						914.13	2	2.82	91-99	IVVVTAGVR
						720.84	1	1.94	113-118	NVNVFK
						958.22	1	1.64	119-126	FIIPQIVK
						1249.35	2	2.59	158-169	VIGSGCNLDSAR
						1284.51	2	3.41	233-243	M*VVESAYEVIK
						960.11	2	2.08	299-307	GLTSVINQK
						1287.49	2	4.09	308-318	LKDDEVAQLKK
35	Cellular retinaldehyde-binding protein	P12271	36.3	4.98	22.15					
						1032.17	2	2.70	19-27	AQLEQLTTK
						975.04	2	2.79	47-54	AKDELNER
						1326.48	2	2.64	87-97	VQEKDSGFFLR
						1865.08	2	4.39	121-136	LQYPELFDSLSPEAVR
						1510.67	2	3.92	137-150	CTIEAGYPGVLSSR
						1442.65	2	3.39	222-233	M*VDM*LQDSFPAR
36	Inorganic pyrophosphatase	Q15181	32.7	5.54	11.76					
						1486.76	2	3.04	58-70	M*EIATKDPLNPIK
						1267.46	2	2.46	64-74	DPLNPIKQDVK
						1178.36	2	3.16	212-221	DKDFAIDIIK
36	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	P62873	37.2	5.60	6.49					
						1018.15	2	2.72	68-77	LLVSASQDGK
						1226.35	2	3.33	197-208	LFVSGACDASAK
37	Inorganic pyrophosphatase	Q15181	32.7	5.54	14.88					
						1486.76	2	3.02	58-70	M*EIATKDPLNPIK
						1267.46	2	2.30	64-74	DPLNPIKQDVK
						1807.06	2	4.83	140-155	VLGILAM*IDEGETDWK
						1178.36	2	3.34	212-221	DKDFAIDIIK
37	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	P62873	37.2	5.60	11.50					
						1018.15	2	2.65	68-77	LLVSASQDGK
						805.99	1	1.98	89-95	VHAIPLR
						1226.35	2	3.55	197-208	LFVSGACDASAK
						1010.09	2	2.25	304-313	AGVLAGHDNR
38	Malate dehydrogenase, mitochondrial	P40926	35.5	8.92	19.82					
						993.14	2	2.46	177-185	ANTFVAELK

						1471.62	2	3.51	242-257	AGAGSATLSM*AYAGAR
						1344.56	2	2.72	258-269	FVFSLVDAM*NGK
						1154.29	2	2.44	270-279	EGVVECSFVK
						1133.34	2	2.78	315-324	M*ISDAIPELK
38	Glyceraldehyde-3-phosphate dehydrogenase	P04406	35.9	8.58	10.78					
						910.01	2	2.38	107-116	AGAHLQGGAK
						1764.88	2	3.62	309-322	LISWYDNEFGYSNR
						1363.59	2	2.93	323-334	VVDLM*AHM*ASKE
39	14-3-3 protein epsilon	P62258	29.2	4.63	14.51					
						1464.63	2	2.39	30-42	VAGM*DVELTVEER
						1418.58	2	2.48	62-73	IISSIEQKEENK
						1385.51	2	2.67	131-142	YLAEFATGNDRK
40	14-3-3 protein zeta/delta	P63104	27.7	4.73	14.69					
						1549.58	2	3.39	28-41	SVTEQGAELSNEER
						1280.41	2	3.05	128-139	YLAEVAAGDDKK
						1206.44	2	2.96	213-222	DSTLIM*QLLR
40	14-3-3 protein theta	P27348	27.8	4.68	14.69					
						1533.58	2	3.62	28-41	AVTEQGAELSNEER
						1321.46	2	2.66	104-115	YLIANATNPESK
						1206.44	2	2.96	213-222	DSTLIM*QLLR
40	14-3-3 protein gamma	P61981	28.2	4.80	13.82					
						1644.72	2	3.64	28-41	NVTELNEPLSNEER
						1081.20	2	2.67	132-141	YLAEVATGEK
						1206.44	2	2.96	217-226	DSTLIM*QLLR
41	Recoverin	P35243	23.0	5.06	10.05					
						1455.64	2	3.18	43-54	ITQQQFQSIYAK
						949.11	2	2.47	131-138	M*ITPEDVK
42	Alpha crystallin A chain	P02489	19.9	5.77	16.76					
						981.21	2	2.09	71-78	FVIFLDVK
						1173.30	2	2.45	79-88	HFSPEDLTVK
						1287.40	2	3.22	89-99	VQDDFVEIHGK
43	Nucleoside diphosphate kinase A	P15531	17.1	5.83	18.42					
						985.21	2	2.11	19-27	GLVGEIIKR

