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Assessment of Negative Symptoms in Clinical Trials of Acute Schizophrenia: Test of a Novel Enrichment Strategy

Seth C. Hopkins*[,1], Sasagu Tomioka[1], Ajay Ogirala[1], Antony Loebel[1], Kenneth S. Koblan[1], and Stephen R. Marder[2]

[1]Translational Medicine, Sunovion Pharmaceuticals Inc., Marlborough, MA, USA; [2]Department of Psychiatry, Semel Institute for Neuroscience and Human Behavior at UCLA and the VA Desert Pacific Mental Illness Research, Education and Clinical Center, Los Angeles, CA, USA

*To whom correspondence should be addressed; 84 Waterford Drive, Marlborough, MA 01752, USA, e-mail: seth.hopkins@sunovion.com

Drug trials for negative symptoms in schizophrenia select patients based on the severity and stability of negative symptoms, using criteria that are not suitable for trials of acute exacerbation of schizophrenia. Here we present a method to prognostically enrich subjects having a predefined factor structure in PANSS and apply it to the measurement of negative symptoms specifically in trials of acute schizophrenia. A vector of 1335 elements based on between- and within-item variances, covariances, and differences of PANSS items was created to calculate an index of heterogeneity and to enrich for a predetermined symptom construct in PANSS. Using prerandomization PANSS scores across *N* = 4876 subjects in 13 trials of acute schizophrenia, we demonstrate an ability to select for a subpopulation having the greatest amount of variance explained across the 7-items of the Marder PANSS negative symptom (MPNS) construct. Network analyses on subjects enriched for MPNS construct confirm that negative symptoms were most influential in overall psychopathology, distinct from subjects without the MPNS construct. As expected for D2 antagonists, drug-placebo differences on negative symptoms with lurasidone were not specific to the subpopulation having the MPNS construct. In contrast, the novel TAAR1 agonist ulotaront demonstrated specific improvements in negative symptoms which were greatest in the MPNS subpopulation. These results demonstrate the utility of a novel prognostic enrichment strategy that can address heterogeneity in clinical trials, where patients can be selected on the basis of a greater likelihood of having the measured symptom construct (negative symptoms) related to the disorder (schizophrenia).ClinicalTrials.gov Identifiers: NCT0296938, NCT00088634, NCT00549718, NCT00615433, NCT00790192

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Introduction

Schizophrenia is a heterogeneous psychiatric disorder characterized by distinct symptom domains.[1,2] Antipsychotic medications that are dopamine (D2) antagonists have demonstrated effectiveness for treating positive symptoms of psychosis including delusions and hallucinations. However, these agents are relatively ineffective for treating other symptom domains, most notably negative symptoms which include deficit of expression, apathy/avolition, and social withdrawal.[3–7] Interest in identifying drugs with specific effects on negative symptoms stems from research indicating that the presence of negative symptoms is strongly associated with impairment in functioning; and given the lack of specific negative symptom efficacy in drugs acting via a dopamine antagonist mechanism, it has become evident that the development of drugs acting via non-D2 mechanisms may be needed to effectively treat negative symptoms.[8–12]

The development of drugs for negative symptoms has been hampered by several methodologic challenges.[13–15] First is differentiating primary negative symptoms (attributable to the underlying neurobiology of schizophrenia) from secondary negative symptoms. Negative symptoms are frequently secondary to (1) the acute effects of positive symptoms, (2) the presence of depression or anxiety, and/or (3) the adverse effect of D2 antagonist drugs.[7,16] Each of these three factors may result in negative symptoms (e.g., blunted effect, alogia, apathy, avolition, asociality) that are both difficult to distinguish from primary negative symptoms, and whose improvement may result in the incorrect inference that a drug has specific efficacy in treating negative symptoms (an inference that the FDA has characterized as being due to "pseudospecificity").[17] Also, within a population of acutely psychotic patients only a proportion, perhaps 50–60%, have prominent negative symptoms.[3,8,18,19] In an attempt to identify an appropriate

target population with primary negative symptoms, the few clinical trials that have been conducted have generally restricted study entry to stable outpatients with persistent negative symptoms who have low levels of positive symptoms.[7,20] These trials have been difficult to implement.[13–15] Limiting negative symptom trials to stable patients with low-grade symptomatology and persistent negative symptoms may be useful for differentiating primary versus secondary negative symptoms, however excluding acutely psychotic patients from treatment studies of negative symptoms is likely to exclude a clinically important population, and brings no clear benefit in measurement of adjusted negative symptom change, nor reduces the correlations between negative and positive symptom change.[21] Currently, studies of negative symptoms in acutely psychotic patients have employed a post hoc analysis strategy that examined efficacy in the subgroup of patients with "predominant" negative symptoms, where the severity of negative symptoms is greater than the severity of positive symptoms.[7]

Valid measurement of negative symptoms also represents an important methodologic challenge. Over the past 15 years, several scales measuring negative symptoms have been validated, for example, the Brief Negative Symptom Scale (BNSS), which has demonstrated good levels of discriminant validity versus the PANSS Positive subscale score (r = 0.09).[22] Nonetheless, the newer negative symptom scales have not been widely used in registration trials. Furthermore, they were not designed to discriminate between primary and secondary negative symptoms.

Since the Positive and Negative Syndrome Scale (PANSS) continues to be, by far, the most widely used primary outcome measure in clinical trials, we have developed a we have developed an Uncorrelated PANSS Score Matrix (UPSM) that exhibit low levels of between-factor correlation (with r-values in the range of 0.04-0.10 for UPSM negative symptoms versus both the positive and depression/anxiety factors), and yet have high face validity and show minimal loss of information when compared to the original Marder PANSS factors.[23,24] Drug vs. placebo effect sizes based on UPSM-transformed PANSS factors are specific for individual symptom domains because each UPSM factor has minimal correlation with other UPSM PANSS factors.

