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The Spectrum of *ROBO3* Mutations in Horizontal Gaze Palsy with Progressive Scoliosis: An Update

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Abstract

Horizontal Gaze Palsy with Progressive Scoliosis is a rare, congenital autosomal recessive disorder caused by mutations in the *ROBO3* gene. It is characterized by the absence of conjugate horizontal eye movements with preserved vertical gaze, variable convergence, and progressive scoliosis, developing in childhood and adolescence. *ROBO3* gene mutations are causative of the lack, or at least reduction, of crossing of the descending corticospinal and ascending lemniscal sensory tracts in the medulla. To date, 39 different mutations, including missense, nonsense, frameshift, and splice site mutations have been described in the *ROBO3* gene and related to Horizontal Gaze Palsy With Progressive Scoliosis. In addition, a lot of variants of uncertain pathological significance have been reported for the first time by Illumina Clinical Services. Here we report an update on mutations of the *ROBO3* gene and some information on the pathogenesis but much remains to be investigated on the consequences of mutations on *ROBO3* expression and function. Therefore, further detailed functional analyses are necessary to clarify a possible role of the variants of uncertain pathological significance as the cause of the disease. In conclusion, we hope that this article will help in molecular screening for the *ROBO3* gene and will contribute to enlargement of the *ROBO3* gene variation database.

Keywords

ROBO3, Mutations, HGPPS, Scoliosis

1. Introduction

Understanding of the pathogenesis of axonal guidance diseases in recent years

has improved greatly, and many molecular genetic conditions related to these pathologies have been found. Among them, there is strong interest in Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS; OMIM 607313), firstly described by Dretakis and Kondoyannis in 1974 in consanguineous Greek pedigrees [1], and subsequently reported both in consanguineous pedigrees and unrelated parents of many different ethnicities [2].

2. Clinical and Genetic Aspects

HGPPS is a rare, congenital autosomal recessive disorder [3] [4] in which affected individuals are characterized by the absence of conjugate horizontal eye movementswith preserved vertical gaze, variable convergence, and progressive scoliosis. This last disability, starting in infancy or childhood and adolescence, is the most common reason for medical advice in patients with HGPPS and is the most serious condition in terms of function and appearance by which affected patients undergo surgical intervention early in life [5] [6] [7] [8]. No other associated neurological or behavioral abnormalities have been highlighted. To date, 39 different mutations, including missense, nonsense, frameshift, and splice site mutations, in the human *ROBO*3 gene, have been described and related to HGPPS [5] [9] [10]. This gene is a member of the Roundabout (*ROBO*) gene family that controls neurite outgrowth, growth cone guidance, and axon fasciculation. *ROBO*3 gene, encompassing 28 exons and located on chromosomal region 11q23-25, encodes an axon-guidance protein of 1386 amino acid, analogous to mouse Rig1/Rob3, involved in the promoting of midline crossing of neurons in the medulla during brain development [11] [12]. This proteinis predicted to contain an extracellular segment with five immunoglobulin-like domains (Ig1-5) and three fibronectin III-like domains (FnIII1-3), a transmembrane segment (Tm), and an intracellular segment with three cytoplasmic signalling motifs (CC0-3) (**Figure 1**) [6]. Human *ROBO*3 shares homology with the superfamily of immunoglobulin transmembrane receptors important in axon guidance and neuronal migration, including decussation of developing nerve fiber tracts in the brainstem [5] [13]. Slit proteins 1-3, a family of secreted chemorepellants, are ligands for *ROBO* proteins and Slit/*ROBO* interactions regulate myogenesis, leukocyte migration, kidney morphogenesis, angiogenesis, and vasculogenesis in addition to neurogenesis (**Figure 2**). *ROBO*3 gene mutations are causative of the lack, or at least reduction, of crossing of the descending corticospinal and ascending lemniscal sensory tracts in the medulla [2] [7] [13]-[20]. Standard MRI T1- and T2-weighted imaging findings consist of pons and cerebellar peduncles hypoplasia, absence of the facial colliculi, butterfly configuration of the medulla and a deep midline pontine cleft [3] [4] [21]. Although scoliosis in HGPPS patients is usually severe, it remains unclear whether the physiopathology of scoliosis is musculoskeletal or neurogenic. Playing *ROBO*3 a critical role for hindbrain axons to appropriately cross the midline [22], a neurogenic mechanism has been postulated by Jen *et al.* in 2004 [5]. Nevertheless to date, since no

genotype-phenotype correlation has not been elucidated in HGPPS, possibly because of intra-familial variability of the cardinal features [5] [11] [16], and whereas HGPPS with scoliosis has been described without detectable mutations in *ROBO3* gene [14] [23], it is not possible to state that scoliosis is linked to *ROBO3* mutations [24].

