Citalopram	Couturier and Nicolson 2002[32]	PDD 小児-思春期 4-15歳 17例	retorospective M：7.4ヶ月		M：19.7mg./日	10/17 (59%)	anxiety	agitation aggression insomnia tic
	Namerow et al. 2003[34]	PDD 小児-思春期 6-16歳 15例	retorospective chart review M：218日	CGI-S CGI-I	M：16.9mg/日 5～40mg/日	11/15 (73%)	repetitive behavior anxiety irritability	33% with mild headaches sedation aggressiveness agitation lip dyskinesia
Escitalopram	Owley 2005[33]	PDD 小児-思春期 6-17歳 (M：10.4歳) 28例	open-label prospectove 10週間	ABC-CV CGI	M：11.1mg/日 開始2.5mg/日 最高20mg.日	17/28 (61%)	irritable	irritability hyperactivity

Abbreviations：ABC=Aberant Behavior Checklist, ABC-CV=ABC-Community Version, BAS=Behavior Assesment Scale, Brown=Brown Aggression Scale, CGI=Clinical Global Impressions scale, CYBOCS=Childrens's Yale-Brown Obsessive Scale HAM-A=Hamilton Rating Scale for Anxiety MR=Mental Retardation, PDD=Pervasive Developmental Disorders, Ritvo-Freema=Ritovo-Freeman Real-Life Rating Scale, SCARED=Screen for Child Anxiety Related Emotional Disorders, Vineland=Vineland maladaptive behavior subscales, YBOCS=Yale-Brown Obsessive Compulsive Scale,

れば，より有用性が得られることが示唆される[26)28)]。小学生以下の年齢の幼児を含む報告は5報告ある。うち2報告で言語の改善が示されている。言語能力の改善に関する効果は幼少時の方が期待できる可能性があるが，小児では成人とは異なり「発達」途中であることを十分に考慮する必要があり，さらなる検討が必要であろう。

薬剤投与前の全血中セロトニン値が薬剤効果を予測できるかどうかにについては，今回の結果も含めて難しいと考えられた[21)]。薬剤投与前の全血中セロトニン値が高い方が言語の改善がみられ，低い方が指差しの改善が認められたが，例数が少なくまだ不明である。

薬剤の効果または副作用には個人差が認められる。この個人差の原因を説明し，薬の副作用を減らし，より効率の良い治療をめざすには遺伝子多型が有用ではないかと期待されている。SSRIsの反応と*SLC6A4*多型との関係を調査した論文は，主として成人うつ病で多く検討されている。自閉症との関連の報告は調べた範囲では見当たらない。Mood Disordersにおける*SLC6A4*の多型とSSRIsの効果についてのレビューが最近では一番纏まっているので紹介する[36)]。*SLC6A4*の多型は，5-HTTLPRについての報告がほとんどである。白人に関する12報告中，反応性が良いまたは反応が速いのが，1アレルの5報告，l/l遺伝子型の3報告，l/l遺伝子型の副作用が少ないが2報告，s/s遺伝子型の反応性が悪いまたは遅いが2報告となっており，圧倒的に1アリルを含む多型に反応例が多い。しかし，東洋人に関する9報告では，反応性が良いのが，1アリルの2報告，l/l遺伝子型の2報告，sアリル1報告，s/s遺伝子型の2報告，関係なし2報告で一定せず，日本人における反応性の良さについても1アリルとsアリルは1報告ずつであり一定しない。これには，5-HTTLPRの多型頻度には人種差が大きく，l/l遺伝子型頻度は白人で高く（ドイツ人36％[37)]），東洋人で少ない（韓国人[38)]≒日本人2～6％[39)40)]）ことと，1アリル内のG/A多型の存在（LGのmRNAレベルはsアリルに近い）[41)]と関係していると思われる。*5-HTR2A*に関しても，成人うつ病に対するSSRIsの反応からの検討がいくつか報告されている。-1438A/G多型と102T/C多型はほぼ完全な連鎖不平衡を示すとされるが[10)]，GG遺伝子型が特にfluvoxamine治療で反応良好[42)]，paroxetine治療中の副作用がCC遺伝子型で多い[43)]，fluvoxamine治療中の嘔気は-1438A/G多型と無関係[44)]，胃腸症状はGG遺伝子型と関係する[45)]などであり，一定しない。

ABC-J行動評価と遺伝子多型の結果からは，TTの症例では治療前から，易興奮性，多動が強い傾向があり，SSRIの使用により興奮，多動，高いところに登りたがるという副作用が強調されたのではないかと推測している。特にTTの症例では使用量をさらに少なめに抑えることにより，副作用を軽減できる可能性を考えている。逆に興奮が強い症例には選択的な5-HTR2Aブロッカーの使用のほうが使いやすいのではないかと推測された。先の考察で，副作用の発現に2群あるとの報告[25)]があったが，少量でも出現してくるタイプの副作用は，このような薬剤反応性に関与する遺伝子多型が関与している可能性が考えられた。

6．ま と め

ASDに対するSSRIs治療について，我々の使用経験とこれまでの報告のレビューを行った。日常生活に困難を来たすASD患者の示す異常行動に対して，ある程度効果が期待できるが，副作用を軽減するためには，少量からゆっくりと増量することが重要である。5-HTTLPRと*5HTR2A*遺伝子多型はASD患者へのSSRIs治療の反応に影響を与えていると思われるが，まだ一定の結論が得られていない。

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シンポジウム

自閉症スペクトラム障害における周生期および新生児期関連要因

杉江　陽子（浜松医科大学小児科）
杉江　秀夫（自治医科大学小児科）

自閉性スペクトラム障害（ASD）の発症には遺伝が関与しているが，環境要因との相互作用も注目されており，ASDの発症時期を考慮すると，周生期は環境因子の中でも重要な時期のひとつと考えられる．我々は，日本人のASDにおいて，周生期に関わるいくつかの要因について報告しているが，さらに検討を加えその結果をここに紹介する．

対象は，DSM-IVにより診断したASD 339例，男278例，女61例で，その臨床型内訳は，自閉性障害（AD）200例，高機能自閉症（HF-PDD）79例（アスペルガー症候群も含む），非定型自閉症を含む特定不能の広汎性発達障害（PDD-NOS）60例である．対象の誕生年は1981年5月から2000年5月で，全例日本人で，明らかな基礎疾患を有する症例は除外した．対照は対象と誕生年がほぼ同年代で，すくなくとも3歳健診のころにはASDの特徴がみられず，健康と判断した小児である．周生期因子の調査は母子手帳の記載により，ASD児出生時の両親年齢，在胎週数，出生時体重，新生児期異常の項目について調査を行った．ただし両親年齢は厚生省の人口動態統計を参考とした．

結果は，母年齢は35歳以上の占める割合はASD，ADで有意に高く，出生時平均父の年齢はPDD-NOSを除いて高かった．何らかの新生児期異常を有する症例が高頻度であり，強度黄疸の症例も高頻度の傾向であった．以上より，新生児期のそれほど致命的ではない非特異的なストレスが，脆弱な遺伝子に作用することにより，エピジェネティクスな変化をもたらし，ASD発症のリスクが増す可能性が考えられた．また，ASDにおける父親年齢が高くなると遺伝子の新生変異または遺伝子刷り込みの変化，遺伝子コピー数のエラーが起き易くなり，ASDのリスクが高くなる可能性が考えられた．

