機能障害の原因として注意すべき状態と考えられた。心奇形ではTOFが10%に認められた。多重合併奇形の外科治療の優先順位を考える上でこのような症例は慎重に治療戦略がたてられるべき症例と考えられた。

主に解剖学的異常に着目して、調査を行った。今後、機能的な予後について解析を進める必要がある

E. 結論

日本人 VATER 症候群患者における小児外科的症状の分布を初めて明らかにすることができた。食道気管瘻の頻度が比較的高く、鎖肛と同程度であることが明らかになった。鎖肛と食道気管奇形が VATER 症候群の主要な合併症であることが確認された。食道気管奇形についてはGross C型が多く、VATER症候群の一部ではない食道気管奇形と同様であることが確認された。十二指腸閉鎖を示した症例を10例に認め、注意すべき合併症と考えられた。鎖肛は中間位以上が多い傾向がみられた。心奇形の重症度によっては、外科治療の治療戦略を総合的に評価して進めていく必要があると考えられた。

F. 研究発表

論文・学会未発表

G. 知的財産権の出願・登録状況

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

A					
鎖肛　有・無	高位鎖肛	低位鎖肛	直腸会陰瘻	直腸尿道瘻	直腸腟瘻
有	有	有	有	有	有
80	28	33	6	20	12
71.5%	25.0%	29.5%	5.4%	17.9%	10.8%
無	無	無	無	無	無
31	37	35	56	44	50
27.7%	33.1%	31.3%	50.0%	39.3%	44.7%
	不明	不明	不明	不明	不明
	3	3	2	2	3
	2.7%	2.6%	1.8%	1.8%	2.6%
無回答	無回答	無回答	無回答	無回答	無回答
1	44	41	48	46	47
0.8%	39.2%	36.6%	42.8%	41.0%	41.9%

表1　水頭症合併症例と母体糖尿病合併症例を除いた112例の解析結果

TE						
消化管奇形　有・無	食道気管瘻	食道閉鎖	Gross	十二指腸閉　鎖	後鼻孔閉鎖	咽頭狭窄
有	有	有	A	有	有	有
89	71	84	7	10	2	3
79.5%	63.4%	75.0%	6.3%	9.0%	1.8%	2.7%
無	無	無	B	無	無	無
21	20	8	1	74	77	78
18.8%	17.9%	7.2%	0.9%	66.1%	68.7%	69.7%
	不明	不明	C	不明	不明	不明
	0	0	73	4	7	5
	0.0%	0.0%	65.2%	3.5%	6.3%	4.4%
無回答	無回答	無回答	D	無回答	無回答	無回答
2	21	20	2	24	26	26
1.7%	18.7%	17.8%	1.8%	21.4%	23.2%	23.2%
			無回答			
			29			
			25.8%			

C					
心奇形 有・無	VSD	ASD	TOF	PDA	単一臍帯 動脈
有	有	有	有	有	有
77	40	26	12	20	11
68.7%	35.8%	23.3%	10.8%	17.9%	9.9%
無	無	無	無	無	×
33	36	43	52	42	39
29.5%	32.2%	38.4%	46.5%	37.5%	34.8%
	不明	不明	不明	不明	不明
	1	1	2	4	12
	0.8%	0.8%	1.7%	3.6%	10.7%
無回答	無回答	無回答	無回答	無回答	無回答
2	35	42	46	46	50
1.8%	31.2%	37.5%	41.0%	41.0%	44.6%

R				
腎無・低形成	水腎症	膀胱尿道管逆流	馬蹄腎	尿管腎盂結合部閉塞
有	有	有	有	有
43	22	24	13	5
38.4%	19.7%	21.5%	11.7%	4.5%
無	無	無	無	無
32	47	43	55	61
28.6%	42.0%	38.3%	49.1%	54.5%
不明	不明	不明	不明	不明
1	0	4	0	0
0.9%	0.0%	3.6%	0.0%	0.0%
無回答	無回答	無回答	無回答	無回答
36	43	41	44	46
32.1%	38.3%	36.6%	39.2%	41.0%

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書
VATER 連合患者に関する iPS 細胞に関する研究
研究分担者　赤松 和土
慶應義塾大学医学部　講師

研究要旨

VATER連合はV=脊椎、A=肛門、T=気管、E=食道、R=腎臓　を主徴とする先天奇形症候群である。C=先天性心疾患、L=四肢奇形の合併も高頻度に認め、これらを伴うものをVACTERL連合という。循環器・呼吸器という生命維持に必須の臓器の障害に運動器の障害（橈骨奇形・側彎）を伴う、慢性的かつ持続的な疾患であり、生活面での長期にわたる支障を来たす。本分担研究班では、VATER連合患者由来のiPS細胞を用いて、VATER連合の病態解明、治療方法の開発を目指す。本年度は、iPS細胞の作成条件について最適化をおこなった。また、作成されたiPS細胞を迅速に分化誘導する方法について検討を行った。

A．研究目的

VATER 症候群は V=脊椎、A=肛門、T=気管、E=食道、R=腎臓　を主徴とする先天奇形症候群である。C=先天性心疾患、L=四肢奇形の合併も高頻度に認め、これらを伴うものを VACTERL 連合という。循環器・呼吸器という生命維持に必須の臓器の障害に運動器の障害（橈骨奇形・側彎）を伴う、慢性的かつ持続的な疾患であり、生活面での長期にわたる支障を来たす。

VATER 症候群が多系統に渡る先天異常を発症する機序は不明である。しかし異常を持つ臓器の発生時期の多くが、原腸形成期であることから、この時期に胚の広い範囲に障害が起きていると推測されている。母体糖尿病やトリソミー18 の部分症状として VATER 連合の症状を呈する場合がある事から、催奇形因子や遺伝子異常など、複数の異なる原因により類似する病態を呈すると考えられている。このため、「症候群」という用語の代わりに「連合」という用語で呼ばれる場合がある。ここで連合とは、高頻度に併存する奇形の組み合わせを指す。現時点では、外科手術や症状に応じた療育上の対応が行われるが、現在、根治療法は開発されていない。

本研究では、VATER 症候群患者由来の iPS 細胞(induced pluripotent stem cell)を健常人の皮膚線維芽細胞由来の iPS 細胞と比較することで、VATER 症候群で特有に認める発生初期の変化を再現することを試みたいと考える。iPS 細胞は、山中らにより開発された多分化能、自己複製能を持った ES 細胞(embryonal stem cell)様の細胞である。iPS 細胞は成体の皮膚線維芽細胞から樹立されるため、ES 細胞で生じる受精卵を使用することに関する倫理的問題や拒絶反応の問題を回避できるようになり、患者由来の細胞を用いた病気の研究や治療の実現可能性が高まると期待されている。

遺伝性疾患に罹患する患者の皮膚線維芽細胞より iPS 細胞を樹立し、さらに種々の組織・臓器に分化させる事により、今までは剖検時以外には入手する事が困難であった、組織や臓器を研究の対象とすることが可能となった。病態が明らかになる事により、治療効果のある低分子化合物のスクリーニングや、培養細胞を用いた治療法の研究が可能となると期待されており、病態解明や新たな治療方法の糸口になると考える。

B．研究方法

本年度はVATER症候群iPS細胞作成のための患者選定および協力が得られず、疾患解析に最適な患者iPS細胞樹立に向けた条件検討を行った。引き続き患者のリクルートを積極的に行っていく。

