Supplemental Materials and Methods

Cell Lines
HEK239 cells were purchased from ATCC; Sanger sequencing confirmed an APOL1 G0 genotype, as reported in the whole genome sequence at hek293genome.org. HEK293 cells were cultured in DMEM (Corning) supplemented with 10% FBS (Atlanta Biologicals) and 2% pen-strep (Gibco). Stable T-Rex HEK293 cells were created using the Flp-In T-Rex 293 system (Life Technologies) by inserting an APOL1 cDNA of interest into the same single copy locus of every cell. A control cell line containing the CAT (Chloramphenicol Acetyltransferase) gene was also created. Cells were cultured in DMEM supplemented with 10% TET-tested FBS (Atlanta Biologicals), 1% pen-strep (Gibco), and 15ug/mL blasticidin (Gibco). Cells were selected polyclonally for resistance to 200ug/mL hygromycin B (Corning) and surviving populations tested for tetracycline-induced APOL1 expression, Zeocin sensitivity, and lack of β-galactosidase activity.

Plasmids
Untagged APOL1 was overexpressed using a pCMV6 plasmid construct. Mutagenesis was performed using the Qiagen Quick Change Lightning kit. pCDNA5-frt was used for generating Flp-In cell lines.

Cytotoxicity/Viability Assays
For fluorescent assays, HEK293 cells or stable T-Rex 293 cells were plated on a black-walled clear bottom 96 well plates (Corning) at 30,000 cells/well. The next day, cells were transfected with Lipofectamine2000 (Life Technologies) at 100ng of APOL1-encoding DNA/well or induced with 100ng/mL of tetracycline. Cytotoxicity/viability ratios were measured using the MultiTox-Fluor Multiplex Cytotoxicity Assay (Promega) at the indicated time points. For MTT assays, Stable T-Rex 293 cells were plated on clear 24- well plates at 125,000 cells/well. Each cell line was induced with 100ng/mL tetracycline for 24h or 48h and with a parallel no-tetracycline control for each cell line. For 48h experiments, culture media was changed at 24h with addition of fresh tetracycline. MTT (Life Technologies) was then incubated at 2.5 mg/mL for 3h at 37°C, after which cells were homogenized with the addition of isopropanol with 4mM HCl and 0.1% NP-40. The absorbance of the clarified solution was measured at 590nm. The ratio of the absorbance of the solution from tetracycline-induced cells to that of the corresponding no-tetracycline control was calculated as % Viability.

qPCR
RNA from induced T-Rex HEK293 cell lines was isolated using the RNeasy Kit (Qiagen). Cellular RNA was then converted to cDNA using the High Capacity RNA to cDNA Kit (Applied Biosystems). Relative transcript level was measured using the TaqMan Gene Expression system (Life Technologies) with probes for APOL1 (Hs01066280_m1) and endogenous beta-actin (Hs99999903_m1).

SDS-Page
Cells were lysed in RIPA buffer and the lysate centrifuged at 17,000g. Clarified supernatant was prepared in SDS-Sample Buffer (Boston BioProducts) and boiled at 95°C for 5 min.

Samples were run on 4-20% gels (Bio-Rad) and transferred onto PVDF membranes (Millipore). Blots were probed with commercial Apol1 antibody (ProteinTech 66124-1-Ig, mouse), custom designed rabbit anti-G0 antibody generated to recognize I384 but not M384), vinculin (abcam ab129002, rabbit), and GAPDH (GeneTex GT239HRP, mouse). HRP linked mouse or rabbit secondary (Cell Signaling 7076 7074) was used and chemiluminescent images were acquired on a FluorChem E (ProteinSimple).

	rs41297245	rs2239785	rs116136671	rs136173	rs136176	rs73885316	rs16996616	rs73885319	rs60910145	rs71785313	
Amino Acid Postion	**96**	**150**	**176**	**228**	**255**	**264**	**337**	**342**	**384**	**388-389**	**Freq. (%)**
Ref	G	E	N	*M*	*R*	N	D	S	I	in	15.6
G0	G	*K*	N	I	K	N	D	S	I	in	65.3
G0 G96R	*R*	*K*	N	I	K	N	D	S	I	in	3.1
G0 N264K	G	*K*	N	I	K	*K*	D	S	I	in	0.5
G0 K150E	G	E	N	I	K	N	D	S	I	in	3.2
G0 K150E D337N	G	E	N	I	K	N	*N*	S	I	in	1.1
G0 K150E D337N N176S	G	E	*S*	I	K	N	*N*	S	I	in	1.0
G1	G	E	N	I	K	N	D	*G*	*M*	in	4.9
G2	G	E	N	I	K	N	D	S	I	*del*	2.0