1. はじめに

自閉性スペクトラム障害（ASD）の発症には遺伝が関与しているが，環境要因との相互作用も注目されている．ASDの発症が幼児期早期であるため，周生期は環境因子としての関心が高く，これまでに多くの先行研究がなされている．先行研究で，妊娠，分娩，新生児期に関わる因子が，ASDにおいて頻度が高いと報告されているが必ずしも一定した結果ではない[4~7,11]．研究年代，症例数，対照の取り方，比較方法などが異なっていることは大きな一因であろう．全体の傾向として，2000年以前は症例数が少なく，疫学研究よりも，臨床研究であり，選択バイアスがかかりやすく，対象項目や比較基準も多様である．2000年以降になり，規模が大きい登録やコホートや，出生時の記録による前方視的な情報収集に基づく研究が増えている[12]．これらの研究結果を表1にまとめた[1,2,5,8,9,13,14,15,18,19,21]．これらは診断基準にInternational Classification of Diseases (ICD)-8~10 RevisionやDiagnostic and Statistical Manual (DSM)-IIIまたはIVを使用しており，分析にはロジスティック解析を用いている．危険因子として一番共通して認められる項目は，男性であること，ついで出生時の母親年齢が高いことであるが，父親年齢の高いことも多く注目される．在胎週数37週未満，アプガースコア低値も比較

表1

著者 論文発表年 調査対象地	対象 対照 誕生年	危険因子	修正後危険因子	非危険因子
Eaton 2001 デンマーク	自閉症 116 ASD MR 201 コホート 102905 1973～1993	出生時母若年 低出生時体重 アプガースコア低値 妊娠歴 流産歴 在胎36週未満 帝王切開 胎盤不全 分娩時間延長 子癇	低出生時体重 母年齢35歳以上 アプガースコア低値 流産歴	胎児切迫仮死 母体疾病
Croen 2002 カリフォルニア	自閉症 4381 コホート 3551306 1989～1994	出生時母高齢 多胎 母高学歴	出生時母高齢 多胎 母高学歴	出生時体重 出産順位 母人種 母出生地
Hultman 2002 スウェーデン	自閉症 408 対照 2040 1974～1993	帝王切開 在胎37週未満 出生時体重2500g以下 SFD アプガー7以下 母出生欧州か北米外	帝王切開 SFD アプガー7以下 先天奇形 出血 妊娠中喫煙 母出生国	頭囲 母糖尿病 双子 出産季節
Glasson 2004 西オーストラリア	自閉症 314 PDD-NOS 84 ASP 67 一般対照 1313 非罹患兄弟 481 1980～1997	出生時母高齢 出生時父親年齢 切迫流産 胎児切迫仮死 帝王切開 分娩後出血 アプガー1分7以下 新生児特別ケア 分娩歴	出生時母高齢 切迫流産 胎児切迫仮死 帝王切開 誕生年	在胎週数 出生時体重 早期破水 分娩前出血 分娩時間 臍帯巻絡(首) 自発呼吸開始までの時間
Larsson 2005 デンマーク	自閉症 698 対照 17450 1973～1994	出生時母高齢 出生時父高齢 アプガー5分7以下 出生時体重2500g以下 在胎35週未満 骨盤位 両親精神病歴 両親低経済	出生時母年齢20歳未満 出生時父高齢39歳 アプガー5分7以下 SFD 在胎35週未満 骨盤位 両親精神病歴	多胎 子癇前症 妊娠中受診回数 既妊娠回数 初回受診時喫煙 母学歴
Lauritsen 2005 デンマーク	ASD 818 コホート 943664 1984～1998		父年齢35歳以上 母精神病歴 兄弟ASD 出生地の都市化 母欧州以外出生 両親出生国異	出生時母年齢 嫡子，非嫡子 父精神病歴
Reichenberg 2006 イスラエル	ASD 319 コホート 378891 1980s	出生時母年齢39歳以上 出生時父年齢39歳以上	出生時父年齢39歳以上	
Maimburg 2006 デンマーク	自閉症 473 対照 4730 1990～1999	出生時母年齢35歳以上 出生時母年齢25歳未満 出生時父年齢35歳以上 妊娠中内服 胎児体位 帝王切開 在胎37週未満 出生時体重2500g以下 アプガースコア5分8以下 NICU入院 先天奇形 母外国籍	出生時母年齢35歳以上 出生時体重2500g以下 NICU入院 先天奇形 母外国籍	母初回受診時喫煙 母妊娠中発熱 分娩様式 誘発分娩 早期破水 分娩時アシドーシス 胎児心モニター異常 羊水混濁 父外国籍
Williams 2007 オーストラリア	ASD 182 コホート 85628 1990～1999		在胎37週未満 出生時母35歳以上 アプガースコア1分5以下 母出生地 多胎出産	出生時体重 アプガースコア5分 SFD，LFD 出産順位
Croen 2007 北カリフォルニア	ASD 593 (AD 277 ASP&PDD-NOS 316) コホート 132251 1995～1999		両親年齢高くなるほど 母高学歴 母人種	出産順位 父学歴 父人種
Schendel 2008 アトランタ	自閉症 617 コホートから抜粋 434091 1981～1993	出生時体重2500g以下 在胎32週以下	女子でのみ 出生時体重2500g以下 在胎32週以下 特に合併症（MR，CP，HL，VI）	出生時体重についてのみ在胎週を加味有で

略：ASD，自閉症スペクトラム障害；MR，精神発達遅滞；PDD-NOS，特定不能の広汎性発達障害；ASP，アスペルガー障害；AD，自閉性障害；SFD，small for date；LFD，large for date；NICU，新生児集中治療室；CP，脳性まひ；HI，聴力障害；VI，視覚障害

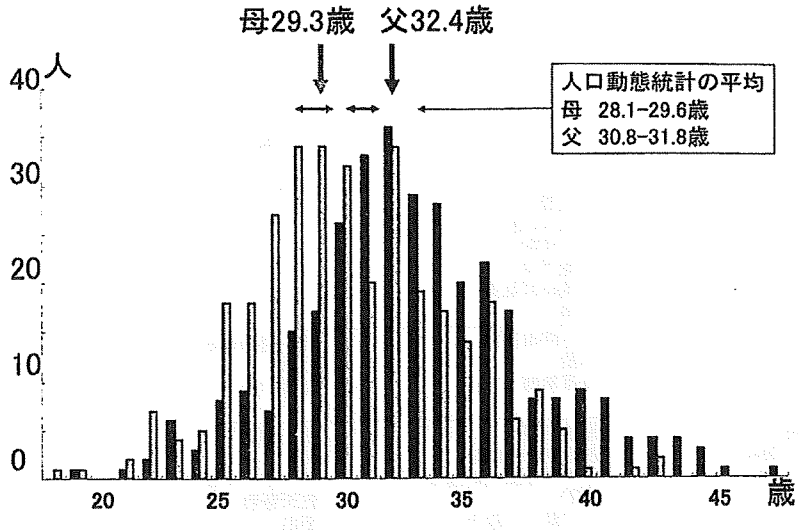

図1　出生時両親年齢分布
ASD出生時における両親の年齢分布．棒グラフの黒は父親，灰色は母親である．縦向きの矢印は，ASD父（黒）と母（灰色）の平均年齢，横向きの矢印は人口動態統計による1980年から2000年の間の平均年齢の幅を示している．黒は父親，灰色は母親である．

