Materials and Methods:

Electrophysiology

Glass capillary patch electrodes with resistance of 2-4 MΩ when filled with internal solution were made using a vertical two-stage puller (PP-830, Narishige, Tokyo, Japan). The internal solution contained (in mM): 120 potassium gluconate, 5 EGTA, 10 HEPES, 20 KCl, 1.5 Mg-ATP, pH 7.3 with KOH. Cells were superfused with external solution containing (in mM): 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 12.5 Glucose, 5 KCl, 130 NaCL, 0.1% dimethyl sulfoxide (DMSO), pH 7.4 with NaOH. The calculated junction potential for these solutions was -15mV, which was corrected for in all experiments. Cells were voltage clamped in whole cell mode using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Current signals were digitized at 5 kHz, filtered at 1 kHz and stored on an IBM-compatible PC interfaced with a Digidata 1440A analogue-digital convertor (Molecular Devices). Series resistance was compensated by at least 80% in all experiments. Data analysis was performed using Clampfit 10.2 software (Molecular Devices, Sunny Vale, CA) and Prism (GraphPad 6.01, La Jolla, CA).

Drugs (purchased from Sigma-Aldrich, Australia, except Aripiprazole, purchased from IS Chemical Technology, China) were prepared as stock solutions in DMSO and subsequently diluted as required in external solution such that the maximum final DMSO concentration was 0.1%v/v. To measure drug block, cells were depolarized from a holding potential of -80mV to 0mV. After the current reached a steady-state level (~20s) drugs were applied for 20s (with the exception of paliperidone and aripiprazole, which were applied for 40s and ~300s, respectively due to slower drug wash-on rates) (1).

CATIE Cohort

Patients with schizophrenia were initially randomly assigned to receive olanzapine (7.5 to 30 mg/day), perphenazine (8 to 32 mg/day), quetiapine (200 to 800 mg/day), risperidone (1.5 to 6 mg/day) or ziprasidone (40 to 160 mg/day) under double-blind conditions and followed up for 18 months or until treatment was discontinued or switched to another antipsychotic (i.e. Phase 2). Positive and Negative Syndrome Scale (PANSS) ratings (Positive, Negative and General Psychopathology) were performed every three months until the end of the study at 18 months, or when patients were switched to another drug (end of Phase 1/1A).

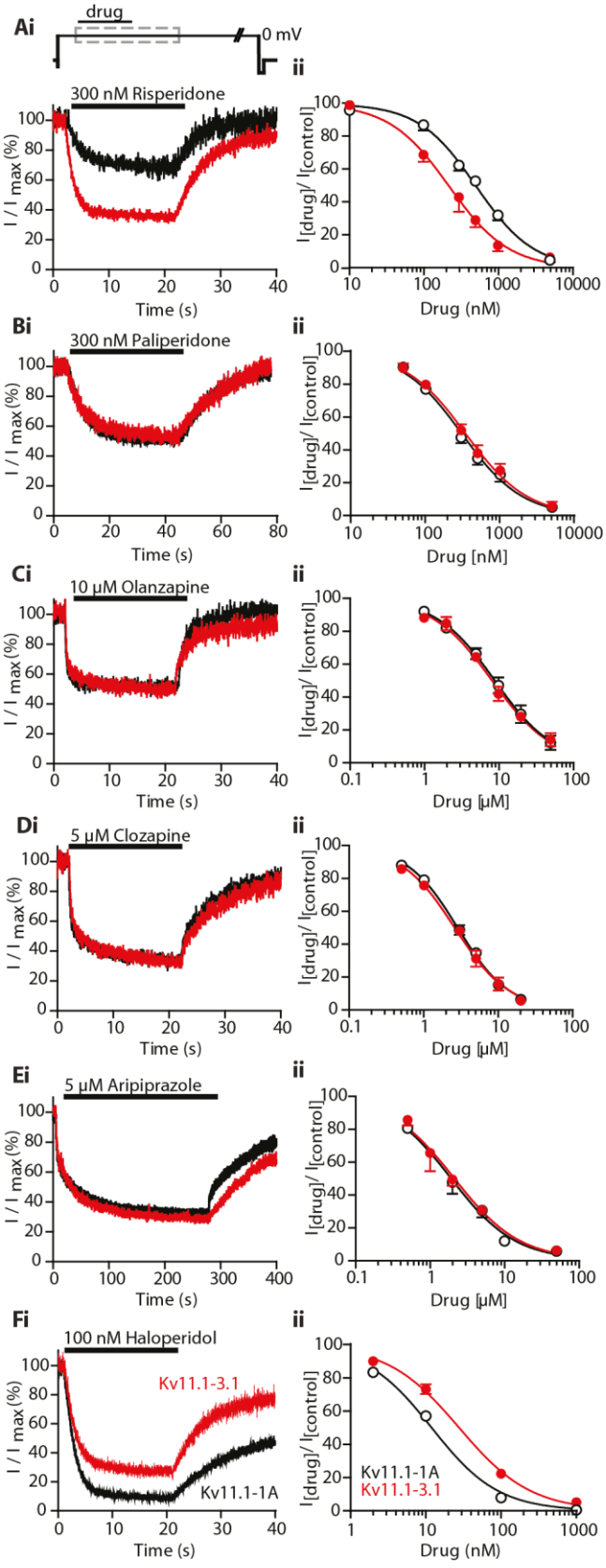
Genotyping and construction of diplotype groups

