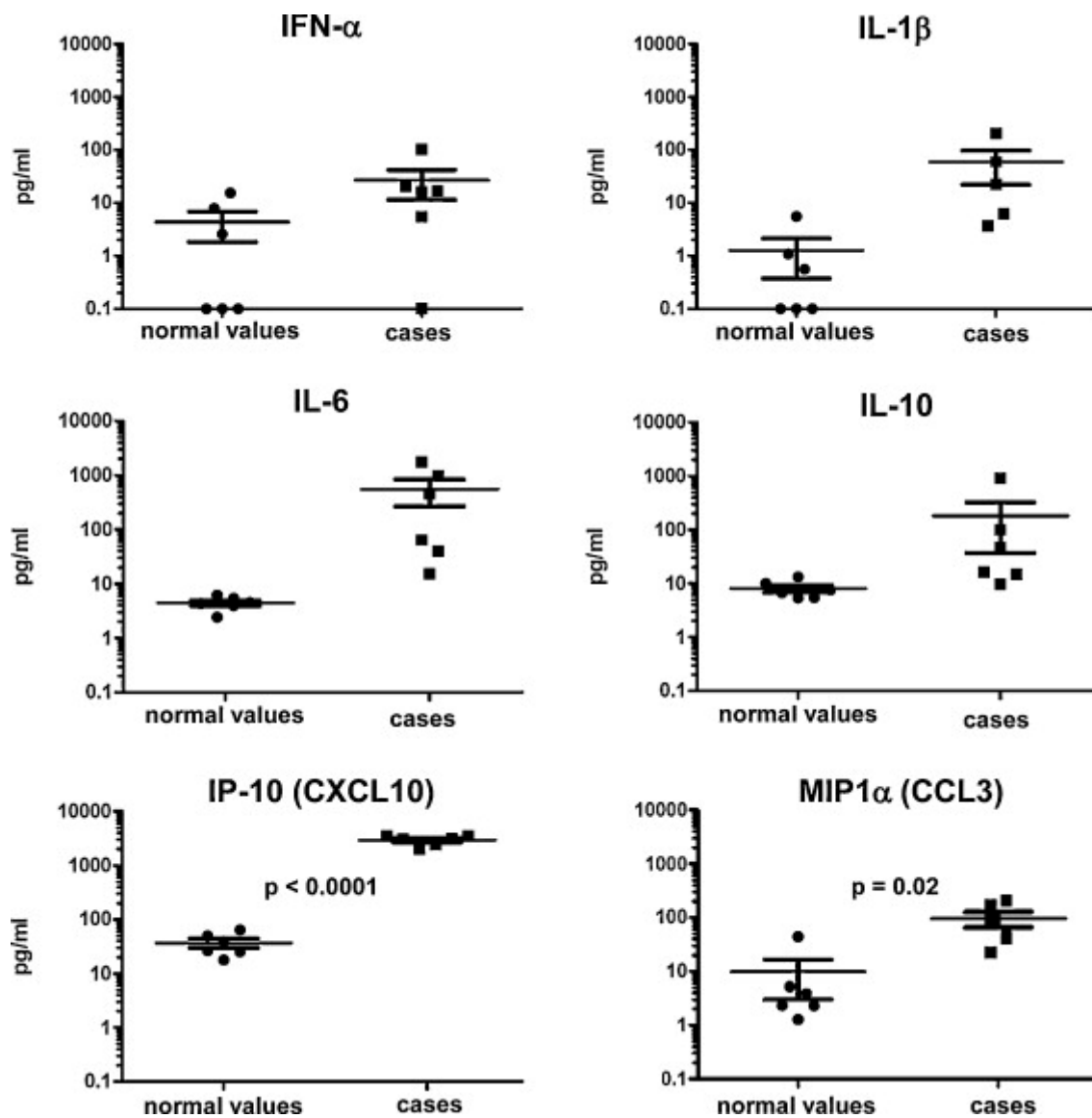


Fig. 2. Cytokine and chemokine levels after 6 h vaccine stimulation of infant/child PBMCs ( $n = 22$ ). Data show individual responses along with mean, standard error of the mean and  $p$  value, comparing responses between the three TIVs. Responses to LPS (innate immune agonist) and SEB (mitogen) from the same subjects are shown for comparison.