的多いが，低出生体重は少なかった．これらは主として，北欧，北米，オーストラリアからの研究である．我々は，日本人のASDにおいて，周生期に関わるいくつかの要因についてすでに報告したが[20]，さらに検討を加えその結果をここに紹介する．

2. 対象と方法

対象は，DSM-IVにより診断したASD 339例，男278例，女61例で男女比は4.6対1である．その臨床型の内訳は，自閉性障害（AD）200例，高機能自閉症（HF-PDD）79例（アスペルガー症候群も含む），非定型自閉症を含む特定不能の広汎性発達障害（PDD-NOS）60例である．対象の誕生年は1981年5月から2000年5月である．全例日本人で，レット症候群や結節性硬化症など明らかな基礎疾患を有する症例は除外している．対照は対象と誕生年がほぼ同年代で，すくなくとも3歳健診の頃にはASDの特徴がみられず，健康と判断した小児である．

周生期因子の調査は母子手帳の記載により，ASD児出生時の両親年齢，在胎週数，出生時体重，新生児期異常の項目について調査を行った．対照の両親年齢は調査されていなかったため，厚生省の人口動態統計を参考とした．比較は，各項目において，①対照との比較を全症例と男女別々に，②男女間の比較，③3臨床病型間の比較を全症例と男女別々に検討した．統計学的分析は項目により，t検定，Kruskal-Wallis法，χ^2検定を用いた．さらに，ASDの危険因子の予測をlogistic regression分析〔性，誕生年，在胎週数（3カテゴリー），体重（3カテゴリー），新生児期異常，強度黄疸，光線療法〕を行い，修正オッズ比（OR），95％信頼区間（CIs）を求めた．有意水準は，$p<0.05$とした．

3. 結　　果

症例の精神発達程度は，精神発達遅滞なし79例，軽度遅滞88例，中等度遅滞121例，重度遅滞47例であった．2世代以内の精神疾患の家族歴を持つ症例は20％であった

図1にASD全症例の出生時における両親年齢分布を示した．母親の平均年齢は29.3歳，父親の平均年齢は32.4歳であった．人口動態統計か

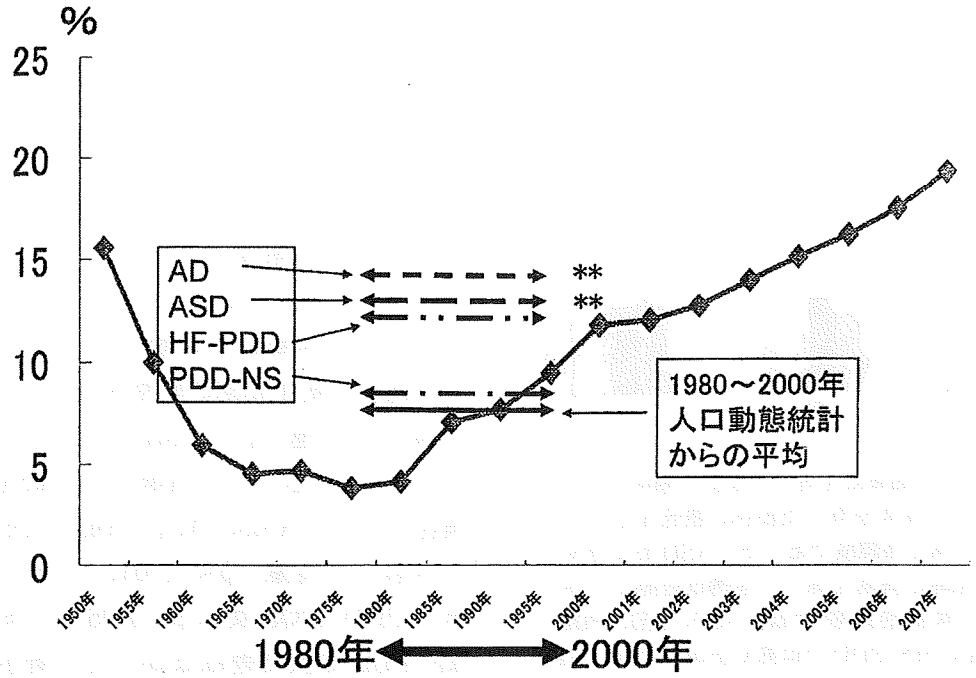

図2　35歳以上の母親の割合：年次別

ASD児出生時の母親の年齢が35歳以上である症例の割合．折れ線グラフは1950年から2007年までの人口動態統計に基づき，35歳以上に出産した母親の割合を年次ごとに示した．我々の調査年代の1980年から2000年の間の人口動態統計（平均頻度8％）と比較するとASD（12.8％）と3臨床病型の中のAD（14.4％）が有意に高頻度であった．＊＊；$p<0.01$

（人口動態調査より）

ら調査年度間の母の平均年齢は28.1～29.6歳で，父年齢は30.8～31.8歳であり，比較すると対象では特に父親で高めであった．病型別では，出生時の母親の平均年齢はAD 29.5歳，HF-PDD 29.6歳，PDD-NOS 28.6歳で病型間差はなく，各病型で男女差はなかった．出生時の父親の平均年齢は，AD 32.7歳，HF-PDD 32.5歳，PDD-NOS 31.5歳で，病型間差はなく，各病型での男女差もなかった．しかし，両親年齢ともADとHF-PDDは高めであり，PDD-NOSは人口動態統計の平均と近似していた．ASD児出生時の母親の年齢が35歳以上である症例の割合を検討した．図2の折れ線グラフは1950年から2007年までの人口動態統計に基づき，35歳以上に出産した母親の割合を年次ごとに示している．その年次ごとの推移は母の年齢が35歳を過ぎても複数の子どもを出産していた時代と一人の母が産む子どもの数が少なくなった時代，そして，第一子を産む年齢が高くなった時代を反映しているようである．我々の調査年代は1980年から2000年で，その間の人口動態統計（調査年代の平均頻度は8％）と比較するとASD（12.8％）では35歳以上で出産した母親の割合が高頻度（$p<0.01$）であり，特に3臨床病型の中ではAD（14.4％）が有意（$p<0.01$）に高頻度であった．

平均在胎週数は対照39.0週，ASD 39.1週，AD 39.1週，HF-PDD 39.0週，PDD-NOS 39.0週であった．対照との比較，男女比較，3病型別比較いずれにおいても有意な差は認められなかった．しかし，対照との男女別比較ではASD男（$p<0.01$）とAD男（$p<0.01$）は対照男に比較して，有意に長い在胎週数であった．また，37週未満の早産児はASDで多い傾向（$p=0.07$）であった．42週以上の過期産はADで有意（$p<$

