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 for Novel Therapeutics  
 of HIV-associated cognitive disorders



**ABSTRACT**

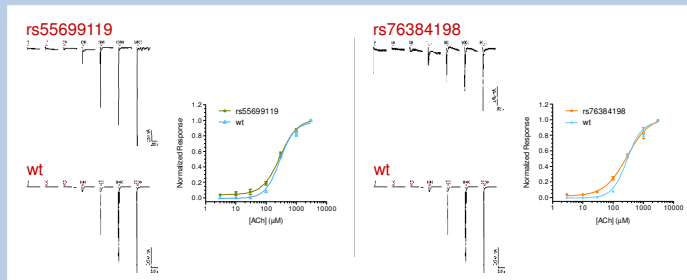
The  $\alpha 7$  nicotinic acetylcholine receptor (nAChR), a ligand gated ion channel characterized by its high calcium permeability, was shown to be upregulated in SH-SY5Y cells exposed to HIV-1 glycoprotein gp120 leading to  $Ca^{2+}$  overloading and cell death. These results suggest that HIV-associated neurocognitive disorders (HAND) could be related to increased neuronal death as result of excessive  $Ca^{2+}$  influx through the upregulated  $\alpha 7$  nAChR. Furthermore, these results also suggest that  $\alpha 7$  nAChR antagonists, such as bupropion, can be exploited for neuroprotection in HIV-1 seropositive patients. The rationale for using antagonists is that by interfering with the upregulated  $\alpha 7$  nAChR activity, antagonists should reduce  $Ca^{2+}$  overloading and the concomitant cell death. Genetic variants of the *CHRNA7* gene (encodes  $\alpha 7$  nAChR) that result in ion channels with increased expression and/or functionality could thus underlie increased risk of HAND. In addition, *CHRNA7* genetic variants could also result in receptors with diminished response to antagonists. **The hypothesis of this study is that non-synonymous single nucleotide polymorphisms (SNPs) located in the *CHRNA7* gene could result in  $\alpha 7$  nAChRs with enhanced functionality, and with altered responses to drugs aimed at interfering with the excessive  $Ca^{2+}$  flow.** Bupropion was studied as a model  $\alpha 7$  nAChR antagonist. Herein we present data demonstrating that genetic variants of the *CHRNA7* gene may result in  $\alpha 7$  nAChRs with altered functionality and response to bupropion.

**INTRODUCTION**

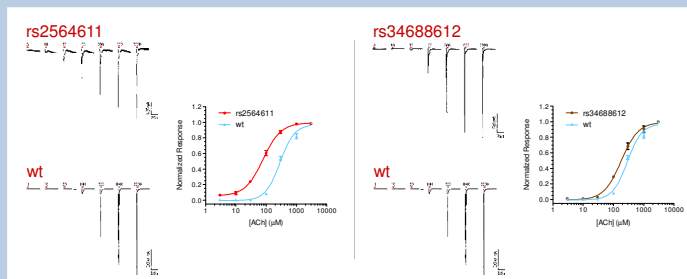
Only a fraction of HIV-1 seropositive patients develop HAND suggesting that genetic factors may contribute to the risk of developing this disorder. Thus, considering the potential role of the gp120-induced  $\alpha 7$  upregulation in the etiology of HAND (Ballester et al., 2011), this suggests that  $\alpha 7$  nAChR variants may be associated with an elevated risk of developing HAND. In addition,  $\alpha 7$  variants could also alter its response to therapeutics aimed at interfering with the excessive  $Ca^{2+}$  influx through the upregulated  $\alpha 7$  nAChR. The following *CHRNA7* non-synonymous SNPs were characterized to probe the hypothesized differences in functionality and response to bupropion of  $\alpha 7$  variants.

rs number	Amino Acid Change	Minor Allele Frequency (%)
rs55699119	A248V	N.A.
rs76384198	I145T	0.116
rs2564611	A280D	N.A.
rs34688612	E41D	N.A.
rs2229956	S489L	3.6 / 23.0