①皮膚からの健常人由来iPS細胞の樹立

昨年に引き続きインフォームド・コンセントを得た後、健康成人の皮膚組織から線維芽細胞を樹立した。本年度は従来のレトロウイルスに加えてセンダイウイルス、プラスミドによる樹立を行った。

②樹立した iPS 細胞の line 選定

昨年と同様に、樹立した iPS 細胞において、iPS 細胞樹立とともに導入した 4 遺伝子が発現低下し、内因性の転写因子が発現していることを定量 PCR にちより比較し、発現が抑えられている line を選定した。

③ **末梢血からの iPS 細胞の樹立と神経分化**

健康成人から末梢血を採取し、CD3陽性のT細胞を純化し、センダイウイルスを用いて遺伝子導入を行いiPS細胞を樹立した。

樹立したT細胞由来のiPS細胞を神経分化誘導を行い、疾患解析に用いることが可能かを検討した。

C. 研究結果

1.レトロウイルス以外の方法を用いて線維芽細胞から樹立したiPS細胞も、従来のiPS細胞と同様に神経分化を誘導することが出来た。

2.センダイウイルスを用いてT細胞から誘導したiPS細胞は神経分化誘導が可能であり、従来の細胞と同様に神経系の細胞が分化誘導された。

D. 考察

末梢血由来の iPS 細胞は線維芽細胞由来の iPS 細胞とほぼ同様の分化誘導能力を示し、十分に疾患解析に用いることが出来るのではないかと考えられる。

E．結論

今後、VATER症候群のiPS細胞の樹立を目指すにあたり、末梢血由来の細胞からのiPS細胞樹立を用いることにより、線維芽細胞採取に比べると患者の負担も大きく減り、研究協力を得やすいのではないかとも思われた。

F．研究発表

1. 論文発表

1) Matsui T, Takano M, Yoshida K, Soichiro Ono, Fujisaki C, Matsuzaki Y, Toyama Y, Nakamura M, Okano H, **Akamatsu W.**: Neural stem cells directly differentiated from partially reprogrammed fibroblasts rapidly acquire gliogenic competency. ***Stem Cells.*** 2012 In press

2) Lee EK, Kim W,Tominaga K,Martindale JL, Yang Xl, Subaran SS, Carlson, OD, Mercken EM, Kulkarni RN, **Akamatsu W,** Okano H, Perrone-Bizzozero NI, de Cabo R, Egan JM, Gorospe M. RNA-binding protein HuD controls insulin translation. ***Molecular Cell.*** 2012 Feb 29. [Epub ahead of print]

3) Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, Yamanaka S, Okano H, Suzuki N. Modeling familial Alzheimer's disease with induced pluripotent stem cells. ***Hum Mol Genet.*** 2011 Dec 1;20(23):4530-9.

2. 学会発表

（国内）

1) Naoko Kuzumaki, Michiko Narita, Yusuke Hamada, Atsumi Nagasawa , Yohei Okada, **Wado Akamatsu** , Hirotaka J Okano, Hideyuki Okano, Minoru Narita :Multiple analyses of G-protein coupled receptor (GPCR) expression in the neural differentiation from embryonic stem cells 第34回　日本神経科学大会、　2011年9月15日 *会期9/14-17　（横浜）

2) Shigeki Ohta, Aya Misawa, Hironobu Okuno, Kimiko Fukuda, **Wado Akamatsu,** Yutaka Kawakami, Hideyuki Okano :Generation of Neural Crest Progenitor cells from human induced pluripotent stem cells by a simple method第34回　日本神経科学大会、　2011年9月15日 *会期9/14-17　（横浜）

3) Takeshi Matsui, Morito Takano, Kenji Yoshida, Soichiro Ono, Yumi Matsuzaki, Masaya Nakamura, **Wado Akamatsu,** Hideyuki Okano : Direct induction of safe neural stem cells from adult mouse fibroblasts、第34回　日本神経科学大会、 2011年9月16日　*会期9/14-17　（横浜）

(国際)

Takeshi Matsui, Morito Takano, Kenji Yoshida, Soichiro Ono, Yumi Matsuzaki, Masaya Nakamura, **Wado Akamatsu,** Hideyuki Okano : "Direct induction of safe neural stem cells from adult mouse fibroblasts.", ISSCR 2011　International Society for Stem Cell Research, Toronto, Canada 2011 6.17

H．知的財産権の出願・登録状況

1. 特許取得

【海外】

（1）発明の名称　神経幹細胞製造方法
出願番号　アメリカ　13/127, 566
出願日　2011年5月4日
出願人　学校法人慶應義塾
発明者　岡野栄之、赤松和土

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書
VATER 症候群様の副作用を示すアドリアマイシンの新規標的候補タンパク質スクリーニングに関する研究

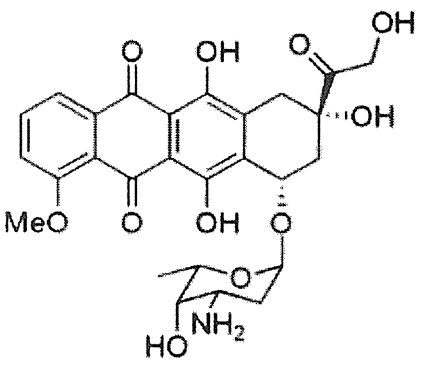
研究分担者　加部泰明
慶應義塾大学医学部医化学教室　　講師

研究要旨

抗ガン剤として用いられるアドリアマイシンは心肥大などの心機能異常や催奇形成などの副作用を示す事が知られるが、その作用メカニズムについては全く不明である。本研究では低分子化合物に対する受容体をスクリーニング出来る独自のアフィニティ精製システムを駆使して、アドリアマイシンに特異的に結合するタンパク質の同定に成功した。この標的候補タンパク質の機能情報を基盤として、VATER症候群の作用発現の分子機構の解明を目指す。

A．研究目的

アドリアマイシン（図）は、放線菌株から得られたアントラサイクリン系抗生物質の1種で、2本鎖DNAのintercalatorとして働いてガン細胞種の増殖阻害効果を示し、臨床において現在でも、悪性リンパ腫などに対する化学療法に用いられている。しかし、その一方でアドリアマイシンは、心肥大などの心機能異常や催奇形成誘導などの重篤な副作用を示すことが知られるが、その副作用発現の分子メカニズムについては全く不明である。我々は、ナノスケールの担体を用いた独自のアフィニティ精製技術の開発を行い、薬剤やホルモンなどの低分子化合物に選択的に結合するタンパク質の精製システムを確立してきた([Chemical Biology/Chemical Genetics] CMC press, 2009)。このアフィニティスクリーニング技術を駆使してアドリアマイシンの未知の結合タンパク質を同定し、その副作用発現の分子機構の解明に繋げることを目的としている。



B．研究方法

薬剤をナノアフィニティビーズに固定化するために、薬剤の官能基を利用してビーズ表面上に共有結合によりカップリングを行う必要がある。そこで、カルボン酸修飾型ビーズと、アドリアマイシン中に存在するアミノ基をアミドカップリングにより反応を行い、共有結合させた薬剤固定化ビーズを作製した。

また、rebamipide分子中のカルボキシル基がその薬理効果発現に必須である可能性があるため、上記とは固定化位置の異なるフェノール性水酸基を導入した誘導体OPC22285を用いて、エポキシ環を導入したアフィニティビーズにカップリングを行った。

また、アドリアマイシンは、実験レベルにおいてニワトリの胚発生時に添加すると奇形誘導することから、ニワトリ受精卵の初期胚状態のものを集め、タンパク質成分を抽出してアフィニティスクリーニングに用いた。