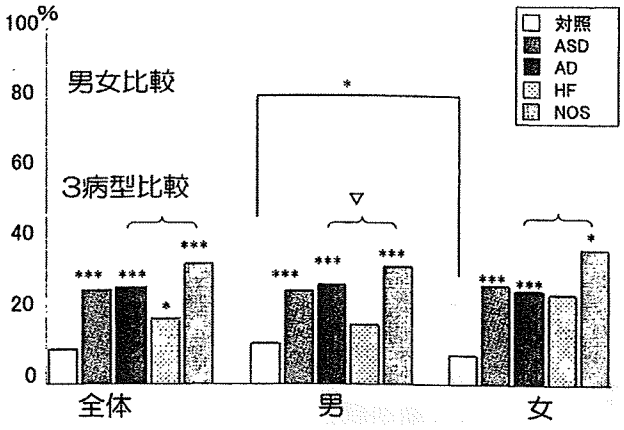

図3　新生児期異常を有する症例の頻度
新生児期に何らかの異常を有した症例の割合を示す．対照に男女差あり，男で高頻度であった．ASDおよびすべての病型で新生児期の異常を有した症例は対照より多かった．対照との男女別比較では，ASD，AD，PDD-NOSでは男女共にHF-PDDでは男で多かったが，HF-PDDの女で差はなかった．3病型間の差はなかった．
＊＊＊；p<0.001，＊；p<0.01，▽；p<0.05

0.05）に高頻度であり，男女別に比較するとASD男（p<0.01）とAD男（p<0.001）で有意であった．

平均出生体重は，対照3037 g，ASD 3089 g，AD 3124 g，HF-PDD 3044 g，PDD-NOS 3028 gであった．対照に比較し，ADでは有意に出生時体重は重かった．本来男女差のある項目であり，当然，対照，ASD（p<0.001），AD（p<0.01）においては，有意な男女差が認められたが，PDD-NOSでは差がなかった．対照との男女別比較では，AD男で出生時体重が有意（p<0.01）に重い症例が多く，ASD男で重い傾向であった．しかし，女では有意差は認められなかった．3臨床病型間の差も認められなかった．出生時体重が2500 g以下の低出生体重児の症例の割合は対照と比較して有意差は認められなかった．また，出生時体重が4000 g以上の巨大児の症例の割合は対照（0.8％）と比較して，ASD（2.2％），AD（3.3％）で高頻度ではあるが有意差は認められなかった．出生時体重と在胎週数との関係から，ある在胎週数の出生時体重基準値の10％タイル以下と体重の少ないSFD（small for date）と10％タイル以上体重の重いLFD（large for date）の検討では，ADでLFDの症例の頻度が多いがSFDの症例の頻度には差は認められなかった．

新生児期に何らかの異常を有した症例の割合を図3に示した．対照において男女差が認められ，男が有意に高頻度であった．しかし，ASDおよびASDの各病型においては，男女差は認められなかった．対照との比較では，ASDおよびすべての病型で新生児期の異常を有した症例は対照より多く，有意（p<0.001ただし，HF-PDDはp<0.05）であった．対照との比較を男女別々に検討すると，ASD，AD，PDD-NOSでは男女共に対照より有意（p<0.001，ただし，HF-PDD男とPDD-NOS女はp<0.05）に多かったが，HF-PDDの女で差はなかった．新生児期異常の内訳を図4に示した．重複して示しており，1〜2例しか認められない項目はその他としてまとめた．特に症例数の多い強度黄疸を示した症例と光線療法を受けた症例の頻度は，対照と比較してASD，ADともに有意（p<0.001）に高頻度であった．仮死Ⅰ度，切迫仮死も高頻度であった．また，ADで強度黄疸が目立ち，PDD-NOSでその他の項目が多く，ADとPDD-NOSでは新生児期異常の内容が異なる印象であった．

ASDの危険因子として，男性，修正OR，4.301；CIs（3.15-5.88）；p<0.001，新生児期異常を有する症例OR，6.295；CIs（3.81-10.41）；p<0.001，であり，強度黄疸はOR，2.21；CIs（0.997-4.88）；p=0.051であった．

以上より，我々の症例において，母年齢は35歳以上の占める割合はASD，ADで有意に高く，出生時平均父の年齢はPDD-NOSを除いて高かった．何らかの新生児期異常を有する症例が高頻度であり，強度黄疸の症例も高頻度の傾向であった．

4. 考　　案

ASDでは何らかの新生児期異常を有する症例の頻度が男女共に高く，強度黄疸（修正では有意傾向），仮死Ⅰ度，胎児切迫仮死が対照より多か

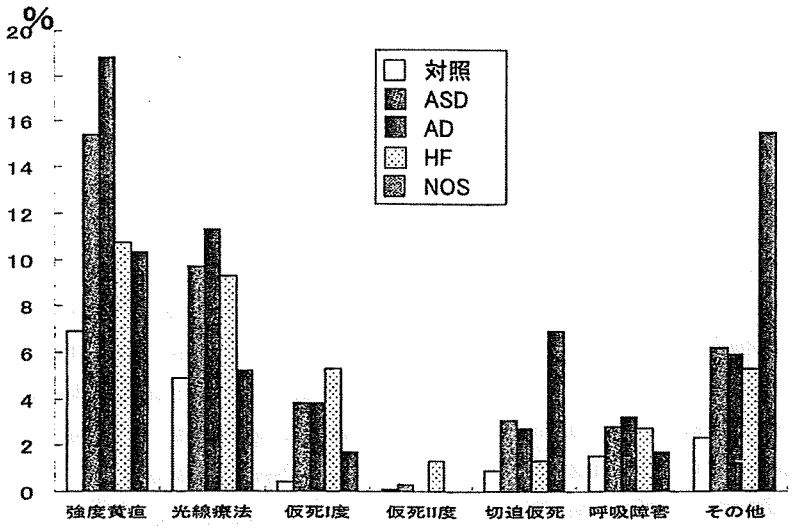

図4 新生児期異常内訳

新生児期異常の内訳を示す．重複あり，1～2例のみの項目はその他としてまとめた．特に症例数の多い，強度黄疸の症例と光線療法を受けた症例，仮死I度，切迫仮死の頻度は対照ASD，ADともに高頻度であった．ADで強度黄疸が目立ち，PDD-NOSでその他の項目が多かった．

