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ABCB1 polymorphisms and neuropsychiatric adverse events in oseltamivir-treated children during influenza H1N1/09 pandemic

Aims: To examine the safety profile of oseltamivir in children and evaluate the impact of P-glycoprotein polymorphisms on the incidence of neuropsychiatric adverse events (NPAE) in oseltamivir-treated children. **Subjects & methods:** This prospective cohort study was conducted in our tertiary care pediatric hospital (University Hospitals of Geneva, Switzerland) during the H1N1 pandemic, between 1 October 2009 and 31 January 2010. All newborn to 18 year-old patients presenting at the emergency department with a flu-like illness were eligible for inclusion. Adverse events were systematically recorded by pediatricians and/or by parents at home using a diary card, with a 30-day follow-up period. The causality assessment of oseltamivir in NPAE was performed by two clinical pharmacologists. After informed consent, enrolled patients were also genotyped for *ABCB1* 3435C>T (rs1045642) and 2677G>T/A (rs2032582) polymorphisms. **Results:** Among the 42 H1N1-infected, oseltamivir-treated children who were genotyped for *ABCB1* 3435C>T and 2677G>T/A variants, 36% presented NPAE. When examining the association between the diplotype and the development of NPAE, we observed that the frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends. A total of 11% of the 2677GG–3435CC individuals (wild-type homozygous) presented NPAE, compared with 39% of the individuals being heterozygous for at least one variant allele and 67% of the 2677TT–3435TT individuals (homozygous variants) ($p = 0.149$, nonsignificant). **Conclusion:** These observations suggest a potential influence of *ABCB1* polymorphisms in oseltamivir-related NPAE, maybe as a result of an enhanced permeability of the blood–brain barrier to oseltamivir.

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KEYWORDS: adverse events ■ anti-infective ■ pediatrics ■ pharmacogenetics ■ psychiatric

A new human H1N1 influenza virus first appeared in Mexico in the spring of 2009 [1] and a Phase 6 pandemic was declared by the WHO on 11 June of the same year. In Switzerland the epidemic threshold was reached during week 43, peaked at week 49, and lasted until the end of March 2010.

According to national recommendations published in August 2009 [101], oseltamivir, an inhibitor of neuraminidase, was used to treat potentially infected patients at risk of complications (including infants under 12 months), those with severe symptoms or those in close contact with at-risk patients [102]. Oseltamivir is a pro-drug that is readily absorbed from the GI tract and extensively converted to the active metabolite oseltamivir carboxylate (OC) by hepatic carboxylesterases [2]. Two recent reviews summarize the current knowledge of the pharmacokinetic characteristics of oseltamivir [3,4], one of them focusing on pediatric data [4]. The pharmacokinetics of oseltamivir carboxylate after oral administration of oseltamivir is characterized by mean

bioavailability of 79% [3]. The extent of exposure has been shown to be reduced when oseltamivir was dissolved in milk. It is therefore plausible that the relative peroral bioavailability of oseltamivir is reduced in neonates and young infants receiving regular feeds with human milk and/or milk-based infant formulas compared with adults [4]. OC is largely eliminated through the kidneys. Approximately 60–70% of an oral dose appears in the urine as the active metabolite, and less than 5% as oseltamivir. Values reported for the apparent oral clearance of the renally eliminated OC are in excess of glomerular filtration, suggesting that elimination occurs via filtration and active tubular secretion. The average elimination half-life of OC varied around 7.4 h [3]. Published pharmacokinetic data in children show a reduced bioavailability and an expanded distribution volume in neonates and young infants coupled with enhanced renal clearance in infants and young children. The half-life of OC appears to demonstrate a degree of age-dependence (<2 years: 14.9 h; 3–5 years: 11.3 h 13–18 years: 8.1 h) [4].