The method of UPSM is also able to isolate subpopulations of patients in acute schizophrenia trials at baseline who are expressing specificity of symptom predominance within each of the 5 symptom domains.[25] Combining the specificity at the dimensional (symptom domains) and typological (subpopulations) levels, UPSM is able to doubly dissociate specificity of drug-placebo treatment effects.[25] However, the UPSM methods of analysis rely on the relatively large sample sizes which are only available via pooled analyses of drug registration trials.

We currently lack symptom-based, data-analytical approaches to relate properties of individual subjects at study entry to the known heterogeneity of the disorder. We sought to adapt factor analytic methods, previously only available at the population level, to the individual subject level, in order to enrich clinical trial populations for the study of targeted symptom domains (e.g., negative symptoms). The development of such enrichment strategies,[26] applicable in real-time during clinical trial enrollment, can target specific study populations to facilitate the development and characterization of novel treatments in schizophrenia.

We hypothesized that sufficient information on the factor structure in PANSS would be contained within the PANSS assessments from a single subject, and between two time points (e.g., screening and baseline), such that subpopulations enriched for a specified PANSS factor can be identified and enrolled one subject at a time. Factor analyses of PANSS data in trials of acute schizophrenia typically identify 7 items of PANSS as the Marder Negative Symptom Factor.[1,2] In trials of acute schizophrenia at baseline, the amount of variance explained by the Marder negative factor in a 5-factor model of PANSS is typically 10–20%. Here we sought to develop an a priori method to identify subjects, one at a time, prior to randomization, that when taken as a clinical trial population, are presenting with a maximal amount of variance explained by the Marder Negative Symptom Factor. We evaluated the response of this subpopulation in existing clinical trials of two schizophrenia treatments: lurasidone, a dopamine antagonist and ulotaront, TAAR1 agonist in development with a mechanism of action unrelated to dopamine blockade.

Methods

Subject-level PANSS item scores between two assessments (e.g., screening and baseline) were encoded in a variance-covariance difference (VCD) vector. The VCD vector captures the intra-item variance, between-item covariance, and between-item differences of PANSS items between two assessment time points from a single subject. Briefly, for each subject h, a variance-covariance matrix of 30 PANSS items was defined as

$$V = \begin{bmatrix} \sigma^2_{s_1} & \dots & \sigma_{s_{1,30}} \\ \vdots & \ddots & \vdots \\ \sigma_{s_{30,1}} & \dots & \sigma^2_{s_{30}} \end{bmatrix}$$

where

$$\sigma^2_{s_j} = \sum_{t=1}^{2} \left(s^j_t - \overline{s^J}\right)^2$$

is the unbiased estimator of variance of s^j, $j = 1, 2, \dots, 30$, and

$$\sigma_{s_{i,j}} = \sum_{t=1}^{2} \left(s^i_t - \overline{s^J}\right)\left(s^j_t - \overline{s^J}\right),$$

is the unbiased estimator of covariance of PANSS items i and j, and $\overline{s^j} = \frac{\sum_{t=1}^{2} s_t^j}{2}$. Note that the denominator of the unbiased estimator of variance and the unbiased estimator of covariance are is 2-1 = 1 for two time points. The unique elements of V for subject h were kept in vector u_{cov_h}, consisting of elements of V on and below the main diagonal.

Separately, a difference matrix for 30 PANSS items was defined as

$$D = \begin{bmatrix} d_{s_{1,1}} & \cdots & d_{s_{1,30}} \\ \vdots & \ddots & \vdots \\ d_{s_{30,1}} & \cdots & d_{s_{30,30}} \end{bmatrix}$$

where $d_{s_{i,j}} = s^i - s^j$for scores of items i and j. Note that the diagonal elements of D are 0. The unique elements of D for subject h at time point t are kept in vector $d_t\,(h)$, consisting of elements of D below the main diagonal.

Together, the VCD vector of Subject h for 2 time points (e.g., screening and baseline) is therefore defined as

$$VCDV_{h\,(t=1,t=2)} = \left[u_{covh} d_{1(h)}, d_{2(h)}\right] \rightarrow \mathbb{R}^{1,1335}$$

and VCD vector for N subjects is

$$VCDV_{(t=1,t=2)} = \left[VCDV_{h1(t=1,t=2)} \quad VCDV_{h2(t=1,t=2)} \cdots \quad \cdots \quad VCDV_{hN(t=1,t=2)}\right] \rightarrow \mathbb{R}^{N,1335}$$

Using the PANSS-defined VCD vector, and the 7 items of the Marder PANSS negative symptoms factor, a new vector of 84 elements per subject was used to define a Marder negative heterogeneity index (MNHI). Table 1 lists the parameters used to derive the Marder Negative Heterogeneity index.

Marder 7 negative items in PANSS are congruent based on Marder factor model.1 Therefore, $\sigma_{s_i}^2 - \sigma_{s_j}^2$ is expected to be small for all p combinations. Similarly, $\Delta\sigma_{s_{(i,j)}\,t}^2$ is expected to be smaller for all p combinations at t = screening and t = baseline. Furthermore, $C(\sigma_{s_{i,j}}| < 0)$ is expected to be smaller for all Marder negative symptoms. Hence, the raw Marder Negative Heterogeneity Index (rMNHI) of subject h was defined as the sum of L1 norm of variance differences, count of negative covariance, L1 norm of between item differences at screening and baseline. It can be expressed as

$$rMNHI_h = \left\|\Delta\sigma_{s_P}^2\right\|_1 + \sum_{\mathrm{p}=1}^{21} C(\sigma_p| < 0) + \left\|d_{s_{P,\ t=1}}\right\|_1 + \left\|d_{s_{P,\ t=2}}\right\|_1$$

then min-max scaling (min = 0, max = 223) was applied to rMNHI to derive MNHI of subject h. In principle, the methods may be applied to enrich for any other item-level construct as a universal approach to any psychometric assessment scale.

PANSS assessments prior to randomization (screening, baseline) were pooled for ITT populations of 13 studies in acute exacerbation of schizophrenia (table 2) for a total of 4,868 subjects.