For this analysis, we focused on three SNPs rs3800779 (SNP1), rs748693 (SNP2) and rs1036145 (SNP3) in *KCNH2*, which have been associated with increased expression of the novel Kv11.1-3.1 isoform in human post-mortem brain samples (2) and overall response to treatment in the CATIE trial (3). Genotypes were determined using the 5'-exonuclease fluorescent Taqman assay and the allelic discrimination was read on an ABI 7900 SDS system (Applied Biosystems, Foster City, CA) (4). Since the three SNPs were in moderate to strong linkage disequilibrium, in order to reduce multiple testing and to gain statistical power for detecting association, we constructed three-SNP diplotypes to be used for testing diplotype by risperidone interaction on treatment response. Haplotype construction was performed and phased diplotypes were assigned using the *Phase* program (5) to individual subjects with probability for treatment response analysis. Diplotypes for all individuals, except two, were assigned with a good confidence probability of at least 92.7%; the two subjects whose assigning probability was below 92.7% were removed from further analysis. Because SNP2 and SNP3 were in strong LD (r-squared = 0.9), diplotype was grouped into three categories according to the number of minor alleles that a diplotype contains at SNP1 and SNP3: 0 = non-minor allele of either SNP1 or SNP3, 1 = one or two copies of minor alleles, 2 = three to four copies of minor allele. The distribution of diplotypes in individuals with drug clearance data was consistent with the total European ancestry sample in the Phase 1/1A CATIE trial, suggesting minimal selection bias (Table S2).

Drug comparison studies

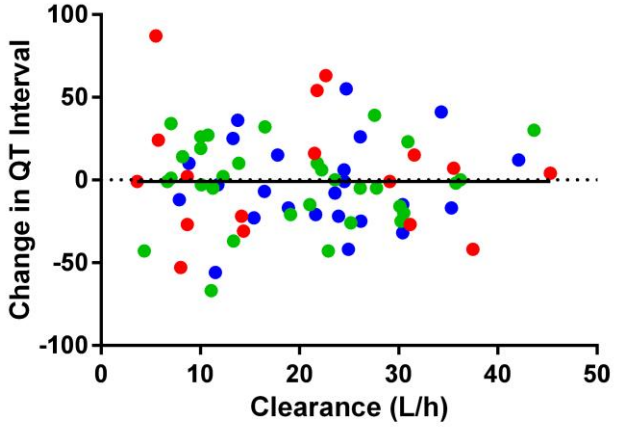
To specifically test the hypothesis that diplotype group 2 patients had a differential response to risperidone compared to other drugs, we grouped all other drugs together. We did this, first, because in our *in vitro* assays all drugs other than risperidone showed no differential affinity for Kv11.1-3.1 compared to Kv11.1-1A so from a functional point of view with respect to Kv11.1 activity, all drugs other than risperidone can be grouped together. Second, none of the other drugs when used as a reference drug to compare all others to showed any significant differential effect on the PANSS ratings. We therefore concluded it was reasonable to include all the other drugs in one group for our subsequent analyses. It is also worth pointing out that as the assignment of drugs in the CATIE trial was randomized there should be no intrinsic bias to the drug groupings.