Ref G1	G	E	N	*M*	*R*	N	D	*G*	*M*	in	
Ref G2	G	E	N	*M*	*R*	N	D	S	I	*del*	
Ref G1 M228I	G	E	N	I	*R*	N	D	*G*	*M*	in	
Ref G1 R225K	G	E	N	*M*	K	N	D	*G*	*M*	in	
Ref G2 M228I	G	E	N	I	*R*	N	D	S	I	*del*	
Ref G2 R225K	G	E	N	*M*	K	N	D	S	I	*del*	
G1 N264K	G	E	N	I	K	*K*	D	*G*	*M*	in	
G2 N264K	G	E	N	I	K	*K*	D	S	I	*del*	

Table S1: Differences in the various Apol1 constructs at amino acid positions along the protein sequence. The upper table lists known common human Apol1 haplotypes while the lower table lists constructs created for this research. Above each amino acid position is the SNP IDs for each individual nucleotide mutation. Global haplotype frequencies for the natural human constructs were obtained from 1000 Genome Project phase 1 data (UCSC Genome Browser) and listed in the rightmost column.

Haplotype	Freq. % (European)	Freq. % (African)	Freq. % (East Asian)
Ref	22.2	3.3	18.9
G0	72.3	30.3	78.8
G0 G96R	4.6	4.5	0.2
G0 N264K	0.0	2.0	0.0
G0 K150E	0.0	13.4	0.0
G0 K150E D337N	0.0	4.5	0.0
G0 K150E D337N N176S	0.0	3.9	0.0
G1	0.0	21.1	0.0
G2	0.0	8.5	0.0
Other	0.9	8.5	2.1

Table S2: Population frequencies of the common human Apol1 haplotypes tested in this study, as represented among subjects of European, African, and East Asian ancestry. Frequencies were obtained from 1000 Genome Project phase 1 data (UCSC Genome Browser) for Apol1.