PANSS factor models were evaluated in subpopulations using confirmatory factor analysis (CFA). Confirmatory factor analysis (CFA) was performed using a R package lavaan, using maximum likelihood estimation (MLE) with robust Huber-White standard errors and a scaled test statistic that is asymptotically equal to the Yuan-Bentler T2-star test statistics.[27] The estimation was selected to reduce the deleterious effects of multivariate non-normality. The Wishart likelihood approach was used in which the covariance matrix is divided by N-1, and both standard errors and test statistics are based on N-1. Goodness of fit indices, comparative fit index (CFI > 0.95 indicating good fit), root mean square error of approximation (RMSEA < 0.08), and Tucker-Lewis index (TLI > 0.95) were computed.[28]

PANSS unidirected network models were built using regularized partial correlations between PANSS items at screening and baseline and were selected using the Extended Bayesian Information Criterion (EBIC) graphical lasso.[29,30] Network properties of average density, path lengths, and clustering coefficients were calculated based on edge weights and paths between nodes. A microscopic measure of 2-step expected influence was calculated as

$$\sum_{i,j} a_{ij} w_{ij} + \sum_{j=1} a_{ij} w_{ij} \sum_{k=1} a_{jk} w_{jk}$$

where a_{ij} is an adjacency matrix with elements either 1 or 0 (presence = 1) between nodes i and j, and where $a_{jk} w_{jk}$is the weighted edge between node j and all other

Table 1. Parameters to Derive the Marder Negative Heterogeneity Index (MNHI)

$\sigma_{s_i}^2$	Variance of PANSS item i between screening and baseline of subject h
cov_{i_s,i_b}	Covariance of PANSS item i between screening and baseline of subject h
$C(x)$	Count of x
$d_{s_{i,j}}$	Difference between PANSS item i and PANSS item j of subject h
P	Set of combinations of two Marder negative items
$\Delta\sigma_{s_{(i,j)}\,t}^2$	$\sigma_{s_i}^2 - \sigma_{s_j}^2$ of subject h at visit = t

Table 2. Randomized, Double-Blind, Placebo-Controlled Studies in Acute Schizophrenia (ITT Population)

Study ID	NCT	*N*	Weeks	Active treatment
D1050006		146	6	lurasidone
D1050049	NCT00044044	349	6	lurasidone, haloperidol
D1001002	NCT00711269	455	6	lurasidone, risperidone
D1050196	NCT00088634	180	6	lurasidone
D1050229	NCT00549718	489	6	lurasidone
D1050231	NCT00615433	473	6	lurasidone, olanzapine
D1050233	NCT00790192	482	6	lurasidone, quetiapine
D1050301*	NCT01911429	326	6	lurasidone
D1050303	NCT01821378	411	6	lurasidone
D1001056	NCT01614899	450	6	lurasidone
D1001066	(EudraCT# 2016-000060-42)	478	6	lurasidone
D1070004	NCT02002832	384	6	lurasidone
SEP361201	NCT02969382	245	4	ulotaront
Total (pool)		**4868**		

*Adolescents ages 13–17; Note: NCT is not available for D1050006.

nodes in the network *k*. Accuracy and stability of the network models were determined by bootstrapped samples and case-dropping according to Epskamp et al.[31] The weighted clique percolation method detected the overlapping community structure of symptom network,[32] with the number of clique and intensity threshold were optimized through entropy strategy based on Shannon information. The results of clique percolation algorithm are presented in figure 2.

Treatment effect sizes for lurasidone and ulotaront were calculated for the UPSM-transformed PANSS scores and for PANSS total scores using PROC MIXED procedure in SAS 9.4 adjusted for baseline and country. Drug-placebo treatment effect sizes were calculated as the LS mean difference divided by the pooled standard deviation, obtained as the standard error of the LS mean difference divided by the square root of the sum of inverse treatment group sample sizes.

Patients were considered to have Predominant Negative Symptoms based on 1) presence of at least moderate for at least 3 symptoms or at least moderately severe for at least 2 symptoms, or 2) any score on PANSS negative subscale but at least 6 points greater than the PANSS positive subscale score, or 3) PANSS Negative subscale score of at least 21 and at least 1 point greater than the PANSS positive subscale score, or 4) PANSS negative subscale score greater than the PANSS positive subscale score (Box 5A of Galderisi et al.[14])

Results

Individual subjects (N = 4863) in acute schizophrenia trials were sorted (figure 1) by their Marder Negative Heterogeneity Index. The index is calculated from each subject's PANSS-defined variance, covariance, and difference vector, prior to randomization at the screening and baseline PANSS assessments. Confirmatory factor analysis (CFA) using a 5-factor PANSS model on each equally populated bins (N = 253 subjects per bin) demonstrated that the amount of variance explained by the Marder Negative Symptom Factor was maximal (20%) for a subset (20%) of subjects in acute schizophrenia trials (figure 1). The variance explained by the other PANSS factors remained constant as a function of the negative symptom heterogeneity index (figure 1). The ability to sort subjects based on individual heterogeneity on a single PANSS factor remained similar among demographic subgroups (figure 1).

The ability to sort subjects based on amount of variance explained on negative symptoms was retained even after splitting the population according to criteria for Predominant Negative Symptoms (figure 1B). The population distribution of negative symptom heterogeneity was similar whether having predominant negative symptom criteria or not. The severity of the negative symptom factor scores, as expected, was greater among the Predominant Negative Symptom subjects (figure 1C). In contrast, severity of negative symptoms was not different when splitting the population by variance explained on negative symptoms. High factor loadings (above ~0.5) and low unique variances (below 0.3) were evident in the enriched subpopulation (table 3) with excellent indices of fit (CFI 0.99, TLI 0.98, and RMSEA of 0.07) indicative of a congruent Marder negative symptom factor structure (table 3). The enriched subpopulation retained excellent indices of fit to also the PANSS two factor model of negative symptoms (CFI 0.99, TLI 0.98, and RMSEA 0.076). The N = 4863 subjects have 40% variance explained by the 7 Marder negative items in a one-factor PANSS model, whereas the enriched subpopulation this amount of variance increases up to 69% and the remaining "de-enriched" subjects had only 37% variance explained by the negative symptoms factor model. We defined the enriched subpopulation as having a Marder PANSS Negative Structure (MPNS) construct. The subjects meeting criteria for Predominant Negative