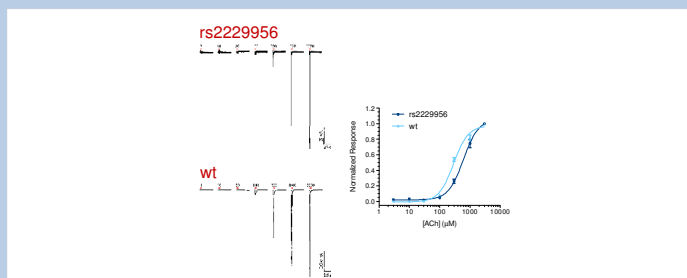
**RESULTS**



**Figure 1. Two-electrode voltage clamp experiments using oocytes expressing wt  $\alpha 7$  or genetic variants rs55699119 and rs76384198.** Dose-response curves reveal that  $\alpha 7$  mutations A248V and I145T that result from rs55699119 and rs76384198, respectively, do not change the EC50 values obtained with acetylcholine (ACh) as agonist. EC50 values for the wt, rs55699119 and rs76384198 are 286.5  $\mu M$ , 286.6  $\mu M$ , and 299.8  $\mu M$ , respectively.

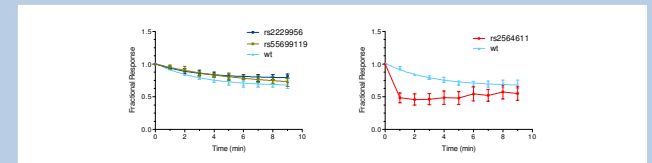


**Figure 2. Two-electrode voltage clamp experiments using oocytes expressing wt  $\alpha 7$  or genetic variant rs2564611 and rs34688612.** Dose-response curves reveal that  $\alpha 7$  mutations A280D (rs2564611) and E41D (rs34688612) result in decreases in EC50 obtained with acetylcholine (ACh) as agonist. EC50 values for the wt, rs2564611 and rs34688612 are 286.5  $\mu M$ , 79.1  $\mu M$ , and 179.8, respectively.

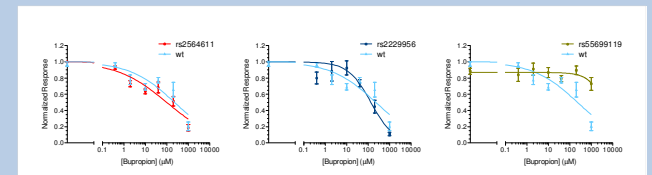


**Figure 3. Two-electrode voltage clamp experiments using oocytes expressing wt  $\alpha 7$  or genetic variant rs2229956.** Dose-response curves reveal that  $\alpha 7$  mutation S489L (rs2229956) results in a remarkable increase (2.16-fold) in the EC50 obtained with acetylcholine (ACh) as agonist. EC50 values for the wt and rs2229956 are 285.6  $\mu M$  and 609.2  $\mu M$ , respectively.

**RESULTS**



**Figure 4. Desensitization of genetic variants rs2229956, rs55699119 and rs2564611 of the  $\alpha 7$  nAChR.** The desensitization curves were obtained by means of periodical (0.017 Hz) stimulation of the  $\alpha 7$  genetic variants with 1 mM ACh. Genetic variant rs2564611 leading to mutation A280D in the  $\alpha 7$  ion channel results in an outstanding increase in the rate at which the  $\alpha 7$  enters a desensitized state.



**Figure 5. Bupropion inhibition of ACh-evoked  $\alpha 7$  currents.** Inhibition curves were constructed by determining current amplitudes in response to 1 mM ACh while co-applying increasing concentrations of bupropion. IC50 values for wt, rs2229956, and rs2564611 are 234.1  $\mu M$ , 135.4  $\mu M$ , and 102.1  $\mu M$ , respectively. IC50 values were not statistically different when compared to wt (wt vs. rs2229956  $P=0.3750$ ; wt vs. rs2564611  $P=0.2114$ ). The curve corresponding to rs55699119 is essentially straight and we were unable to fit the data using the log(inhibitor) vs. response, variable slope model.

**CONCLUSIONS**

Our experiments demonstrate that variants of the *CHRNA7* gene can result in gain-of-function (rs2564611 and rs34688612) and loss-of-function (rs2229956)  $\alpha 7$  mutations. We hypothesize that such differences in functionality could exacerbate/ameliorate the effects of the gp120-induced  $\alpha 7$  upregulation and lead to elevated/reduced risk of HAND. In addition, these results suggest that rs55699119 is essentially unresponsive to bupropion. While bupropion could be used as adjunctive therapy to avoid/treat HAND in HIV-1 seropositive patients, these results suggest that individuals carrying the rs55699119 variant may not respond to bupropion treatment.

**REFERENCES**

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