Reference	Experimental System	Sequence Source Cited	Haplotype (Provided or Inferred)	Cytotoxicity and Related Phenotypes
Anderson BR, et al. PLoS Genet. **11** 2015	Zebrafish	GenBank: BC112943 (G0)	Fig. 1. C-terminus only. Haplotypes cannot be inferred.	G2 (but not G1) promotes developmental kidney defects.
Beckerman P, et al. Nat. Med. **23** 2017	HEK293 Mouse podocyte Mouse kidney	APOL1-CDS-NM-003	Sup. Fig. 1. C-terminus only. Haplotypes cannot be inferred.	G1 and G2 expressing mice develop more proteinuria than G0 expressing mice.
Bruggeman LA, et al. J. Am. Soc. Nephrol. **27** 2016	Mouse Mouse podocyte	GenBank: NM003661 (Ref)	G2 deletion generated by 6bp deletion on G0 plasmid.	Transgenic G2 expression in podocytes leads to lower podocyte density and more severe preeclampsia phenotype than G0 expression.
Cheng D, et al. J. Lipid Res. **56** 2015	Mouse Rat hepatoma COS7	pIRES2-EGFP-APOL1 vector Genotype not provided	Not Available	All genotypes cytotoxic with overexpression in rat hepatoma cells (G1>G0=G2).
Fu Y, et al. J. Am. Soc. Nephrol. **28** 2017	Drosophila Nephrocyte	G0 cDNA - GenBank: AAI43040 (Ref) G1 cDNA - Patient podocytes	Likely Ref vs. G1 on natural background haplotype.	G1 expression causes increased lethality in Drosophila compared to G0. G1 expression causes larger nephrocyte size and lower nephrocyte number relative to G0.
Granado D, et al. J. Am. Soc. Nephrol. **28** 2017	Human podocyte HEK293	Human cDNA library derived from AB8 podocytes	Not Available	G1 and G2 stably expressed in HEK293 cells cause more toxicity than G0. G1 and G2 stably expressed in podocytes cause more toxicity than G0.
Hayek SS, et al. Nat. Med. **23** 2017	Podocyte Mouse kidney	GenBank: NM001136540 (Ref TV3) GenBank: NM003661 (Ref)	Not Available	In vivo gene delivery of G1 and G2 but not G0 cause proteinuria and foot process effacement in mice.
Kruzel-Davila E, et al. J. Am. Soc. Nephrol. **28** 2017	D. melanogaster Nephrocyte S. cerevisiae	GenBank: NM145343 (Ref TV2)	Fig. 1. C-terminus only. Haplotypes cannot be inferred.	G1 and G2 cause increased nephrocyte cell death and lethality in fly, and increased toxicity in yeast compared to G0.
Lan X, et al. Am. J. Phys. Renal Phys. **307** 2014	Human podocyte	Not Available	Not Available	G1 and G2 expressing podocytes cause increased podocyte swelling, LDH release, and necrosis compared to G0 expressing podocytes.
Lan X, et al. Exp. Mol. Pathol. **98** 2015	Human podocyte Co-cultured with human smooth muscle cells	Not Available	Not Available	More podocyte death when co-cultured with G1 or G2 expressing smooth muscle cells than with G0 expressing smooth muscle cells.
Lan X, et al. Exp. Mol. Pathol. **99** 2015	HEK293T	Not Available	Not Available	Overexpression of G1 and G2 more toxic than G0 in HEK239T cell.
Ma L, et al. J. Am. Soc. Nephrol. **28** 2017	HEK293	pIRES2-EGFP-APOL1 vector Genotype not provided	Not Available	G1/G2 expression causes mitochondrial dysfunction. G1/G2 moderately more toxic than G0.
Nichols B, et al. Kidney Int. **87** 2015	HEK293 HUVEC	GenBank: NM003661 (Ref)	G0: Ref (E150 M228 R255). G1 and G2: generated on natural haplotype backgrounds (E150 I228 K255). Data not provided in original paper.	More cytotoxicity with G1 and G2 vs G0 overexpression by transient transfection in HEK293 cells.
Okamoto K, et al. Commun. Biol. **1** 2018	HEK293 Human podocyte Human kidney bioposy	HEK/Mice - NM001136540 (Ref TV3) Mice - NM003661 (Ref)	Sup. Fig. 1 & 8. Small part of C-terminus. Haplotype cannot be inferred.	G1 and G2 cause increased cytotoxicity when stably overexpressed in HEK293 cells compared to G0. G1 BAC transgenic mice have more albuminuria than G0 BAC transgenic mice in a glomerular injury model.
Olabisi OA, et al. Proc. Natl. Acad. Sci. **113** 2016	HEK293	RefSeqORF: 1197 (Ref)	G0: G0 (K150 I228 K255). G1 and G2: generated on K150 background (K150 I228 K255). Data not provided in original paper.	G1 and G2 cause more cytotoxicity than G0 when expressed after stable transfection in HEK293 cells (Tet-induced).
Olabisi O, et al. Clin. Neph. **86** 2016	Zebrafish	Not Available	Not Available	Podocyte specific expression of RV leads to increased glomerular histological defects compared to G0 but no overt phenotypes observed.

O'Toole JF, et al. J. Am. Soc. Nephrol. **29** 2018	HEK293	GenBank: NM003661 (Ref)	Sup. Fig. 5. G0: Ref, G1 and G2 on Ref background (E150 M228 R255).	No differences in cytotoxicity when comparing G0 vs RV overexpression.
Thomson R, et al. Proc. Natl. Acad. Sci. **111** 2014	Mouse liver	GenBank: NM003661 (Ref)	Sup. Fig. 1 (Ref).	Hydrodynamic gene delivery of G1 and G2 but not G0 cause liver injury in mice.
Wen H, et al. Biosci. Rep. **38** 2018	Human podocyte	GenBank: NM145343 (Ref TV2)	Fig. 2. C-terminus only. Haplotypes cannot be inferred.	G1 and G2 cause increased ER stress, decreased nephrin expression, and more injury compared to G0 when overexpressed in podocytes.
Zhang J, et al. Proc. Natl. Acad. Sci. **115** 2018	HEK293	RefSeqORF: 1197 (Ref)	G0: G0 (K150 I228 K255). G1 and G2: generated on natural haplotype backgrounds (E150 I228 K255). Data not provided in original paper.	G1 and G2 stably expressed in HEK293 cells cause more toxicity than G0.

