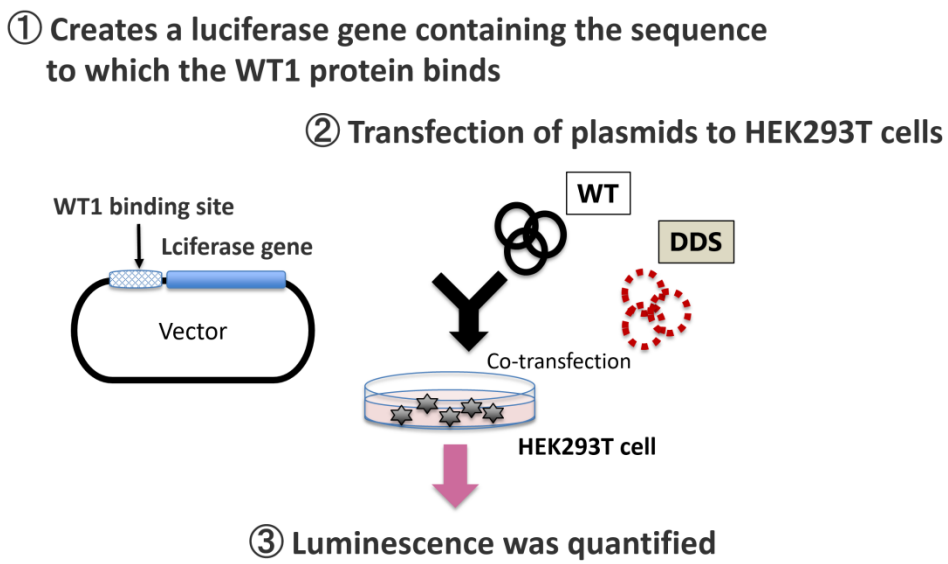
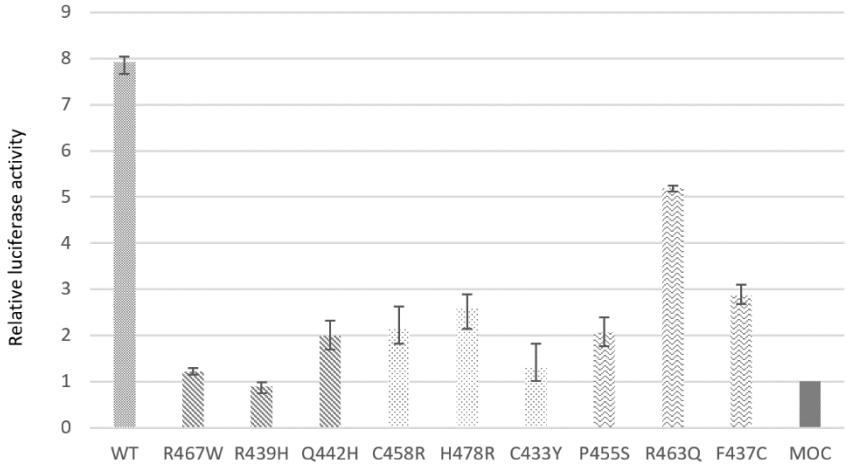
Supplemental Material Table of Contents

