Impact of oral Cannabis on driving skills and genetic vulnerability to psychotic symptoms

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Abstract

In a previous study designed to assess the effects of an oral intake of cannabis or dronabinol on driving capability, 2 out of 8 healthy male subjects, all of them occasional cannabis smokers, over-reacted to medium doses of Δ^9-tetrahydrocannabinol by developing transient psychotic symptoms. Since some candidate genes associated with vulnerability to drug side-effects have been suggested, a pilot study involving genetic investigations was carried out. Genetic polymorphisms of CYP2C9 and 2C19 were analyzed. One volunteer who experienced psychotic symptoms was the only one to display a CYP2C9 *2/*2 genotype suggesting a poor metabolizer phenotype. The THC/11-OH-THC concentration ratio was slightly higher than those calculated for the other volunteers. Because very few is known about the identity of the UGT enzymes involved in THCCOOH glucuronidation, in vitro experiments were performed allowing to identify UGT1A3 and 1A1 as the main enzymes catalyzing this reaction. The same volunteer was found to have a Val/Val COMT polymorphism suggesting an increased dopamine metabolism and a dopamine receptor D2 A1/A2 polymorphism possibly causing a brain hypodopaminergic state. Alteration of dopaminergic neurotransmission has been reported to increase the risk of psychiatric disorders after drug exposure. In conclusion, our findings suggest that the contribution of genetic polymorphisms in cannabis vulnerability and THC metabolism warrants further investigations.

1. Introduction

In a previous study that was designed to evaluate the effects of ingestion of cannabis on car-driving capability, we have observed two cases of "cannabis acute psychosis" whose cause has not yet been clearly established (Favrat et al 2005; Menetrey et al 2005). These unwanted side effects occurred during a double blind controlled administration study with placebo. The 8 male subjects who were recruited for this study were occasional cannabis smokers without known psychiatric history. Two of them developed transient psychotic symptoms (depersonalization, paranoid feelings and derealization) following oral administration of a cannabis milk decoction containing a medium dose of THC (15.8 mg) or dronabinol (20 mg). The participants reported a strong feeling of intoxication. Their willingness to drive was more or less diminished according to the importance and emotional load of the fictive job entrusted to them. Their tracking ability assessed with a driving simulator was also strongly diminished. Some genetic and environmental conditions may differentially predispose individuals to drug adverse

effects and psychosis. This could explain why only 2 out of 8 subjects experienced psychotic symptoms after ingestion of the same cannabis or dronabinol preparation. The genetic mechanisms which may contribute to inter individual differences in cannabinoids vulnerability are numerous: first, polymorphism of enzymes involved in THC metabolism can influence the pharmacokinetic of cannabinoids and indirectly modulate drug effects. Secondly, polymorphisms of brain receptors, transporters and enzymes constituting the main pharmacological targets of cannabinoids may also contribute to drug vulnerability. The objectives of this pilot study was to investigate possible association between acute cannabis psychosis and genetic forms or polymorphisms of a few selected targets. Enzymes, transporters or receptors involved in brain neurotransmission or THC metabolism constitute the most likely candidates.

2. Experimental

2.1 Design and participants

The study was approved by the ethics committee of the Department of Internal Medicine of the University of Lausanne. The study used a double-blind crossover design with placebo. Volunteers were occasional cannabis smokers. Drug and breath alcohol screens were performed prior to experimental sessions. Subjects ingested a cannabis milk decoction or dronabinol capsules. The doses were 16.5 mg THC/decoction or 4 capsules containing a total amount of 20 mg dronabinol. Blood was sampled at regular time intervals through a Venflon® for THC, 11-OH-THC and THCCOOH determination by GC-MS. Blood specimens were also taken for genetic investigations.

2.2 Genetic polymorphism of cytochrome 2C9/2C19

SNP analyses was performed using a real-time PCR CYP2C9 and CYP2C19 allelic discrimination TaqMan® assay (Applied Biosystems).

2.3 Genetic polymorphism of catechol-O-methyltransferase enzyme and D2 dopamine receptor genes

Two genes involved in dopamine metabolism and neurotransmission were also examined using the TaqMan® allelic discrimination technology: the functional catechol-O-methyltransferase (COMT) polymorphism (Val158Met), the C957T and *Taq*IA polymorphisms of the dopamine D2 receptor (DRD2) gene.