C．研究結果

上記のように調整したニワトリ由来のタンパク抽出液を用いて、アドリアマイシン固定化ビーズと混合してこれに結合するタンパク質のスクリーニングを行った。なお、今回の実験では、アドリアマイシンとは異なる作用で催奇形成誘導を引き起こすと考えられる抗てんかん薬カルバマゼピンを固定化したもので同時に検証し、これらの薬剤にタンパク質結合特異について検討した。

このスクリーニングの結果、図に示したように、薬剤を固定化したものにおいていくつかの結合してくるバンドが見られた。この中で特に矢印で示した 75kDa 付近の位置に、カルバマゼピンでは結合せず、アドリアマイシン特異的に結合するバンドが見られた。

この SDS ポリアクリルアミドゲル上のバンドを切り出して、trypsin による in gel digestion 法でペプチド分解し、MS スペクトル（Hitachi Nano-Frontier）でペプチド同定を行った結果、これが選択的な塩基配列を認識する RNA 結合性のタンパク質の一つである事が分かった。

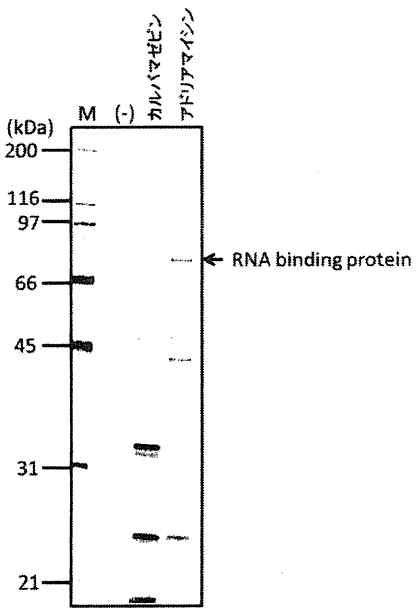


図 アドリアマイシン結合タンパク質のアフィニティ精製

D．考察

これまでに、アドリアマイシンの分子標的としては、2本鎖DNAにintercalateしたアドリアマイシンがDNA topoisomerase IIなどの酵素を阻害して、染色体DNA複製阻害によるガン細胞増殖抑制効果が知られていたが、このような作用は細胞・組織特異性が無いため、アドリアマイシンによる心機能障害や催奇形成誘導などの局所特異的な副作用発現の作用メカニズムとは異なると考えられている。

本研究の解析で同定されたアドリアマシン特異的に結合する因子は、特定の塩基配列を認識する RNA 結合性のタンパク質であり、特異的遺伝子の RNA のプロセシングやタンパク質翻訳修飾に関わっていると考えられている。今後、薬剤によるこの標的候補タンパク質の機能制御について解析するとともに、心臓や奇形形成部位での遺伝子発現の影響などについて解析することにより、これまで未知であった VATER 症候群様の作用メカニズムの解明に繋がる可能性がある。

E．結論

本研究の解析で、VATER 症候群様の副作用を引き起こすアドリアマイシンの新たな結合タンパク質の同定に成功した。この機能情報を基盤として、未知の病態形成の作用機構の解明に繋げて行きたい。

F．研究発表

1. 論文発表

1) Morikawa T, Kajimura M, Nakamura T, Hishiki T, Nakanishi T, Yukutake Y, Nagahata Y, Ishikawa M, Hattori K, Takenouchi T, Takahashi T, Ishii I, Matsubara K, Kabe Y, Uchiyama S, Nagata E, Gadalla MM, Snyder SH, Suematsu M. Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. Proc Natl Acad Sci U S A. 109(4):1293-8,2012.

2) Nishiyama Y, Goda N, Kanai M, Niwa D, Osanai K, Yamamoto Y, Senoo-Matsuda N, Johnson RS, Miura S, Kabe Y, Suematsu M. HIF-1α induction suppresses excessive lipid accumulation in alcoholic fatty liver in mice. J Hepatol. 56(2):441-7, 2012.

2. 学会発表

無し

G．知的財産権の出願・登録状況（予定を含む。）

1. 特許取得

無し

2. 実用新案登録

無し

3. その他

無し

〔Ⅳ〕

刊行に関する一覧表

研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
小崎里華	ＶＡＴＥＲ症候群	大関武彦他	今日の小児の治療指針	医学書院	東京	2011	190
水野誠司	1p36欠失症候群	大関武彦	今日の小児治療指針第15版	医学書院	東京	2012年	P180
水野誠司	レックリングハウゼン病	大関武彦	今日の小児治療指針第15版	医学書院	東京	2012年	P190
松井　健・赤松和土・岡野栄之	神経を創る－ Direct conversion による神経系細胞の誘導と医薬応用	御子柴克彦	in vivo 実験医学によるヒト疾患解明の最前線	羊土社	東京	2011	増　刊 Vol.30 No.2

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoshihashi H, Ohki H, Torii C, Ishiko A, Kosaki K.	Survival of a male mosaic for *PORCN* mutation with mild focal dermal hypoplasia phenotype. Pediatric Dermatology	Pediatric Dermatology	28	550-554	2011
Soneda A, Teruya H, Furuya N, Yoshihashi H, Enomoto K, Ishikawa A, Matsui K, Kurosawa K.	Proportion of malformations and genetic disorders among cases encountered at a high-care unit in a children's hospital.	Eur J Pediatr.	171	301-305	2012
Kurosawa K, Masuno M, Kuroki Y	Trens of occurrence of twin births in Japan.	Am J Med Genet Part A	158A	75-77	2012
Shimizu H, Migita O, Kosaki R, Kasahara M, Fukuda A, Sakamoto S, Shigeta T, Uemoto S, Nakazawa A, Kakiuchi T, Arai K	Living-related liver transplantation for siblings with progressive familial intrahepatic cholestasis 2, with novel genetic findings.	Am J Transplant.	11 (2)	394-8	2011
Kosaki R, Fujita H,Ueoka K, Torii C, Kosaki K.	Overgrowth of prenatal onset associated with submicroscopic 9q22.3 deletion.	Am J Med Genet A.	155 (4)	903-5	2011
Kosaki K, Saito H, Kosaki R, Torii C, Kishi K, Takahashi T.	Branchial arch defects and 19p13.12 microdeletion: defining the critical region into a 0.8 M base interval.	Am J Med Genet A	155A (9)	2212-4	2011