Supplemental Figure S1. Schema of functional analysis

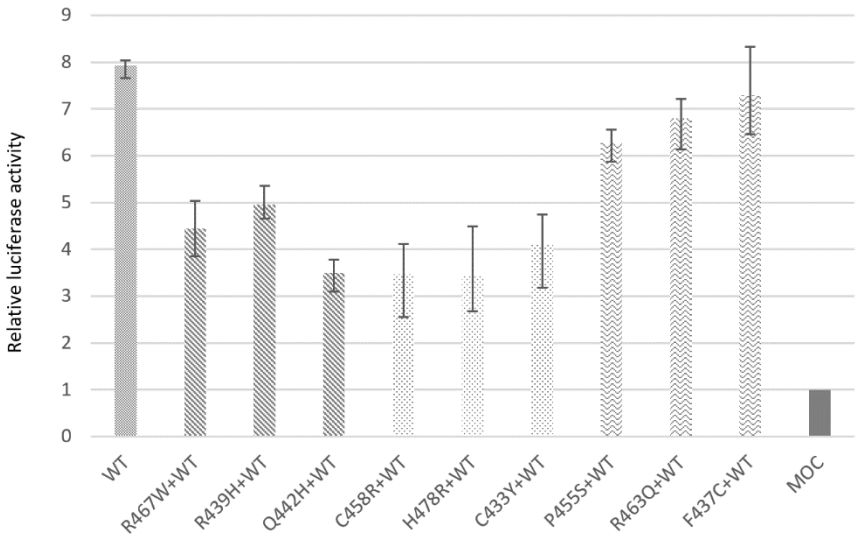


Supplemental Figure S2. Luciferase analysis

A) The promoter activities associated with all mutations upon transfection with wild-type or mutant vectors are shown. R467W (p.Arg467Trp), R439H (p.Arg439His), and Q442H (p.Gln442His) were classified as the DBS group. C458R (p.Cys458Arg), H478R (p.His478Arg), and C433Y (p.Cys433Tyr) were classified as the C2H2 group. P455S (p.Pro455Ser), R463Q (p.Arg463Gln), and F437C (p.Phe437Cys) were classified as the Others group.

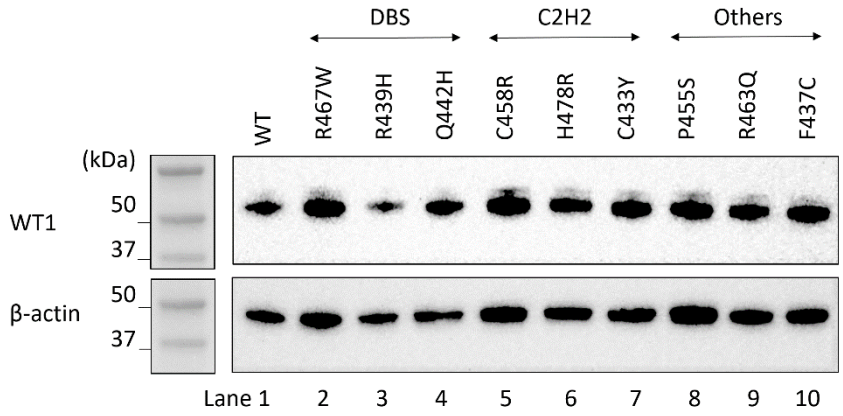


B) The promoter activities for all mutations upon (co-)transfection with wild-type and mutant vectors are shown.



