Supplementary Tables

Table S1. SCAR16 patient variables.

ID	HZ	Genotype	Mut1	Mut2	Ex1	Ex2	AA1	AA2	Sex	AOO (y)	Ancestry	SARA	HG	CD	TR
1_04	N	c.82G>A c.430A>T	E28K	K144*	1	3	28	144	F	33	SAS	23‡	Y	Y	N
1_01	Y	c.194A>G	N65S	N/A	2	N/A	65	N/A	M	2	MENA	29‡	N	Y	Y
1_02	Y	c.194A>G	N65S	N/A	2	N/A	65	N/A	M	0.01	MENA	29‡	N	Y	Y
1_03	Y	c.194A>G	N65S	N/A	2	N/A	65	N/A	F	0.7	MENA	11‡	N	Y	Y
2_03	N	c.235G>A c.236C>A	A79T	A79D	2	2	79	79	M	49	MENA	14.5	N	N	Y
2_04	N	c.235G>A c.236C>A	A79T	A79D	2	2	79	79	M	29	MENA	14.5†	N	N	Y
3_01	N	c.335C>T c.880A>T	R119*	I294F	2	7	119	294	M	12	EUR	36	Y	Y	Y
2_01	Y	c.367C>G	L123V	N/A	3	N/A	123	N/A	M	2	EUR	10	N	N	N
4_05	N	c.389A>T c.441G>T	N130I	W147C	3	3	130	147	M	20	EAS	4	N	N	N
5_01	N	c.433A>C c.687-690delCTAC	K145Q	Y230Cfx*8	3	6	145	N/A	M	23	EUR	20†	N	Y	Y
5_02	N	c.433A>C c.687-690delCTAC	K145Q	Y230Cfx*8	3	6	145	N/A	M	25	EUR	20†	N	Y	Y
3_02	N	c.433A>C c.728C>T	K145Q	P243L	3	6	145	243	M	12	EUR	40	N	Y	Y
3_03	N	c.433A>C c.728C>T	K145Q	P243L	3	6	145	243	F	20	EUR	40	N	Y	Y
4_01	Y	c.493C>T	L165F	N/A	3	N/A	165	N/A	F	17	EAS	34	N	Y	N
4_02	Y	c.493C>T	L165F	N/A	3	N/A	165	N/A	F	17	EAS	15	N	N	Y
4_03	Y	c.493C>T	L165F	N/A	3	N/A	165	N/A	F	14	EAS	21	N	Y	Y
4_04	Y	c.493C>T	L165F	N/A	3	N/A	165	N/A	M	19	EAS	15	N	N	Y
4_06	N	c.621C>G c.707G>C	Y207X	S236T	5	6	207	236	F	16	EAS	5	N	N	Y
6_01	N	c.633G>A c.712G>T	M211I	E238*	5	6	211	238	F	25	EUR	34†	N	Y	Y
6_02	N	c.633G>A c.712G>T	M211I	E238*	5	6	211	238	M	22	EUR	30†	N	Y	Y
2_02	Y	c.719T>C	M240T	N/A	6	N/A	240	N/A	F	16	MENA	12	N	Y	N
7_01	Y	c.737C>T	T246M	N/A	6	N/A	246	N/A	F	19	EAS	13	Y	Y	N
7_02	Y	c.737C>T	T246M	N/A	6	N/A	246	N/A	F	17	EAS	15	Y	Y	Y
8_01	N	c.612+1G>C c.823C>G	syn	L275V	i 4-5	7	N/A	275	M	15	AMR	17	N	Y	Y

ID is unique identifier comprised of the referenced study (see Supplementary References) and a number in the format ref_##, HZ = homozygous mutation, Mut = mutation, Ex = exon, AA = amino acid, AOO = age of onset, AMR = Admixed American, SAS = South Asian, MENA = Middle Eastern North African, EUR = European, EAS = East Asian, SARA = scale for the assessment and rating of ataxia, HG = hypogonadism, CD = cognitive dysfunction, TR = increased tendon reflex, Syn = synonymous mutation, N/A = not applicable.

[a] imputed from the clinical report

[b] calculated from Disability Scale

[c] imputed from clinical report

Supplementary Tables (cont.)

Table S2. Bivariate analysis of AOO and SARA with SCAR16 patient phenotypes.