Numabe H, Sawai H, Yamagata Z, Muto K, Kosaki R, Yuki K, Kosaki K.	Reproductive success in patients with Hallermann-Streiff syndrome	Am J Med Genet A	155A (9)	2311-3	2011
Tsutsumi Y, Kosaki R, Itoh Y, Tsukamoto K, Matsuoka R, Shintani M, Nosaka S, Masaki H, Iizuka Y	Vein of Galen Aneurysmal Malformation Associated With an Endoglin Gene Mutation.	Pediatrics	12 (5)	1307-10	2011
Tonoki H, Harada N, Shimokawa O, Yosozumi A, Monzaki K, Satoh K, Kosaki R, Sato A, Matsumoto N, Iizuka S	Axenfeld-Rieger anomaly and Axenfeld-Rieger syndrome: Clinical, molecular-cytogenetic, and DNA array analyses of three patients with chromosomal defects at 6p25.	Am J Med Genet A	155 A(12)	2925-32	2011
Hayashi S, Imoto I, Aizu Y, Okamoto N, Mizuno S, Kurosawa K, Okamoto N, Honda S, Araki S, Mizutani S, Numabe H, Saitoh S, Kosho T, Fukushima Y, Mitsubuchi H, Endo F, Chinen Y, Kosaki R, Okuyama T, Ohki H, Yoshihashi H, Ono M, Takada F, Ono H, Yagi M, Matsumoto H, Makita Y, Hata A, Inazawa J.	Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies.	J Hum Genet.	56 (2)	110-24	2011
Okamoto N, Hatsukawa Y, Shimojima K, Yamamoto T.	Submicroscopic deletion in 7q31 encompassing CADPS2 and TSPAN12 in a child with autism spectrum disorder and PHPV.	Am J Med Genet A.	155A	1568-73	2011
Shimizu K, Okamoto N, Miyake N, Taira K, Sato Y, Matsuda K, Akimaru N, Ohashi H, Wakui K, Fukushima Y, Matsumoto N, Kosho T.	Delineation of dermatan 4-O-sulfotransferase 1 deficient Ehlers-Danlos syndrome: Observation of two additional patients and comprehensive review of 20 reported patients.	Am J Med Genet A.	155A	1949-58	2011
Misako Naiki,Seiji Mizuno,Kenichiro Yamada, Yasukazu Yamada, Reiko Kimura, Makoto Oshiro, Nobuhiko Okamoto, Yoshio Makita, Mariko Seishima, and Nobuaki Wakamatsu	MBTPS2 mutation causes BRESEK/BRESHECK syndrome	Am J Med Genet	158A	97-102	2012
Okamoto N, Tamura D, Nishimura G, Shimojima K, Yamamoto T. Submicroscopic d	Submicroscopic deletion of 12q13 including HOXC gene cluster with skeletal anomalies and global developmental delay.	Am J Med Genet A.	155	2997-3001	2011

Seiji Mizuno, Daisuke Fukushi, Reiko Kimura, Kenichiro Yamada, Yasukazu Yamada, Toshiyuki Kumagai, Nobuaki Wakamatsu	Clinical and genomic characterization of siblings with a distal duplication of chromosome 9q (9q34.1-qter)	Am J Med Genet A,	155 (9)	224-2280	2011
Liang JS, Shimojima K, Takayama R, Natsume J, Shichiji M, Hirasawa K, Imai K, Okanishi T, Mizuno S, Okumura A, Sugawara M, Ito T, Ikeda H, Takahashi Y, Oguni H, Imai K, Osawa M, Yamamoto T.	CDKL5 alterations lead to early epileptic encephalopathy in both genders.	Epilepsia	5(10)	1835-42	2011
Matsui T, Takano M, Yoshida K, Soichiro Ono, Fujisaki C, Matsuzaki Y, Toyama Y, Nakamura M, Okano H, Akamatsu W.	Neural stem cells directly differentiated from partially reprogrammed fibroblasts rapidly acquire gliogenic competency	Stem Cells		In press	2012

Eur J Pediatr (2012) 171:301–305
DOI 10.1007/s00431-011-1534-2

ORIGINAL PAPER

Proportion of malformations and genetic disorders among cases encountered at a high-care unit in a children's hospital

Akiko Soneda · Hideki Teruya · Noritaka Furuya · Hiroshi Yoshihashi · Keisuke Enomoto · Aki Ishikawa · Kiyoshi Matsui · Kenji Kurosawa

Received: 5 May 2011 / Accepted: 5 July 2011 / Published online: 16 July 2011

Abstract Genetic disorders and birth defects account for a high percentage of the admissions in children's hospitals. Congenital malformations and chromosomal abnormalities are the most common causes of infant mortality. So their effects pose serious problems for perinatal health care in Japan, where the infant mortality is very low. This paper describes the reasons for admissions and hospitalization at the high-care unit (HCU) of a major tertiary children's referral center in Japan. We retrospectively reviewed 900 admission charts for the period 2007–2008 and found that genetic disorders and malformations accounted for a significant proportion of the cases requiring admission to the HCU. Further, the rate of recurrent admission was higher for patients with genetic disorders and malformations than for those with acquired, non-genetic conditions. Over the past 30 years, admissions attributed to genetic disorders and malformations has consistently impacted on children's hospital and patients with genetic disorders and malformations form a large part of this facility. These results reflect improvements in medical care for patients with genetic disorders and malformations and further highlight the large proportion of cases with genetic disorders, for which highly specialized management is required. Moreover, this study emphasizes the need for involvement of clinical geneticists in HCUs at children's hospitals.

Keywords Malformation · Genetic disease · High-care unit · Children's hospital · Mortality

Grant sponsor The Ministry of Health, Labor and Welfare, Japan

A. Soneda · N. Furuya · H. Yoshihashi · K. Enomoto · A. Ishikawa · K. Kurosawa (✉)
Division of Medical Genetics,
Kanagawa Children's Medical Center,
2-138-4 Mutsukawa, Minami-ward,
Yokohama 232-8555, Japan
e-mail: kkurosawa@kcmc.jp

H. Teruya
Division of Critical Care Medicine,
Kanagawa Children's Medical Center,
Yokohama, Japan

K. Matsui
Division of General Pediatrics,
Kanagawa Children's Medical Center,
Yokohama, Japan

H. Teruya
Department of Pediatrics, Yokohama Rosai Hospital,
Yokohama, Japan

K. Kurosawa
Clinical Research Institute, Kanagawa Children's Medical Center,
Yokohama, Japan

Introduction

Genetic disorders and birth defects account for a high percentage of the admissions to children's hospitals [4, 13]. In 2008 [5], the Ministry of Health, Labor and Welfare in Japan reported that congenital malformations, chromosomal abnormalities, and genetic diseases are the leading causes of death in children during the first year of life. As per that report, 999 infants under the age of 1 year died of congenital malformations and chromosomal abnormalities; this corresponds to 35.7% of the total number of deaths in this age group. Since 1985, congenital malformations and chromosomal abnormalities have remained the leading causes of infant mortality in Japan [5]. Indeed, in USA it

has been found that patients with genetic disorders had a greater need for hospital admission and were hospitalized for longer durations than were those without genetic disorders [14].

However, recent advances in treatment are likely to improve the survival of individuals with congenital malformations, which, in turn, is likely to increase the rates of readmission to pediatric intensive care units (PICUs) [16]. Several studies have assessed the role of genetic disorders in pediatric mortality and hospitalization [2, 6, 7, 16]. Congenital malformations and chromosomal abnormalities pose serious challenges for perinatal health care in this country, as they are the leading contributors to the infant mortality rate in Japan.

In this study, we assessed the reasons for admissions and hospitalization to the high-care unit (HCU) of a major tertiary children's referral center in Kanagawa Prefecture, Japan, and compared our findings to those of a study of this unit 30 years ago. To elucidate the impact and contribution of birth defects and genetic diseases on pediatric hospitalization, we studied the reason for hospitalization, underlying diagnoses, and duration of hospitalization in this children's hospital in Japan.

Materials and methods

Permission for the study was obtained from the Ethical Committee of our medical center.

We retrospectively analyzed the cases of children hospitalized at the HCU of Kanagawa Children's Medical Center (KCMC) between June 2007 and December 2008. KCMC is a major tertiary children's referral center for pediatric cardiology, surgery, and cancer cases and serves a large area in Kanagawa Prefecture, Japan. It has an institute for the severely handicapped, a PICU, a neonatal intensive care unit, and an HCU. In contrast to the PICU, which admits patients who have undergone cardiovascular or neurosurgery, the HCU specializes in pediatric patients with other acute conditions. All of the patients were included if they were admitted to the HCU from the emergency room, operating room, or inpatient ward. KCMC, with 419 beds, is the only specialized pediatric hospital in Kanagawa Prefecture, where the total number of births is 80,000 annually [8, 9]. About 8,500 patients (male/female, 1:1) were admitted to KCMC in 2007, and the average of hospital stay was 15.3 days.