2.4 Genetic variants of UGT enzymes

Because nothing is known about the identity of the UGT enzymes involved in THCCOOH glucuronidation, we made in a first attempt an investigation to determine which UGT isoform is responsible for THCCOOH conjugation. Determination of genetic variants of UDP-Glucuronosyl Transferases (UGT) involved in THCCOOH glucuronidation were carried out *in vitro* with human UGTs using the baculovirus-insect cell-expression system from BD

Biosciences™. The following human UGT 1A and 2B BD Supersomes™ enzymes were tested: 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10 and 2B4, 2B7, 2B15 and 2B17. Incubations were carried out at 37°C for up to 3 hours according to the recommendations of the manufacturer. THCCOOH was added as a DMSO solution in increasing concentrations ranging from 25 to 400 µM. No THCCOOH-glucuronide formation was observed after incubation with a control made of transfected cells without added UGTs. The reaction was initiated with 2 mM UDPGA cofactor. A pore forming protein, alamethicin was included in the reaction buffer. The reaction was stopped by adding an ice-cold acetonitrile solution containing 1 µM THCCOOH-d_9 as internal standard. After mixing, centrifugation and evaporation of the supernatant, the residue was reconstituted in 200-1000 µl of the HPLC mobile phase. The extract was analyzed by LC-MS using a Waters XTerra® MS C_{18} 3.5 µM 2.1x150 mm column, a linear acetonitrile/2 mM formate buffer gradient, a Lee TCMA HP mixing chamber interconnected to a Series 200 Perkin Elmer dual pumps HPLC system. A PE Sciex (Applied BioSystems) 150EX LC-MS equipped with a Turbo Ionspray® was used for quantification. The LC-MS was operated in negative mode ionization and the following ions were monitored, m/z: 343.2 (THCCOOH), 352.2 (THCCOOH-d9) and 519.2 (THCCOOH-glucuronide). An uncalibrated qualitative reference standard provided by Alltech (no more available) was used for THCCOOH-glucuronide identification. Enzymatic velocities were expressed in relative units, i.e. by calculating the area ratio of THCCOOH-glucuronide to THCCOOH-d_9. The extracts were diluted to avoid ion suppression effects.

3. Results

Cytochrome P450 (CYP) catalyzed hydroxylation appears to be the most important phase one biotransformation undergone by THC. Allylic hydroxylation at C-11 by CYP2C9 and CYP2C19 to give 11-OH-THC is the major metabolic route. Further oxidation via the aldehyde yields the corresponding acid THCCOOH. CYP 2C9 is the major isoform found in the liver of four known members of the CYP2C family. Although the wild-type protein, CYP2C9*1 (Arg144, Ile359, Asp360), is most commonly expressed, several variants caused by single nucleotide polymorphisms in the CYP2C9 gene, have been associated with reduced metabolic activity and altered pharmacokinetic profiles.

The most common variants, which include CYP2C9*2 (Cys144), and CYP2C9*3 (Leu359) (Bland et al 2005) were investigated. The results presented in Table I show that the subject 107 who experienced psychotic symptoms after drinking the cannabis decoction displays a CYP2C9 *2/*2 genotype suggesting a poor metabolizer (PM) phenotype.

Table I. CYP2C9 genotyping and predicted phenotype. Genotyping suggests that subject 107 is a CYP2C9 poor metabolizer. acute cannabis psychosis symptoms. EM = extensive, IM = intermediate, PM = poor metabolizer. Psychotic symptoms experienced after ingestion of 4 Marinol™ capsules containing each 5 mg of dronabinol ( ). Psychotic symptoms felt after drinking a cannabis milk decoction containing 16.5 mg THC ( ). All subjects received both treatments and placebo in separate sessions.

		Subjects							
		101	102	103	104	105	106	107	108
Psychotic symptoms		-		-	-	-	-		-
CYP 2C9	Genotype	✶1/✶2	✶1/✶1	✶1/✶1	✶1/✶3	✶1/✶3	✶1/✶1	✶2/✶2	✶1/✶1
	Predicted phenotype	**IM**	**EM**	**EM**	**IM**	**IM**	**EM**	**PM**	**EM**
Treatment									
Frequency [%] in general population		20.4	65.3	65.3	11.6	11.6	65.3	0.9	65.3

This phenotype suggests a low transformation rate of THC into 11-OH-THC and, consequently, a high THC/11-OH-THC concentration ratio. When considering the blood concentration time-profiles and the highest cannabinoid levels achieved after ingestion of the cannabis drink, it appeared that the maximal THC concentration ranged in the upper part of maximal values observed for the 8 volunteers while the 11-OH-THC concentration showed a reversed trend (Table II).