Table S3: APOL1 sequence and haplotype data from previously published papers. We surveyed the literature through 2018 for papers that directly compared G0 and Risk Variants (RV) for APOL1-induced cytotoxicity and related phenotypes. The starting DNA source cited by the authors is presented in column 3 ("Sequence Source Cited"); GenBank entries almost exclusively contain the Reference APOL1 sequence and may not accurately reflect the sequence used in experiments. Column 4 reports either sequence data provided by the authors or the APOL1 haplotypes inferred where possible from sequence data and mutagenesis steps described in the paper's methods. In general, APOL1 plasmids available from commercial sources encode the Reference sequence and RV engineered onto this background will generate non-toxic RV unless the amino acids at positions 228 and 255 are also engineered to match the RV background found in nature. Abbreviations: Transcript Variant (TV).

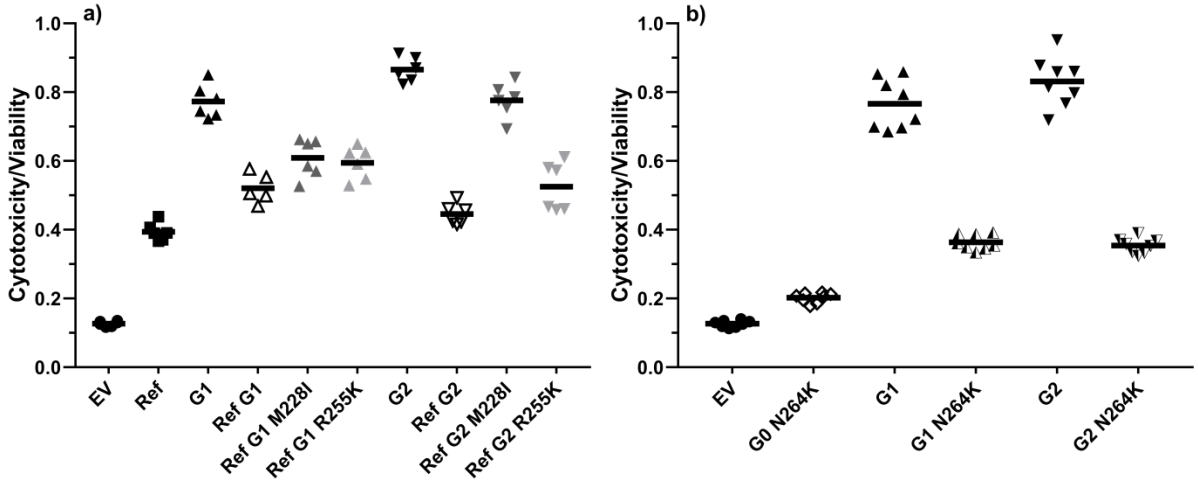

Figure S1: APOL1-induced cytotoxicity in transiently transfected HEK293 cells at longer incubation times. Cells were transfected using Lipofectamine 2000 and cytotoxicity to viability ratio was measured after 48 hours using the MultiTox-Fluor Multiplex Cytotoxicity Assay. Background toxicity has increased for all experimental conditions including EV suggesting either transfection reagent or bacterial DNA may cause some toxicity in longer experiments. a) G1 and G2 mutations on a Ref background show no difference in cytotoxicity compared to Ref APOL1 (M228/R255). Mutating either amino acid 228 (M to I) or 255 (R to K) in these artificial Ref G1 and Ref G2 variants partially restores toxicity. $P<0.001$ for G1 vs Ref G1, Ref G1 M228I, and Ref G1 R225K. $P<0.001$ for G2 vs. Ref G2, and Ref G2 R225K. b) A N264K mutation on a G1 or G2 background attentuates risk variant toxicity. $P<0.001$ for G1 vs G1 N264K and for G2 vs. G2 N264K.