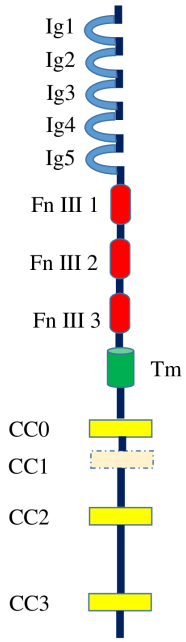

Figure 1. *ROBO3* receptor structure. *ROBO3* receptor contains an extracellular segment with five immunoglobulin-like domains (Ig1-5) and three fibronectin III-like domains (Fn III 1-3), a transmembrane segment (Tm), and an intracellular segment with three cytoplasmic signalling motifs (CC0-3).

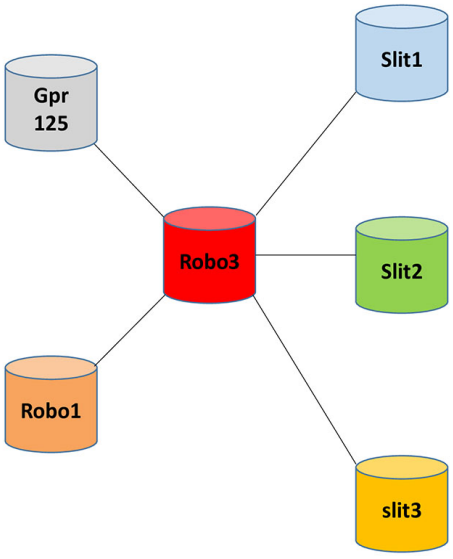

Figure 2. Interacting Proteins for *ROBO3* gene. Slit proteins 1-3 and Gpr125 are ligands for *ROBO* proteins. In particular, Slit proteins 1-3 are a family of secreted chemorepellants and Gpr125 belongs to the family of Adhesion G protein-coupled receptors (GPCRs).

3. Spectrum of Mutations in HGPPS

The purpose of our study is to group all HGPPS-related mutations described to date, both pathogenic and uncertain significance. Mutations are listed in Table 1. HGPPS-related mutations occur in all *ROBO3* gene exons and exon-intron boundaries, mostly located on the extracellular part of the protein, inherited both in affected members of consanguineous families harboring homozygous *ROBO3* mutations and in individuals from non-consanguineous families harboring compound heterozygous mutations [2]. Most reported *ROBO3* mutations are scattered throughout the *ROBO3* gene without a specific region or domain that can be considered a hot-spot area for mutations [13], although an important accumulation of missense and frameshift mutations in the last exons coding for the C-terminal part of the receptor has been described [25]. This difference, contrasting with HGPPS-associated variants, suggests a critical role of the extracellular domain in *ROBO3* function. The mutations are highly diverse, and indistinguishable phenotypes result from *ROBO3* nonsense, frame-shift, splice-site, or missense mutations, supporting a complete loss of *ROBO3* function [26]. Some mutations are predicted to induce a premature stop-codon, associated with expression of a truncated protein or mRNA degradation vianonsense mediated decay (NMD) [27]. In addition, we report in Table 1 a lot of variants of uncertain pathological significance, described for the first time by Illumina Clinical Services (Laboratory Illumina, 2016). It cannot be excluded that they could cause the pathological phenotype, even though experiments are necessary to determine whether these variations underlie the pathogenesis of HGPPS.

Table 1. Currently known mutations in *ROBO3* gene linked to HGPPS.

Variant ID	Nucleotide change	Exon	Amino acid change	Clinical significance	Control screened	Ethnicity	References
rs121918275	c.14T > C	1	L5P	pathogenic	106	Italian	Jen *et al.*, 2004
rs121918276	c.196A > C	2	I66L	pathogenic	175	Greek	Jen *et al.*, 2004
rs121918274	c.955G > A	6	E319K	pathogenic	197	Greek	Jen *et al.*, 2004
rs121918271	***c.2108G > C***	14	R703P	pathogenic	150	Turkish	Jen *et al.*, 2004
rs121918272	c.2113T > C	14	S705P	pathogenic	116	Saudi	Jen *et al.*, 2004
rs121918270	c.1082G > A	7	G361E	pathogenic	95	Indian	Jen *et al.*, 2004
rs121918273	c.1366G > T	9	G456X	pathogenic	95	Turkish	Jen *et al.*, 2004
CI041652	c.2310 + 1C	15	frameshift	pathogenic	106	Pakistani	Jen *et al.*, 2004
CI041653	c.3325 + 1G	23	frameshift	pathogenic	116	Saudi	Jen *et al.*, 2004
CS041545	c.IVS13 + 1G > A	13	frameshift	pathogenic	93	Saudi	Jen *et al.*, 2004
rs121918277	**c.733C > T**	4	R245W	pathogenic	87	Irish/English	Chan *et al.*, 2006
rs121918278	**c.2317C > T**	15	Q773X	pathogenic	87	Irish/English	Chan *et al.*, 2006
CD061464	**c.1886_1887delTT**	12	frameshift	pathogenic	87	Irish/German	Chan *et al.*, 2006
CD061465	**c.1844_1845delCA**	12	frameshift	pathogenic	87	Irish/German	Chan *et al.*, 2006