Arnaud G L'Huillier*,
Kuntheavy Ing
Lorenzini*, Pierre-Alex
Crisinel, Michela C
Rebsamen, Joel Fluss,
Christian M Korff, Remy
P Barbe, Claire-Anne
Siegrist, Pierre Dayer,
Klara M Posfay-Barbe,
Jules A Desmeules[†]
& The H1N1 Pediatric
Epidemiology Study
Group of Geneva

[†]Author for correspondence:
Geneva Medical Faculty & University
Hospitals of Geneva, Division of
Clinical Pharmacology & Toxicology,
University Hospitals of Geneva, Rue
Gabrielle-Perret-Gentil 4, 1211 Genève
14, Switzerland

Tel.: +41 223 829 942

Fax: +41 223 829 945

jules.desmeules@hcuge.ch

*Authors contributed equally

For a full list of affiliations please see
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Oseltamivir was generally well-tolerated during clinical studies involving children, the most frequently reported adverse event being vomiting (approximately 15% of children) [5]. The majority of reports of neuropsychiatric adverse events (NPAE) (including delirium, convulsions and encephalitis) in children taking oseltamivir have mainly originated from Japan [6]. The precise mechanism is not known; the events could be caused by oseltamivir, OC, or by the disease itself [7].

Animal studies have demonstrated that oseltamivir penetrates through the blood–brain barrier, but that P-glycoprotein (P-gp) limits its uptake in the brain [8,9]. Indeed, a pharmacokinetic study in healthy human volunteers showed a low penetration of oseltamivir into the cerebrospinal fluid, as well as of OC [10]. P-gp, the encoded product of the human *ABCBI* (*MDR1*) gene, is a membranous transporter, which is expressed at the luminal membrane in brain capillaries [11] and which presents several genetic SNPs. Variants in exons 26 (3435C>T) and 21 (2677G>T/A) are the most extensively studied and are associated with differences in expression and/or function [12,13]. *In vitro* and *in vivo* studies on P-gp over-expressing cells and on MDR1a/1b knock-out mice showed that oseltamivir, but not OC, is a substrate of P-gp [8,9]. The carboxylate metabolite has been shown to be a substrate MRP4/*ABCC4* and of the OAT3/*SLC22A8*. The MRP4 is an ATP-binding cassette transporter localized in brain capillary endothelial cells that accepts anionic drugs as substrates. The OAT3 is a transporter which is expressed on the abluminal membrane of the brain capillary endothelial cells in rodents, but its functional importance at the human blood–brain barrier has not yet been established. The OAT3 may act in the efflux of OC from inside the endothelial cells into the brain, as well as in its uptake from the brain to the endothelial cells (bidirectional transport) [14]. Moreover, the transporters MRP4 and OAT3, as well as OAT1, play a role in the renal tubular secretion of OC [3]. The interaction with MRP4, in conjunction with the hydrophilic property of OC results in a low penetration of OC into the CNS. However, given this low permeability, OC formed from oseltamivir by cerebral carboxylesterase could accumulate in the brain. Nonetheless, this conversion of oseltamivir to OC in the brain seems to be limited compared with the liver [15].

During the H1N1 pandemic, we conducted a prospective cohort study in children. Our study was part of a larger study, which concerned 109 patients during the H1N1 pandemic and

aimed to compare the clinical presentation of febrile respiratory tract infections in H1N1/09 positive and negative patients [16]. The primary objective of our study was to examine the safety profile of oseltamivir in the pediatric population, with a focus on NPAE. Our secondary objective was to assess if the presence of *ABCBI* 3435C>T and 2677G>T/A variants could increase the risk of NPAE in oseltamivir-treated children.

Subjects & methods

The protocol of this cohort study was approved by the ethics committee of our institution (protocol number: 09-192). The trial was registered prior to patient enrolment at ClinicalTrials.gov (clinicaltrials.gov identifier: NCT01022931) and conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice and Swiss regulatory requirements. Written informed consent was obtained from a parent, and the child whenever possible, prior to participation. From 1 October 2009 to 31 January 2010, newborn to 18 year-old patients presenting at the emergency department of our tertiary care pediatric hospital (University Hospitals of Geneva, Switzerland) with a flu-like illness were eligible for inclusion in the study, according to national recommendations for usage of oseltamivir. Demographical and clinical data were recorded in a standardized questionnaire. Oseltamivir (Tamiflu®, Roche, Reinach, Switzerland) was prescribed at an oral dose of 2–3 mg/kg for infants younger than 12 months, and 30–75 mg (depending on the weight) for older children, twice a day for 5 days, if H1N1 infection was confirmed by PCR. Oseltamivir was stopped in the case of a negative PCR result. Adverse events were systematically recorded, regardless of severity, either by pediatricians during hospitalization, and/or by parents at home using a diary card, with a 30-day follow-up period. To collect the data, parents were systematically contacted by telephone at days 2 and 7, and seen again at day 30 after oseltamivir treatment onset. NPAE (seizures, agitation, irritability, behavior modifications, nightmares, hallucinations, perception disorders and sleeping disorders) were actively elicited using a standardized screening questionnaire, the Safety Monitoring Uniform Report Form developed by the National Institute of Mental Health-sponsored network of the Research Units on Pediatric Psychopharmacology [17], adapted by child psychiatrists and neurologists from our institution. Other recorded adverse events were systematically searched for: gastrointestinal