Interestingly, the THC/11-OH-THC ratio obtained for this psychotic 2C9 poor metabolizer subject was the highest of the participants (1.6 compared to 0.3-1.3). Since both 11-OH-THC and THC have similar psychoactive profiles, although 11-OH-THC is more potent than THC (Lemberger et al 1973), changing their ratio should have only limited effect on appearance of psychotic symptoms. Indeed, the sum of THC and 11-OH-THC concentrations measured for this case (10.1 ng/ml of whole blood) was not very different from that of the other participants (5.8-14.7). Channeling the THC metabolism to other pathways, because of impaired formation of 11-OH-THC, is also likely. Cytochrome 2C19 is also contributing to the oxidation of THC into 11-OH-THC, mitigating the significance of CYP2C9 mutations. Both participants who experienced psychotic side effects were characterized by the wild type ✶1/✶1 genotype with a predicted extensive metabolizer phenotype (Table III). We can hypothesize that for subject 107, CYP2C19 could partially compensate for CYP2C9 deficiency and significantly contribute to the formation of 11-OH-THC

Table II. Highest blood concentrations of THC,11-OH-THC and THCCOOH and maximal subjective "high"values reported on a linear Visual Analog Scale (VAS) scale rated from 0 to 10 after administration of a cannabis milk decoction containing 16.5 mg of THC. Acute cannabis psychosis symptoms felt by subject 107.

Subject	Maximal blood concentrations [ng/ml] and weight ratios					
	THC	11-OH-THC	free THCCOOH	THC/11-OH-THC	THC+11-OH-THC	Max. feeling of intox. VAS scale 0→10
101	3.0	3.8	30.3	0.8	6.8	5.5
102	2.3	3.8	39.9	0.6	6.1	8.2
103	1.9	7.0	40.1	0.3	8.9	7.8
104	5.5	4.7	29.2	1.2	10.2	8.2
105	2.3	3.5	15.1	0.7	5.8	6.7
106	1.5	5.6	18.9	0.3	7.1	4.7
107	6.2	3.9	31.8	**1.6**	10.1	9.8
108	8.3	6.4	42.4	1.3	14.7	7.0

Table III.CYP2C19 genotyping and predicted phenotype. Genotyping suggests for both subjects 102 and 107 with psychotic feelings a CYP2C19 extensive metabolizer phenotype. acute cannabis psychosis symptoms. EM = extensive, IM = intermediate metabolizer. Psychotic symptoms experienced after ingestion of 4 Marinol™ capsules containing each 5 mg of dronabinol ( ). Psychotic symptoms felt after drinking a cannabis milk decoction ( )containing 16.5 mg THC.

		Subjects							
		101	102	103	104	105	106	107	108
Psychotic symptoms		-		-	-	-	-		-
CYP 2C19	Genotype	✶1/✶1	✶1/✶1	✶1/✶1	✶1/✶3	✶1/✶3	✶1/✶1	✶2/✶2	✶1/✶1
	Predicted phenotype	**EM**	**EM**	**EM**	**IM**	**EM**	**IM**	**EM**	**EM**
Treatment									
Frequency [%] in general population		61.9	61.9	61.9	17.2	61.9	17.2	61.9	61.9

The acyl glucuronide conjugate of THCCOOH made by UGT enzymes is the end compound which is excreted into bile and urine. UGT enzymes belong to a super-family of enzymes, each of them having several isoenzymes. The enzymes

show either broad overlapping specificities or strict substrate preference. For instance, UGT1A1 is very active for bilirubin conjugation. As far as we know, only limited data have been reported on the conjugation of THCCOOH and the identity of the involved UGT isoforms. All the UGT1A proteins are localized in the liver with the exception of UGT1A7, 1A8 and 1A10, which are expressed in gastrointestinal tissue. The UGTs belonging to the 2B family are expressed in the liver as well as extra-hepatic tissues. Similarly to the CYP enzymes, the UGT activity is changed because of induction or inhibition, or genetic polymorphism. Generally, glucuronidation is considered as a detoxification mechanism. A toxification reaction may also occur because acyl glucuronides can form reactive intermediates which can bind irreversibly to plasma proteins (Spahn-Langguth & Benet 1992) and form potentially toxic adducts. A whole series of UGT isoforms belonging to two different families (1A and 2B) were tested for their capability to conjugate THCCOOH. Figure 1 shows the formation of THCCOOH-glucuronide by 12 different UGT isoforms. In the tested conditions, it appears that UGT1A3 and 1A1 were the most active while UGT1A9 and 2B4 displayed less activity.