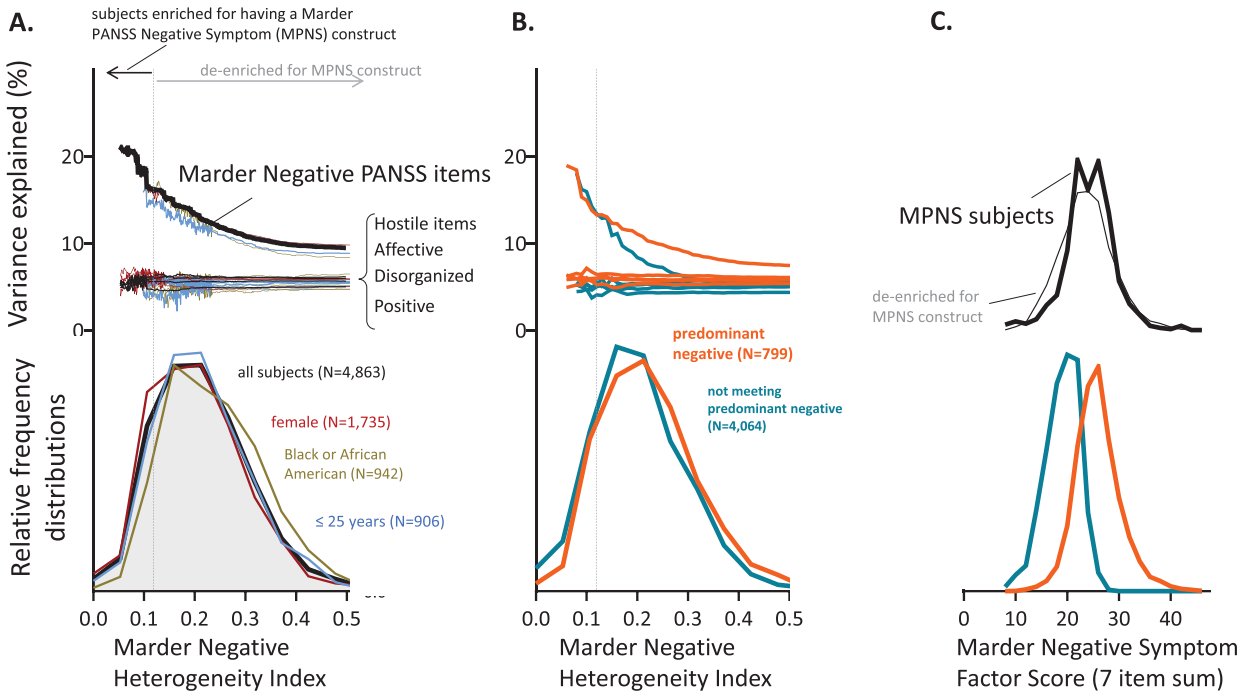

Fig. 1. Subjects in acute schizophrenia trials were rank ordered by their degree of heterogeneity on negative symptoms using PANSS assessments prior to randomization (screening and baseline). A) Variance explained by a 5-factor PANSS model is plotted as a function of negative heterogeneity index, for each of the 5 factors, and according to distinct demographic groups (*upper panel*) together with their frequency distributions (*lower panel*) for different demographic subpopulations. B) Negative symptoms explain slightly more variance among subjects meeting criteria for Predominant Negative symptoms (*orange*) versus subjects who do not meet criteria (*blue*), as a function of heterogeneity index (*upper panel*) with a similar overall frequency distribution (*lower panel*). C) MPNS subjects (*upper panel*) have a similar severity on the 7-item sum of Marder Negative Symptom Factor Score as the subjects who are de-enriched for MPNS construct. In contrast, the subjects who meet criteria for Predominant Negative Symptoms have greater negative symptom severity than subjects who do not (*lower panel*).

Table 3. Factor Loadings for Marder PANSS Negative Symptom (MPNS) Construct in Acute Schizophrenia Trials. The Degree of Factor Loading Is Represented Qualitatively as a Heat-Map, With Darker Green Indicating a Higher Level of Factor Loading

ITT population		**Enriched for MPNS**	**De-enriched (not MPNS)**	**Predominant Negative**	**Not Predominant Negative**
***N* = 4863**	**PANSS Items in MPNS Construct**	***N* = 929**	***N* = 3934**	***N* = 3401**	***N* = 1462**
0.77	N02 Emotional withdrawal	0.88	0.75	0.71	0.46
0.71	N04 Passive/Apathetic social avoidance	0.88	0.69	0.63	0.43
0.69	N01 Blunted affect	0.85	0.67	0.58	0.53
0.67	N06 Lack of spontaneity and flow of conversation	0.84	0.65	0.56	0.56
0.62	N03 Poor rapport	0.82	0.59	0.55	0.44
0.48	G07 Motor retardation	0.78	0.46	0.42	0.51
0.40	G16 Active social avoidance	0.77	0.34	0.39	0.26
40%	Variance explained (1-factor model)	69%	37%	31%	22%
2.8	Eigenvalue	4.8	2.6	2.2	1.5
0.88	CFI	0.99	0.88	0.83	0.69
0.82	TLI	0.98	0.82	0.74	0.53
0.135	RMSEA	0.071	0.130	0.134	0.143

Symptoms had only 31% variance explained by MPNS construct, and their counterparts not meeting criteria had even less variance explained at 22%. The low amount of variance explained, and the low fit indices, indicated poor construct validity for MPNS in either subpopulation (table 3).

