カルマン症候群に関する研究の紹介

1．アメリカのオーランド（フロリダ）で2017年３月29－31日に開催された第15回国際下垂体学会で岡本新悟院長はカルマン症候群の遺伝子解析について発表しました。

2．本研究は岡本内科こどもクリニックのカルマン症候群11例と低ゴナドトロピン性性腺機能低下症8例について３１の候補遺伝子を解析し、その結果それぞれ5例と3例に遺伝子異常を認めました。

3．カルマン症候群で遺伝子異常が明らかでない例が60％以上あり、今後原因遺伝子のさらなる解明が必要であると報告しました。

4．本研究に興味を持ってくれた外国のドクター達との交流もあり有意義な学会でした。

5．本研究は浜松医科大学小児科の緒方　勤教授と国立成育医療センターの遺伝子解析研究チームとの共同研究で今後もカルマン症候群では診断から遺伝子解析など研究についても日本でトップの臨床施設としての自覚をもって治療に当たりたいと考えています。

学会発表の抄録

Genetic analysis in Japanese Kallmann syndrome and idiopathic hypogonadotropic hypogonadism

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We examined the genetic abnormalities in 11 cases of Japanese Kallmann syndrome(KS) and 8 cases of idiopathic hypogonadotropic hypogonadism without anosmia(IHH). These patients had accessed our KS support web site "http://kallmannsyndrome.jp/" and/or visited our endocrine department from areas all over Japan. Patients were diagnosed as having the isolated hypogonadotropic-hypogonadism due to the results of hypothalamic GnRH deficiency with or without anosmia. Gene abnormalities were examined by next generation sequencer (MiSeq) and abnormal sites were confirmed by Sanger methods. The 29 candidate genes, *CHD7, FGF8, FGFR1, FSHB, GNRH1, GNRHR, HESX1, HES6ST1, ANO1/KAL1, KISS1, KISS1R, LEP, LEPR, LHB, LHX3, LHX4, NELF, NROB1, OTX2, POU1F1, PROK2, PROK2R, PROP1, SEMA3A, SOX2, SOX3, TAC3, TACR3, WDR11,* were analyzed in all patients (*ethical approval was obtained*). We found gene abnormalities in 4 of 11cases in KS and 3 of 8 cases in IHH. Compared with the clinical findings in each group with or without gene abnormality, no specific difference was found. In this study, the gene abnormality ratio in KS cases was 36% and in IHH cases was 37%.

In conclusion, KS and IHH are genetically heterogeneous and pathologically complex syndrome. In our study of over 60% patients with KS or IHH, no genetical abnormality was found. This result shows that, we must progress our study for searching other candidate genes or examine the cause of abnormalities other than genetic abnormalities.

Genetic analysis in Japanese Kallmann syndrome and idiopathic hypogonadotropic hypogonadism

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1) introduction:

Kallmann syndrome(KS) is defined as a syndrome of hypothalamic hypogonadism with anosmia. In such cases, some gene abnormalities were found such as *KAL1, KAL2*・・・. Recently a number of candidate genes relevant to KS were found and listed, as shown in this poster. Even by increasing the number of candidate genes of KS, there are some cases which cannot clarify the gene abnormalities. It shows that KS is a genetically or a non-genetically heterogeneous disease. We are interested in the clinical differences of KS between the cases with genetic abnormalities and the cases without genetic abnormalities. We studied genetic analysis in Japanese KS and hypogonadotropic hypogonadism without anosmia(IHH) and compared with their clinical characteristics.