Continued

CM086900	c.2312C > T	15	P771L	pathogenic	100	Saudi	Khan *et al.*, 2008
CM090356	c.271C > T	2	P91S	pathogenic	50	Sudanese	Abu-Amero *et al.*, 2009
CM090354	c.335G > C	2	R112P	pathogenic	120	Saudi	Abu-Amero *et al.*, 2009
rs771613910	c.1379A > G	9	Q460R	pathogenic	120	Saudi	Abu-Amero *et al.*, 2009
HM070122	c.1726T > C	11	W576R	pathogenic	120	Saudi	Abu-Amero *et al.*, 2009
CD090357	c.571delC	2	frameshift	pathogenic	120	Saudi	Abu-Amero *et al.*, 2009
CM095023	c.1450T > C	9	W484R	pathogenic	NR	Tunisian	Amouri *et al.*, 2009
CM095022	c.283T > C	2	I95T	pathogenic	100	Tunisian	Amouri *et al.*, 2009
CD095024	c.1618delG	10	R539fsX574	pathogenic	NR	Tunisian	Amouri *et al.*, 2009
rs121918277	c.733C > T	4	R245W	pathogenic	NR	Tunisian	Amouri *et al.*, 2009
CD118835	c.2_16 delTGCTGCGCTACCTGC	1		pathogenic	100	Saudi	Abu-Amero *et al.*, 2011
CX115641	c.913delAinsTGC	6	I305CfsX13	pathogenic	144	Caucasian/Turkish	Volk *et al.*, 2011
CS115643	c.3319A > C	22	skip + frameshift	pathogenic	144	Caucasian/Turkish	Volk *et al.*, 2011
CG115642	c.2769_2779del11, 2779+1_+20del20	17		pathogenic	144	Caucasian/Turkish	Volk *et al.*, 2011
NA	G > T♦	17	●E-X	pathogenic	NR	Indian	Ng *et al.*, 2011
rs34519996	c.541dup	3	E181GfsX71	pathogenic	NR	Kosovar	Kurian *et al.*, 2013
NA	c.2663T > C	17	L888P	pathogenic	NR	Saudi	Khan and Abu-Amero, 2014
NA	**c.767_776delAGCGTCCCTC**	5		pathogenic	NR	Portuguese	Pina *et al.* 2014
NA	**c.767-2_767-1delAG**	5		pathogenic	NR	Portuguese	Pina *et al.* 2014
NA	c.2392C > T	15	Q798X	pathogenic	NR	Japanese	Yamada *et al.*, 2015
NA	c.2576del	16	P859fs	pathogenic	NR	Austrian	Arlt *et al.*, 2015
NA	c.1158G > C	7	Q386H	pathogenic	NR	Spanish	Fernández-Vega Cueto *et al.*, 2016
NA	***c.416G > T***	2	G139V	pathogenic	NR	Switzerland	Hackenberg *et al.*, 2017
NA	c.2524C > T	16	R842X	pathogenic	NR	Turkish	Bozdoğan *et al.*, 2017
NA	**IV55-5delCATAG**	2		pathogenic	NR	Cape Verde	Mendes Marques *et al.*, 2017
NA	**c.767_775delAGCGTCCCT**	5		pathogenic	NR	Cape Verde	Mendes Marques *et al.*, 2017
NA	**c.1433C > T**	9	P478L	pathogenic	100	Italian	Ungaro *et al.*, 2018
NA	**c.3321-G > A**	int 22	Splice site mut	pathogenic	100	Italian	Ungaro *et al.*, 2018