(nausea, vomiting and diarrhea) and cutaneous (rash and toxidermia). For each adverse event, including NPAE, the following information was recorded: onset, duration, outcome (resolved, persistent, sequelae and death), severity (mild, moderate or severe). The causality assessment of oseltamivir in NPAE was performed by two clinical pharmacologists, using the WHO-Uppsala Monitoring Centre (UMC) system [103]. The clinical pharmacologists were not blinded to the genetic data (*ABCBI* genotype). However, the genetic data were provided by the laboratory once the causality assessment of oseltamivir in adverse events had already been performed.

ABCBI genotyping was performed in our center's toxicogenetic and molecular clinical chemistry laboratory. Genomic DNA was extracted from whole blood (200 µl) using the QIAamp DNA blood mini kit (Qiagen, Hombrechtikon, Switzerland). *ABCBI* 3435C>T (rs1045642) and 2677G>T/A (rs2032582) polymorphisms were determined in a single multiplex PCR, with fluorescent probe melting temperature analysis on a LightCycler (Roche, Rotkreuz, Switzerland) as previously described [18]. Pairwise linkage disequilibrium analysis was calculated from estimated haplotype frequencies using the SHEsis software [19]. No other genetic polymorphisms were investigated in the study participants.

The number of patients presenting to our hospital during the study period determined the sample size. Given the setting of our study, an *a priori* calculation of sample size was not performed, as the number of children presenting with a flu-like illness could not be planned.

The primary outcome was the systematic assessment of adverse effects, with a focus on NPAE. The secondary outcome was the association between the development of NPAE defined as possibly related to oseltamivir by the clinical pharmacologists and the presence of *ABCBI* 3435C>T and 2677G>T/A variants. We also assessed for a correlation between the development of NPAE, age and gender.

Standard descriptive statistics were used. For group comparison, χ^2 or Fisher's test was used in the case of binary outcomes. p-values < 0.05 were considered statistically significant. Statistical analyses were performed with SPSS statistical software (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 109 patients were enrolled in the cohort study [16], from which our study was a part of (median age of 7.02 years old, interquartile range

[IQR] of 6.99). A total of 69% (75/109) had H1N1 disease confirmed by PCR, of which 72% (54/75) were treated with oseltamivir. Thus, 54 patients were in our study. Among oseltamivir-treated patients, 96% (52/54) responded to our 30-day follow-up questionnaire and 78% (42/54) had a genetic analysis. FIGURE 1 illustrates the study flowchart. The two patients who did not respond to the questionnaire did not have a genetic analysis. Twelve patients had no genetic analysis owing to parental refusal. Demographics are described in TABLE 1. Oseltamivir-treated patients were older (p = 0.022), had more risk factors (p = 0.001) and were more frequently receiving antibiotics (p = 0.018) than untreated patients. Risk factors among the oseltamivir-treated patients were asthma or wheezing history in seven, immunosuppression in ten, sickle cell disease in three, cerebral palsy in three and prematurity in one patient. One patient stopped oseltamivir treatment after four days because of adverse gastrointestinal discomfort.