We conducted network analysis to evaluate the extent to which subjects enriched for the MPNS construct differ from the subpopulation without, using methods that are independent of the assumptions of Confirmatory Factor Analyses. The baseline PANSS items formed a network of mutually connected communities (figure 2). Subjects

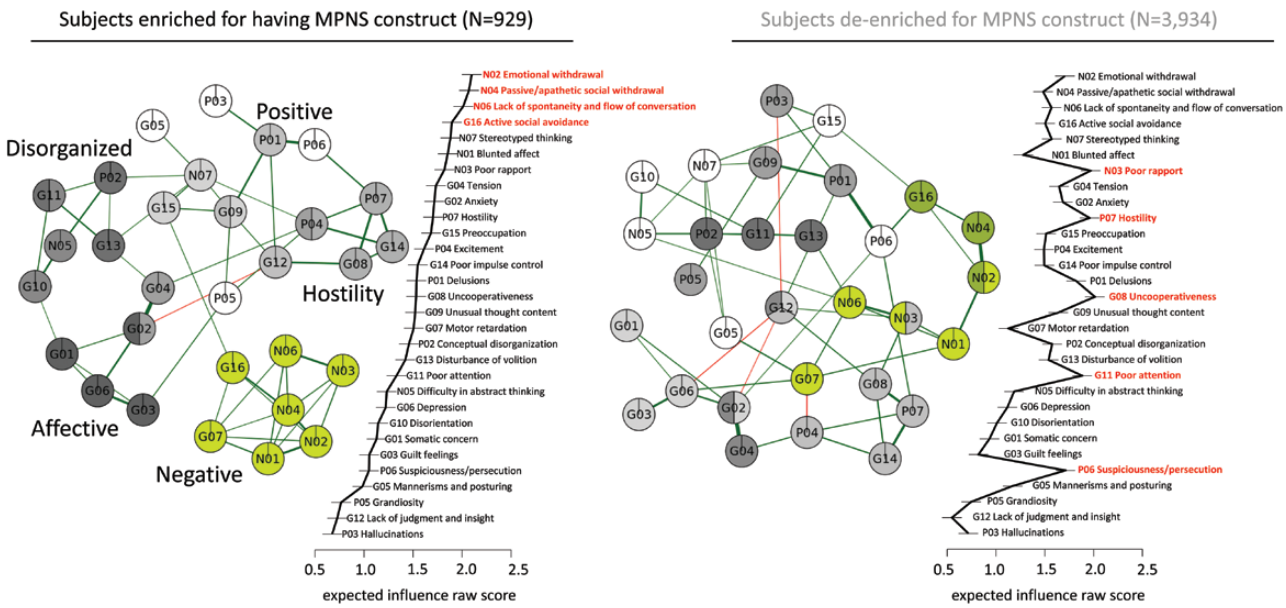

Fig. 2. PANSS network models for subjects enriched and de-enriched for the Marder PANSS Negative Symptom (MPNS) construct prior to randomization. Each circled PANSS item is a node. Each line connecting 2 nodes is an edge of varying thicknesses denoting weights between two symptoms. Edge weights are estimated as partial correlations, given all other symptoms. Green and red edge lines indicate positive and negative associations, respectively. The coloring of each node identifies individual symptoms belonging to a local community of mutually related symptoms, with some nodes belonging to 2 communities. In MPNS subjects, negative symptoms formed a distinct community (*green nodes*), but in the de-enriched subpopulation negative symptoms were split and dispersed among overlapping communities. The influence of each node on the overall network was calculated by a 2-step expected influence score and plotted for the 2 network graphs. High-scoring nodes are core symptoms and most-influential to the overall psychopathology. The top-ranking symptoms are highlighted in red. Negative symptoms in MPNS subjects were the most influential to the overall network with apathy/avolition items identified as the core symptoms in these subjects. In contrast, the de-enriched subjects did not have negative symptoms as core symptoms in the network.

enriched for MPNS demonstrated a distinct community of negative symptoms. In the MPNS subpopulation, PANSS items of apathy/avolition items were the most highly influential symptoms determining overall psychopathology in these subjects. On the other hand, the subpopulation de-enriched for MPNS construct did not demonstrate a distinct community of negative symptoms and was instead influenced by a more-dispersed collection of hostility and positive symptoms.

Current criteria for negative symptoms specify a threshold of severity for negative items versus positive items. During symptom change, the classification of subjects (as Predominant Negative or not) can change as total symptoms change (decrease) during the acute treatment phase. We sought to examine whether the approach of defining negative symptoms as variance explained (MPNS subjects), rather than as total symptoms (e.g., Predominant Negative Symptoms), would provide a more-stable classification than current definitions, which rely on symptom severity. Figure 3A shows that the subjects enriched for having MPNS construct retained the higher amount of variance explained postrandomization and even as total symptoms change. The variance explained by subpopulations with and without Predominant Negative Symptoms, remained low postrandomization. Subjects enriched for having the MPNS construct *before* randomization were more likely to convert to subjects meeting criteria for Predominant Negative Symptoms *after* randomization (figure 3B).

Negative symptoms are a target of treatment, especially for development-stage compounds having mechanisms of action that do not block dopamine D2 receptors. However, overall improvements in total symptoms obscure the specificity of improvements in negative symptoms, due to correlated improvements among items in PANSS. Here we reanalyzed drug-placebo differences of 2 compounds from 2 different pharmacological classes, ulotaront (TAAR1 agonist) and lurasidone (D2-antipsychotic). We hypothesized that specific effects on negative symptoms may be more accurately determined in a subpopulation enriched for having the MPNS construct versus the larger population de-enriched for the MPNS construct, depending on the pharmacological mode of action. In subjects having the MPNS construct, versus those without, there was a similar benefit of drug treatment on total symptoms (PANSS total score effect sizes, figures 3 and 4). In all subjects, drug-placebo separation was evident on the Negative Symptom Factor Score, as expected from the overall