FIGURE S1. Block of Kv11.1a and Kv11.1-3.1 channels by anti-psychotic drugs



Ai-Fi. Typical current traces for Kv11.1-1A (black) and Kv11.1-3.1 (red) during application of 300 nM risperidone (A), 300 nM paliperidone (B) 10 μM olanzapine (C), 5 μM clozapine (D), 5 μM aripiprazole (E) and 100 nM haloperidol (F). The voltage protocol is shown on top of panel A: cells were depolarized from a holding potential of -80mV to 0mV and drugs applied after the current reached a steady-state level. Aii-Fii. Hill curves for Kv11.1-1A (black) and Kv11.1-3.1 (red). IC_{50} values are 508±27 nM (n=6) and 220±56 nM (n=6) respectively for risperidone (Aii), 307±29 nM (n=4) and 346±29 nM (n=4) for paliperidone (Bii), 8.9±0.3 μM (n=6) and 8.2±0.3 μM (n=6) for olanzapine (Cii), 2.8±0.2 μM (n=6) and 2.6±0.2 μM (n=6) for clozapine (Dii), 1.9±0.1 μM (n=6) and 2.1±0.1 μM (n=5) for Aripiprazole (Eii) and 11.9±2.2 nM (n=4) and 27.8±4.1 nM (n=4), for haloperidol (Fii,).

FIGURE S2. Change in QT interval is independent of risperidone metabolizer status



Change in QT versus clearance rate for 141 patients with ECG recordings. Diplotype 0 (blue), diplotype 1 (green), diplotype 2 (red).
r^2 for linear regression (all genotypes, =0.003, P = 0.9971). Dashed line indicates the zero value.

TABLE S1. Linkage disequilibrium of three SNPs in *KCNH2* previously associated with risk for schizophrenia.

Chromosome	SNP	Chromosome location	SNP1 rs3800779	SNP2 rs748693	SNP3 rs1036145
7	rs3800779	150302147	1	0.793	0.728
7	rs748693	150302370	0.793	1	0.928
7	rs1036145	150305363	0.728	0.928	1

r^2-values for the correlation between three different SNPs, from intron 2 of *KCNH2*.

TABLE S2: Diploptype distribution and grouping in samples with or without drug clearance data

	With clearance		Total		
	N	%	N	%	Diplotype Group
GAC|GAC	142	39.23	167	40.05	0
GAC|GAT	3	0.83	3	0.72	1
GAC|GGT	16	4.42	19	4.56	1
GAC|TAC	5	1.38	6	1.44	1
GAC|TGC	5	1.38	6	1.44	1
GAC|TGT	130	35.91	144	34.53	1
TAC|TGT	8	2.20	8	1.92	1
TGC|TGC	0	0.00	1	0.24	1
TGC|TGT	1	0.28	2	0.48	1
TGT|TGT	43	11.88	51	12.44	2
GGT|GGT	1	0.28	1	0.24	2
GGT|TGT	8	2.21	9	2.16	2
Total	362	100.00	417	100.00	

Distribution of diplotype groups within the study population. Note: two individuals were excluded due to diplotype assigning probability below 92.7%. Highlighted are the proportions of each diplotype in samples with drug clearance and overall sample as well as the three major diplotypes, one in each of three diplotype groups

TABLE S3. Summary of IC_{50} values and Hill co-efficients for all drugs and channel mutants

Anti-Psychotic	Kv11.1-1A		Kv11.1-3.1	
	IC_{50} [nM]	Hill	IC_{50} [nM]	Hill
Haloperidol	11.9 ± 2.2#	-0.98 ± 0.04	27.8 ± 4.1	-0.91 ± 0.06
Clozapine	2830 ± 200	-1.26 ± 0.06	2580 ± 200	-1.19 ± 0.08
Olanzapine	8940 ± 300	-1.09 ± 0.09	8210 ± 300	-1.07 ± 0.08
Risperidone	508 ± 27#	-1.13 ± 0.09	220 ± 56	-1.08 ± 0.16
Paliperidone	307 ± 29	-1.06 ± 0.07	346 ± 29	-1.05 ± 0.08
Aripiprazole	1890 ± 100	-1.00 ± 0.14	2090 ± 100	-0.98 ± 0.13

= $p < 0.05$, Tukey's method used for multiple comparisons test for 1A and 3.1

TABLE S4. Treatment effect of risperidone vs all other drugs

Effect (PANSS)	Num DF	Den DF	F-value	Pr > F
Positive	1	360	0.04	0.8468
Negative	1	360	0	0.9806
General Psychopathology	1	360	0.45	0.5025

Num DF: Numerator degrees of freedom; Den DF: Denominator degrees of freedom, F: S-statistic,
Pr > F: the p-value associated with the F statistic.
88 Patients were treated with risperidone compared to 274 patients treated with other drugs.