The distribution of *ABCBI* 3435C>T and 2677G>T/A genotypes is described in TABLE 2. In two patients, the 3435C>T genotype could

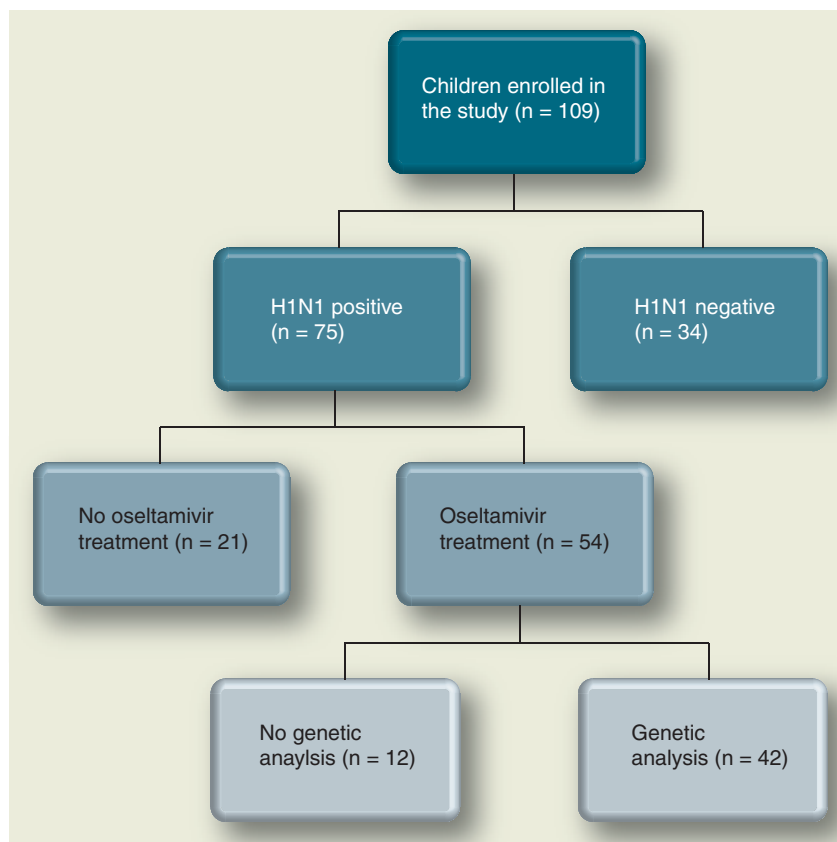


Figure 1. Enrollment of study subjects. Children presenting with a flu-like illness were included in the cohort study. H1N1 positive children who were treated with oseltamivir according to the Swiss recommendations were proposed to participate in the genetic study.

Table 1. Demographics of H1N1-positive patients, according to oseltamivir treatment.

Characteristic	Total (n = 75)	Oseltamivir treatment (n = 54)	No oseltamivir treatment (n = 21)	p-value
Median age, years (IQR)	8.2 (5.9)	9 (7.1)	7.2 (6.1)	0.022
Female gender, % (n)	47 (35)	46 (25)	48 (10)	NS
Caucasian ethnicity, % (n)	73 (55)	72 (39)	76 (16)	NS
Risk factor, % (n)	33 (25)	44 (24)	5 (1)	0.001
Median delay of consultation, days (IQR)	1 (1)	1 (1)	2 (4.5)	NS
Comedication with paracetamol, % (n)	60 (45)	61 (33)	57 (12)	NS
Comedication with nonsteroidal anti-inflammatory drugs, % (n)	37 (28)	41 (22)	29 (6)	NS
Comedication with antibiotics, % (n)	16(12)	22 (12)	0 (0)	0.018

IQR: Interquartile range; NS: Nonsignificant.

not be determined with the PCR method used in our laboratory. A strong linkage disequilibrium was observed between 3435C>T and 2677G>T/A ($D' = 0.916$, coefficient of linkage disequilibrium).

The distribution of adverse events, including NPAE, was similar whether the ten patients without genetic analysis were included or not. Thus, only NPAE in patients with genetic analyses are described (TABLE 3). A total of 25 out of the 42 patients (60%) reported adverse events with oseltamivir treatment, and 36% (15/42) of the patients presented NPAE, possibly related to oseltamivir. A patient could present several NPAE; indeed three patients presented more than one NPAE. Nightmares were described in 6/15 patients (40%), agitation in six patients (40%), irritability in five patients (33%), sleeping disorders in one patient (7%), and seizures in one patient (7%). After exclusion of sleeping disorders (including patients presenting only nightmares), 21% (9/42) of the treated patients presented NPAE. The majority of NPAE started 12–36 h after the beginning of oseltamivir treatment, and resolved before day 7. No correlation was observed between the development of NPAE, age and gender ($p = 0.756$ and $p = 0.621$, respectively). The other adverse events were gastrointestinal symptoms (nausea, vomiting, or diarrhea, $n = 9$) and rash ($n = 1$).