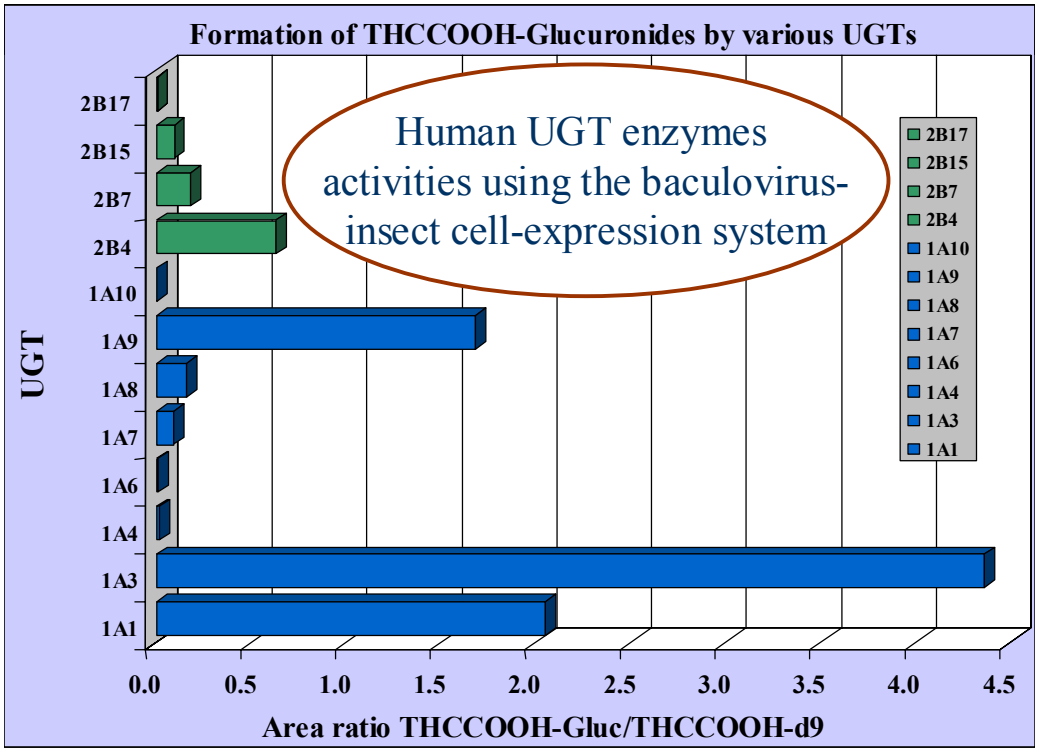

Fig 1. Formation of THCCOOH-glucuronide by 12 different UGT isoforms belonging to the 1A and 2B UGT enzyme families

Good estimates of the initial rate of UGT relative activities were obtained with one hour incubation time-period. In the presence of 100 µM THCCOOH, a linear curve was obtained for the kinetics of UGT1A9 and 2B4 up to 2 hours incubation-

time. For estimation of UGT kinetic data, substrate concentration and relative velocity data were fitted to the simple Michaelis-Menten model. K_m values were calculated from double reciprocal plots.