Supplemental Figure S3. Western blot analysis of WT1 proteins

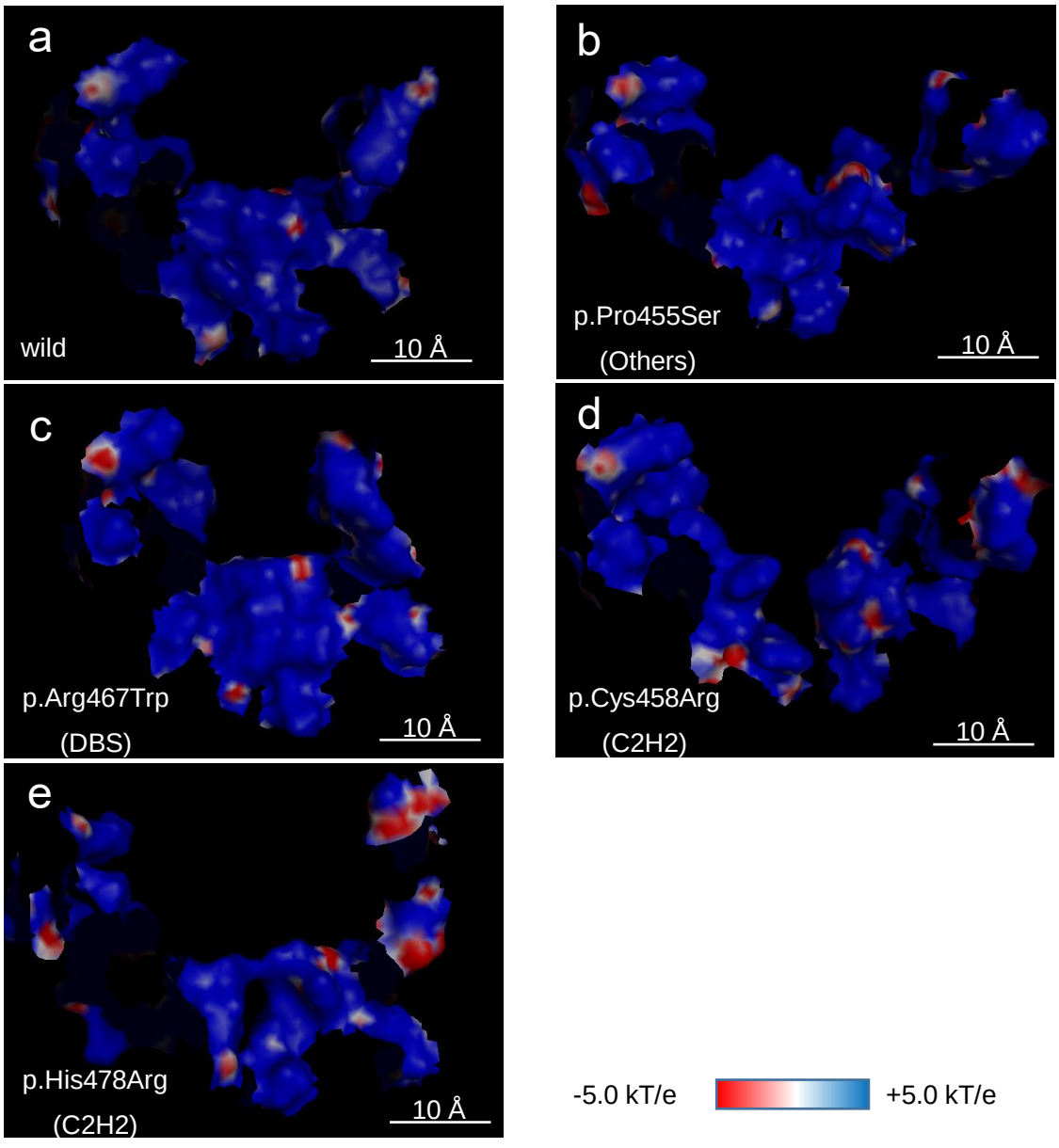
Protein (2 μg) was fractionated on 4%–20% Mini-PROTEAN® TGX™ Precast Protein gels (Bio-Rad) and processed for western blotting with chemiluminescence detection. The wild-type WT1 is shown in lane 1, DBS is shown in lanes 2–4, C2H2 is shown in lanes 5–7, and Others are shown in lanes 8–10.



Supplemental Figure S4. Electrostatic surface potential of DNA binding region

Electrostatic surface potential of only the DNA binding region in the zinc finger domain is shown. Positive to negative alterations of the surface charge in the DNA binding region were increased (more red-colored areas in mutants), and there was a tendency for a reduction of the region under these amino acid substitutions. These changes reduce the capacity for DNA binding.

a, wild type; b, p.Pro455Ser (Others); c, p.Arg467Trp (DBS); d, p.Cys458Arg (C2H2); e, p.His478Arg (C2H2); bar, 10 Å.