2) Methods:

We examined the genetic abnormalities in 11 cases of Japanese Kallmann syndrome(KS) and 8 cases of hypogonadotropic hypogonadism without anosmia(IHH). These patients had accessed our KS support web site "http://kallmannsyndrome.jp/" and/or visited our endocrine department from areas all over Japan. Gene abnormalities were examined by next generation sequencer (MiSeq) and were confirmed by Sanger methods. The 31 candidate genes such as *ANO1, FGFR1, PROKR2, PROK2, CHD7, FGF8, GLI2, POU1F1, FSHB, GNRH1, HESX1, LEP, LEPR, LHB, LHB3, LHX3, LHX4, NELF, NROB1, OTX2, PROP1, SOX2, SOX3, WDR11, GNRHR, HS6ST1, KISS1, KISS1R, SEMA3A, IL17RD, TAC3 and TACR3* genes were analyzed in all patients (*ethical approval was obtained*).

Furthermore, we examined the brain image of each group by MRI with genetic abnormality or not, specifically the area of the olfactory sulcus and bulb, and compared with KS and IHH.

31 *Candidate* genes and these characteristics analyzed in this study

relevant to Kallmann syndrome | relevant to Hypogonadotropic Hypogonadism | relevant to both diseases

	Candidate Genes	Encoding proteins	Chromosomal Location	Diseases relevance of genetic abnormalities
Relevant to Kallmann syndrome				
1	***ANO1/KAL1*** gene	anosmin 1	Xp2w2.3 b.p: 8,528,874 to 8,732,187	Kallmann syndrome
2	***FGFR1/KAL2*** gene	fibroblast growth factor receptor 1	8p11.23 b.p: 38,411,138 to 38,468,834	1. Kallmann syndrome 2. Encephalocraniocutaneous lipomatosis
3	***PROKR2/KAL3*** gene	prokineticin 2 receptor	20p12.3 b.p: 5,299,874 to 5,317,547	Kallmann syndrome
4	***PROK2/KAL4*** gene	prokineticin 2	3p13 b.p: 71,771,655 to 71,785, 206	Kallmann syndrome
5	***CHD7/KAL5*** gene	chromodomain helicase DNA binding protein 7	8q12.2 b.p: 60,678,744 to 60,868,028	1. Kallmann syndrome 2. CHARGE syndrome
6	***FGF8/KAL6*** gene	fibrobrast growth factor 8	10q24.32 b.p: 101,770,130 to 101,780,369	1. Kallmann syndrome 2. Nonsyndromic holoposencephaly
Relevant to Hypogonadotropic Hypogonadism				
7	***POU1F1/PIT-1***gene	POU class 1 homeobox 1	3p11.2 b.p: 87,259,633 to 87,276,587	Pituitary hormone deficiency, combined 1 (Pit-1 deficiency)
8	***GLI2*** gene	GLI family zinc finger 2	2q14.2 b.p: 120,797,291 to 120,992,653	1. Nonsyndromic holoprosencephaly 2. Combined pituitary hormone deficiency
9	***FSHB*** gene	follicle stimulating hormone beta subunit	11p14.1 b.p: 30,231,016 to 30,235,277	Hypogonadotoropic hypogonadism 24 without anosmia (HH24)
10	***GNRH1*** gene	gonadotropin releasing hormone 1	8p21.2 b.p: 25,419,258 to 25,425,040	Hypogonadotoropic hypogonadism 7 without anosmia (HH7)
11	***HESX1*** gene	HESX homeobox 1	3p14.3 b.p: 57,197,838 to 57,227,643	1. Combined pituitary hormone deficiency 2. Septo-optic dysplasia
12	***LEP*** gene	leptin	7q32 b.p: 128,.241,201 to 128,258,629	1. Congenital leptin deficiency 2. Hypogoandotrpic hypogonadism