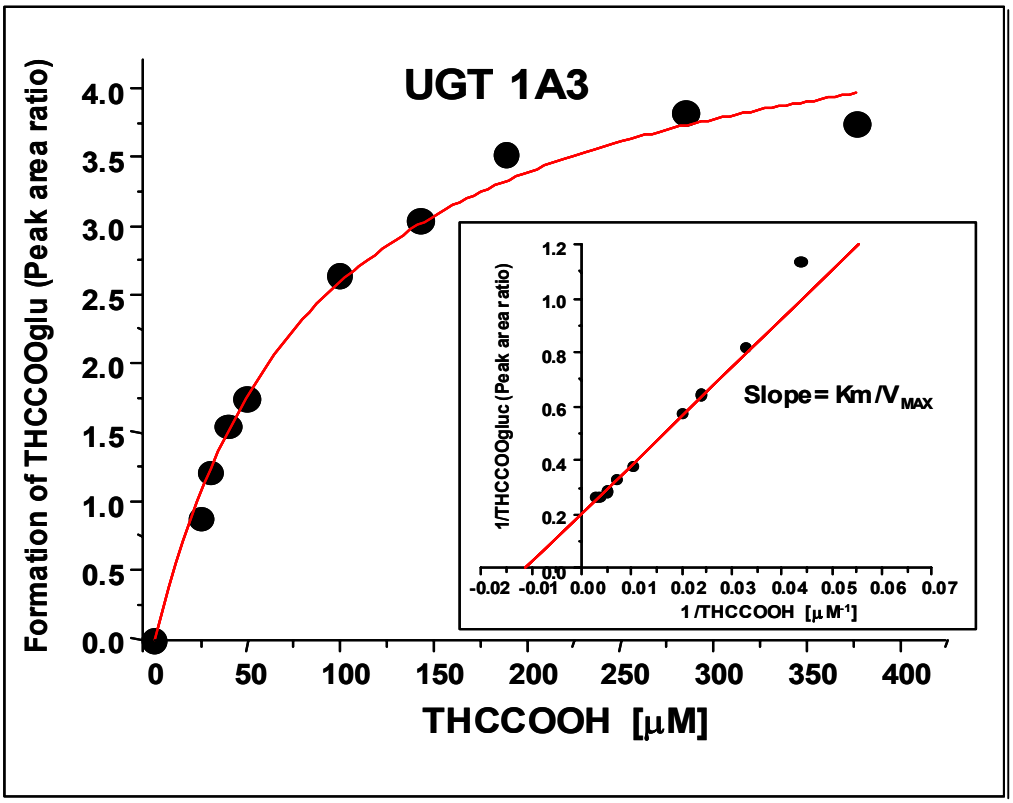

Fig 2. Michaelis-Menten kinetics of UGT1A3. Reaction velocity is plotted against THCCOOH substrate concentration. Lineweaver-Burk double reciprocal plots (figure insert) were used to estimate enzymatic parameters.

For UGT1A9 and UGT 2B4, substrate inhibition was observed at high THCCOOH concentration. For K_m estimation, we considered only the first part of the curve, i.e. the low THCCOOH concentration range. The main kinetic constants for the four UGTs which show significant THCCOOH conjugation activity are presented in table IV. This synoptic table shows that apparent clearance of UGTs, a parameter which is related to enzymatic efficiency, was highest for UGT1A3.

Similarly to many enzymes, the apparent K_m values were found to be much higher than typical free THCCOOH concentrations measured by GC-MS for a few *postmortem* cases (see table V). Total THCCOOH concentrations were measured after basic hydrolysis and THCCOOH-glucuronide levels were estimated by subtracting the free THCCOOH level from this value and taking account of the glucuronide moiety. These results suggest a poor affinity of UGT enzymes for their substrate.

Table IV. Kinetic constants for THCCOOH-glucuronide formation in vitro for the 4 main UGT enzymes showing significant THCCOOH conjugation capability

Substrate	UGT isoform	incubation time (min)	K_m [μM]	V_{max} Relative units	Apparent Cl_{int} V_{max}/K_m
THCCOOH	UGT1A1	60	118.3±20.4	1.10±0.06	8.5
	UGT1A3	60	77.1±8.7	2.27±	**29**
	UGT1A9	180	15.7±2.4	0.100±0.05	6.3
	UGT2B4	180	52.6±16.4	0.06±0.01	1.1

Table V. Comparison of Km values with postmortem liver concentrations of THCCOOH and THCCOOH-glucuronide

K_m [μM]	Free liver THCCOOH [μM]	Liver THCCOOH-glucuronide [μM]
15.7 – 18.3	0.006 – 0.06	0.05 – 0.35

Several genetic polymorphisms with associated decreased UGT activity are known for UGT1A1. These defects may lead to more (Crigler-Najjar syndrome) or less (Gilbert's syndrome) severe disease. Genetic variants of human UGT1A3 with a remarkably lower or much higher activity than the wild-type have been also recently detected (Chen et al 2006). The inactivation of estrone, an estrogen precursor, depends on UGT1A3 activity. Combination of cannabis exposure and mutation of the UGT1A3 gene may increase estrogens toxicity and alter pharmacokinetic parameters of THC.

Catechol-O-methyltransferase (COMT) is an enzyme that metabolizes dopamine in the extracellular space and terminates dopamine activity. COMT activity in the human striatum is not critical, since dopamine reuptake proteins largely terminate dopamine activity in that region. COMT activity in the frontal cortex is important because dopamine reuptake proteins are sparse and COMT functions there to terminate dopamine action in the synaptic cleft. The frontostriatal dopaminergic network is strongly implicated in neuropsychiatric disorders, such as schizophrenia, depression and other psychiatric disorders. This network is also modulated by cannabis exposure which increases the risk of schizophrenia in vulnerable people (Di Forti et al 2007). Several genetic polymorphisms have been found for the COMT gene. Two main genetic forms exist for the gene. A common functional polymorphism resulting in a valine to methionine substitution at codon 158 of the membrane-bound transcript. Enzyme variants with Met158 have 2-4-fold lower activity because of reduced thermostability at body temperature.