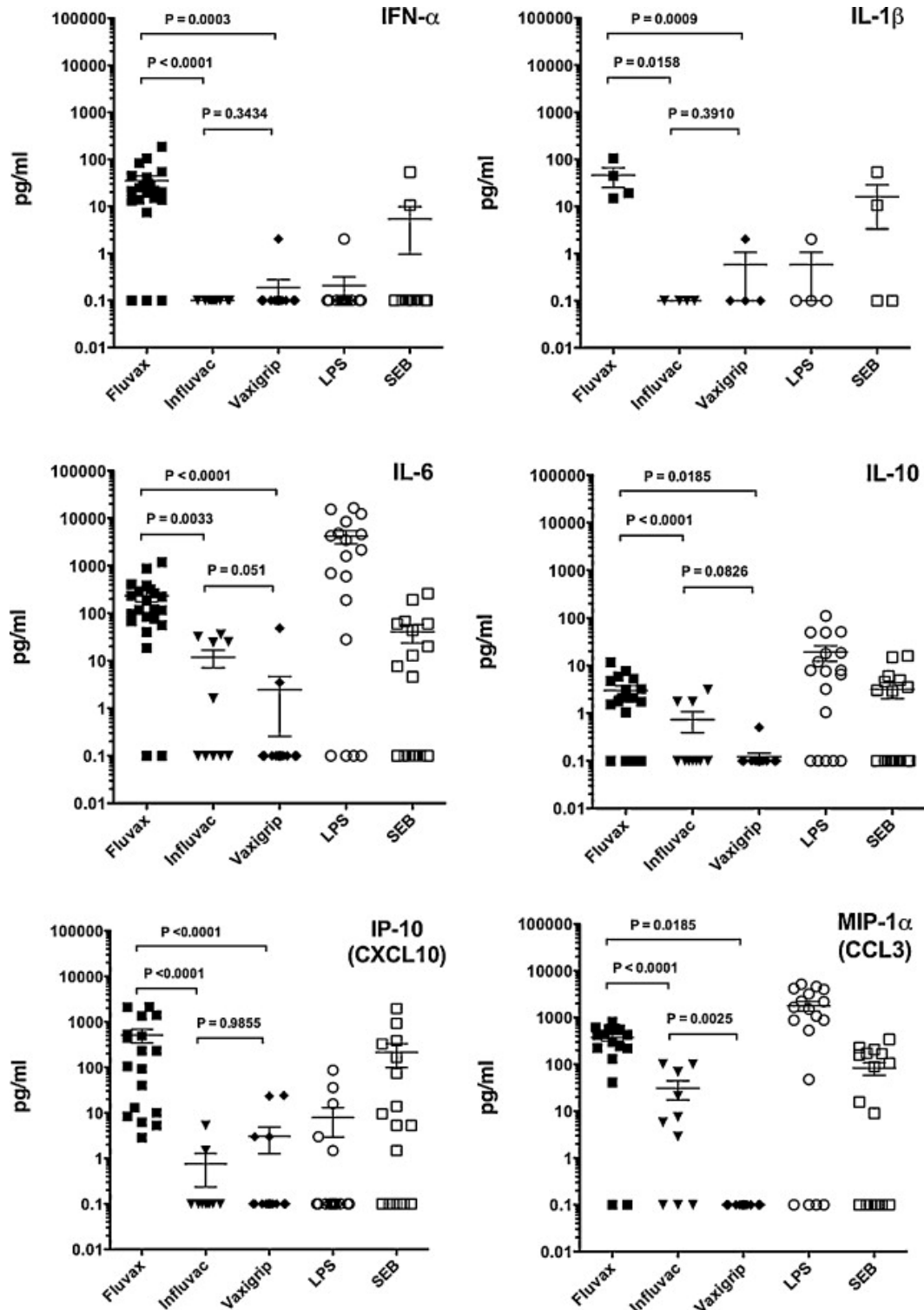


Fig. 3. *In-vitro* stimulatory potency of the influenza A/H1N109-containing (2010) and influenza A/H1N109-negative (2009) formulations of Fluvax<sup>®</sup>. Data show mean IL-6 and IP-10 responses (6 h)  $\pm$  standard error of the mean ( $n = 22$ ).