						1052.16	2	2.22	106-114	GDFCIQVGR
44	Hemoglobin beta subunit	P68871	15.9	6.81	22.60					
						953.08	2	2.67	1-8	VHLTPEEK
						1315.42	2	3.36	18-30	VNVDEVGGEALGR
						1150.36	2	3.22	133-144	VVAGVANALAHK
45	Hemoglobin beta subunit	P68871	15.9	6.81	22.60					
						953.08	2	2.26	1-8	VHLTPEEK
						1315.42	2	2.52	18-30	VNVDEVGGEALGR
						1150.36	2	2.94	133-144	VVAGVANALAHK
46	Hemoglobin alpha subunit	P69905	15.1	8.73	23.40					
						1530.62	2	3.16	17-31	VGAHAGEYGAEALER
						1088.30	2	2.11	32-40	M*FLSFPTTK
						1088.29	2	2.68	91-99	LRVDPVNFK

Table 2

Proteins identified from spots detected only macular retina.

Proteins from macular unique spots by LC-MS/MS and listed.

[(a)]Spot ID corresponds to the numbers on gel images in figure 1 - 4.

[(b)]Accession no. corresponds to UniProtKB/Swiss-Prot database (Release 48.8).

[(c)]MW and pI are theoretical scores by Bioworks ver.3.1.

*Oxidation of methonine.

Spot no.[a]	Protein name	Database Accession no.[b]	MW (kDa)[c]	pI[c]	Sequence coverage (%)	Precursor ion MH+	Charge	XC	Residue	Sequence
M1	Pyruvate kinase, isozymes M1/M2	P14618	32.7	4.69	18.11					
						1198.35	2	3.10	32-42	LDIDSPPITAR
						1360.53	2	4.43	43-55	NTGIICTIGPASR
						1194.43	2	2.70	56-65	SVETLKEM*IK
						914.08	2	2.50	106-114	PVAVALDTK
						1119.25	2	2.86	125-135	GSGTAEVELKK
						1463.70	2	4.32	173-185	IYVDDGLISLQVK
						1780.91	2	4.61	188-205	GADFLVTEVENGGSLGSK
						1142.28	2	2.96	294-304	GDLGIEIPAEK
M2	Tropomyosin 1 alpha chain	P09493	32.7	4.69	13.73					
						1400.56	2	2.95	91-101	RIQLVEEELDR
						1728.89	2	3.24	92-105	IQLVEEELDRAQER
						1315.54	2	3.69	168-178	KLVIIESDLER
						1672.86	2	2.58	169-182	LVIIESDLERAEER
						1121.21	2	2.35	190-198	CAELEEELK
M2	Heterogeneous nuclear ribonucleoproteins C1/C2	P07910	33.7	4.95	16.67					
						1317.60	2	4.08	18-29	VFIGNLNTLVVK
						1330.47	2	4.35	51-61	GFAFVQYVNER
						1700.00	2	4.50	74-89	M*IAGQVLDINLAAEPK
						1416.60	2	3.49	205-216	QKVDSLLENLEK
M3	Transaldolase	P37837	37.5	6.36	16.62					
						1051.20	2	2.82	11-19	M*ESALDQLK
						1792.15	2	4.16	82-97	NAIDKLFVLFGAEILK
						1269.45	2	2.86	111-121	LSFDKDAM*VAR
						1234.34	2	2.50	205-215	SYEPLEDPGVK
						998.18	2	2.78	231-239	TIVM*GASFR
M3	3'(2'),5'-bisphosphate nucleotidase 1	O95861	33.4	5.46	10.06					
						1151.34	2	3.98	11-21	LVASAYSIAQK
						1243.43	2	4.16	29-40	VIAEGDLGIVEK
						914.13	2	2.84	225-232	IIQLIEGK
M4	Poly(rC)-binding protein 1	Q15365	37.5	6.66	16.85					
						1289.37	2	3.24	47-57	INISEGNCPER
						925.13	2	2.25	71-78	AFAM*IIDK