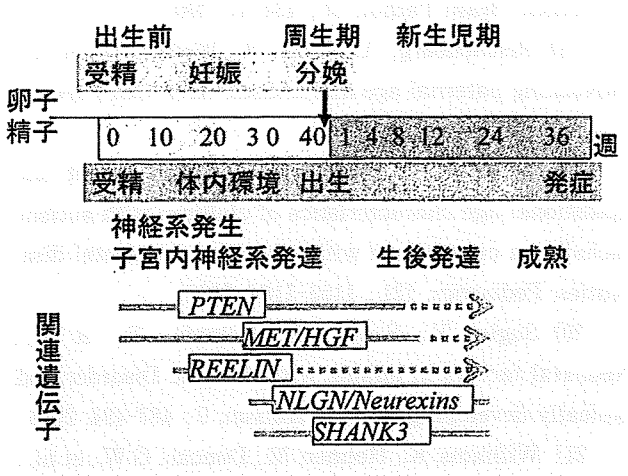

図5 Pardoら[17]より改変

った．先行研究では，アプガースコアに関してリスクがあるとされる報告が多く[5,9,13,21]，低酸素症の中枢神経系に及ぼす影響が示唆される．黄疸あるいは高ビリルビン血症は，リスクに関して一定していない[3,11,16]．しかし，このように特定項目が関連しているというより，ASDにおける周生期因子のリスクの意味合いについて考察する場合（参考Pardoら[17]の図より；図5），胎内環境は，神経系の発生，発達の重要な場である．誕生，新生児期早期から生後1～2年にかけては軸索樹状突起のsprouting，シナプス形成，髄鞘化の進行している時期であり，これらの時期に重要なASD関連遺伝子の候補もいくつか挙げられている．新生児期のそれほど致命的ではない非特異的なストレスが，脆弱な遺伝子（必ずしもここで挙げられた候補遺伝子とは限らない）に作用することにより，エピジェネティクスな変化をもたらし，ASD発症のリスクが増す可能性は充分に考えられる．どの遺伝子との組み合わせが重要なのかは今後の課題であろう．また，ASDにおける両親年齢の高さは，母親が高齢であると妊娠や分娩における合併症は高率となることもあるが，染色体の変化がおきやすくなることは良く知られている．父親年齢が高くなると遺伝子の新生変異または遺伝子刷り込みの変化，遺伝子コピー数のエラーが起き易くなり[10]，ASDのリスクが高くなる可能性が考えられる．

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Original article

Study of *HOXD* genes in autism particularly regarding the ratio of second to fourth digit length

Yoko Sugie [a,*], Hideo Sugie [b], Tokiko Fukuda [b], Junko Osawa [a]

[a] *Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan*
[b] *Department of Pediatrics, Jichi Medical University and Jichi Children's Medical Center, Tochigi, Japan*

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Abstract

Multiple genes are involved in the pathogenesis of autism. To study the causative gene, the relationship between autism endophenotypes and their closely related genes has been analyzed. There is a subgroup of autism spectrum disorder (ASD) in which the ratio of second digit length to fourth digit length (2D/4D) is low (short digit group, SDG). We studied the relationship between ASD and *HOXD* genes, which are located in the candidate locus for ASD and are associated with digit morphogenesis, with a particular focus on SDG. We analyzed 25 SNPs of *HOXD11*, *HOXD12*, and *HOXD13* in the subject of 98 ASD, 89 healthy controls, and 16 non-autistic patients (non-ASD). There was no significant difference in the genotype frequencies between the ASD and the healthy controls. However, the G-112T heterozygote in the promoter region of *HOXD11* was observed in only four patients with ASD and in none of the healthy controls or non-ASD subjects. Moreover, this *HOXD11* G-112T was observed in three of 11 SDG with ASD but in none of the 15 non-SDG patients with ASD. There were eight SDG patients among the non-ASD ones, but this polymorphism was observed in none of them. Considering the above results, it is expected that candidate genes will be further identified, using *HOXD11* G-112T polymorphism as a marker, by analyzing genes located near 2q in a larger number of ASD subjects with clinical signs of SDG.

Keywords: Autism; *HOXD*; 2D/4D; Endophenotype; Genetic polymorphism

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1. Introduction

Autism is basically characterized by severely impaired social interaction and communication, and a limited range of activities and interests. As the diagnosis of autism is made on the basis of patients' behavioral characteristics, the disorder is not caused by only one factor. It is considered that various genetic and environmental factors are involved in the occurrence of autism, and their interactions are complex. In 1998, the International Molecular Genetic Study of Autism Consortium (IMGSC) reported their genome-wide linkage analysis of families in which there was more than one member with idiopathic autism [1]. On the basis of the results of a subsequent large-scale genome-wide scan, candidate

* Corresponding author. Address: Department of Pediatrics, Hamamatsu University School of Medicine, 1-20-1, Handayama, Higashi-ku, Hamamatsu 431-3192, Japan. Tel.: +81 53 435 2312; fax: +81 53 435 4311.
E-mail addresses: y-sugie@umin.ac.jp (Y. Sugie), sugie@jichi.ac.jp (H. Sugie), toki-fukuda@jichi.ac.jp (T. Fukuda), yusosawa@nifty.com (J. Osawa).

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gene loci, including 7q21.2–q36.2, 16p12.1–p13.3, 6q14.3–q23.2, 2q24.1–q33.1, 17q11.1–q21.2, 1q21–q44, and 3q21.3–q29, were identified [2]. In an attempt to increase the linkage, a nearly homogeneous group was selected among patients with autism of heterogeneous causes. Autism patients were classified into subgroups or subsets in accordance with the phenotype of autism [3], such as through a quantitative trait locus (QTL) analysis of the constituent elements of endophenotypes in autism [4], and an ordered-subset analysis [5] was carried out. The ratio of second digit (2D) length to fourth digit (4D) length (2D/4D) is very low in some autism patients [6,7]. The *homeo box D (HOXD)* gene family is involved in skeletal morphogenesis, and correlations between digit length and the expression levels of *HOXD11*, *HOXD12*, and *HOXD13* have been observed [8,9]. In addition, *HOXD* genes form a cluster at 2q24.1–q33.1, which has been found to be a candidate locus by a genome-wide scan [3]. Therefore, we considered that digit length is one of the small physical signs of autism. Hence, we investigated the relationships between autism and polymorphism of *HOXD11*, *HOXD12*, and *HOXD13*. Moreover, we classified autism patients into two categories: patients with a low 2D/4D formed the short digit group (SDG), while the remaining patients formed the non-short-digit group (non-SDG). We also examined the genetic polymorphism of these three genes between SDG and non-SDG with autism and also between SDG with and without autism. No analysis of autism focusing on these relationships has been reported to date.

2. Subjects and methods

Seven patients with autism in the SDG were screened for the presence or absence of gene mutations in the exon and intron of *HOXD11*, *HOXD12*, and *HOXD13*, and for gene polymorphisms. The genotypic frequencies of the detected polymorphisms and the polymorphisms already listed in the GenBank were compared between the autism patients and the controls. Finally, the genotypes of the above polymorphisms of the autism patients in SDG were investigated.

2.1. Subjects

The subjects examined by genetic analysis in this study were 98 patients who visited the Department of Pediatrics, Hamamatsu University School of Medicine and Hamamatsu City Medical Center for Developmental Medicine, and who were diagnosed as having autism, PDD-NOS, and Asperger syndrome on the basis of the criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV [10]). Patients with clear underlying diseases such as chromosomal abnormalities, tuberous sclerosis, and Fragile X syndrome were excluded from the study. The patients were of 82 males and 16 females with ages ranging from 5 years and 2 months to 31 years and 10 months (mean age: 12 years and 7 months). In terms of ethnicity, 95 patients had Japanese parents, 2 had Japanese fathers and Filipino mothers, and 1 had Bangladeshi parents. Eighty-nine subjects without any neurological abnormality served as healthy controls for gene analysis; all of them were Japanese and their sex and age were not determined. Thirty patients were also examined as disease controls, including 16 non-autistic patients, 14 mentally retarded patients, and 2 AD/HD patients, all of whom were Japanese.