Supplemental Table S1. Primers for cloning and mutagenesis

		5’ 3’
Oligonucleotides	F	GTACCTGAGCTCGCTAGTGTGGGAGAGACACTAGAGGG TATATAATGGAAGCTCGACTTCCAG
	R	TGATATCCTCGAGGCCTGGAAGTCGAGCTTCCATTATA TACCCTCTAGTGTCTCTCCCACACC
p. Pro455Ser	F	TGTGAAATCATTCCAGTGTAAAACTTG
	R	TGGAATGATTTCACACCTGTATGTC
p. Cys458Arg	F	ATTCCAGCGTAAAACTTGTCAGCGA
	R	GTTTTACGCTGGAATGGTTTCACACC
p. Arg467Trp	F	GTTCTCCTGGTCCGACCACCTGAAG
	R	TCGGACCAGGAGAACTTTCGCTGACA
p. His478Arg	F	AGGACTCGTACAGGTGAAAAGCCCTTC
	R	ACCTGTACGAGTCCTGGTGTGGGTC
p.Arg439His	F	TTTTCTCATTCAGACCAGCTCAAAAG
	R	GTCTGAATGAGAAAACCTTCGTTCA
p.Gln442His	F	CAGACCATCTCAAAAGACACCAAAG
	R	TTTTGAGATGGTCTGAACGAGAAAA
p.Cys433Tyr	F	AAGGACTATGAACGA AGGTTTTCTCG
	R	TCGTTCATAGTCCTTGAAGTCACACTG
p.Arg463Gln	F	TGTCAGCAAAAGTTCTCCCGGTCCGA
	R	GAACTTTTGCTGACAAGTTTTACACTG
p.Phe437Cys	F	CGAAGGTGTTCTCGT TCAGACCAGCT C
	R	ACGAGAACACCTTCGTTCACAGTCCTTG

Supplemental Table S2. Characteristics of 161 cases

No.	Sex	Age at onset (y)	Age at ESKD (y)	Extra-renal symptom	Wilms' tumor	Exon	Gene variants		Binding site	reference
1	M	6.30	6.30		No	8	1270A>G	Lys424Glu	Others	1
2	F	1.00		No	Yes	8	1282T>G	Cys428Gly	C2H2	2
3	F	1.00	1.00		Yes	8	1283G>A	Cys428Tyr	C2H2	3
4	F	1.00	1.00		Yes	8	1283G>A	Cys428Tyr	C2H2	4
5	F	1.30	10.00	No	No	8	1297T>A	Cys433Ser	C2H2	5
6	F	0.40	0.50	Yes		8	1297T>C	Cys433Arg	C2H2	6
7	F	2.50	2.80		Yes	8	1298G>A	Cys433Tyr	C2H2	6
8	F	2.70	2.70	No	No	8	1298G>A	Cys433Tyr	C2H2	7,8
9	F	0.17				8	1298G>A	Cys433Tyr	C2H2	9
10	M	1.75	1.75		No	8	1304G>C	Arg435Pro	Others	11
11	M	0.50	0.75		No	8	1309T>C	Phe437Leu	Others	12
12	M	11.00		No		8	1310T>G	Phe437Cys	Others	13
13	F	0.08	0.08		No	8	1315C>T	Arg439Cys	DBS	10
14	F	0.00	0.08		No	8	1315C>T	Arg439Cys	DBS	10
15	F	0.08	0.08		No	8	1315C>T	Arg439Cys	DBS	14
16	M	0.08		Yes		8	1315C>T	Arg439Cys	DBS	9
17	M	0.02	0.17	No		8	1315C>T	Arg439Cys	DBS	15
18	F	0.00	0.10			8	1316G>A	Arg439His	DBS	6
19	F	0.01	0.01	No	No	8	1316G>A	Arg439His	DBS	12
20	F	2.00				8	1316G>A	Arg439His	DBS	16
21	M	prenatal		No		8	1316G>A	Arg439His	DBS	9
22	F	0.17		Yes		8	1316G>A	Arg439His	DBS	9
23	F	0.08		Yes		8	1316G>A	Arg439His	DBS	9
24	M	0.02		Yes		8	1316G>A	Arg439His	DBS	9
25	F	0.08		Yes		8	1316G>A	Arg439His	DBS	9
26	F	0.08				8	1316G>A	Arg439His	DBS	17
27	F	0.00	0.10		No	8	1316G>A	Arg439His	DBS	18
28	M	0.04		No		8	1316G>A	Arg439His	DBS	15