M5	Crk-like protein	P46109	33.8	6.26	7.59	1087.17	2	2.54	315-325	IANPVEGSSGR
						1229.45	2	3.50	254-265	TALALEVGDIVK
						1318.33	2	2.79	293-303	IFDPQNPDENE
M6	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	4.53	1800.00	2	4.85	23-38	LFIGGLSFETTEESLR
M7	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	11.90	1928.18	2	4.47	22-38	KLFIGGLSFETTEESLR
						1189.34	2	3.72	138-147	IDTIEIITDR
						1696.80	2	3.17	154-168	GFGFVTFDDHDPVDK
M8	Voltage-dependent anion-selective channel protein 2	P45880	38.1	6.32	9.80	941.03	2	2.25	193-200	NNFAVGYR
						1722.92	2	3.22	263-278	VNNSSLIGVGYTQTLR
						1017.20	2	2.81	283-292	LTLSALVDGK
M9	Voltage-dependent anion-selective channel protein 1	P21796	30.6	8.63	7.80	1415.52	2	2.03	224-235	YQIDPDACFSAK
						1031.23	2	3.29	256-265	LTLSALLDGK
M10	Voltage-dependent anion-selective channel protein 1	P21796	30.6	8.63	15.60	1960.99	2	5.74	34-52	SENGLEFTSSGSANTETTK
						1529.68	2	3.53	96-109	LTFDSSFSPNTGKK
						1214.35	2	3.32	163-173	VTQSNFAVGYK
M11	Endoplasmic reticulum protein ERp29	P30040	29.0	6.77	27.59	1325.54	2	2.88	37-48	GALPLDTVTFYK
						1248.32	2	2.61	60-69	FDTQYPYGEK
						1321.51	2	2.43	113-122	ESYPVFYLFR
						1609.72	2	3.64	123-137	DGDFENPVPYTGAVK
						1725.86	2	4.72	209-223	ILDQGEDFPASEM*TR
						1135.34	2	3.65	244-253	SLNILTAFQK
M12	Guanylate kinase	Q16774	21.6	6.11	13.27	1069.24	2	2.84	5-16	PVVLSGPSGAGK
						1675.78	2	3.85	44-57	PGEENGKDYYFVTR
M13	Guanylate kinase	Q16774	21.6	6.11	19.39	1069.24	2	3.39	5-16	PVVLSGPSGAGK
						1675.78	2	4.23	44-57	PGEENGKDYYFVTR
						1387.60	2	3.71	96-107	ICVLDVDLQGVR

						1674.84	2	4.65	81-96	TVEEAENIAVTSGVVR
M15	Fatty acid-binding protein, epidermal	Q01469	15.0	6.80	21.64					
						928.11	2	2.22	24-32	ELGVGIALR
						1272.38	2	2.94	61-71	TTQFSCTLGEK
						1026.04	2	2.25	72-80	FEETTADGR
M16	Arrestin-C	P36575	42.8	5.53	14.95					
						1402.72	2	3.74	48-58	KLFVM*LTCAFR
						1654.85	2	4.47	59-72	YGRDDLEVIGLTFR
						1654.75	2	3.41	145-158	SFCAENPEETVSKR
						1952.20	2	4.11	167-185	KVQFAPPEAGPGPSAQTIR
M17	Arrestin-C	P36575	42.8	5.53	4.64					
						1824.03	2	4.66	168-185	VQFAPPEAGPGPSAQTIR
M18	Isocitrate dehydrogenase [NAD] subunit alpha	P50213	39.6	6.46	22.40					
						1607.90	2	2.90	101-115	TPIAAGHPSM*NLLLR
						1392.58	2	3.70	135-146	TPYTDVNIVTIR
						975.13	2	2.74	170-178	LITEGASKR
						1217.36	2	3.94	179-188	IAEFAFEYAR
						1055.23	2	2.38	206-214	M*SDGLFLQK
						1896.29	2	4.58	300-316	DM*ANPTALLLSAVM*M*LR
						1124.30	2	3.03	327-336	IEAACFATIK
M18	Transaldolase	P37837	37.5	6.36	15.73					
						1051.20	2	2.54	11-19	M*ESALDQLK
						1250.55	2	2.65	87-97	LFVLFGAEILK
						1234.34	2	2.34	205-215	SYEPLEDPGVK
						998.18	2	2.35	231-239	TIVM*GASFR
						1393.60	2	3.77	246-258	ALAGCDFLTISPK
M19	Tropomyosin 1 alpha chain	P09493	32.7	4.69	14.79					
						1885.07	3	4.05	91-105	RIQLVEEELDRAQER
						1977.23	3	5.13	134-149	AQKDEEKM*EIQEIQLK
						1315.54	2	3.66	168-178	KLVIIESDLER
M19	Heterogeneous nuclear ribonucleoproteins C1/C2	P07910	33.7	4.95	12.75					
						1317.60	2	3.87	18-29	VFIGNLNTLVVK
						1330.47	2	3.69	51-61	GFAFVQYVNER
						1700.00	2	5.06	74-89	M*IAGQVLDINLAAEPK
M20	Pyruvate dehydrogenase E1 component beta subunit	P11177	39.2	6.20	31.75					