2.2. Measurement of second and fourth digit lengths

A digital camera providing three-megapixel images was used for the measurement of the 2D and 4D lengths. Each subject's right hand was placed palm-up on a flat desk, and was photographed with the camera 20 cm above the hand. Three pediatric neurologists separately measured the 2D and 4D lengths from the line of the base to the tip of the digits three times using the image analyzing software Scion Image (NIH). The mean ratio of 2D length to 4D length (2D/4D) was calculated. In this study, patients with lower than the mean 2D/4D of the autism patients reported by Osawa et al., that is, a 2D/4D of 0.94 or lower, were classified as SDG [7].

2.3. Gene analysis

Seven patients with autism (6 males and 1 female) in the SDG were screened for the presence or absence of gene mutations and gene polymorphisms by the direct sequencing method. *HOXD11*, *HOXD12*, and *HOXD13* – each consisting of two exons and one intron – were searched for in a region from approximately 500 bp upstream, including a promoter, to approximately 500 bp downstream of the gene. Genomic DNA extracted from lymphocytes using a DNA extraction kit (Takara Co., Shiga, Japan) was used. DNA was amplified by PCR using a Taq PCR Core kit (QIAGEN Co., CA, USA), and the base sequence was obtained by the direct sequencing method. Genotypes were determined for single nucleotide polymorphism (SNP) in five loci that were newly found by this method in this study and for SNP in 20 loci that are listed in the online database GenBank (NCBL dbSNP). Genotypes in some loci were also determined by real-time PCR analysis using a TaqMan allelic discrimination assay (Applied Biosystems).

2.4. Statistical analysis

Genotypic frequency and allelic frequency of the autism patients were compared to those of the healthy con-

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trol group using a χ^2 test or Fisher's exact test with SPSS 12.0J for a Windows-based System. A statistical significance level of $p \leqq 0.05$ was set.

3. Results

2D/4D was determined in 28 patients (24 males and 4 females) out of the 98 autism patients. Eleven patients (9 males and 2 females) of these 28 patients were classified as SDG. The clinical features of these patients, including sex, age, and the severity of mental retardation, are shown in Table 1. A high percentage of patients with severe mental retardation were observed in SDG with autism, whereas no patients with severe mental retardation were observed in non-SDG with autism. We also measured 2D/4D in 16 non-autistic patients in the disease control group, and 8 patients were classified as SDG and 8 patients as non-SDG. The results of the 2D/4D values of the 28 ASD and 30 non-ASD patients are shown in Fig. 1.

The results of the polymorphism analysis are shown in Table 2. No significant difference in polymorphism was observed between the autism patients and the healthy control group. However, with regarding to SNP in the promoter region of *HOXD11* G-112T, heterozygosity was observed in 4 autism patients, but not in the healthy or disease control group. The SNP in the promoter region of *HOXD12* -C226A and the SNP in

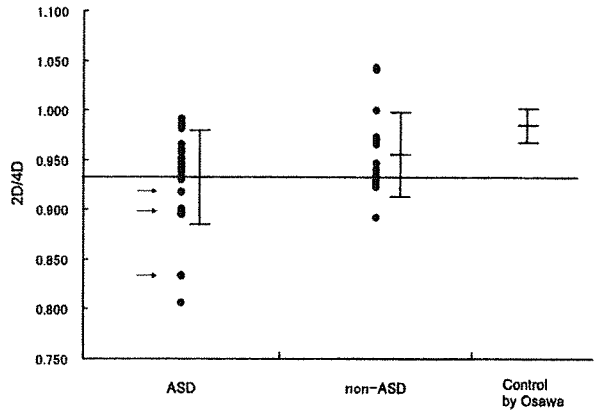

Fig. 1. 2D/4D values for the ASD28 cases and the non-ASD30 cases. Mean ± SD was presented. The M-ASD line is the average for the ASD cases; at or below this line is the SDG. As a reference, we showed the mean ± SD for normally healthy children as calculated by Osawa et al. [7]. The arrow indicates cases with *HOXD11* heterogeneity.

Table 1
Clinical features of patients.

	All the autistic disorder patients	Patients with 2D/4D determined		
		Total	SDG	NSDG
Number of patients	98	28	11	17
Sex				
Males:females	82:16 (5.1:1)	24:4 (5.5:1)	9:2 (4.5:1)	15:2 (7.5:1)
Age	5 y 2 m–31 y 10 m	5 y 4 m–31 y 10 m	8 y 1 m–31 y 10 m	5 y 4 m–16 y 7 m
Median	11 y 6 m	12 y 0 m	14 y 4 m	9 y 2 m
Mean	12 y 7 m	12 y 11 m	16 y 6 m	10 y 4 m
Family history: (3 generations)				
With[a]	22 (22.4%)	10 (35.7%)	3 (30.0%)	7 (41.2%)
Those with autism	7 (7.3%)	5 (17.9%)	2 (20.0%)	3 (17.4%)
Without	69 (70.4%)	16 (57.1%)	7 (70.0%)	7 (41.2%)
Mental retardation				
Without	10 (10.3%)	7 (25.0%)	2 (18.2%)	5 (29.4%)
Minor	21 (21.6%)	6 (21.4%)	2 (18.2%)	4 (23.5%)
Moderate	44 (45.4%)	10 (35.7%)	2 (18.2%)	8 (47.1%)
Severe	22 (22.7%)	5 (17.9%)	5 (45.5%)	0
Age at walk alone	9–48 m (91 cases)	9–48 m (26)	11–48 m (10)	9–18 m (16)
Median	13 m	12 m	12 m	12 m
Mean	13.9 m	14.3 m	18 m	12.9 m
Age at first word	10 m–6 y 10 m (80 cases)	11 m–6 y 10 m (25)	11 m–6 y 10 m (10)	1 y 3 m–3 y 5 m (15)
Median	1 y 6 m	1 y 6 m	1 y 6 m, 1 y 11 m	1 y 10 m
Mean	1 y 9 m	2 y 1 m	2 y 4 m	1 y 11 m
No. of patients 2 y or over	28	10	4	6
Age at first phrase	1 y 6 m–5 y 0 m (31 cases)	1 y 7 m–5 y 0 m (13)	2 y 6 m–5 y 0 m (5)	1 y 7 m–4 y 0 m (8)
Median	2 y 11 m	2 y 11 m	3 y 0 m	2 y
Mean	2 y 10 m	2 y 9 m	3 y 2 m	2 y 5 m
No. of patients 3 y or over	16	5	3	2

[a] Family history with psychiatric disorders including major depression, autism etc.

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Table 2
Results of analysis of gene polymorphisms.