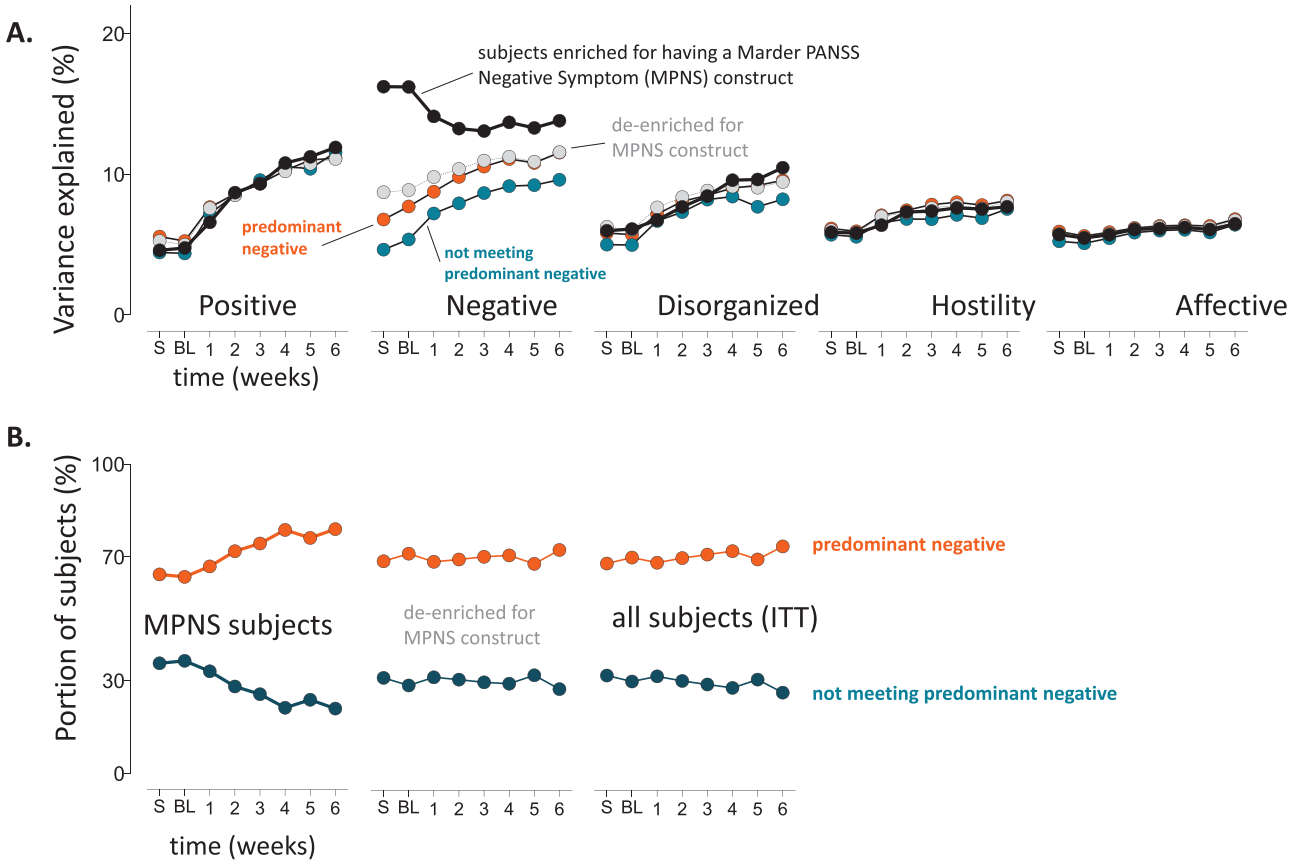

Fig. 3. A) Subjects in acute schizophrenia trials, enriched for having Marder PANSS Negative Symptom (MPNS) construct (*black circles*) prior to randomization, continue to explain more variance for the Marder Negative PANSS factor, as a function of time post baseline. Subjects meeting criteria for Predominant Negative Symptoms (*orange circles*) have slightly more variance explained by negative symptoms compared to those subjects who do not meet criteria (*blue circles*) but MPNS subjects explained the most variance in negative symptoms. B) The proportion of subjects meeting criteria for Predominant Negative Symptoms increased postbaseline among MPNS subjects to a greater degree than in the subpopulations de-enriched for MPNS construct, or in the ITT population as a whole.

improvement in PANSS total score (PANSS Negative Symptom factor scores, figure 4). We next tested for the specificity of the treatment effect on each of the domains of schizophrenia, using an Uncorrelated PANSS Score Matrix (UPSM) to transform PANSS, as described by Hopkins et al.[24] As reported by Koblan et al.,[11] ulotaront, a non-D2 compound, demonstrated specific treatment effects on negative symptoms, using the UPSM transformation of PANSS. In contrast, the subjects de-enriched for the MPNS construct did not exhibit specificity in their negative symptom improvements on ulotaront. There was a specific effect of ulotaront on UPSM Affective Anxiety scores in subjects having MPNS construct. Lurasidone a D2-based antipsychotic lacked overall specific effect on negative symptoms and did not appear to distinguish between the subpopulations of subjects (figure 4).

Discussion

When starting with an acutely psychotic patient population, it is difficult to attribute symptom change in schizophrenia to improvements in specific symptom domains (e.g., negative symptoms). Clinical trials designed to evaluate negative symptoms of schizophrenia seek to define stability with respect to the construct being measured. In contrast, here we propose a prognostic enrichment strategy for clinical trials of negative symptoms in schizophrenia, targeting a population more likely to have a predefined psychopathological construct. Here we enriched for subjects having a construct defined by the 7-items of the Marder PANSS negative factor, where a specific drug effect on negative symptoms might be more readily demonstrated. Using clinical trial data of the trace amine-associated receptor 1 (TAAR1) agonist ulotaront[11,33] and dopamine D2-based antagonist lurasidone[34] we propose analytical methods to demonstrate specificity of treatment effects in trials of patients with an acute exacerbation of schizophrenia.