29	F	0.02	died at 1 mo			8	1316G>A	Arg439His	DBS	15
30	F	0.17	died at 13 mo			8	1316G>A	Arg439His	DBS	15
31	M	0.06		No		8	1316G>A	Arg439His	DBS	15
32	F	0.00	0.08		Yes	8	1316G>A	Arg439His	DBS	3
33	F	0.17	1.08		No	8	1316G>A	Arg439His	DBS	19
34	F	0.04	0.17	Yes	No	8	1316G>A	Arg439His	DBS	20
35	F		0.08		Yes	8	1316G>A	Arg439His	DBS	4
36	F	0.15	0.15			8	1316G>C	Arg439Pro	DBS	21
37	F	0.17	0.19		Yes	8	1316G>C	Arg439Pro	DBS	21
38	M	0.92		No	No	8	1316G>T	Arg439Leu	DBS	22
39	F	0.83	2.50			8	1326G>C	Gln442His	DBS	17
40	M	1.00	3.00		No	8	1326G>T	Gln442His	DBS	23
41	F	0.90	2.70		No	8	1326G>T	Gln442His	DBS	6
42	M	3.50		No	No	8	1336C>T	His446Tyr	C2H2	24
43	F	0.33	1.08		No	8	1337A>G	His446Arg	C2H2	11
44	F	5.80	11.90		No	8	1338C>A	His446Gln	C2H2	18
45	F	5.70	12.00	No	No	8	1338C>A	His446Gln	C2H2	18
46	F	1.60		No	No	8	1340A>C	Gln447Pro	Others	25
47	ambiguous	0.25	0.25		Yes	8	1348C>A	His450Asn	C2H2	26
48	F	0.50	3.83		No	8	1348C>T	His450Tyr	C2H2	10
49	M	0.25		No	Yes	8	1349A>T	His450Leu	C2H2	27
50	M	0.23		No	Yes	8	1349A>T	His450Leu	C2H2	28
51	M	1.30	1.30	No	Yes	8	1352C>G	Thr451Arg	Others	6
52	F	5.00				8	1352C>G	Thr451Arg	Others	9
53	M	3.00		No		8	1352C>T	Thr451Ile	Others	29
54	F	0.25	0.25		No	9	1354G>T	Gly452Cys	Others	12
55	F	0.25	0.25		No	9	1354G>T	Gly452Cys	Others	30
56	F	10.70	no (at 10.7)	No	No	9	1363C>T	Pro455Ser	Others	7
57	M	0.67	3.00		No	9	1366T>C	Phe456Leu	Others	10
58	M	2.00	2.00		No	9	1372T>C	Cys458Arg	C2H2	3
59	M	0.60	1.00	No	No	9	1372T>C	Cys458Arg	C2H2	6
60	M	1.00		No		9	1372T>C	Cys458Arg	C2H2	9
61	M	3.17	4.33	No	Yes	9	1372T>C	Cys458Arg	C2H2	12