	SARA			AOO		
Variable	Statistic	*P*	Q	Statistic	*P*	Q
Cognitive dysfunction (yes)	**13.8**	**0.002**	**0.011**	-5.67	0.252	0.756
Ancestry (MENA, EUR, EAS)	**4.13**[a]	**0.032**	**0.097**	0.03[a]	0.735	0.999
Increased tendon reflex (yes)	6.56	0.199	0.398	-0.18	0.972	0.999
Homozygosity (yes)	-4.38	0.328	0.491	**-11.9**	**0.005**	**0.027**
AOO (year)	-0.08[b]	0.721	0.792		N/A	
Sex (male)	1.19	0.792	0.792	-0.01	0.999	0.999
SARA		N/A		-0.08[b]	0.721	0.999

Student's t tests were used to calculate the difference (statistic), *P*, and Q values between scale for the assessment and rating of ataxia (SARA) or age of onset (AOO) and each variable, unless otherwise indicated. Super populations for ancestry: MENA = Middle Eastern North African, EUR = European, EAS = East Asian.

[a]ANOVA was used and the F ratio is provided as the statistic
[b]Linear regression was used and the beta is provided as the statistic

Table S3. Biochemical properties of mutant CHIP proteins.

Mut	AA#	Ex	Domain	Oligomer	T_m	%E	%Ub chain	%HSP70 Ub	K_D	B_{max}
WT	N/A	N/A	N/A	dimer	39.0	100	100	73	2.9	203.6
E28K	28	1	TPR	oligomer	35.4	50	120	46	10.1	204.8
N65S	65	2	TPR	dimer	39.0	40	25	42	N/A	N/A
A79T	79	2	TPR	dimer	37.5	60	120	47	3.9	192.4
A79D	79	2	TPR	oligomer	31.4	25	110	47	9.2	188.2
L123V	123	3	TPR	oligomer	32.1	40	75	46	N/A	N/A
N130I	130	3	CC	dimer	37.6	0	90	47	16.2	215.9
K145Q	145	3	CC	dimer	36.7	0	95	49	3.9	202.7
W147C	147	3	CC	oligomer	37.2	90	90	45	3.3	216.7
L165F	165	3	CC	dimer	36.7	10	95	57	3.9	207
M211I	211	5	CC	dimer	41.1	90	100	63	2.5	206.7
S236T	236	6	Ubox	dimer	38.2	80	25	49	4.3	214.5
M240T	240	6	Ubox	oligomer	41.1	60	15	25	17.5	243.7
T246M	246	6	Ubox	oligomer	27.1	15	15	7	17.8	238.6

AA = amino acid, T_m = thermal melting temperature (°C), %E = percentage of steady-state protein expression relative to wild-type (WT) CHIP, %Ub chain = percentage of E2-mediated ubiquitin chain formation relative to CHIP-WT, %HSP70 Ub = percentage of HSP70 that was modified by ubiquitination, K_D = equilibrium dissociation constant (μM), B_{max} = amount of HSP70 binding (μmol•min^{-1}). TPR = tetratricopeptide repeat, CC = coiled-coil, Ubox = U-box domain containing. N/A = saturation not reached.

Table S4. Multivariate analysis of CHIP biochemical properties.

Variable 1	Variable 2	r	P	Q
B_{max}	%Ub chain	**-0.856**	**0.0004**	**0.006**
K_D	%HSP70 Ub	**-0.760**	**0.0042**	**0.032**
%HSP70 Ub	%Ub chain	**0.641**	**0.0135**	**0.068**
B_{max}	%HSP70 Ub	**-0.678**	**0.0154**	**0.058**
B_{max}	K_D	**0.658**	**0.0199**	**0.060**
%HSP70 Ub	T_m	0.521	0.0559	0.140
%E	T_m	0.488	0.0770	0.165
K_D	%Ub chain	-0.521	0.0824	0.155
K_D	%E	-0.445	0.1468	0.245
%HSP70 Ub	%E	0.380	0.1801	0.270
K_D	T_m	-0.312	0.3231	0.441
%Ub chain	T_m	0.055	0.8511	1.000
B_{max}	T_m	0.045	0.8898	1.000
B_{max}	%E	-0.031	0.9227	1.000
%Ub chain	%E	0.026	0.9309	1.000

The Pearson correlation coefficient (r), *P*, and Q values of the pair-wise comparisons of biochemical properties of CHIP proteins. B_{max} = amount of binding, K_D = equilibrium dissociation constant, T_m = thermal melting temperature, %Ub chain = E2-dependent ubiquitin chain formation, %E = steady-state expression levels relative to wild-type CHIP, %HSP70 Ub = amount of ubiquitinated HSP70 relative to wild-type CHIP.