TABLE 4 summarizes the frequency of NPAE according to the presence of variant alleles. For 3435C>T polymorphism, 40% (12/30) of the patients carrying at least one 3435C>T variant allele (CT or TT genotype) presented a NPAE, compared with 20% (2/10) of wild-type patients (wild-type for the 3435C>T polymorphism, CC carriers, $p =$ nonsignificant [NS]). When detailing the results by the number of variant alleles, we found that 22% (2/9) of carriers of two variant alleles (TT genotype) presented a

NPAE, compared with 48% (10/21) of carriers of one variant allele (CT genotype, $p =$ NS) and 20% (2/10) of wild-type patients (wild-type for the 3435C>T polymorphism, CC genotype, $p =$ NS). In one of the patient presenting NPAE, the 3435C>T could not be determined.

For 2677G>T/A polymorphism, 38% (10/26) of the patients carrying at least one 2677G>T/A variant allele (GT, GA, TT or TA genotype) presented a NPAE, compared with 31% (5/16) of wild-type patients (wild-type for the 2677G>T/A polymorphism, GG genotype, $p =$ NS). When detailing the results by the number of variant alleles, we found that 50% (2/4) of carriers of two variant alleles (TT or TA genotype) presented a NPAE, compared with 36% (8/22) of carriers of one variant allele (GT or GA genotype, $p =$ NS) and 31% (5/16) of wild-type patients (wild-type for the 2677G>T/A polymorphism, GG genotype, $p =$ NS).

We also examined the association between the development of NPAE and diplotype (FIGURE 2). Given the small size of our population and the number of possible diplotypes (seven diplotypes), we defined only three groups, 2677GG–3435CC (wild-type homozygous), 2677TT–3435TT (homozygous variants) and a third group consisting of individuals being heterozygous for either 2677G>T/A or 3435C>T or both. A total of 11% (1/9) of the wild-type homozygous presented a NPAE, compared with 39% (11/28) of the heterozygous and 67% (2/3) of the variant homozygous ($p = 0.149$, NS). This analysis did not include the two patients in which the 3435C>T genotype could not be determined.

Discussion

The distribution of *ABCBI* 3435C>T and 2677G>T/A polymorphisms observed in this study is similar to other reports [12,20]. Conversely, we observed one or more NPAE in

Table 2. Distribution of ABCB1 3435C>T and 2677G>T/A genotypes.

Number of alleles	2677G>T/A			3435C>T			2677–3435 diplotype				
	Genotype	%	n	Genotype	%	n	Diplotype subgroup	Diplotype	%	n	
No variant allele	GG	38	16	CC	24	10	Wild-type	GG CC	21	9	
One variant allele	All	52	22	CT	50	21	Heterozygous [†]	All	67	28	
	GT	95	21					GT CT	36	15	
	GA	5	1					GT TT	14	6	
Two variant alleles	All	10	4	TT	21	9		Homozygous variants	GG CT	12	5
	TT TA	75	3						GA CC	2	1
		25	1						TA CT	2	1
Undetermined		0	0		5	2	Undetermined [‡]			5	2
Total		100	42		100	42	Total			100	42

[†]The ‘heterozygous’ group was defined as individuals being at least heterozygous for either 2677G>T/A or 3435C>T variants, or both. In our study population, five diplotypes were considered in this group.

[‡]For two individuals, the 3435C>T genotype could not be determined with the PCR method used in our laboratory. Therefore, the 2677–3435 diplotype was defined as ‘undetermined’ in these two patients.