Although treatment of schizophrenia, historically, has largely focused on reducing positive symptoms, a substantial portion of the psychopathology is accounted for by negative symptoms. Across the $N = 4863$ subjects in our dataset of 13 acute schizophrenia trials, negative symptoms consistently explained the greatest variance

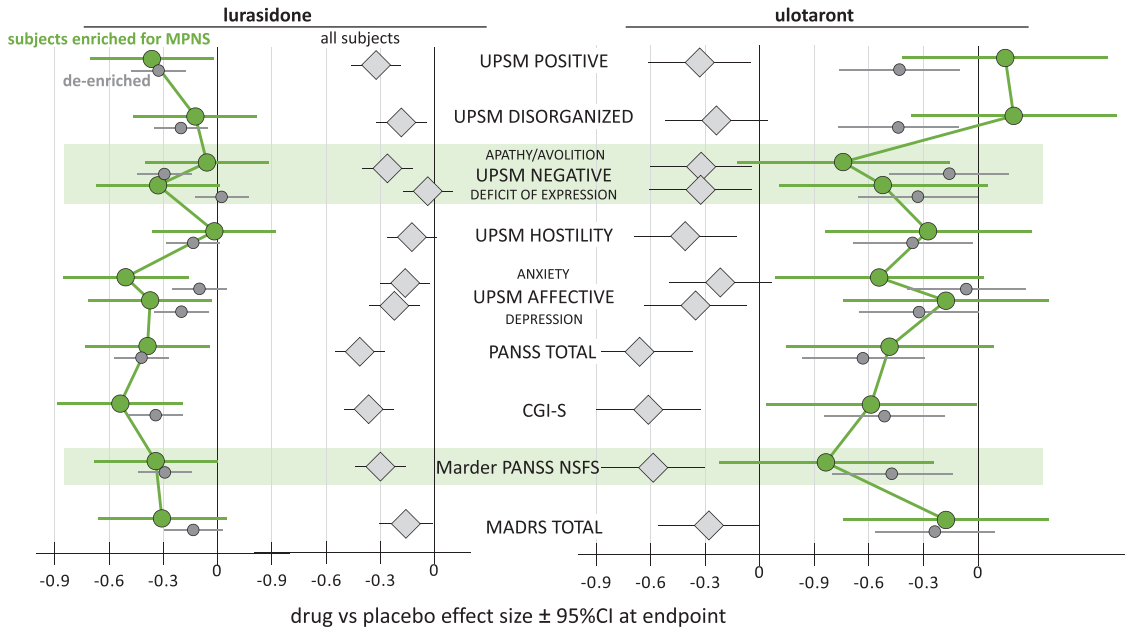

Fig. 4. Drug treatment effect sizes for two drugs of different pharmacological classes in acute schizophrenia. Dopamine D2-based antipsychotic lurasidone (N = 1532) effect sizes for a total population pooled from 5 similarly-designed studies (*grey diamonds*) are as reported in Hopkins et al.,[24] and here separated into subpopulation of subjects having Marder PANSS Negative Symptom (MPNS) construct prior to randomization (*green circles, N* = 218) versus not (*grey, N* = 1,314). TAAR1 agonist ulotaront effect sizes are from a single study as reported in Koblan et al.,[11] and here separated into subjects having the MPNS construct (N = 63) versus not (N = 182). MPNS subjects demonstrated robust effect sizes for negative symptoms with ulotaront for specific improvements in UPSM Apathy/Avolition by an Uncorrelated PANSS Score Matrix (UPSM) transformed of PANSS, as well as the standard Marder PANSS Negative Symptom Factor Score (NSFS).

in symptoms both at baseline and during treatment, compared to the other symptom domains in PANSS. We postulated that there might be a subpopulation of patients who have substantially more symptom variance explained by the 7 Marder negative PANSS items, than is observed for a population taken as a whole. To identify such patients, where the construct of negative symptoms might best match the psychometric scale used to assess them, we developed a mathematical, vector-based approach to analyze PANSS items from individual subjects to quantify their heterogeneity. The method requires only the within-subject PANSS data between two sequential assessment periods prior to randomization (screening and baseline). We postulated that information on the structure of schizophrenia symptoms might also be contained in the item scores of individual subjects, assessed at two time points prior to randomization (screening and baseline). The idea to capture information on the structure of schizophrenia symptoms within a single individual is an extension of a previously described heuristic observation: that the structure of schizophrenia symptoms at baseline appear related to the structure of symptom change apparent over time postbaseline.[23,24] In our dataset of 13 RCTs in acute exacerbation of schizophrenia, confirmatory factor analyses showed that Marder negative symptom factor accounted for 16% of the variance between subjects at baseline, which is similar to the 13% of the variance within subjects' change scores over six weeks of treatment, respectively. Using such an a priori rationale, and the desire to quantify heterogeneity along a single symptom domain, we developed a mathematical index, a PANSS heterogeneity detector, to quantify heterogeneity along a single symptom dimension in PANSS.

The PANSS heterogeneity detector was designed to be applied before randomization, based only on symptom presentation, and one subject at a time. The PANSS heterogeneity detector was designed to meet 3 criteria: (1) to capture factor-analytical properties of PANSS based on analysis of subject-level data, in a manner suitable for specifying inclusion/exclusion criteria in clinical trial protocols, without reliance on postbaseline data, and in contrast to the large sample sizes required by standard factor analysis; (2) to rank-order subjects by level of symptom heterogeneity along a single PANSS factor (e.g., negative symptoms); (3) to enrich for subjects who have a large variance explained by the prespecified factor (e.g., the 7 items of the Marder PANSS negative factor structure). The heterogeneity detector was applied here to identify a subpopulation that maximally adhered to a prespecified factor structure. In principle, a heterogeneity detector can be used enrich for any item-level construct in any psychometric scale, as a universal approach to trials in psychiatry.

The PANSS heterogeneity detector is a novel approach to enrich for specific subpopulations of schizophrenia expressing variance along a specified symptom dimension. Symptom dimensions emerge from factor analyses of large samples of PANSS data based on the variance-covariance matrix of PANSS item scores. The PANSS heterogeneity detector also relies on a variance-covariance concept but defined on individual subjects. For each subject's individual variance-covariance-difference vector, the 7 Marder negative items in PANSS are mathematically combined into a single index. The index of negative symptom heterogeneity is robust enough to rank-order subjects based on the amount of variance explained by their negative symptoms. The threshold value of Marder negative symptom heterogeneity index (0.119) identified here was selected based on maximizing the amount of variance explained on the MPNS construct in a selected subpopulation. Such a subpopulation has minimal variance from other symptom domains contributing to the measurement of the targeted negative items.