Table S5. Analysis of SCAR16 mutation location with biochemical properties of the encoded mutant CHIP protein

Biochemical property	F ratio	*P*	Q
%Ub chain	9.3	0.0053	0.0165
B_{max}	10.7	0.0055	0.0165
%HSP70 Ub	5.2	0.0281	0.0562
K_D	1.5	0.2871	0.4307
T_m	0.6	0.5430	0.6516
%E	0.1	0.8658	0.8358

The F ratio, *P*, and Q values of from ANOVA testing of the three domains of CHIP with the variance in biochemical properties. B_{max} = amount of binding, K_D = equilibrium dissociation constant. T_m = thermal melting temperature, %Ub chain = E2-dependent ubiquitin chain formation, %E = steady-state expression levels relative to wild-type CHIP, HSP70 Ub = amount of ubiquitinated HSP70 relative to wild-type CHIP.

Table S6. Analysis of cognitive dysfunction and increased tendon reflex with biochemical properties of CHIP.

	CD			TR		
Variable	Statistic	*P*	Q	Statistic	*P*	Q
%Ub chain	**-34.5**	**0.0086**	**0.060**	9.0	0.5328	0.622
B_{max}	**14.3**	**0.0232**	**0.081**	**-17.7**	**0.0058**	**0.020**
%HSP70 Ub	-9.51	0.0772	0.180	9.87	0.0787	0.138
K_D	3.01	0.2003	0.351	**-6.21**	**0.0079**	**0.020**
T_m	0.82	0.5666	0.678	1.61	0.2756	0.386
%E	-5.39	0.5811	0.678	-5.06	0.6195	0.620
Oligomeric (yes)		0.7160[a]	0.716		**0.0018**[a]	**0.013**

Student's t tests were used to calculate the difference (statistic), *P*, and Q values between cognitive dysfunction (CD) or increased tendon reflex (TR) and each variable, unless otherwise indicated.

[a]Fisher's Exact Test (2-tailed)

Table S7. Analysis AOO and SARA with biochemical properties of CHIP.

	SARA			AOO		
Variable	Statistic	*P*	Q	Statistic	*P*	Q
%Ub chain	0.046	0.2865	0.334	**0.182**	**0.0001**	**0.001**
B_{max}	-0.190	0.1014	0.178	**-0.286**	**0.0044**	**0.016**
K_D	**-0.715**	**0.0228**	**0.080**	-0.148	0.6203	0.740
Oligomeric (yes)	**-9.083**[a]	**0.0070**	**0.049**	4.066[a]	0.3372	0.740
%HSP70 Ub	0.204	0.0559	0.130	0.071	0.5862	0.821
T_m	0.665	0.1081	0.178	-0.503	0.3170	0.821
%E	-0.056	0.3643	0.364	0.020	0.7916	0.821

Linear regression was used to calculate the beta (statistic), *P*, and Q values between SARA or age of onset (AOO) with the indicated variables, unless otherwise indicated. Variables that were included in the regression modeling are indicated as well as the beta coefficients from the initial fit. SARA = Scale for the Assessment and Rating of Ataxia, B_{max} = amount of HSP70 binding, K_D = equilibrium dissociation constant. T_m = thermal melting temperature.

[a]T test was used and the difference reported as the statistic

Supplementary Figures

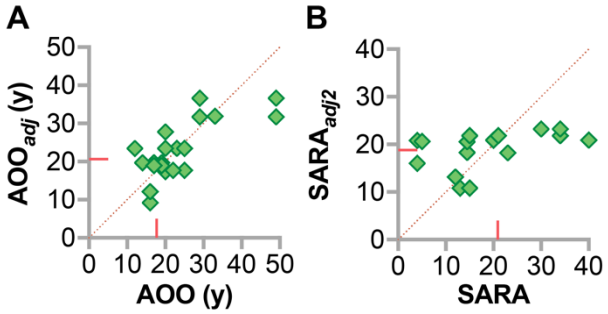

Figure S1. Regression model performance of age of onset and SARA as a function of the biochemical properties of mutant CHIP proteins.

(**A**) Scatter plot of actual age of onset (AOO) by predicted onset (AOO_{adj}) via the PLS model. (**B**) Scatter plot of actual SARA (Scale for the Assessment and Rating of Ataxia) by predicted SARA ($SARA_{adj2}$) plot of the PLS model. The mean values of the adjusted (y-axis) and actual (x-axis) are indicated by the red tick marks.

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