Continued

rs185584218	c.-120G > C		5' UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047914	c.*68C > G		3'UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs114572753	c.1189C > A	8	P397T	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs114572753	c.1189C > T	8	P397S	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs543770866	c.-60G > T		5' UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs774646580	c.43T > C	1	F15L	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs189616702	c.46G > A	1	A16T	uncertain-significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs145440217	c.160 + 12C > T	int		uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
NA	c.568C > T		P190S	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs192622083	c.592G > A	3	V198M	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs765531515	c.716C > T	4	S239F	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs747047729	c.764T > C	4	L255P	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs200451819	c.769C > T	15	R257C	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs142090631	c.850G > A	5	D284N	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs151168595	c.968C > T	20	T323M	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047908	c.1034-13C > G	7		uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs200197609	c.1542G > A	10	M514I	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016

Continued

rs550454340	c.1957A > C	13	S653R	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs778522624	c.2048C > T	13	P683L	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs184921255	c.2102G > T	14	G701V	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs189303564	c.2183G > A	14	S728N	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs372821877	c.2427G > A	16	W809X	pathogenic	NR	NR	EGL Genetic Diagnostics, Eurofins Clinical Diagnostics, 2017
rs536588537	c.2504T > C	16	L835P	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs199932669	c.2621T > A	17	L874Q	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs756785207	c.2779 + 9C > T	int		uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs763859563	c.2931A > T	20	E977D	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs765206958	c.2947T > C	20	C983R	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs75098003	c.2993G > T	21	G998V	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047909	c.3091A > C	21	T1031P	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047910	c.3139T > G	21	W1047G	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs761311616	c.3320+12C > T	int		uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047912	c.3706G > A	25	G1236R	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047913	c.3707G > T	25	G1236V	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016

Continued

rs575251327	c.3886C > G	26	R1296G	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs752717878	c.3922G > A	26	V1308M	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs139835890	c.4116C > A	27	S1372R	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs200324766	c.4150-11T > C	int		uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs189881000	c.*67C > T		3'UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047914	c.*68C > G		3'UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047915	c.*111C > T		3'UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016
rs886047916	c.*159_*162delCTTT		3'UTR variant	uncertain significance	NR	NR	Illumina Clinical Services Laboratory, Illumina, 2016

♦ The nucleotide number where the mutation was located was not mentioned by the authors. ● The codon number was not mentioned by the authors. Mutations in **bold** were inherited in heterozygous status; mutations in ***Italics and bold*** were inherited both in heterozygous status as compound and in heterozygous status. The remaining mutations were inherited in homozygous status. NA = Not available. NR = Not reported by authors.

4. Discussion and Conclusion

ROBO3 is a transmembrane receptor that plays an important role in axon guidance and neuronal migration and is critical for long ascending medial lemniscal and descending corticospinal tracts in the medulla. In fact, in HGPPS contralateral axon projections of inter-nuclear neurons that co-ordinate the activity of oculomotor and abducens neurons fail to form, resulting in defects in horizontal eye movements. Mutations in *ROBO3* are associated with noncrossing of selected paths in the central nervous system that are normally subjected to midline crossing during embryonic development [20]. A clinical misdiagnosis of HGPPS seems unlikely because the association of horizontal gaze palsy and severe scoliosis is considered pathognomonic of HGPPS [5] [11]. Diffusion tensor imaging (DTI) and DTI tractography have to be performed to evaluate the corticospinal pathways and to confirm the presence of uncrossed corticospinal tracts, being a useful adjunct to the structural magnetic resonance imaging (MRI) in confirming the clinical features suggestive of HGPPS. Since an early diagnosis of HGPPS is possible by analysis of the *ROBO3* gene, this is of great importance with respect to genetic counselling of HGPPS families [19]. A genotypic assessment of

ROBO3 mutations presents challenges because relatively little is known about the function of various *ROBO3* domains or actions of alternative splice forms of *ROBO3* in the human Brainstem [5] [13] [28]. The action of *ROBO3* or its protein product might be inhibited by environmental or epigenetic factors in the developing brainstem; furthermore, a phenotype identical to HGPPS might be caused by abnormalities in *ROBO3* splice variant expression. Moreover,although most reported *ROBO3* mutations are equally distributed along the *ROBO3* sequence, it would be interesting to determine whether specific mutation types are associated with a more disease phenotype and/or whether other disease genes for patients with horizontal gaze palsy with or without scoliosis who do not harbor mutations in *ROBO3* are engaged. Consequentially, a Targeted Next Generation Sequencing (NGS) could be useful to elucidate the possible contribute of other genes. In fact, despite the advances in understanding about this condition much remains to be investigated on the consequences of mutations on *ROBO3* expression and function. Further detailed functional analyses are necessary to clarify a possible role of the variants of uncertain pathological significance as causative of the disease. In conclusion, we hope that this article will help in molecular screening for the *ROBO3* gene and will contribute to enlargement of the *ROBO3* gene variation database.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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