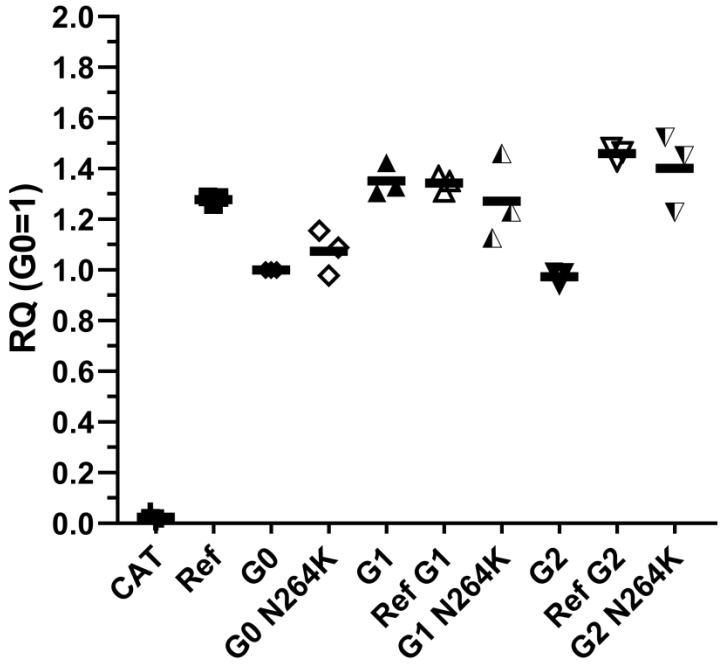

Figure S2: Stable APOL1-producing cell lines express similar levels of APOL1 mRNA. Stable T-Rex 293 cells induced with 100ng/mL tetracycline for 6h. Each measurement reflects 3 technical replicates and transcript levels were normalized to G0 with beta actin acting as an endogenous control. All APOL1-expressing inducible cell lines show similar APOL1 transcript levels, whereas a control cell producing CAT (Chloramphenicol Acetyltransferase) at the Flp-in locus shows minimal transcript APOL1 levels.

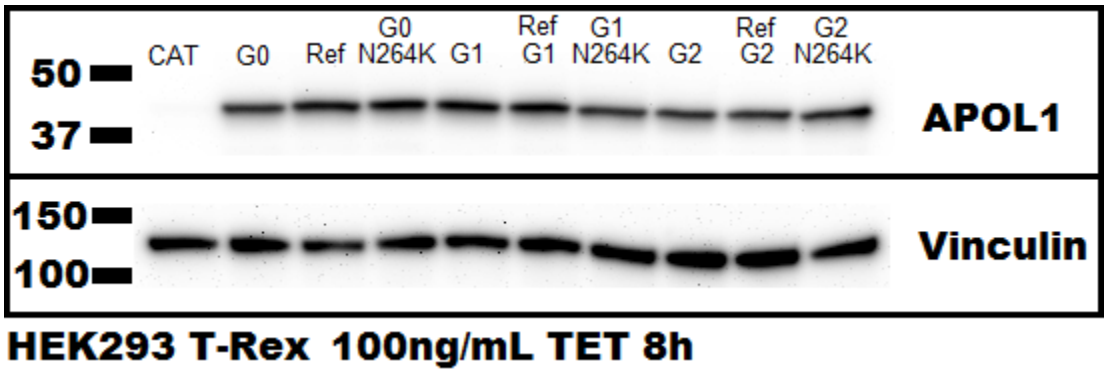

Figure S3: Western blot showing APOL1 protein levels for stable T-Rex 293 cells induced with 100ng/mL tetracycline for 8h. All APOL1 inducible cell lines show similar ApoL1 protein levels while a control cell producing CAT (Chloramphenicol Acetyltransferase) shows minimal ApoL1 protein levels. Protein ladder on left shows protein size in kDa.