Gene	Location in gene	dtSNP ID	Allele	Frequency		Genotype	Frequency	
				Autism	Control		Autism	Control
HOXD11	Promoter		G	0.979	1	GG	0.959	1
			T	0.021	0	GT	0.042	0
						TT	0	0
	Intron	rs84746	A	0.711	0.721	AA	0.571	0.561
			C	0.289	0.288	AC	0.230	0.371
						CC	0.133	0.067
	Exon 2	rs863678	G	0.541	0.567	GG	0.316	0.292
			T	0.459	0.443	GT	0.449	0.551
						TT	0.235	0.157
	Exon2	rs6745764	A	0.214	0.18	AA	0.031	0.011
			G	0.786	0.82	AG	0.367	0.337
						GG	0.602	0.652
HOXD12	Promoter		A	0.041	0.028	AA	0	0
			C	0.959	0.972	AC	0.082	0.056
						CC	0.918	0.944
	Promoter		G	0.929	0.955	GG	0.929	0.955
			T	0.071	0.045	GT	0.071	0.045
						TT	0	0
	Exon 1	rs847151	A	0.041	0.028	AA	0	0
			G	0.959	0.972	AG	0.082	0.056
						GG	0.918	0.944
HOXD13	Promoter	rs847196	C	0.893	0.938	CC	0.786	0.876
			G	0.107	0.061	CG	0.214	0.124
						GG	0	0
	Promoter		A	0.082	0.107	AA	0	0
			T	0.918	0.893	AT	0.163	0.213
						TT	0.837	0.787
	Exon 1		C	0.985	0.989	CC	0.969	0.978
			T	0.015	0.011	CT	0.031	0.022
						TT	0	0
	Exon 1	rs2518053	A	0.408	0.455	AA	0.173	0.235
			G	0.592	0.545	AG	0.469	0.438
						GG	0.357	0.326
	Intron	rs847194	A	0.684	0.657	AA	0.459	0.404
			C	0.316	0.343	AC	0.449	0.506
						CC	0.092	0.09

SNP with no polymorphism detected in the present cases analyzed among the SNPs listed at the GenBank

HOXD11	Promoter	rs2736846	*HOXD13*	Exon 1	rs847195
	Intron	rs2736847		Exon 1	rs13392701
	Exon 2	rs12995279		Intron	rs847193
	Exon2	rs12995280		Intron	rs847192
HOXD12	Exon 1	rs2551807		Exon 2	rs28928892
	Exon2	rs2553776		Exon 2	rs28933082
				Exon 2	rs28928891

exon 1 of *HOXD12* (rs847151, G364A) showed a nearly complete linkage disequilibrium. Heterozygosity for both *HOXD12* -C226A and *HOXD12* G364A was observed in five healthy controls and eight autism patients. Furthermore, all of the five controls heterozygous for *HOXD12* -C226A and *HOXD12* G364A were homozygous for *HOXD11* -G112G. On the other hand, of the eight autism patients heterozygous for both *HOXD12* -C226A and *HOXD12* G364A, four were homozygous and four were heterozygous for *HOXD11* G-112T. Taken together, heterozygosity in all the three loci *HOXD11* G-112T, *HOXD12* -C226A, and *HOXD12* G364A was found in four autism patients but not in the healthy controls. Table 3 shows the relationships between the polymorphisms in these three loci for two cases: SDG and non-SDG with autism and SDG and non-SDG without autism. Of the four patients heterozygous for *HOXD11* G-112T, three in whom digit length was measured were classified into SDG with autism and the rest was unknown. The clinical type of ASD of the patients with having *HOXD11* heterogeneity was classified as autistic disorder in all cases. No patients

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Table 3
Frequency of *HOXD* gene polymorphisms between SDG and NSDG patients with or without autism.

Gene	dbSNP ID	Genotype	Autistic patients			Normal	Non-autistic	
			Total	SDG	NSDG	Control	SDG	NSDG
HOXD11		GG	94	8	17	89	8	8
		GT	4	3	0	0	0	0
HOXD12		CC	90	8	17	84	8	8
		AC	8	3	0	5	0	0
HOXD12	rs847151	GG	90	8	17	84	8	8
		AG	8	3	0	5	0	0

heterozygous for *HOXD11* G-112T were observed among the 16 non-autistic disease controls including the eight patients with SDG.

4. Discussion

In genetic research for autism, some studies have been conducted that focused mainly on language development skills (e.g., age at first word, age at first phrase, onset of first phrase >36 months, and nonverbal communication) skill. Other studies have focused on the establishment of motor language development, bladder and bowel control milestones, developmental regression, repetitive/stereotyped behavior, restricted behavior, interest, and activity [2–4,11–13].

Manning et al. [6] reported that 2D/4D is low in autism and Asperger syndrome. In Japan, Osawa et al. [7] reported a higher incidence of low 2D/4D in autism patients than in healthy children. From their report, we assumed that it is possible to consider a low 2D/4D as a specific feature in some autism patients. Such patients formed part of a group of subjects (SDG) for investigation in our study. It was assumed that SDG in autism may express one of the common features; hence, 2D/4D may be associated with one of the etiological genes of autism. Manning et al. [14] reported the findings of their 2D/4D measurement as follows: (1) there is a gender difference in 2D/4D measurements (2D/4D is lower in males than in females); (2) a low 2D/4D is observed across races and countries; (3) 2D/4D is closely related to fetal growth, sperm count, family size, myocardial infarction, and breast cancer; and (4) 2D/4D is related to sexual differentiation, the production of sex hormones in the fetal stage, and disease programming in the fetal stage. In addition, there is an inverse correlation between 2D/4D and testosterone concentration at the fetal stage, and 2D/4D correlates with the CAG repeat number in the androgen receptor gene [15].

A study of female twins conducted by Paul et al. [16] showed that the concordance rate of 2D/4D is higher in monozygotic twins than in dizygotic twins, that the heritability of 2D/4D is approximately 66%, and that the genetic contribution to 2D/4D in females may be more influential than the effects of prenatal environmental factors. Although it is uncertain whether these findings differ significantly between males and females in the absence of any report for males, it seems possible that 2D/4D is affected by both hereditary and secondary perinatal environmental factors.

One study showed that the mean 2D/4D did not change with gestational age from the 9th week to the 40th week [17]. In addition, there was a small increase in 2D/4D with age, which was lowest in the right hand [18]. This study indicates that 2D/4D is probably established in the uterus and that this ratio remains almost constant until adult life.

Because 2D/4D, an easily measurable physical feature, is already determined in utero and remains constant until adult life, it can be used regardless of age differences among subjects and is universal; moreover, its measurement is noninvasive. Therefore, 2D/4D is an excellent parameter for evaluating a group of autistic patients.

In genomic scans of families having more than one member with autism, the susceptibility loci for autism were investigated, and identified; these included 2q21–q33 [3,4]. In the candidate genes located here, the NRP2 gene is reported as one of the genes related to autism [19]. In addition, specific polymorphism has been found in distal-less 2 (DLX2) and cAMP guanine nucleotide exchange factor II (cAMP-GEFII) in a few cases of autism [20]. On the other hand, no significant correlation has been reported between autism and distal-less 1 (DLX1) [20,21] and DLX2 [20]. With regard to *HOXD* genes, Bacchlli et al. reported that there is no relationship between *HOXD1* and autism [20]. There has been no report on *HOXD11*, *HOXD12* or *HOXD13* to date.

It seems that these genes may be found to be significant in the development of autism when cases as a study subject have been carefully chosen and classified by the specific characteristics of presenting behavior or phenotypic clinical presentations.