We summarized and reviewed the medical charts of all patients admitted to the HCU. The charts and summaries were reviewed for age, sex, duration of hospitalization, underlying disease, and reason for admission. Subcategories were created for the underlying diseases and reason for admission.

The underlying disease was classified into two main categories: genetic conditions and acquired (non-genetic) conditions. Genetic conditions were considered to include chromosomal abnormalities, recognizable malformation and dysplasia, multiple malformations, isolated malformations (e.g., those related to the heart, central nervous system (CNS), and respiratory and gastrointestinal tracts), other single-gene defect-related conditions, mitochondrial diseases, and metabolic disorders (Table 1). All cases of chromosomal abnormalities and multiple malformations were examined using standard karyotyping. Cases of recognizable malformation/dysplasia were ascertained by clinical dysmorphologists (H.Y., N.F., and K.K.). Acquired conditions were considered to include perinatal complications, trauma, neoplasm, and sequelae of severe infectious conditions.

The reasons for admission were classified as problems of the respiratory system, CNS, heart, gastrointestinal tract, kidneys and urinary tract, infectious diseases, post-operative management, and unknown condition. Those cases that did not fall into these categories were placed into a category called "others."

Statistical analyses were performed to compare the duration of hospitalization and the age distribution, using StatView version 5.0 (SAS Institute, Inc; Cary, NY). Categorical data were reported as counts and percentages, and continuous data as mean (SD) or median values. Statistical differences for categorical variables were determined by using chi-squared analyses. Median differences were compared by Mann–Whitney *U* test.

Results

A total of 900 admissions, consisting of 687 individual cases with 200 recurrent admissions, were reviewed. Sixteen admissions were excluded from the study because of insufficient information regarding the underlying causes for admission.

The median age at admission was 3.5 years (range, 1 day–32.5 years), and the sex ratio was 1.36 (396 males and 291 females). The median lengths of hospitalization in the HCU were 4 days. Table 2 shows the distribution of the 884 admissions across the different categories of causes for admission. Most patients were admitted for common medical problems, including respiratory problems, post-operative management, and CNS problems. Of the 298 admissions for respiratory problems, most cases involved respiratory infection, including pneumonia and bronchitis. Admissions for post-operative management accounted for 30.7% cases (271 of 884 admissions), while CNS problems such as convulsions, encephalitis, and meningitis accounted for 16.3% (144 of 884 admissions).

Table 1 Definitions of categories

Category	Examples
Chromosomal syndromes	Down syndrome, trisomies 13 and 18, cri du chat syndrome, and Wolf–Hirschhorn syndrome
Recognizable malformation/dysplasia	22q11.2 deletion syndrome, CHARGE syndrome, and VATER association, Lowe syndrome, achondroplasia, Crouzon syndrome, Noonan syndrome, and Treacher–Collins syndrome
Multiple malformations	
Isolated malformations	
Congenital heart diseases	VSD ASD, AVSD, TGA, and DORV
Central nervous system malformations	Schistorrhachis, hydrocephalus, and meningoencephalocele
Gastrointestinal malformations	Diaphragmatic hernia, biliary atresia, and congenital intestinal obstruction
Respiratory system malformations	CCAM and tracheal stenosis
Other isolated malformations	Cleft palate and cleft lip
Single-gene defect	Metabolic diseases, spinal muscular atrophy, and spinocerebellar degeneration
Mitochondrion	

The classification of the underlying conditions of the 687 patients is shown in Table 3. In 13 cases, the data for identifying the underlying disease were insufficient (e.g., charts were missing). These cases were categorized as "unknown condition." Of the total 687 patients, 372 (54.1%) had genetic disorders and the remaining 302 (44.0%) had acquired conditions unrelated to genetic disorders, including perinatal complications, neoplasm, and trauma. Among the 372 patients with genetic disorders, 72 had chromosomal abnormalities, with Down syndrome (29 cases) being the most common underlying disorder. Seventy patients had recognizable malformations and dysplasia, with conditions such as osteogenesis imperfecta, 22q11.2 deletion syndromes, CHARGE syndrome, and VATER association. Multiple malformations with unrecognizable patterns were present in 38 cases while isolated malformations, including CNS malformation, congenital heart disease, and gastrointestinal malformation were present in 160 cases.

We also summarized the reasons for the total of 884 admissions, according to the underlying condition (genetic or acquired). Of these admissions, 200 were readmissions. Patients with genetic disorders and malformations had a greater tendency to be hospitalized repeatedly as compared with those with acquired conditions (Fig. 1). In both genetic and acquired condition categories, respiratory disease, post-operative management, and CNS problems were the major medical problems leading to admission.

We further compared age distribution and the lengths of hospitalization between the groups with genetic and acquired disorders (Table 4). The patients with genetic

Table 2 Medical problems for admission (N=884)

Causes for admission	Number	Percent
Respiratory problems	298	33.7
Post-operative management	271	30.7
CNS problems	144	16.3
Gastrointestinal problems	35	4.0
Cardiac diseases	23	2.6
Other infectious state	23	2.6
Examination	21	2.4
Kidney and urinary tract problems	14	1.6
Other	55	6.2
Total	884	100.0

Table 3 Classification of underlying diseases in 678 patients

Underlying diseases	Number	Percent
Genetic disorders and malformations (subtotal)	372	54.1
Chromosomal abnormalities	(72)	10.5
Recognizable malformation/dysplasia	(70)	10.2
Multiple malformations	(38)	5.5
Isolated malformations	(subtotal:160)	23.3
Central nervous system malformation	(71)	10.3
Congenital heart disease	(35)	5.1
Gastrointestinal malformation	(32)	4.7
Respiratory system malformation	(9)	1.3
Other isolated malformations	(13)	1.9
Single-gene defect	(26)	3.8
Mitochondrion	(6)	0.9
Acquired non-genetic conditions (subtotal)	302	44.0
Perinatal complications	(66)	9.6
Neoplasm	(38)	5.5
Trauma(non-accidental and accidental)	(27)	3.9
Infection	(16)	2.3
Other	(155)	22.6
Unknown	13	1.9
Total	687	100.0

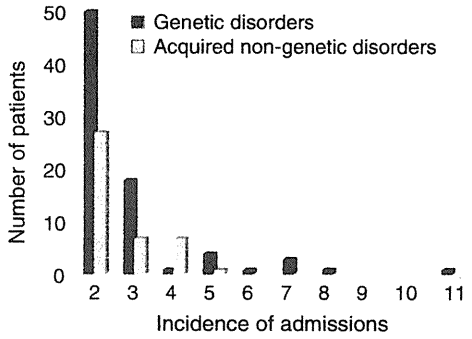


Fig. 1 Comparison of the incidence of admission between the groups with genetic disorders and acquired disorders. In both groups, a total of 200 patients were readmitted. The group with genetic disorders generally required frequent readmission

disorders were significantly younger than those with acquired conditions (median age, 2.0 vs. 4.9 years; $P<0.0001$). There is no significant difference in the length of hospitalization between the patients with genetic disorders and those with acquired conditions (median, 4 vs. 4 days; $P=0.26$), but some patients with genetic disorders had much longer hospitalization (mean, 13.0 vs. 7.0 days; $P=0.007$; range, 1–979 days). Among the reasons for admission, respiratory problems tended to have a longer duration of hospitalization for patients with genetic disorders than for those with acquired conditions (median, 7 vs. 5 days; $P=0.17$).