62	M	0.58	1.25		No	9	1372T>C	Cys458Arg	C2H2	31
63	M	2.92	4.17		Yes	9	1372T>C	Cys458Arg	C2H2	31
64	F		2.00	No	No	9	1372T>C	Cys458Arg	C2H2	4
65	F	0.08	1.67		Yes	9	1381T>C	Cys461Arg	C2H2	32
66	F	0.10	0.20		Yes	9	1381T>C	Cys461Arg	C2H2	6
67	F	0.10	1.80	No	Yes	9	1381T>C	Cys461Arg	C2H2	33
68	M	0.25		Yes		9	1381T>C	Cys461Arg	C2H2	9
69	F	0.42	1.08		Yes	9	1382G>A	Cys461Tyr	C2H2	34
70	F	30.00				9	1388G>A	Arg463Gln	Others	35
71	M	24.00	27.00			9	1388G>A	Arg463Gln	Others	35
72	M	28.00	33.00			9	1388G>A	Arg463Gln	Others	35
73	M	16.00	17.00			9	1388G>A	Arg463Gln	Others	35
74	F	10.00				9	1388G>A	Arg463Gln	Others	9
75	F	30.00				9	1388G>A	Arg463Gln	Others	6
76	M	1.50	no (at 9)	No	No	9	1395C>G	Phe465Leu	Others	36
77	F	7.00	no		No	9	1397C>T	Ser466Phe	Others	37
78	F	0.60	0.90		Yes	9	1399C>G	Arg467Gly	DBS	6
79	F	0.90	0.90	Yes	No	9	1399C>T	Arg467Trp	DBS	6
80	M	3.50	5.80		Yes	9	1399C>T	Arg467Trp	DBS	6
81	M	1.50	1.70	Yes	No	9	1399C>T	Arg467Trp	DBS	6
82	F	0.60	no (at 2.7)		Yes	9	1399C>T	Arg467Trp	DBS	6
83	F	1.50	1.50	Yes	No	9	1399C>T	Arg467Trp	DBS	6
84	M	1.50	1.50		Yes	9	1399C>T	Arg467Trp	DBS	6
85	F	0.30	0.80		No	9	1399C>T	Arg467Trp	DBS	6
86	F	0.80	no (at 2)		No	9	1399C>T	Arg467Trp	DBS	6
87	M	2.00		No	Yes	9	1399C>T	Arg467Trp	DBS	9
88	M	7.00			Yes	9	1399C>T	Arg467Trp	DBS	9
89	M	0.60		Yes		9	1399C>T	Arg467Trp	DBS	9
90	M	3.00		Yes		9	1399C>T	Arg467Trp	DBS	9
91	F	0.00				9	1399C>T	Arg467Trp	DBS	9
92	M	0.02		No		9	1399C>T	Arg467Trp	DBS	9
93	F		no (at 7)	No	Yes	9	1399C>T	Arg467Trp	DBS	38
94	F	0.50	1.75		Yes	9	1399C>T	Arg467Trp	DBS	39

95	F	0.33	1.67	No	Yes	9	1399C>T	Arg467Trp	DBS	12
96	M	2.67	5.58	No	Yes	9	1399C>T	Arg467Trp	DBS	12
97	F	1.50	1.50		Yes	9	1399C>T	Arg467Trp	DBS	12
98	F	0.50	0.50		No	9	1399C>T	Arg467Trp	DBS	12
99	M	0.01	no (at 1.2)		No	9	1399C>T	Arg467Trp	DBS	40
100	F	1.17	1.17		Yes	9	1399C>T	Arg467Trp	DBS	40
101	M	0.83	1.08		Yes	9	1399C>T	Arg467Trp	DBS	40
102	M	4.92	4.92		No	9	1399C>T	Arg467Trp	DBS	40
103	F	0.58	0.58		No	9	1399C>T	Arg467Trp	DBS	40
104	M	0.58	0.58		No	9	1399C>T	Arg467Trp	DBS	40
105	F	4.08	4.33		No	9	1399C>T	Arg467Trp	DBS	40
106	F	0.08	no (at 0.38)		No	9	1399C>T	Arg467Trp	DBS	40
107	M	1.42	no (at 6.6)		Yes	9	1399C>T	Arg467Trp	DBS	40
108	F	0.67	0.67		No	9	1399C>T	Arg467Trp	DBS	40
109	M	1.83	5.17		Yes	9	1399C>T	Arg467Trp	DBS	40
110	F	0.58	0.58		No	9	1399C>T	Arg467Trp	DBS	40
111	F	1.33	7.83		Yes	9	1399C>T	Arg467Trp	DBS	40
112	F	0.42	1.75		Yes	9	1399C>T	Arg467Trp	DBS	40
113	F	0.60	0.60			9	1399C>T	Arg467Trp	DBS	16,41
114	F	1.00				9	1399C>T	Arg467Trp	DBS	16,41
115	ambiguous	3.17	4.42		No	9	1399C>T	Arg467Trp	DBS	10
116	ambiguous	0.01	0.05		No	9	1399C>T	Arg467Trp	DBS	10
117	F	0.25	2.08	No	Yes	9	1399C>T	Arg467Trp	DBS	10
118	M	3.50	4.00	No	No	9	1399C>T	Arg467Trp	DBS	16
119	M	1.00	2.50	No		9	1399C>T	Arg467Trp	DBS	15
120	M		0.92	No	No	9	1399C>T	Arg467Trp	DBS	4
121	M			No		9	1399C>T	Arg467Trp	DBS	42
122	M	0.03	0.07	No	No	9	1400G>A	Arg467Gln	DBS	12
123	M	0.01	0.33		No	9	1400G>A	Arg467Gln	DBS	14
124	M	0.17	0.42		No	9	1400G>A	Arg467Gln	DBS	14
125	M	0.04	0.01		No	9	1400G>A	Arg467Gln	DBS	14
126	F	0.00	0.10		No	9	1400G>A	Arg467Gln	DBS	6
127	M	0.00	0.10	No		9	1400G>A	Arg467Gln	DBS	6