						1803.01	2	3.07	53-68	VFLLGEEVAQYDGAYK
						1845.16	2	4.59	130-145	TYYM*SGGLQPVPIVFR
						1352.49	2	2.48	259-269	EGVECEVINM*R
						1901.22	2	3.37	270-285	TIRPM*DM*ETIEASVM*K
						1765.03	2	4.39	309-324	IM*EGPAFNFLDAPAVR
						1265.46	2	3.43	325-336	VTGADVPM*PYAK
						1256.43	2	3.25	337-347	ILEDNSIPQVK
M21	Glucose-6-phosphate 1-dehydrogenase	P11413	59.1	6.44	5.84					
						1174.34	2	2.75	182-191	LSNHISSLFR
						1265.53	2	2.37	205-214	EM*VQNLM*VLR
						1192.30	2	2.39	498-507	VGFQYEGTYK
M22	Glucose-6-phosphate 1-dehydrogenase	P11413	59.1	6.44	9.73					
						1139.33	2	2.58	95-103	LKLEDFFAR
						1174.34	2	3.07	182-191	LSNHISSLFR
						1265.53	2	2.35	205-214	EM*VQNLM*VLR
						1274.41	2	3.75	246-256	GGYFDEFGIIR
						1192.30	2	2.42	498-507	VGFQYEGTYK
M23	Glucose-6-phosphate 1-dehydrogenase	P11413	59.1	6.44	12.65					
						1011.16	2	2.36	81-88	KQSEPFFK
						1174.34	2	2.91	182-191	LSNHISSLFR
						1265.53	2	2.24	205-214	EM*VQNLM*VLR
						1274.41	2	3.44	246-256	GGYFDEFGIIR
						1944.13	3	3.81	370-385	LQFHDVAGDIFHQQCK
						1192.30	2	2.72	498-507	VGFQYEGTYK
M24	26S proteasome non-ATPase regulatory subunit 11	O00231	47.3	6.09	23.04					
						1517.58	2	4.24	33-45	DIQENDEEAVQVK
						1401.63	2	3.41	46-58	EQSILELGSLLAK
						1158.33	2	3.13	59-70	TGQAAELGGLLK
						1324.55	2	3.14	71-81	YVRPFLNSISK
						1086.26	2	2.30	132-140	LVSLYFDTK
						1342.61	2	3.28	163-174	ALLVEVQLLESK
						1731.99	2	4.86	258-273	IM*LNTPEDVQALVSGK
						1267.50	2	3.15	344-354	VQIEHISSLIK
M25	Elongation factor Tu	P49411	49.5	7.26	10.62					
						1674.84	2	4.58	105-120	GITINAAHVEYSTAAR