Discussion

Our study shows that genetic disorders and malformations account for a significant proportion of cases requiring admission to the HCU. Additionally, the rate of recurrent admission was higher among patients with genetic disorders and malformations than among those with acquired non-genetic conditions. This finding is in agreement with those of previous reports for other countries [4, 13].

Several studies from different countries have previously suggested that genetic conditions and malformations and the associated mortality and morbidity have a significant impact on the cost burden for society and the patients' families. Cunniff et al. reported that 19% of deaths in a PICU were in cases of heritable disorders [1]. Stevenson and Carey reported that the 34.4% of deaths in a children's hospital were due to malformations and genetic disorders [15]. On the basis of a population-based study, Yoon et al. reported that the overall rate of hospitalization was related to birth defects and genetic diseases, and varied with age and race/ethnicity [16]. McCandless et al. reported the enormous impact of genetic disease on inpatient pediatrics and the health care system in both admission rates and the total hospital charges [11]. These studies emphasize the importance of understanding the impact that genetic diseases have on mortality and healthcare strategies [15]. Furthermore, it is also clear that early recognition of the underlying disorders is necessary for optimal management of patients with genetic disorders.

Our study highlights another aspect related to the impact of genetic disorders and malformations. In 1981, Matsui et al. analyzed the cases of 18,736 children of total admission during 1975–1979 to KCMC and found that 44% had genetic disorders and malformations [10]. Although our study period and ward are limited to those in the HCU, the patients with genetic disorders and malformations had consistently significant impact in KCMC during the ensuing three decades. Further, it emphasizes that medical care for acute conditions and surgical procedures frequently requires highly specialized knowledge of unusual disease conditions and should be provided in consultation with specialists such as clinical geneticists.

Table 4 Comparison of patients with genetic disorder vs. acquired condition on ages at admission and lengths of stay

	Genetic disorders		Acquired conditions		
	Median (range)	*n*	Median (range)	*n*	*P*
Ages	2.0 years (1 day–27.0 years)	372*	4.9 years (9 days–32.5 years)	302*	<0.0001
Length of hospitalization (days)					
Respiratory problem	7 (1–979)	182	5 (1–97)	109	0.17
CNS	4 (1–54)	73	4 (1–207)	68	0.61
Cardiovascular	4 (2–11)	13	4 (2–24)	8	0.94
Gastrointestinal	5.5 (1–37)	22	5 (2–15)	12	0.60
Kidney and urinary tract	3 (2–12)	5	8 (2–12)	9	0.32
Sepsis	3.5 (2–9)	14	7 (2–20)	9	0.19
Post-operative care	2 (1–49)	174	2 (1–62)	93	0.18
Total	4 (1–979)	518	4 (1–207)	366	0.26

*For the patients who have recurrent admissions, the only first admission was calculated

Although the strategies for management of respiratory infection, by means of newly developed antibiotics and mechanical ventilators, and surgical intervention for infants with malformations, have improved, the general strategies for the medical treatment of genetic disorders and malformations remain to be clarified. Hall commented on the report by Yoon et al. [16] and emphasized the significance of basic research on the human genome and developmental genetics [3]. As shown in Table 2, genetic disorders and malformations include rare diseases, which, although uncommon, remain an important public-health issue and a challenge for the medical community [12].

Our study had the limitations of genetic studies and evaluation in cases with multiple malformations and other isolated malformations. The underlying conditions of most patients in this study were ascertained by clinical geneticists, but high-resolution genome analysis with arrays using comparative genomic hybridization was applied in only limited cases. Recently, research attention has focused to a large extent on rare genetic disorders and Mendelian diseases, because of their significant effect on human health, with the aim of identifying disease-related genetic variations. Re-evaluation and classification of underlying disorders, especially in the case of multiple congenital anomalies in undiagnosed patients, are required for further analysis.

Another limitation of our study is estimation of the financial burden of the group of patients with a genetic background. McCandless et al. showed that the disorders with genetic determinant account for 81% of the total hospital charges [11]. Their results are consistent with those of Hall et al. in 1978 [4]. Further analysis of financial burden in our study may provide useful information for improvement of health care systems.

In conclusion, we report here the proportion of genetic disorders and malformations among cases encountered at the HCU of a tertiary children's medical center in Japan. Over 30 years, the proportion of admissions attributed to genetic disorders and malformations has impact and currently accounts for more than half of admissions to this facility. These results firstly indicate improvements in medical care for patients with genetic disorders and malformations and further highlight the large proportion of cases with genetic disorders. As these cases require highly specialized management, the involvement of clinical geneticists in HCUs at children's hospitals is crucial. Eventually, a better fundamental understanding of genetic disorders and malformations may lead to further improvements in medical care and may reduce the impact of these conditions on the patients and their families.

Acknowledgments The authors are grateful to Dr. Hiroyuki Ida (Tokyo Jikei University) for his valuable comments. This research was supported in part by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan.

Conflict of interest The authors declare no conflict of interest.

References

1. Cunniff C, Carmack JL, Kirby RS, Fiser DH (1995) Contribution of heritable disorders to mortality in the pediatric intensive care unit. Pediatrics 95:678–681
2. Garrison MM, Jeffries H, Christakis DA (2005) Risk of death for children with Down syndrome and sepsis. J Pediatr 147:748–752
3. Hall JG (1997) The impact of birth defects and genetic diseases. Arch Pediatr Adolesc Med 151:1082–1083
4. Hall JG, Poweres EK, McIlvaine RT, Ean VH (1978) The frequency and financial burden of genetic disease in a pediatric hospital. Am J Med Genet 1:417–436
5. Health and Welfare Statistics Association (2010) Mortality rate of infant. J Health Welfare Statist 57:63–65, in Japanese
6. Heron M, Sutton PD, Xu J, Vetura SJ, Strobino DM, Guyer B (2010) Annual summary of vital statistics: 2007. Pediatrics 125:4–15
7. Hudome SM, Kirby RS, Senner JW, Cunniff C (1994) Contribution of genetic disorders to neonatal mortality in a regional intensive care setting. Am J Perinatol 11:100–103
8. Kuroki Y, Konishi H (1984) Current status and perspectives in the Kanagawa Birth Defects Monitoring Program (KAMP). Cong Anom 24:385–393
9. Kurosawa K, Imaizumi K, Masuno M, Kuroki Y (1994) Epidemiology of limb-body wall complex in Japan. Am J Med Genet 51:143–146
10. Matsui I, Naito K, Hanawa Y et al (1981) Impact of the congenital birth defects on children's health care. J Jpn Pediatr Soc 85:889–897, in Japanese
11. McCandless SE, Brunger JW, Cassidy SB (2004) The burden of genetic disease on inpatient care in a children's hospital. Am J Hum Genet 74:121–127
12. Schieppati A, Henter J-I, Daina E, Aperia A (2008) Why rare diseases are an important medical and social issue. Lancet 371:2039–2041
13. Scriver CR, Neal JL, Saginur R, Clow A (1973) The frequency of genetic disease and congenital malformation among patients in a pediatric hospital. Can Med Assoc J 108:1111–1115
14. Sever L, Lynberg MC, Edmonds LD (1993) The impact of congenital malformations on public health. Teratology 48:547–549
15. Stevenson DA, Carey JC (2004) Contribution of malformations and genetic disorders to mortality in a children's hospital. Am J Med Genet 126A:393–397
16. Yoon PW, Olney RS, Khoury MJ, Sappenfield WM, Chavez GF, Taylor D (1997) Contribution of birth defects and genetic diseases to pediatric hospitalizations. Arch Pediatr Adolesc Med 151:1096–1103