Network analyses verified that the MPNS population exhibited negative symptoms as a core domain, but with methods independent of factor analysis. The MPNS subpopulation demonstrated a distinct community of negative symptoms, with items of apathy/avolition having the greatest influence on overall psychopathology. These results are consistent with the notion of avolition as a core symptom of schizophrenia.[6,35,36] In contrast, positive and hostility symptoms were core in the subpopulation de-enriched for MPNS construct. The ability to identify a subpopulation of subjects, whose negative symptoms explain the greatest variance (by confirmatory factor analysis) and are a core domain of greatest influence (by network analysis), even in the setting of acute exacerbation of psychosis, is a novel approach to the study of negative symptoms. Instead of focusing on the clinical features that facilitate the measurement of negative symptoms with a given rating scale (eg, severity and stability), this approach focuses on matching the psychometric properties of a given rating scale (variance explained) to the enrichment of the population itself (for the MPNS construct). Optimizing the match between instrument, trial population, and endpoint will increase the validity and power of clinical trials, especially for the development of new treatments.

The MPNS subpopulation, identified prerandomization, demonstrated continued stability of psychometric properties evaluated postbaseline. MPNS subpopulation continued to have greater amount of variance explained by their negative symptoms postrandomization, as the acute treatment phase subsided, and as total symptoms decreased, indicating that negative symptom psychopathology persists even in the context of change in symptom severity during acute treatment. Although the MPNS subpopulation did not differ on total severity of negative symptoms *before* randomization, this subpopulation demonstrated the greatest relative *increases* in the proportions of subjects meeting criteria for Predominant Negative Symptoms *after* randomization. These results demonstrate the prognostic utility of enriching subpopulations for having a particular symptom construct in a specified psychometric instrument at the level of variance explained, rather than only using symptom severity for inclusion criteria. We suggest that using a subject-level property identified prerandomization (here at the level of PANSS items) to enrich a subpopulation prior to randomization (here MPNS subjects) for studying the effect of treatment on a targeted psychopathology (negative symptoms) can be considered a category of prognostic enrichment in the language of FDA guidance on enrichment.[26] The ability to choose patients whose variance in a specified symptom domain (negative symptoms) is well-described by the selected instrument (e.g., PANSS Negative Symptom Factor Score), improves the psychometric reliability of a selected endpoint in schizophrenia clinical trials.

In clinical trials and in clinical practice, improvements in positive symptoms of schizophrenia likely contribute to improvements in negative symptoms. In drug registration trials of patients with an acute exacerbation of schizophrenia, for example, improvements in PANSS positive and negative subscale (factor) scores are highly correlated in their change from baseline to a 6-week endpoint.[24] Thus any efforts to improve psychometric instruments will have change scores correlated to changes in other symptom domains. One approach to addressing correlated changes among symptom domains, is to use an Uncorrelated PANSS Score Matrix (UPSM) to transform the item scores of PANSS and to describe drug-placebo differences that are independent of correlated change in other domains, as has been applied to lurasidone[24] and ulotaront[11] clinical trial data. In the subpopulation enriched according to the Marder PANSS negative symptom (MPNS) construct, the effects of ulotaront were more specific for negative symptoms, based on the UPSM procedures, than in the de-enriched population. In contrast, lurasidone did not distinguish itself for specific effects on negative symptoms (small to zero UPSM effect sizes) in either population. Due to the small sample size, the effects of ulotaront among subjects having the MPNS construct should be considered a preliminary finding that requires replication.

Heterogeneity at the population level is useful for research studies. Large clinical samples can exploit heterogeneity to reveal the dimensions of underlying psychopathologies.[37] For example, by analyzing the variance explained between subjects in large samples, factor analyses of rating scales can reveal the dimensions of positive, negative, and cognitive symptoms.[2,38–40] Correlations of item severities within a population demonstrate clustering and their shared variance are thought to reflect a shared psychopathology. Such relationships can be determined at the population level

independent from the severity of total symptoms per se. However, at the level of individual subjects, heterogeneity hinders the understanding of individual psychopathology, because item severities within- and between-symptom domains are so highly dependent on total scores. However, if symptom heterogeneity could be characterized at the level of individual subjects using established rating scales, then our increased understanding of schizophrenia symptom measurement could help to facilitate the development of novel treatments. The variability of symptom presentation at screening and baseline has been described as a strong predictor of short-term outcome,[41,42] however the approach here is to our knowledge the first method demonstrating applicability at the individual subject level for understanding the specific psychopathology of a targeted symptom domain, and independent of symptom severity itself.

Several limitations of the current investigation should be noted. The PANSS rating scale itself is a limitation for the measurement of negative symptoms. The approach is limited to finding those subjects having the greatest construct validity of the PANSS negative symptom factor (and its constituent items) for measuring the severity of negative symptoms in patients with schizophrenia, and for the ability to specifically and sensitively measure change in severity during treatment. Although limited to acute schizophrenia, the approach of prognostic enrichment for predefined factor structures would be applicable to more-stable negative symptom populations such as deficit syndrome.

A further limitation of the current investigation is the small sample sizes of the MPNS subjects, together with the need for replication of the drug treatment effects in randomized controlled trials. It should also be noted that the enrichment strategy we report here, utilizing a PANSS heterogeneity detector, has not been prospectively defined as inclusion criteria for studies to test drug effects on negative symptoms. Therefore, the relative advantages and disadvantages of such an enrichment strategy awaits future research.

The analytical approaches piloted here with already-conducted clinical trials, can be prospectively defined as analyses in clinical trials of acute exacerbation of schizophrenia, to facilitate the characterization of compounds with non-D2 mechanisms of action. We believe this is the first demonstration that individual subjects can be selected prior to randomization to enrich study populations with the greatest degree of variance explained by a targeted factor structure, and that its application in clinical trials as an inclusion criterion, and/or as a preplanned analysis, will facilitate the demonstration of specific treatment effects on negative symptoms. In principle, the approach can enrich any predetermined symptom construct as a novel way to address heterogeneity in clinical trials.

In conclusion, the ability to prognostically enrich for a specific dimension of psychopathology, independent of total item scores, and at the level of individual subjects, is a powerful strategy for uncovering specific drug-treatment effects in clinical trials.

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