13	***LEPR*** gene	leptin receptor	1p31.3 b.p: 65,420652 to 65,637,493	1. Leptine receptor deficiency 2. Hypogoandotrpic hypogonadism
14	***LHB*** gene	luteinizing hormone beta polypeptide	19q13.33 b.p: 49,015,980 to 49,017,090	Hypogonadotoropic hypogonadism 23 without anosmia (HH23)
15	***LHX3*** gene	LIM homeobox 3	9q34.3 136,196,259 to 136,205,129	Pituitary hormone deficiency, combined 3 (CPHD3)
16	***LHX4*** gene	LIM homeobox 4	1q25.2 b.p: 180,228,372 to 180,278,982	Pituitary hormone deficiency, combined 4 (CPHD4)
17	***NELF*** gene	nasal embryonic LH-RH factor	9q34.3 b.p: 137,47,570 to 137,459,357	Hypogonadotropic hypogonadism (IHH)
18	***NROB1*** gene	nuclear receptor subfamily 0 groupB member 1	Xp21.2 b.p: 30,304,206 to 30,309,598	1. X-linked congenital adrenal hypoplasia 2. Hypogonadotropic hypogonadism (IHH)
19	***OTX2*** gene	orthodenticle homeobox 2	14q22.3 56,800,707 to 56,810,476	1. Combined pituitary hormone deficiency 2. Septo-optic dysplasia
20	***PROP1*** gene	PROP paired-like homobox 1	5q35.3 b.p: 177,992,235 to 177,996,242	Combined pituitary hormone deficiency
21	***SOX2*** gene	SRY-box 2	3q26.33 b.p: 181,711,924 to 181,714,436	1. Combined pituitary hormone deficiency 2. Septo-optic dysplasia
22	***SOX3*** gene	SRY-box 3	Xq27.1 b.p: 140,502,987 to 140,505,060	1. Panhypopituitarism X-linked 2. 46,XX testicular disorder of sex development
Relevant to Kallmann syndrome and Hypopgonadotropic Hypogonadism				
23	***WDR11*** gene	WD repeat domain 11	10q26.12 b.p: 120,851,175 to 120,909,526	1. Kallmann syndrome 2. Hypogonadotropic hypogonadism 14 (HH 14)
24	***GNRHR*** gene	gonadotropin releasing hormone receptor	4q13.2 b.p: 67,737,375 to 67,756,086	Hypogonadotropic hypogonadism 7 with or without anosmia (HH7)
25	***HS6ST1*** gene	heparin sulfate 6-o-sulfo-trsferase 1	2q14.3 b.p: 128,265,479 to 128,318,596	Hypogonadotropic hypogonadism 15 with or without anosmia (HH15)
26	***KISS1*** gene	Kiss-1 metastasis-suppressor	1q32.1 b.p: 204,190,341 to 204,196,491	Hypogonadotropic hypogonadism 13 with or without anosmia (HH 13)
27	***KISS1R*** gene	Kiss1 receptor	19p13.3 b.p: 916.693 to 921,015	Hypogonadotropic hypogonadism 8 with or without anosmia (HH8)
28	***SEMA3A*** gene	semaphorin 3A	7q21.11 b.p: 83,965,846 to 84,492,724	1. Kallmann syndrome 2. Hypogonadotropic hypogonadism 16 (HH 16)
29	***IL17RD*** gene	interleukin 17 receptor D	3p14.3 b.p: 57,089,982 to 57,170,317	1. Kallmann syndrome 2. Hypogonadotropic hypogonadism 17 (HH 17)
30	***TAC3*** gene	tahykinin 3	12q13.3 b.p: 57,009,997 to 57,016,560	Hypogonadotropic hypogonadism 10 with or without anosmia (HH 10)
31	***TACR3*** gene	tahykinin 3 receptor	4q24 b.p: 103,589,468 top 103,719,816	Hypogonadotropic hypogonadism 11 with or without anosmia (HH 11)