RESEARCH ARTICLE

AMERICAN JOURNAL OF
medical genetics

Trends in Occurrence of Twin Births in Japan

Kenji Kurosawa,[1]* Mitsuo Masuno,[2] and Yoshikazu Kuroki[2]

[1]Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan

[2]Kawasaki University of Medical Welfare, Kurashiki, Japan

Received 1 May 2011; Accepted 3 October 2011

The rise in the rate of multiple births since the 1980s is due to the effect of advanced maternal age and increased use of assisted reproductive technology (ART). To determine the trends of prevalence in twin births, we studied the data of a population-based birth defects monitoring system during 26 years in Kanagawa Prefecture, Japan. A total of 15,380 twins from 7,690 deliveries were ascertained from 990,978 births in the Kanagawa Birth Defects Monitoring Program (KAMP) during 1981–2008. From the start of KAMP in 1981, the incidence of twin births had been consistently increasing from 57.0 to 98.6 per 10,000 deliveries until 2003, but after this time, the incidence declined to 78.5 in 2007. While the rate of monozygotic twins has been stable (~40 per 10,000 deliveries) after 1990, that of dizygotic twins increased from 25.3 to 57.3 per 10,000 deliveries until 2002, and recovered to 40.1 in 2007. These results showed the most recent tendency of twin births and indicated that the single embryo transfer method can provide protection and reduction of perinatal risk caused by multiple births.

Key words: assisted reproductive technology (ART); twin; Kanagawa Birth Defects Monitoring Program (KAMP); zygosity

How to Cite this Article:
Kurosawa K, Masuno M, Kuroki Y. 2011.
Trends in occurrence of twin births in Japan.
Am J Med Genet Part A.

INTRODUCTION

Multiple births including twin births have several implications for maternal and child health care. Twin pregnancy is associated with an increased incidence of anomalies [Bahtiyar et al., 2007; Glinianaia et al., 2008; Hardin et al., 2009a], a higher risk of perinatal mortality, and preterm births with low birth weight [Helmerhorst et al., 2004; McDonald et al., 2005] compared with singleton pregnancy. A tendency for an increasing rate of twin delivery has been observed in 14 out of 16 countries in Europe, Canada, Australia, Singapore, and Hong Kong [Imaizumi 1998]. This tendency has also been observed in Japan [Imaizumi 2000]. The rise in the rate of multiple births is due to the effect of advanced maternal age and increased use of assisted reproductive technology (ART) [Bondel and Kaminski, 2002]. In the USA and Europe, between 20 and 30% of deliveries following ART are twin births compared with 1% following spontaneous conception [Andersen et al., 2008; Wright et al., 2008]. However, the rate of twin pregnancies in the USA has stabilized at 32 per 1,000 births in 2006 [Chauhan et al., 2010]. In Australia, recent data indicated that the proportion of twin deliveries decreased in 2006 [Wang et al., 2008].

To determine the trends of prevalence in twin births, we studied the data of a population-based birth defects monitoring system during 26 years between 1981 and 2008 in Kanagawa Prefecture, Japan. Kanagawa Prefecture, which is adjacent to Tokyo, includes Yokohama City with a total population 3,687,000. To investigate the effects of ART, we analyzed the data of twins according to the zygosity during the study period.

MATERIALS AND METHODS

A total of 15,380 twins from 7,690 deliveries were ascertained from 990,978 births in the Kanagawa Birth Defects Monitoring Program (KAMP). This program has been in operation since October 1981 as the first population-based monitoring system in Japan. Details of KAMP are described elsewhere [Kuroki et al., 1982; Kuroki and Konishi, 1984, 1992; Kuroki, 1988; Kurosawa et al., 1994; Yuan et al., 1995]. KAMP covers one-half of the total births (40,000 births annually) in Kanagawa Prefecture. All live births and stillbirths are screened for 44–48 marker malformations (only surface anomalies), arranged in 10–11 groups, and they are examined by general obstetricians or occasionally by general pediatricians within 7 days after birth. During the study period between 1981 and 2008, the KAMP was divided into four stages according to a minor modification in marker anomalies and registration systems. The first two stages, for 1981–1983 and 1984–1988, had total birth registration systems including all the malformed infants, normal

Grant sponsor: Ministry of Health, Labour and Welfare, Japan.
*Correspondence to:
Kenji Kurosawa, MD, PhD, Division of Medical Genetics, Kanagawa Children's Medical Center, 2-138-4 Mutsukawa, Minami-ku, Yokohama 232-8555, Japan. E-mail: kkurosawa@kcmc.jp
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34362

infants, and all multiple births. However, in the last two stages, 1989–2000 and 2001–2008, all malformed infants as well as all multiple births were registered with two consecutive normal infants. Information on zygosity is not available in the KAMP, and therefore, we used Weinberg's differential rule for zygosity estimation [Fellman and Eriksson, 2006; Hardin et al., 2009b]. The incidence of twin births was defined as the number of twin pairs per total deliveries.

RESULTS

During the period of analysis, the incidence of malformed infants was 0.88% in live births and 17.24% in stillbirths. The sex ratio was 1.05. From the start of KAMP at 1981, the incidence of twin births had been consistently increasing from 57.0 to 98.6 per 10,000 deliveries until 2003 (Fig. 1). This tendency is consistent with the results of previous studies [Imaizumi, 1998, 2000]. The incidence of twin births peaked at 98.6 per 10,000 deliveries in 2003, but after this time, the incidence declined to 78.5 per 10,000 deliveries in 2007. The incidence of monozygotic twins fluctuated during the first 10 years, but after 1990 the incidence was stable at 40 per 10,000 deliveries. The incidence of dizygotic twins increased from 25.3 to 57.3 per 10,000 deliveries in 2002, but rapidly decreased to 40.1 in 2007, while the incidence of monozygotic twins was stable (Fig. 2). These results indicated that the incidence of twins is directly affected by the rate of dizygotic twins, and that the incidence of dizygotic twin births has already reached its peak, at least in the urban area of Japan.

DISCUSSION

Our study found that during the last 20 years, the incidence of twin births increased from 57 to 98 per 10,000 deliveries, but after it reached a peak in 2003, it recovered to 78.5 per 10,000 deliveries in 2007. Our study demonstrated that the trend in twin births was affected by the incidence of dizygotic twins. The incidence of monozygotic twins was stable at 40 per 10,000 deliveries, while that of dizygotic twin births attained a peak in 2002 with 57.3 per 10,000 deliveries, and it declined to 40.1 after this time. To the best of our knowledge, this is the first report describing the trends of a decrease in the rate of twin births in Japan. Because the rates of monozygotic twins are thought to be constant throughout the world, our results on the tendency of the rates of monozygotic twins have implication of the accuracy of the study. In the USA, between 1980 and 2006, the rate of twin pregnancies consistently increased from 18.9 to 32.1 per 1,000 births [Chauhan et al., 2010]. However, the rapid rise appeared to end in 2004 and the rate stabilized in 2006. A rise in the prevalence of twin births has also been observed in Austria, Finland, Norway, Sweden, Canada, Australia, Hong Kong, Israel, and Singapore [Imaizumi, 1997]. The rate of twin births in these countries stabilized between 2004 and 2006, and recent trends of a decreasing rate has been reported in some countries [Wang et al., 2008].