36% of oseltamivir-treated patients, which is considerably higher than previously reported. In an observational retrospective study, the prevalence of NPAE reached 3.84% [7]. The incidence of postmarketing reports of NPAE in the Roche global safety database among Japanese and American children reached 99 and 19 per million, respectively. However, in a prospective study specifically designed to assess oseltamivir-related NPAE (hallucinations, delirious speech, frightening episodes, abrupt anger, abnormal activities leading to accidents, and putting anything unusual into the mouth) in Japanese children, the incidence reached 13% [21]. The higher incidence of NPAE observed in our cohort may be related to the fact that sleeping disorders were not included in the previous studies. When excluding sleeping disorders, the incidence of NPAE in our cohort decreased to 21%. Moreover, we actively sought NPAE whereas the majority of studies were based on spontaneous reports; it is therefore possible that only serious and/or major NPAE were reported by others. It is also likely that some of the reported NPAE were owing to the disease itself or other comorbidities. The H1N1/09 virus has a stronger neurotropism than other influenza viruses, thereby explaining the lower incidence of NPAE reported in previous studies [22,23]. Moreover, some of these studies were not limited to pediatric patients. As oseltamivir and/or H1N1 may be more neurotropic in children than in adults, this could contribute to the higher incidence of NPAE in our cohort [7].

As none of our patients stopped oseltamivir treatment because of NPAE and all patients with NPAE completely recovered at the end of

the treatment, we can assume that their clinical significance is possibly low. However, as there is actually few data concerning oseltamivir treatment in children, we think it is important to report it, until larger studies prove the safety of this drug.

Whether or not oseltamivir could be incriminated in more severe NPAE should be more extensively evaluated. In an industry-sponsored review, Toovey *et al.* demonstrated that there was no clinical or pharmacological evidence to incriminate oseltamivir, or its metabolite, for an increased risk of NPAE [7]. However, in a recent Japanese prospective study with more than 10,000 seasonal influenza-infected patients, the authors demonstrated a higher risk of NPAE in those treated with oseltamivir [21].

One cannot exclude that the potentialization of two variants, one on 3435C>T and one on 2677G>T/A, could alter the expression and/or function of ABCB1, and thereby increase cerebral oseltamivir concentration and adverse CNS events. The molecular mechanisms explaining alteration of P-gp phenotype by ABCB1 3435 and 2677 variants, alone or in combination,

Table 3. Characteristics of NPAE in the 15 patients who presented ≥1 NPAE.

Type of NPAE	%	n
Nightmare	40	6
Agitation	40	6
Irritability	33	5
Sleeping disorder	7	1
Seizure	7	1

NPAE: Neuropsychiatric adverse events.

Table 4. Frequency of NPAE according to the presence of variant alleles.

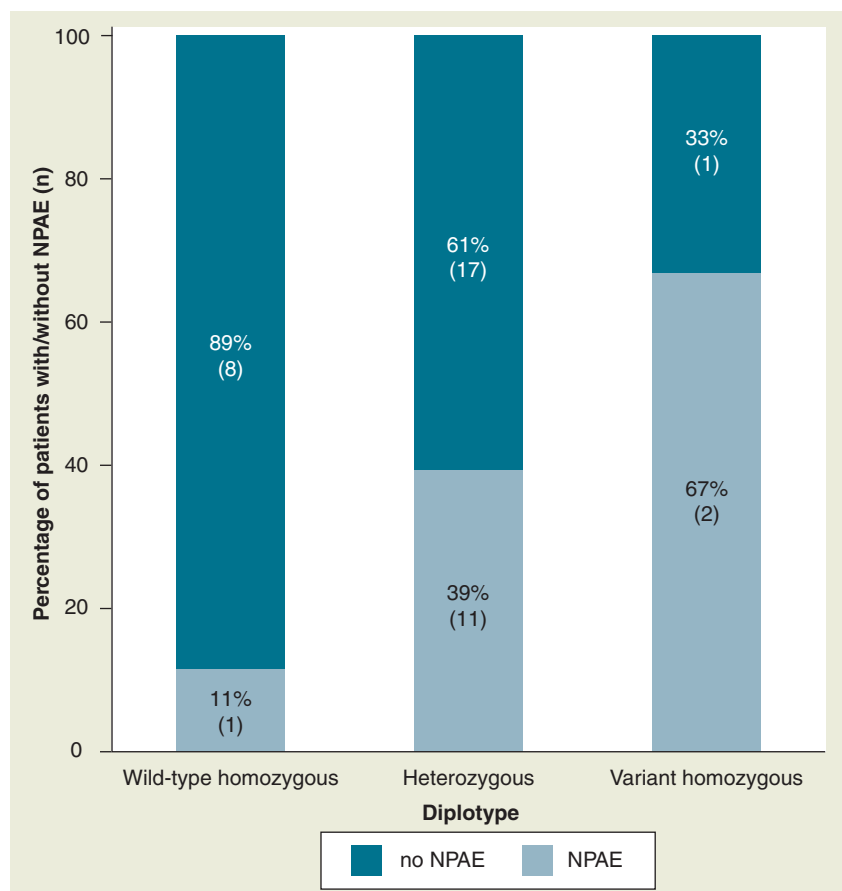
Number of variant alleles	2677G>T/A		3435C>T	
	%	<i>n</i>	%	<i>n</i>
No variant alleles	31	5	20	2
One variant allele	36	8	48	10
Two variant alleles	50	2	22	2
Undetermined	0	0	NA	1