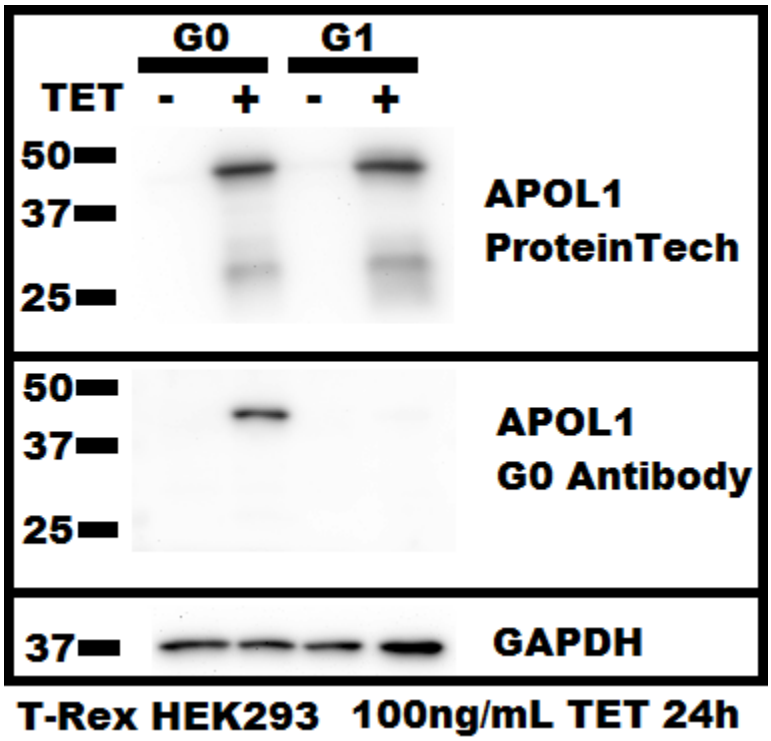

Figure S4: APOL1 transgene expression does not induce endogenous ApoL1 protein. Stable T-Rex 293 cells with either G0 or G1 transgenes were induced with 100ng/mL tetracycline for 24h. Western blots were probed with antibody that detects all APOL1 haplotypes (ProteinTech) and with a second antibody designed to recognize G0 but not G1 (see methods). APOL1 overexpression in T-Rex 293 cells does not induce detectable expression of the endogenous ApoL1 G0. Protein ladder on left shown in kDa.

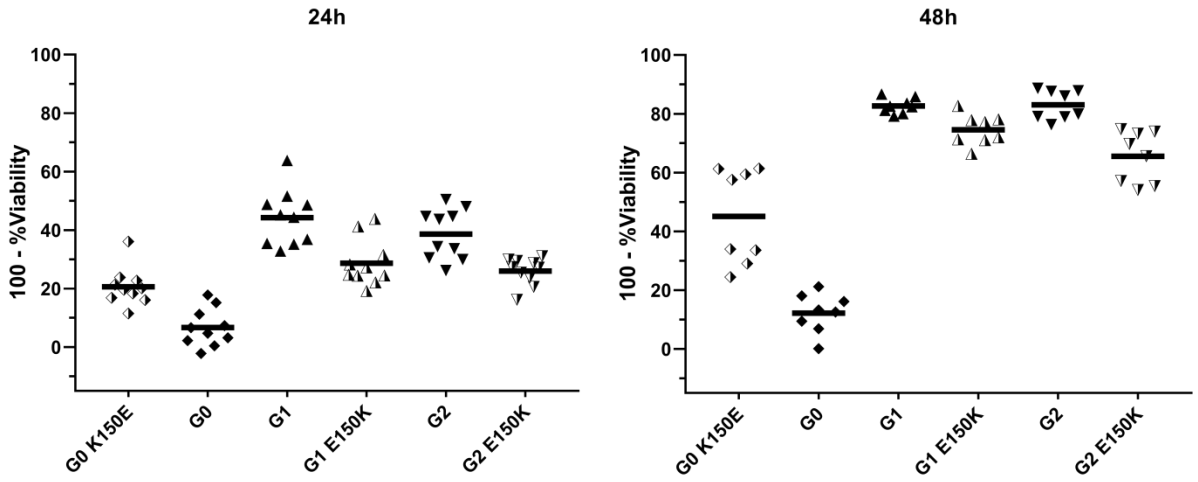

Figure S5: Effect of the E150K polymorphism on risk variant toxicity in HEK293 cells that stably express APOL1. APOL1 expression was induced from T-Rex HEK293 cells with addition of 100ng/ml tetracycline. Cytotoxicity was measured at 24 or 48 hours by MTT assay and expressed relative to its own no-tetracycline control. At 24 hours, cell death in the presence of the E150-containing haplotype is slightly but statistically increased compared to the K150 haplotype (differences much smaller that those between in Reference or N264K haplotype backgrounds): $P<0.001$ for G0 vs. G0 K150E and G1 vs. G1 E150K. $P<0.01$ for G2 vs. G2 E150K. At 48 hours, E150-containing haplotype are more toxic, with substantial differences observed for G0 and small differences for the risk variants: $P<0.001$ for G0 vs. G0 K150E and for G2 vs. G2 E150K. $P<0.01$ for G1 vs. G1 E150K.