TABLE S5. Summary data for PANSS ratings of positive and negative syndromes and general psychopathology by diplotype group based on three SNPs in *KCNH2*

drug	treatment	PANSS	Diplotype Group	n	mean	SD	min	max
others	before	Positive	0	113	15.2	5.7	7	30
		Negative			16.8	6.9	7	38
		General			31.8	9.1	17	59
	after	Positive	0	113	15.0	5.7	7	31
		Negative			17.0	6.2	7	32
		General			32.0	10.2	16	68
risperidone	before	Positive	0	29	16.5	5.5	7	25
		Negative			18.5	5.9	7	33
		General			35.3	8.9	20	54
	after	Positive	0	29	15.9	6.0	7	27
		Negative			18.2	7.1	7	33
		General			36.0	12.1	17	71
others	before	Positive	1	128	15.8	5.9	7	35
		Negative			18.3	6.5	7	38
		General			33.8	9.8	17	57
	after	Positive	1	128	15.8	6.2	7	34
		Negative			18.4	6.9	7	36
		General			33.4	10.2	16	62
risperidone	before	Positive	1	40	18.0	6.1	8	31
		Negative			20.1	6.6	7	38
		General			35.2	9.8	17	62
	after	Positive	1	40	18.4	6.2	10	34
		Negative			20.1	6.2	10	33
		General			36.3	8.9	22	55
others	before	Positive	2	33	17.6	5.6	8	28
		Negative			19.4	5.6	7	29
		General			34.9	10.3	19	60
	after	Positive	2	33	16.1	6.4	7	30
		Negative			17.5	5.1	9	30
		General			32.3	10.1	18	60

risperidone	**before**	**Positive**	**2**	**19**	14. 2	6.0	7	29
		Negative			20.0	7.1	9	33
		General			33.2	8.5	22	50
	after	**Positive**	**2**	**19**	13.5	6.5	7	29
		Negative			19.7	6.0	11	35
		General			31.1	9.5	18	54

PANSS ratings, before and after treatment, for all patients grouped according to diplotype group and whether they are treated with risperidone or other drugs.

TABLE S6. Numbers of patients treated with Risperidone categorized according to clearance and *KCNH2* diplotype

Risperidone Clearance	Diplotype Group				
	0	**1**	**2**	**Total**	**P**
Fast or intermediate	20	26	13	59	0.93
%	33.9	44.1	22.0	100	
Slow metabolizer	9	14	6	29	
%	31.0	48.3	20.7	100	
Total	**29**	**40**	**19**	**88**	

P: p-value for association of drug clearance and *KCNH2* diplotype based on likelihood ratio, χ^2 test

TABLE S7. Summary data for PANSS ratings of positive, negative and general psychopathology ratings in patients on risperidone medication during the Phase1-1A of CATIE study based on clearance rates

Risperidone metabolism	Diplotype Group	Treatment	N	Positive		Negative		General	
				Mean	SD	Mean	SD	Mean	SD
Slow	**0**	**Before**	**9**	18.2	5.2	20.6	7.4	39.0	10.5
		After		15.6	6.5	19.1	8.8	41.3	16.2
	1	**Before**	**14**	16.1	5.6	17.9	5.2	30.1	7.9
		After		15.0	5.5	19. 8	4. 9	34.6	9.5
	2	**Before**	**6**	20.2	5.9	21.5	8.8	39.7	7.6
		After		15.7	7.8	19.5	7.1	34.0	12.3
Intermediate	**0**	**Before**	**10**	15.2	7.0	17.7	5.5	31.7	7.78
		After		16.4	6.6	17.2	7.5	32.3	8.7
	1	**Before**	**13**	19.1	6.7	19.2	6.9	37.0	13.1
		After		20.0	6. 2	19.1	5. 8	36.5	8.5
	2	**Before**	**6**	13.0	4.9	20.0	8.4	37.2	9.4
		After		14.3	7.2	20.8	5.2	34.5	6.9
Fast	**0**	**Before**	**10**	16.2	3.9	17.5	4.9	35.6	7.8
		After		15.8	5.5	18.4	5.6	34.8	9.9
	1	**Before**	**13**	18.4	6.5	22.5	6. 8	37.01	5.8
		After		20.2	6.2	21.0	7. 5	37.3	9.2
	2	**Before**	**7**	11. 3	3. 3	20.1	6.3	27.6	4.9
		After		11.4	4.1	20.0	7.6	26.9	8.8

The 88 patients treated with risperidone were classified into slow (lower 33.3%), fast (upper 33.3%) and intermediate groups and then sub-classified according to diplotype group. When considering just diplotype group 2 (shaded groups) slow metabolisers showed an improvement of 4.5, 2.0 and 5.7 units for positive, negative and general symptoms whereas in fast metabolisers there was no improvement in positive or negative symptoms and only a very small improvement in general symptoms (0.7 units).