NA: Not applicable; NPAE: Neuropsychiatric adverse events.

remain controversial. Among the possible hypothesis, modifications of mRNA folding and stability as well as potential strong linkage disequilibrium with a functional distinct allele have been proposed [24,25]. As linkage disequilibrium exists between SNP in exon 26 (3435C>T) and exon 21 (2677G>T/A), several genotype combinations can be described. Three common genotype combinations are found in more than 70% of the individuals reported in most studies, 2677GT-3435CT, 2677GG-3435CC and 2677TT-3435TT [20]. While comparing NPAE frequency according to the paired genotypes

in our study population (2677GG-3435CC [wild-type homozygous] vs 2677TT-3435TT [homozygous variants]), we observed that the frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends. Wild-type homozygous presented much less NPAE compared with variant homozygous (11 vs 67%), while heterozygous individuals for at least one variant were between wild-type and homozygous variants (39%). However, the small number of patients enrolled in our study is a major limitation of our study and could explain the lack of statistically significant difference. Another limitation could be the high proportion of at-risk patients in our cohort, which might not represent typical flu patients. However, these patients represent the population who will mainly benefit from antiviral treatment. Thus, we think that our results could certainly be extrapolated to other settings. Although careful conclusions should be drawn owing to the small sample size of our study and the lack of control group, our results might suggest that variant homozygous individuals could be more vulnerable to NPAE, maybe as a consequence of a greater penetration of oseltamivir in the CNS. These observations can be considered to provide a signal deserving further confirmation by independent investigations. The association between the development of NPAE and *MDR1* polymorphisms has been demonstrated for the antimalarial drug mefloquine by Aarnoudse *et al.* [26]. The above mentioned study identified the 1236C>T, 2677G>T/A, and 3435C>T *MDR1* polymorphisms as risk factors for neuropsychiatric adverse effects in female mefloquine users. A haplotype-based analysis showed even more pronounced results, the homozygous 1236-2677-3435 TTT genotype being at higher risk. So far 48 SNP have been identified for the *ABCB1* gene [27]. Other variants not tested here could therefore also play a role. However, these other variants have been much less studied and their consequences on the pharmacokinetics of drug substrate and on protein expression/function are hypothetical.

We did not compare oseltamivir-treated and nontreated patients in terms of proportion of NPAE, which constitutes another limitation of our study. The lack of control group is owing to the fact that our study was an observational study with a primary objective to examine the safety profile of oseltamivir in children during the H1N1 pandemic. It is therefore impossible to distinguish the proportion of NPAE that could be related to H1N1 alone.

**Figure 2.** Frequency of neuropsychiatric adverse events according to the *ABCB1* 2677G>T/A-3435C>T diplotype.

NPAE: Neuropsychiatric adverse events.

Conclusion

Our study brings new insight concerning potential neurotoxicity of oseltamivir in children and adolescents. Our results show that NPAE are more frequent than expected when systematically assessed and suggest a possible influence of ABCBI polymorphisms in NPAE pathogenesis. Variants homozygous individuals (2677TT–3435TT) appeared to be more vulnerable to oseltamivir-induced NPAE, maybe as a result of an enhanced permeability of the blood–brain barrier to oseltamivir.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Oseltamivir is a prodrug that is extensively converted to the active metabolite oseltamivir carboxylate.
- Neuropsychiatric adverse events (NPAE) have been described in children taking oseltamivir, mainly in Japan.
- *In vitro* and *in vivo* studies on P-gp over-expressing cells and on mdr1a/1b knockout mice showed that oseltamivir, but not its metabolite, is a substrate of P-glycoprotein.