128	F	0.58	0.58		No	9	1400G>C	Arg467Pro	DBS	43
129	M	0.08	0.08		No	9	1400G>C	Arg467Pro	DBS	43
130	M	0.20	0.40	No	Yes	9	1400G>T	Arg467Leu	DBS	6
131	M	0.42	0.58	No	Yes	9	1400G>T	Arg467Leu	DBS	10
132	M	0.83	no	No	No	9	1405G>A	Asp469Asn	Others	25
133	M	0.25	1.42		No	9	1405G>A	Asp469Asn	Others	40
134	F	1.58	3.92		Yes	9	1405G>A	Asp469Asn	Others	40
135	M	0.33	0.33		No	9	1405G>A	Asp469Asn	Others	40
136	F	1.10	2.50		Yes	9	1405G>A	Asp469Asn	Others	40
137	F	1.50	no (at 5)		Yes	9	1405G>A	Asp469Asn	Others	6
138	M	0.64	0.64	No	No	9	1405G>A	Asp469Asn	Others	12
139	F	1.00	2.25		No	9	1405G>A	Asp469Asn	Others	10
140	F	0.17				9	1405G>A	Asp469Asn	Others	17
141	M	1.17	1.17	No	No	9	1405G>A	Asp469Asn	Others	4
142	F	0.08				9	1405G>A	Asp469Asn	Others	9
143	M	0.25	0.33		Yes	9	1405G>C	Asp469His	Others	44
144	F	0.02	died at 6 mo			9	1405G>T	Asp469Tyr	Others	15
145	F	0.08	0.08			9	1405G>T	Asp469Tyr	Others	19
146	F	0.33	1.00		Yes	9	1406A>G	Asp469Gly	Others	40
147	M	0.75		No		9	1408C>T	His470Tyr	DBS	9
148	F	5.25	5.25		Yes	9	1409A>C	His470Pro	DBS	32
149	F	1.25	9.33		No	9	1409A>C	His470Pro	DBS	32
150	F	1.10	8.70	No	No	9	1409A>C	His470Pro	DBS	6
151	F	1.20	6.10	No	No	9	1409A>C	His470Pro	DBS	33
152	ambiguous	0.67	no (at 6)	No	No	9	1412T>C	Leu471Pro	Others	10
153	M	7.30	11.20		No	9	1412T>G	Leu471Arg	Others	6
154	F	4.83			Yes	9	1420C>T	His474Tyr	C2H2	45
155	F	0.30	3.40		Yes	9	1421A>G	His474Arg	C2H2	6
156	F	0.50	3.80	No		9	1421A>G	His474Arg	C2H2	7
157	ambiguous		before 2	No	No	9	1422C>A	His474Gln	C2H2	46
158	M	16.00	no	No	No	9	1427G>A	Arg476Lys	Others	47
159	F	1.75			Yes	9	1432C>G	His478Asp	C2H2	28
160	F	1.50	1.92	Yes	Yes	9	1433A>G	His478Arg	C2H2	48

161	F	3.00	4.00			9	1434T>A	His478Gln	C2H2	49

Supplemental Table S2.

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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	5-6
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5-6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	7
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	7
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	7
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	7
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Sup Table S2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	11
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	7
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	12
Study characteristics	17	Cite each included study and present its characteristics.	12
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	12
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	12
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	12
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	12-13
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	12-13
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	18
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	7
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	19
Competing interests	26	Declare any competing interests of review authors.	19
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Sup Table S2

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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