TABLE S8. Least-square mean estimates of risperidone clearance and *KCNH2* diplotype interaction on antipsychotic treatment response in individuals on risperidone during Phase 1/1A of the CATIE study

	Diplotype group	Metaboliser group	Estimate	SE	t-value	P	P*
Positive symptoms	0	Slow (9) vs. Med. (10)	3.2	2.6	1.21	0.2276	0.0708
	0	Slow (9) vs. Fast (10)	3.0	2.6	1.15	0.2544	
	1	Slow (14) vs. Med.(13)	-3.4	2.2	-1.53	0.1284	
	1	Slow (14) vs. Fast (13)	-4.5	2. 2	-2.08	0.0399	
	2	Slow (6) vs. Med (6)	7.0	3.3	2.14	0.0349	
	2	Slow (6) vs. Fast (7)	7.2	3.1	2.30	0.0232	
Negative Symptoms	0	Slow (9) vs. Med. (10)	3.9	2.9	1.32	0.1882	0.9165
	0	Slow (9) vs. Fast (10)	3.3	2.9	1.14	0.2557	
	1	Slow (14) vs. Med.(13)	-0.1	2.5	-0.03	0.9757	
	1	Slow (14) vs. Fast (13)	-2.9	2.4	-1.17	0.2457	
	2	Slow (6) vs. Med (6)	1.4	3.7	0.37	0.7089	
	2	Slow (6) vs. Fast (7)	1.1	3.5	0.32	0.7466	
General Psychopathology	0	Slow (9) vs. Med. (10)	8.7	4.1	2.16	0.0332	0.0575
	0	Slow (9) vs. Fast (10)	4.6	4.0	1.14	0.2571	
	1	Slow (14) vs Med (13)	-4.8	3.4	-1.40	0.1636	
	1	Slow (14) vs. Fast (13)	-7.5	3.4	-2.22	0.0283	
	2	Slow (6) vs. Med (6)	7.4	5.1	1.46	0.1482	
	2	Slow (6) vs. Fast (7)	11.6	4.9	2.36	0.0201	

Note: Post-hoc least square mean estimates based on linear-mixed model with a three-way interaction of risperidone clearance, *KCNH2* diplotype group and treatment (i.e. before and after treatment).
P: p value for testing for the change in PANSS rating between metaboliser groups stratified by diplotype group in subjects who took risperidone medication.
P*: p value for testing for a three-way interaction between medication, diplotype and metabolizer status.
The numbers in parentheses indicate the number of patients in each group
Rows highlighted in orange indicate groups where slow metabolisers showed more improvement than their comparator. Slow metabolisers did better than fast metabolisers within diplotype group 2 for both positive and general symptoms and slow metabolisers did better than intermediate metabolisers in group 2 for positive symptoms. Rows highlighted in yellow indicate groups where slow metabolisers showed less improvement than their comparator. Slow metabolisers fared more poorly than fast metabolisers in diplotype group 1 for both positive and general

symptoms. Overall there is a trend for slow metabolisers to do better if they are diplotype group 2 but to do worse if they are not diplotype group 2.

References

1. Hill A, Perrin MJ, Heide J, Campbell T, Mann S, Vandenberg JI. Kinetics of Drug Interaction with the Kv11.1 Potassium Channel. Mol Pharmacol. 2014.
2. Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, et al. A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat Med. 2009;15(5):509-18.
3. Apud JA, Zhang F, Decot H, Bigos KL, Weinberger DR. Genetic variation in KCNH2 associated with expression in the brain of a unique hERG isoform modulates treatment response in patients with schizophrenia. Am J Psychiatry. 2012;169(7):725-34.
4. Livak KJ. Allelic discrimination using fluorogenic probes and the 5 ' nuclease assay. Genet Anal-Biomol Eng. 1999;14(5-6):143-9.
5. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. American journal of human genetics. 2001;68(4):978-89.