Subjects & methods

- During the H1N1 pandemic, we conducted a prospective cohort study to examine the safety profile of oseltamivir in children; to assess if the presence of ABCBI 3435C>T and 2677G>T/A variants could increase the risk of NPAE in oseltamivir-treated children.
- All adverse events were systematically recorded with a 30 days follow-up.

Results

- Among the 109 patients enrolled in the study, 75 patients had H1N1 disease confirmed by PCR, of which 54 were treated with oseltamivir, and of which 42 had a genetic analysis and responded to our 30-days follow-up questionnaire.
- A strong linkage disequilibrium was observed between 3435C>T and 2677G>T/A ($D' = 0.916$).
- A total of 36% (15/42) of the patients presented NPAE, possibly related to oseltamivir, described as nightmares (6/15 patients), agitation (6/15), irritability (5/15), sleeping disorders (1/15) and seizures (1/15).
- We examined the association between the development of NPAE and 2677-3435 diplotype. A total of 11% (1/9) of the wild-type homozygous (2677GG-3435CC) presented a NPAE, compared with 39% (11/28) of the heterozygous and 67% (2/3) of the variant homozygous (2677TT-3435TT; $p = 0.149$, nonsignificant). The frequency of NPAE displayed a 'genotype-trend effect' with the variant and the wild-type subgroups at the two far ends.

Discussion & conclusion

- Our results bring new insight concerning potential neurotoxicity of oseltamivir in children and adolescents, suggesting that variant homozygous individuals could be more vulnerable to NPAE, maybe as a consequence of a greater penetration of oseltamivir in the CNS.
- These observations can be considered to provide a signal deserving further confirmation by independent investigations.

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- of interest
- of considerable interest

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- **Review on neuropsychiatric adverse events associated with the use of oseltamivir.**
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Affiliations

- **Arnaud G L'Huillier**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Geneva, Switzerland
- **Kuntheavy Ing Lorenzini**
Geneva Medical Faculty & University Hospitals of Geneva, Division of Clinical Pharmacology & Toxicology, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland
- **Pierre-Alex Crisinel**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Geneva, Switzerland
- **Michela C Rebsamen**
Geneva Medical Faculty & University Hospitals of Geneva, Service of Laboratory Medicine, Geneva, Switzerland
- **Joel Fluss**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Pediatric Neurology, Pediatric Specialties Service, Geneva, Switzerland
- **Christian M Korff**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Pediatric Neurology, Pediatric Specialties Service, Geneva, Switzerland
- **Remy P Barbe**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Pedopsychiatry Unit, Geneva, Switzerland
- **Claire-Anne Siegrist**
Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Geneva, Switzerland

and

Geneva Medical Faculty & University Hospitals of Geneva, Vaccinology Center, Department of Pathology-Immunology, Geneva, Switzerland

▪ **Pierre Dayer**

Geneva Medical Faculty & University Hospitals of Geneva, Division of Clinical Pharmacology & Toxicology, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland

▪ **Klara M Posfay-Barbe**

Geneva Medical Faculty & University Hospitals of Geneva, Department of Pediatrics, Geneva, Switzerland

▪ **Jules A Desmeules**

Geneva Medical Faculty & University Hospitals of Geneva, Division of Clinical Pharmacology & Toxicology, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland

The H1N1 Pediatric Epidemiology Study Group of Geneva

- Constance Barazzone, Rémy Barbe, Maurice Beghetti, Dominique Belli, Michel Berner, Christophe Combescure, Pierre Dayer, Cristina Delco, Jules Desmeules, Joël Fluss,

Annick Galetto, Alain Gervaix, Eric Girardin, Kuntheavy Ing Lorenzini, Laurent Kaiser, Christian Korff, Arnaud L'Huillier, Valérie McLin, Hulya Ozsahin, Klara Pósfay-Barbe, Michela Rebsamen, Claire-Anne Siegrist, Jean Taguebue, Michela Tempia-Caliera, Noémie Wagner, Barbara Wildhaber and the Pediatric Clinical Research Platform