The present study has limitations because it is a case-control study, rather than a family study, with a small number of subjects enrolled. However, in this study,

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HOXD11 SNP -112G/-112T heterozygosity was specifically observed in autism patients with low 2D/4D. On the basis of this result, we expect that the relationships between autism and the *HOXD* genes or other candidate genes located in 2q will be clarified by studying a larger population with low 2D/4D, that is, by studying patients heterozygous for -112G/-112T in the *HOXD11* promoter.

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Original Article

Early Development of Understanding Words and Equivalence Cognition of Matching Pictures: Children With Severe Motor and Intellectual Disabilities

Toshihide KOIKE*,****, Yuki YOSHIDA**,
Miyoshi KUMOI***, and Kazuo KATAGIRI****

The present study aimed to investigate the relation between early development of the understanding of words and equivalence cognition of matching pictures in children with severe motor and intellectual disabilities (SMID). Equivalence cognition accompanying expectancy was evaluated by measuring expectancy heart rate (HR) responses on a task of sample matching with an S1-S2 paradigm. Using the Japanese MacArthur Communicative Developmental Inventory, mothers of 5 of the 12 participants evaluated their children as being able to understand more than 60 words (Group A). The teachers of 3 of the participants in Group A did not evaluate those participants as being able to understand as many words. The mothers and teachers evaluated 7 participants as understanding fewer than 20 words (Group B). In the task of matching sample shapes with colors, the number of correct choices by the children in Group A was larger than that by the children in Group B. The ratio of occurrence of expectancy heart rate responses in the Group A children was larger than that in the Group B children. Participants in Group A whose understanding of words was evaluated as good by their mothers might have equivalence cognition of matching pictures, which accompanies expectancy.

Key Words: sample matching task, expectancy heart rate responses, Japanese MacArthur Communicative Developmental Inventory, cognition of equivalence, children with severe motor and intellectual disabilities

Introduction

With progress in the technology of augmentative and alternative communication (AAC), methods of teaching children with severe motor and intellectual disabilities (SMID) have been studied (Iino, 2005; Takaizumi & Ishida, 1999). Evaluation of the

*United Graduate School of Education, Tokyo Gakugei University
**Faculty of Education, Tokyo Gakugei University
***Faculty of Education , Kagoshima University
****Graduate School of Human and Socio-Environment Studies, Kanazawa University

early development of the understanding of words in such children is needed for useful application of the technology of augmentative and alternative communication (Oishi, 2006).

In a study of early development of understanding words, Nagasaki and Onosato (1996) examined behavior patterns of young infants at about 8 months and above. They found that young infants can pay attention to objects pointed to by their mothers. Nagasaki and Onosato (1996) also noted that visual discrimination of object shapes develops during early infancy, in that infants can identify objects that their mothers indicate with spoken words.

Development of the understanding of words is delayed in children with severe motor and intellectual disabilities. Such children also have weak responsiveness to visual stimuli. This is considered to be related to the underdevelopment of their visual cognition (Katagiri, Koike, & Kitajima, 1999). Thus, in order to clarify early development of the understanding of words in children with severe motor and intellectual disabilities, it is important to investigate how visual cognition relates to development of the understanding of words.

In a study of development of understanding words in children with motor and intellectual disabilities, Otomo (2005) asked mothers to complete the Japanese version of the MacArthur Communicative Developmental Inventory (Komuku & Watamaki, 2004). The results indicated that children with motor and intellectual disabilities were reported to have good understanding of words related to routine events of daily life. Because all the children's understanding of words was also evaluated by their teachers, it is possible that the evaluations by teachers based on their classroom contacts with the children were different from the evaluations by mothers, based on their experience with their children at home. If so, then for investigation of the understanding of words by children with severe motor and intellectual disabilities, evaluations by their teachers might be needed in addition to evaluations by their mothers. Some children with severe motor and intellectual disabilities might be evaluated by both their teachers and their mothers as having good understanding of words, whereas others might be evaluated by both teachers and mothers as not having a good understanding of words. These children might show an obvious tendency to respond or not respond to spoken words. But other children with severe motor and intellectual disabilities might be evaluated only by their mothers as understanding some words. Their responses might not be as obvious, but they may exhibit behavior that enables their mothers to understand their children's responses to words in their daily care of them. Thus, these children are considered to be early in the process of development of their understanding of words, and they might be able to attain an early stage of cognitive development.

In a study of the development of visual cognition before the attainment of the understanding of words, Odera, Kurai, and Satake (1998) examined the process of training children with severe intellectual disabilities. Odera et al. (1998) suggested that the ability to match pictures on common visual features through recognizing the equivalence of stimuli in the task of sample matching was a prerequisite conditions

for the development of the understanding of spoken words. On the basis of the findings of Odera et al. (1998), children with severe motor and intellectual disabilities who are evaluated by their mothers as having an understanding of words and who are considered to be early in the process of development of the understanding of words, might attain equivalence cognition of matching pictures.

In classroom teaching, when children with severe intellectual disabilities have not showed stable matching behavior, adjustments for each child of the shape and color of the stimuli have been known to be effective for prompting matching behavior. This suggests that children with severe intellectual disabilities might have shape or color preferences. From this, it can be inferred that children with severe motor disabilities as well as intellectual disabilities might also have shape or color preferences and might also show preferences when matching on shape or color.

Because children with severe motor and intellectual disabilities have severe motor disabilities, accurate observation of their matching behavior is difficult, and correct evaluation of equivalence cognition is also uncertain. Thus, in order to study the behavior of children with severe motor and intellectual disabilities, it is necessary to develop a method for evaluating equivalence cognition of matching in such children. Kitajima, Koike, Katada, and Matsuno (1993) found that expectancy heart rate (HR) responses could be observed in an S1-S2 paradigm in children with severe motor and intellectual disabilities whose communication ages were approximately 8 months and above. In a sample matching task, the behavior of choosing a stimulus identical to the sample was reinforced immediately. When children with severe motor and intellectual disabilities were slow to choose a stimulus, a supporter made the choice for them. Thus, using the S1-S2 paradigm, expectancy heart rate responses could be recorded during the sample matching task. In this situation, S1 was the time of the supporter's choice, and S2, the time of the delivery of the reinforcing stimulus. When the chosen stimulus was identical with the sample, the reinforcing stimulus (S2) was presented. When expectancy heart rate responses occurred in the S1-S2 interval on trials in which the stimuli were identical, it was speculated that the children might have judged the pictures to be identical and hence have expected the reinforcing stimulus (S2).

Through the observation of children's correct choices in a sample matching task, equivalence cognitions can be inferred. From the findings with respect to expectancy heart rate responses, equivalence cognitions that accompany expectancy can be inferred. Because equivalence cognition accompanying expectancy is considered to allow children with severe motor and intellectual disabilities to learn relations between objects actively, despite their severe motor dysfunctions, equivalence cognition with expectancy might widen the possibilities for them to be able to understand relations between objects and spoken words.

On the basis of the above, children with severe motor and intellectual disabilities, whose understanding of words is developing and which has been evaluated mainly by their mothers, might show expectancy heart rate responses on trials with identical stimuli and attain the equivalence cognition that accompanies expectancy.