Table VI. Val/Met polymorphism of COMT enzyme and psychotic symptoms

		Subjects							
		101	102	103	104	105	106	107	108
Psychotic symptoms		-	😵	-	-	-	-	😵	-
COMT	Val/Vale	**X**				**X**		⊗	
	Val/Met		⊗						**X**
	Met/Met			**X**	**X**		**X**		

By altering COMT activity, especially in the frontal cortex, Val158Met polymorphism may modify risk for psychiatric disorders involving dopamine metabolism. Caspi et al. have suggested that carriers of the Val allele which result in higher COMT activity and a lower dopamine concentration in the prefrontal cortex are more at risk to develop psychotic symptoms and schizophreniform disorder provided cannabis had been taken during adolescence (Caspi et al 2005). Interestingly, both subjects who experienced psychotic symptoms were carriers of the COMT Val158 allele (table VI). Three subjects who did not experience unwanted psychotic symptoms were homozygous for the Met158 allele.

The dopaminergic neurotransmission systems are generally assumed to account for onset of both schizophrenia and addictive behavior. Abnormal dopaminergic transmission has been suggested in the ethiopathogenesis of both diseases. Five dopamine receptors exist, each one with several genetic polymorphisms. The hypothesis of a shared anomaly in the coding for the dopamine receptor D2 (DRD2) gene has been postulated. The DRD2 receptor is a major target of antipsychotic drugs.

Table VII *Taq*IA and C957T polymorphisms of the DRD2 gene

		Subjects							
		101	102	103	104	105	106	107	108
Psychotic symptoms		-	😵	-	-	-	-	😵	-
DRD2	*Taq*IA	A2A2	A2A2	A2A2	A2A2	A2A2	A2A2	A1A2	A2A2
	C957T	TT	TT	TT	CT	CT	TT	CT	TT

The *Taq*IA polymorphism of the DRD2 results from a C/T SNP. The A1 allele of DRD2 has been shown to be associated with a reduced number of dopamine binding sites in the brain and is hypothesized to play a role in drug addiction by causing a hypodopaminergic state that is alleviated by chronic exposure to several substances of abuse. Psychosis vulnerability and schizophrenia have been reported to be associated with the C/C genotype for the functional C957T DRD2

gene SNP. The results presented in table VII show that no C/C combination could be detected among the participants of the study. However, the subject 107 was the only one to possess a A1/A2 *Taq*IA allelic combination which has been linked to an increased vulnerability to psychotic symptoms. It is worthwhile to notice that DRD2 and COMT Val/Met polymorphisms have been reported to interact to strengthen schizophrenic symptoms. Interestingly, subject 107 who experienced psychotic symptoms after drinking a cannabis milk decoction is carrying two mutations which could cause an increased vulnerability to psychosis and schizophrenia. On the hand, only one gene polymorphism (Val/Met COMT polymorphism) could be detected for the second subject who also experienced unwanted psychotic symptoms.

4. Conclusions

Several metabolic and genetic investigations were carried out in a pilot study to investigate possible associations of different risk factors for psychotic reactions after cannabis exposure. This study yielded some clues as why 2 out of 8 subjects experienced a toxic cannabis psychosis after ingestion of similar doses of THC or dronabinol. Interestingly, three possibly detrimental genetic polymorphisms were detected for one subject. A CYP2C9 *2/*2 genotype suggesting a poor THC metabolizer (PM) phenotype, a Val/Val COMT polymorphism suggesting an increased dopamine metabolism and a dopamine receptor D2 A1/A2 polymorphism possibly causing a brain hypodopaminergic state. Alteration of dopaminergic neurotransmission have been reported to increase the risk of psychotic disorders after drug exposure. Investigations carried out in vitro suggest that UGT1A3 and 1A1 are mainly responsible for THCCOOH glucuronidation. Further studies are needed to delineate the influence of UGT polymorphisms on THC pharmacokinetics. Further controlled administration studies with more subjects are required to unveil the influence of genetic polymorphisms on cannabis metabolism, vulnerability and risks of psychosis.

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