						1186.30	2	3.31	316-327	AEAGDNLGALVR
						1150.35	2	3.50	352-361	VEAQVYILSK
M26	Elongation factor Tu	P49411	49.5	7.26	4.87					
						1186.30	2	3.66	316-327	AEAGDNLGALVR
						1150.35	2	3.14	352-361	VEAQVYILSK
M27	Alpha-centractin	P61163	42.6	6.19	8.78					
						1868.04	2	4.98	239-255	AQYYLPDGSTIEIGPSR
						1684.96	2	3.94	293-308	TLFSNIVLSGGSTLFK
M28	Heterogeneous nuclear ribonucleoproteins C1/C2	P07910	33.7	4.95	16.01					
						1317.60	2	3.56	18-29	VFIGNLNTLVVK
						1124.27	2	3.16	30-39	KSDVEAIFSK
						1330.47	2	4.23	51-61	GFAFVQYVNER
						1700.00	2	4.11	74-89	M*IAGQVLDINLAAEPK
M29	Heterogeneous nuclear ribonucleoproteins C1/C2	P07910	33.7	4.95	22.55					
						1317.60	2	3.72	18-29	VFIGNLNTLVVK
						996.10	2	2.73	31-39	SDVEAIFSK
						1330.47	2	4.45	51-61	GFAFVQYVNER
						1700.00	2	5.44	74-89	M*IAGQVLDINLAAEPK
						1229.45	2	3.27	188-198	LKGDDLQAIKK
						1160.30	2	3.51	207-216	VDSLLENLEK
M30	Heterogeneous nuclear ribonucleoprotein H3	P31942	36.9	6.37	11.27					
						1272.39	2	3.88	56-67	STGEAFVQFASK
						1920.12	2	4.95	206-222	ATENDIANFFSPLNPIR
						1053.15	2	2.76	223-232	VHIDIGADGR
M31	Voltage-dependent anion-selective channel protein 1	P21796	30.6	8.63	19.50					
						1960.99	2	4.52	34-52	SENGLEFTSSGSANTETTK
						1401.50	2	4.13	96-108	LTFDSSFSPNTGK
						1214.35	2	3.33	163-173	VTQSNFAVGYK
						1415.52	2	3.13	224-235	YQIDPDACFSAK
M31	Esterase D	P10768	31.5	6.54	4.61					
						1402.53	2	4.63	186-198	KAFSGYLGTDQSK
M32	Pyruvate kinase, isozymes M1/M2	P14618	57.8	7.95	33.21					
						1198.35	2	3.32	32-42	LDIDSPPITAR
						1360.53	2	3.86	43-55	NTGIICTIGPASR
						1885.03	3	4.35	73-88	LNFSHGTHEYHAETIK

						914.08	2	2.85	106-114 PVAVALDTK
						1214.37	2	2.87	141-150 ITLDNAYM*EK
						1463.70	2	4.50	173-185 IYVDDGLISLQVK
						1780.91	2	6.10	188-205 GADFLVTEVENGGSLGSK
						1766.03	2	4.67	206-223 KGVNLPGAAVDLPAVSEK
						1682.88	2	4.25	279-293 FDEILEASDGIM*VAR
						1142.28	2	3.10	294-304 GDLGIEIPAEK
						1020.12	2	2.56	367-375 GDYPLEAVR
						996.17	2	2.76	489-497 VNFAM*NVGK
						1084.19	2	3.01	516-525 PGSGFTNTM*R
M33	Pyruvate kinase, isozymes M1/M2	P14618	57.8	7.95	29.43				
						1360.53	2	4.10	43-55 NTGIICTIGPASR
						1571.71	2	4.07	92-105 TATESFASDPILYR
						914.08	2	2.69	106-114 PVAVALDTK
						1214.37	2	3.10	141-150 ITLDNAYM*EK
						1463.70	2	4.43	173-185 IYVDDGLISLQVK
						1780.91	2	5.94	188-205 GADFLVTEVENGGSLGSK
						1637.86	2	4.16	207-223 GVNLPGAAVDLPAVSEK
						954.02	2	2.69	270-277 IENHEGVR
						1682.88	2	3.17	279-293 FDEILEASDGIM*VAR
						1142.28	2	3.04	294-304 GDLGIEIPAEK
						1020.12	2	2.57	367-375 GDYPLEAVR
						996.17	2	2.90	489-497 VNFAM*NVGK
						1084.19	2	2.89	516-525 PGSGFTNTM*R
M34	Aspartate aminotransferase	P17174	46.1	6.57	8.74				
						1357.54	2	3.39	86-98 LALGDDSPALKEK
						1428.62	2	4.00	99-113 RVGGVQSLGGTGALR
						1013.09	2	2.45	259-266 NFGLYNER
M35	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	9.92				
						1058.21	2	2.21	4-12 TLETVPLER
						1800.00	2	4.67	23-38 LFIGGLSFETTEESLR
						1189.34	2	2.67	138-147 IDTIEIITDR
M36	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	9.92				
						1058.21	2	2.28	4-12 TLETVPLER
						1800.00	2	4.99	23-38 LFIGGLSFETTEESLR