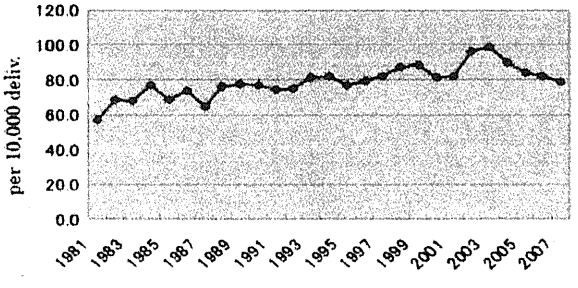


FIG. 1. Trends of the incidence of total births of twin pairs per total deliveries in Japan. The rate of twin births consistently increased from 57.0 per 10,000 deliveries in 1981 to 98.6 in 2003, but after this time, the rate recovered to that of the 1990s.

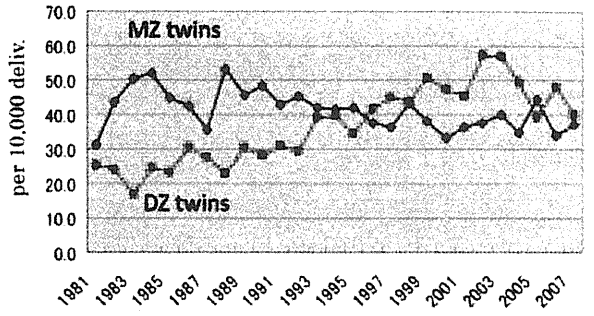


FIG. 2. Comparison of the rate of twin births between monozygotes and dizygotes. In the first 10 years, the fluctuation was remarkable in the rate of births in monozygotic twins for unknown reasons, but during the last 20 years, the rate was stable at approximately 40 per 10,000 deliveries. However, the rate of dizygotic twins consistently increased to reach a peak in 2003, and it then rapidly decreased.

Clearly, the use of ART has contributed to the changes in the rate of twin pregnancies [Wright et al., 2008; Hansen et al., 2009]. ART twins have a greater risk of adverse perinatal outcome including preterm birth, low birth weight, and cerebral palsy compared with spontaneously conceived twins and singletons [Hansen et al., 2009]. The use of single embryo transfers reduces multiple birth rates and the risks of these adverse outcomes following ART. According to a report from the European Society of Human Reproduction and Embryology, compared with the number of cycles in 2003, fewer embryos were transferred in Germany in 2004, but there were still huge differences between countries [Andersen et al., 2008]. This transfer policy had a considerable impact in Belgium, Finland, Sweden, and several other countries [Andersen et al., 2008], and therefore, a reduced rate of twin births may be observed within a few years in these countries. In the case of Japan, the reduction of the rate was rapid, but a stable rate was not observed at the end of the study period. The rate of twin births may be stabilized when there is a balance between maternal age distribution in reproductive generation and establishment of technical standardization of single embryo transfer. Further analysis on the rates of multiple births based on the population-based monitoring system is required to determine the impact of ART.

ACKNOWLEDGMENTS

The authors are grateful to The Association of Obstetrics and Gynecology in Kanagawa Prefecture for participating in the KAMP, which made this work possible. This study was supported in part by a grant from the Ministry of Health, Labour and Welfare, Japan.

REFERENCES

Andersen AN, Goossens V, Ferraretti AP, Bhattacharya S, Felberbaum R, de Mouzon J, Nygren KG, The European IVF-monitoring (EIM) Consortium, for the European Society of Human Reproduction and Embryology (ESHRE). 2008. Assisted reproductive technology in Europe, 2004: Results generated from European resisters by ESHRE. Hum Reprod 23:756–771.

Bahtiyar MO, Dulay AT, Weeks BP, Freidman AH, Copel JA. 2007. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations: A systematic literature review. J Ultrasound Med 26:1491–1498.

Bondel B, Kaminski M. 2002. Trends in the occurrence, determinants, and consequences of multiple births. Semin Perinatol 26:239–249.

Chauhan SP, Scardo J, Hayes E, Abuhamad AZ, Berghella V. 2010. Twins: Prevalence, problems, and preterm births. Am J Obstet Gynecol 203: 305–315.

Fellman J, Eriksson AW. 2006. Weinberg's differential rule reconsidered. Hum Biol 78:253–275.

Glinianaia SV, Rankin J, Wright C. 2008. Congenital anomalies in twins: A register-based study. Human Reprod 23:1306–1311.

Hansen M, Colvin L, Petterson B, Kurinczuk JJ, de Klerk N, Bower C. 2009. Twins born following assisted reproductive technology: perinatal outcome and admission to hospital. Hum Reprod 24: 2321–2331.

Hardin J, Selvin S, Carmichael SL, Shaw GM. 2009a. The estimated probability of dizygotic twins: A comparison of two methods. Twin Res Hum Genet 12:79–85.

Hardin J, Carmichael SL, Selvin S, Lammer EJ, Shaw GM. 2009b. Increased prevalence of cardiovascular defects among 56,709 California twin pairs. Am J Med Genet Part A 149A:877–886.

Helmerhorst FM, Perquin DA, Donker D, Keise MJ. 2004. Perinatal outcome of singleton and twins after assisted conception: A systematic review of controlled studies. BMJ 328:261.

Imaizumi Y. 1997. Trends of twinning rates in ten countries, 1972–1996. Acta Genet Med Gemellol (Roma) 46:209–218.

Imaizumi Y. 1998. A comparative study of twinning and triplet rates in 17 countries, 1972–96. Acta Genet Med Gemellol (Roma) 47:101–114.

Imaizumi Y. 2000. Epidemiology of multiple births in Japan, 1951–1998. In: Yanaihara T, editor. Study on reproductive assisted medical service in Japan. Tokyo: Ministry of Health and Welfare of Japan. pp 776–793 (in Japanese).

Kuroki Y, Konishi H, Tsunoda A, Tadokoro F, Adachi K, Yagi S. 1982. A preliminary report on Kanagawa Birth Defects Monitoring Program (KAMP). Cong Anom 22:223–228.

Kuroki Y, Konishi H. 1984. Current status and perspectives in the Kanagawa Birth Defects Monitoring Program (KAMP). Cong Anom 24:385–393.

Kuroki Y. 1988. Monitoring of congenital anomalies. Cong Anom 28:S89–S99.

Kuroki Y, Konishi H. 1992. Monitoring of congenital anomalies in Japan. International Conference on Radiation Effects and Protection (Proceeding), Japan Atomic Energy Research Institute, pp 59-64.

Kurosawa K, Imaizumi K, Masuno M, Kuroki Y. 1994. Epidemiology of limb-body wall complex in Japan. Am J Med Genet 51:143–146.

McDonald S, Murphy K, Beyene J, Ohlsson A. 2005. Perinatal outcomes of in vitro fertilization twins: A systematic review and meta-analyses. Am J Obstet Gynecol 193:141–152.

Wang YA, Dean JH, Badgery-Parker T, Sullivan EA. 2008. Assisted reproduction technology in Australia and New Zealand 2006. Assisted reproduction technology series no. 12. AIHW cat. no. PER 43. Sydney: AIHW National Perinatal Statistics Unit.

Wright VC, Chang J, Jeng G, Chen M, Macaluso M. 2008. Assisted reproductive technology surveillance-united States, 2005. MMWR CDC Surveill Summ 56:1–22.

Yuan P, Okazaki I, Kuroki Y. 1995. Anal atresia: Effect of smoking and drinking habits during pregnancy. Jpn J Hum Genet 40:327–332.