Kallmann syndrome cases and their characteristics and endocrinological data

No.	Patients & I.D.	Age	Sex	Anosmia	Testes volum R/L ml	clinical problems	Te(ng/dl)/E2(pg/ml)	basal LH	peak LH	basal FSH	peak FSH	other hormonal abnormality	gene abnormality
1	*F. T. 51975*	17	*m*	Anosmia	3ml/3ml	Uncul with Kallmann synd.	17.9	< 0.1	0.7	0.2	1.4	no	*ANOS1(KAL1)*
2	*K.H. 51620*	15	*m*	Anosmia	3ml/3ml	n.p.	16.4	< 0.1	0.7	0.1	1.4	no	*IL17RD*
3	*T.K. 31961*	29	*m*	Anosmia		with complete deafness	already diagnosed as Kallmann syndrome					no	*GLI2*
4	*N.M. 51843*	15	*m*	Anosmia	2ml/3ml	r-renal aplasia	5	0.4	4	0.3	3.2	no	*ANOS1(KAL1)*
5	*D.M. 52428*		*f*	Anosmia	*	already Kauffmann therapy	0.5	0.3		1.4		no	*PROKR2(KAL3)*
6	*Y.S. 51901*	56	*m*	Anosmia	5mm/5ml	with history of Te. Therapy	26.9	0.8	5.8	1.4	3.5	Adult GHD	no abnormality
7	*Y.H. 51699*	45	*m*	Anosmia	4ml/4ml	brother of case No. 6	20.1	0.1	2	0.4	1.4	no	no abnormality
8	*K.K. 36233*	25	*m*	Anosmia	2ml/3ml	n.p.	freeTe <0.6pg/ml	0.6	5.8	1.1	4.1	no	no abnormality
9	*K.N. 51241*	37	*m*	Anosmia	3ml/5ml	with history of Te. Therapy	50.7	0.6	4.9	0.8	3.1	no	no abnormality
10	*K.K. 51375*	17	*f*	Anosmia	*	n.p.	18.6	0.2	4	0.5	2.8	no	no abnormality
11	*U.H. 21720*	27	*m*	Anosmia	4ml/4ml	with history of Te. Therapy	10	0.5	5.2	1.4	3.1	no	no abnormality

Hypogonadotropic hypogonadism without anosmia cases and their characteristics and endocrinological daga

No.	Patients & I.D.	Age	Sex	Anosmia	Testes R/L	othe physical anomaly	Te(ng/dl)/E2(pg/ml)	basal LH	peak LH	basal FSH	peak FSH	other hormonal abnormality	gene abnormality
1	K.K. 51602	32	*m*	no	3ml/3ml		15.5	0.2	2.4	1.3	5.8	no	*KISS1R*
2	T.Y. 52112	23	*f*	no	*	already Kauffmann therapy	5.2	0.07		0.8		no	*FGFR1(KAL2)*
3	N.T. 31274	27	*m*	no	*	already diagnosed as IHH						no	*CHD7(CHD5)*
4	I.A. 50729	14	*m*	no	2ml/2ml		7.8	0.1	2.4	0.1	4.1	no	no abnormality
5	Y.Y. 51272	20	*m*	no	4ml/4ml		37.1	0.3	3.7	1.3	4.3	no	no abnormality
6	T.S. 50496	33	*m*	no	*	already diagnosed as IHH						no	no abnormality
7	T.H. 52218	39	*m*	no	5ml/5ml		17.1	0.1	1.2	0.1	0.8	no	no abnormality
8	M.S. 52462	36	*m*	no	3ml/4ml		13.2	0.3	4.4	21.3	6.4	no	no abnormality

3) Results:

In this study, gene abnormalities were found in 5 of 11cases in KS and 3 of 8 cases in IHH. Compared with the clinical findings in each group with or without gene abnormality, no specific difference was found. In this study, the gene abnormality ratio in the KS cases was 45.5% and in IHH cases was 37.5%.

In brain imaging examination by MRI, the depths of the olfactory sulcus were markedly shallow in KS cases as opposed to the IHH cases.

4) Conclusions:

In over 50% cases of KS and over 60% cases of IHH, we could not find genetic abnormalities examined in 31 candidate genes relevant to KS and IHH.
This means that the KS and IHH are heterogeneous and we must clarify other candidate genes and other causes other than genetic abnormalities.

We found a new radiographical approach, measuring the depth of olfactory sulcus, as the clinical approach to differentiate the KS and IHH.