M37	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	7.37					
						1800.00	2	4.40	23-38	LFIGGLSFETTEESLR
						1189.34	2	2.81	138-147	IDTIEIITDR
M38	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37.4	8.97	7.37					
						1800.00	2	4.06	23-38	LFIGGLSFETTEESLR
						1189.34	2	2.99	138-147	IDTIEIITDR
M39	Phosphoglycerate mutase 1	P18669	28.7	6.75	8.30					
						1060.19	2	2.48	90-99	HYGGLTGLNK
						1151.34	2	3.16	180-190	VLIAAHGNSLR
M40	Superoxide dismutase [Mn]	P04179	24.7	8.35	10.36					
						1425.66	2	3.68	76-89	GDVTAQIALQPALK
						1029.22	2	2.59	115-123	GELLEAIKR

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Expression of Mutated Optineurin Leads to Normal Tension Glaucoma in Mice by the Disruption of Optineurin-Rab8 Interaction

Abbreviated title: Mutated optineurin leads to normal tension glaucoma in mice

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ABSTRACT

Glaucoma is one of the leading causes of blindness, affecting 70 million people worldwide. Glaucoma is characterized by a progressive loss of retinal ganglion cells and is often associated with elevated intraocular pressure (IOP). However, patients with normal tension glaucoma (NTG), a subtype of primary open angle glaucoma (POAG), are affected without IOP elevation. Molecular pathways leading to the pathology of the disease are still unclear mainly due to the lack of animal models. Here, we describe the first animal model of NTG based on the same gene mutation found in NTG patients. The transgenic mice over expressing E50K mutation of optineurin (*OPTN*) developed phenotype which mimics the clinical features of NTG patients including degeneration of the retinal ganglion cells at normal IOP. We demonstrate that the E50K mutation in OPTN disrupts the interaction between OPTN and Rab8, a protein known to regulate vesicle transport from Golgi to plasma membrane. Wild-type OPTN and active GTP-bound form of Rab8 complex were localized adjacent to the Golgi complex. These data provide new information about the initial steps in the development of NTG at the molecular level and a new animal model of NTG suitable for therapeutic development.

INTRODUCTION

Glaucoma is characterized by progressive loss of retinal ganglion cells (RGCs), degeneration of axons in the optic nerve, and visual field defects. POAG is one of the major causes of irreversible blindness leading to vision loss in about 4.5 million people and accounting for 12% of all global blindness (Quigley et al., 1996; 2006). POAG is often associated with elevated IOP, which is one of main risk factors in glaucoma. However, characteristic degenerative changes in the retina and optic nerve, as well as visual filed loss, may occur even in the absence of elevated IOP in a subtype of POAG which is called NTG. Recent epidemiological study in Tajimi city, have resulted with more than 90% of POAG cases diagnosed as NTG (Iwase et al., 2004).

At present, at least 24 different genetic loci have been linked to various forms of glaucoma and over the last decade, four genes, *myocilin, cytochrome P4501B1*, *OPTN*, and *WDR36* have been identified as glaucoma-associated genes (Stone et al., 1997; Stoilov et al., 1997; Rezaie et al., 2002; Monemi et al., 2005). *OPTN* was the first identified gene mutation in which can lead to NTG. The *OPTN* gene contains an initial three 3 non-coding exons followed by 13 exons encoding a protein with a length of 577 amino acids. The *OPTN* mutation at nucleotide 458 (G>A) leading to substitution of glutamic acid by lysine at amino acid 50 (E50K) is tightly linked to patients with a severe NTG phenotype. While this mutation has never been reported

in the normal population (Rezaie et al., 2002; Aung et al., 2005; Alward et al., 2003), mutations in the *OPTN* gene have been observed in 13.5% of NTG families. Chalasani et al. (2007) demonstrated that the *OPTN* E50K mutation selectively induces RGC death. OPTN has been shown to interact with number of proteins including huntingtin (Faber et al., 1998), transcription factor IIIA (Moreland et al., 2000), RAB8 (Hattula and Peranen, 2000; Park et al., 2006), myosin VI (Sahlender et al., 2005), FOS (Miyamoto-Sato et al., 2005), ring finger protein 11 (Colland et al., 2004), and metabotrophic glutamate receptor 1-a (Anborgh et al., 2005).

The molecular pathways leading to NTG from a single gene mutation still remain unclear mainly due to the difficulty of working with patient's eyes at the molecular level and lack of animal models with this particular mutation. Recently, it has been reported that the glutamate transporter-deficient mice exhibit an NTG-like phenotype (Harada et al., 2007). However to date, no NTG animal models have been developed based on the gene mutation found in patients with NTG.

In this paper we show that interaction of OPTN with the GTP-form of Rab8, which occurs adjacent to the Golgi, is significantly reduced by the OPTN E50K mutation. Transgenic mice over expressing this mutant show progressive RGC loss and excavation of the optic nerve head without detectable changes in IOP similar to the phenotype observed in NTG patients. These mice represent the first OPTN-based

animal model of NTG.

MATERIALS AND METHODS

Development of transgenic mice over expressing mutant OPTN

Total RNA was extracted from a fresh C57BL/6N mouse brain tissue using TRIzol (Invitrogen, Carlsbad, CA) and reverse-transcribed into first-strand cDNA using oligo-dT adaptor primer and SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). *OPTN* cDNA was amplified by PCR using oligonucleotides 5'-cggaattccgatgtcccatcaacctctgag-3' and 5'- cggaattccgtcaaatgatgcagtccatca-3' as primers. The amplified DNA fragment was purified using a MinElute gel extraction kit (Qiagen, Hilden, Germany), ligated into pBluescriptII (KS-) (Agilent Technologies, Santa Clara, CA) and sequenced using the M13 primers and ABI PRISM 3130 (Applied Biosystems, Foster City, CA). Site-directed mutagenesis was carried out to produce cDNA corresponding to the deletion of the E50K mutation, the first leucine zipper (1st LZ del), deletion of the second leucine zipper (2nd LZ del) and the H486R mutation. The following primers were used: 5'-cagctcaaactcaactccgg -3' and 5'-atgctccacttcctgctcca -3' for1st LZ del , 5'-aaatgaaggaactcctggttaagaaccaccagctgaaagaa-3' and 5'-ttctttcagctggtggttcttaaccaggagttccttcattt-3' for E50K, 5'-gagaccatggccgtcctc-3' and

5'-caacatcttgtccacctttttctg-3' for 2nd LZ del, and

5'-gcagcaagagagaagattcgtgaagaaaaggagcagc-3' and

5'-gctgctccttttcttcacgaatcttctctcttgctgc-3' for H486R. The identities of all clones were confirmed by sequencing. Plasmids were digested with EcoRI, purified by agarose gel electrophoresis, and recovered using the MinElute gel extraction kit according to the manufacturer's protocol. The cDNA inserts were ligated into EcoRI digested pCMVHA vector (Takara Bio USA, Madison, WI). HA-tagged *OPTN*s were amplified by PCR using oligonucleotides 5'- ccgctcgagcgccaccatgatgtacccatacgatgttcc-3' and 5'-ccgctcgagcggtcaaatgatgcagtccatca-3' as primers. The amplified DNA fragments were purified using a MinElute gel extraction kit (Qiagen), ligated into the pCAGGS vector and sequenced as above. cDNA inserts were released from the pCAGGS vector using SalI and BamHI. These restriction fragments were injected into pronuclear stage BDF1/C57BL6N embryos and transgenic mice were generated at PhoenixBio Co., Ltd (Tochigi, Japan). Offspring were screened for the transgene by isolating genomic DNA from tail biopsies followed by PCR. Primers used for PCR were 5'-ctctagagcctctgctaaccatgt-3' and 5'- ccatggccataagagcgtaa -3'. All experiments with mice were performed in accordance with the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Vision Research.

Fundus photography

Mice were anesthetized by aspiration of isoflurane (Mylan, Canonsburg PA). Optic disk imaging was performed as previously described (Coban et al., 2003). Fundus images were obtained using 2 mm gonio lens (Ocular Instrument, Bellevue, WA) and slit lamp (SR-D7, Topcon, Tokyo, Japan) with digital camera (D100, Nikon, Tokyo, Japan).

Light microscopic histopathology of the optic nerve

After deep anesthesia, mouse eyes were dissected and immersed in Davidson solution fixative overnight at 4°C. The eyes were embedded in paraffin and sectioned at 5 μm thickness along the vertical meridian through the optic nerve head. After deparaffinization, and rehydration, sections were stained with hematoxylin and eosin (HE staining).

Electroretinogram (ERG)

Mice (15 month) were anesthetized by intraperitoneal injection of a mixture of xylazine (10 mg/kg) and ketamine (25 mg/kg). Pupils were dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide. ERGs were recorded by Mayo Co. (Nagoya, Japan). Standard Flash ERG was obtained using flash intensity 2.50cd·