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Tesi di dottorato
Doctoral thesis

Ricerca delle basi molecolari e analisi dei profili cognitivi nelle patologie di sviluppo del Sistema Nervoso Centrale

Identification of the molecular basis and analysis of cognitive profiles in developmental CNS pathologies

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SUMMARY

The analysis of cognitive profiles in specific genetic syndromes is an alternative approach to understand normal and abnormal physiology in developmental neurosciences. Correlating the molecular basis of developmental CNS anomalies with specific neuropsychological phenotypes, in association with neuroradiological (functional MRI, fiber-tracking) and neurophysiological (EEG, EP, cognitive potentials) data, would help determining localisation and function of an altered cerebral region. This would be an important step in future diagnostic and therapeutical approaches to brain pathologies.

Aims of the 4-years doctoral study were the classification and identification of the genetic basis of developmental pathologies of the CNS, with a special focus on the cerebellum (section 1) and the utilisation and validation of cognitive tests in developmental neurosciences (section 2). The final goal was to plan a collaborative multicentric project on the genetic and cognitive aspects of primary microcephalies. Yet, analysing the complex relations among genetic factors that lead to a malfunctioning small brain, when integrated with cognitive data, paves the way to the understanding of mental retardation. The European project on primary microcephalies is still ongoing, and preliminary results are reported in the 3rd section of the doctoral thesis.

In these 4 years, a 2-years period has been spent in France to establish a continuative cooperation in pediatric research, basic and clinical (Pr. P. Evrard; Pr. A. Verloes; Pr. J.-C. Mercier – Robert Debré Hospital, APHP, Paris VII University).

The implementation of laboratory and clinical research tools in the field of pediatric neurosciences has allowed the planning of a post-doc program in Developmental Neurobiology (dr. P. Gressens, Inserm Unit U676, Paris, France).

The publications in peer-reviewed journals that are part of the doctoral thesis are reported in the appendix section.

INDICE

Alle persone che mi sono state particolarmente vicine in questi 4 anni, e a cui devo tanto:

A Generoso Andria, cui va la mia più profonda stima
Ad Ennio Del Giudice, che mi ha iniziato con affetto alla neurologia pediatrica
Ad Alain Verloes, Philippe Evrard e Jean-Christophe Mercier, che mi hanno calorosamente accolto

A Roberto, Giancarlo, Alfonso, Gianfranco, ed ai colleghi con cui ho condiviso ottimi momenti

Alla mia famiglia, che mi ha sempre stimolato a migliorare
A Vania, che ha appoggiato le mie scelte e mi é stata sempre accanto

PREFAZIONE

Il dottorando si è occupato, nel corso degli studi, delle problematiche relative agli aspetti genetico-metabolici delle malattie neurologiche pediatriche.

In particolare, le alterazioni cognitive in queste patologie hanno destato un interesse specifico poiché la neuropsicologia riveste oramai un ruolo importante nell'inquadramento delle malattie neurologiche. Inoltre, non essendo ancora completamente elucidate le correlazione tra anomalie cognitive specifiche e alterazioni molecolari, questa area di ricerca è particolarmente stimolante. Ha partecipato quindi all'attività clinica del Settore di Neuropsichiatria Infantile, e, in particolare dell'Ambulatorio di Neurogenetica del Dipartimento Clinico di Pediatria dell'Azienda Ospedaliera Universitaria Federico II di Napoli, dall'anno 2002 fino all'Aprile 2004.

Dal 1 Maggio 2004 al 30 Aprile 2006 il dottorando ha frequentato il "Service de Neurologie Pédiatrique et des Maladies Métaboliques" dell'Ospedale "R. Debré" di Parigi – Université Paris VII (Francia), diretto dal Prof. Philippe Evrard. Inoltre, ha svolto attività clinica e di ricerca nell' "Unité de Génétique Clinique" (Prof. Alain Verloes) e nel "Service d'Accueil des Urgences Pédiatriques (Prof. Jean-Christophe Mercier) dello stesso Ospedale. Si è attivamente impegnato nello studio di pazienti affetti da patologie neurologiche severe. I campi specifici di interesse sono stati soprattutto la ricerca di alterazioni genetiche nelle anomalie di sviluppo del sistema nervoso centrale (SNC), e l'individuazione di alterazioni cognitive in patologie neurologiche note.

Ciò ha permesso di acquisire una esperienza specifica nella neurobiologia dello sviluppo, necessaria alla programmazione di un progetto di ricerca multicentrico europeo sulle microcefalie primarie. Quest'ultimo progetto, attualmente in corso, si avvale anche della collaborazione con il laboratorio di ricerca INSERM diretto dal Dr. Pierre Gressens (Université Paris VII) per lo sviluppo di un modello animale.

INTRODUZIONE

Il ruolo della ricerca genetica in neuropediatria

Lo sviluppo del cervello è un processo attivo, il cui studio ha permesso, negli ultimi decenni di comprendere le cause di alcune anomalie del suo sviluppo. Complessi processi molecolari regolano infatti la sequenza di eventi che portano al cervello adulto, ma molti di questi sono ancora sconosciuti.

Il ruolo della genetica nella ricerca in neurologia pediatrica è quindi di notevole importanza, specie dopo l'enorme impulso dato alla genetica, negli ultimi anni, dallo sviluppo di tecniche di analisi sofisticate. L'identificazione dei geni responsabili ha portato a un cambiamento consistente dell'approccio clinico e della cura, poiché sono stati scoperti i meccanismi di base e approntati specifici protocolli terapeutici (in relazione alla causa) per molte malattie neurologiche. Ad esempio, il trapianto di midollo osseo (vedi il trattamento di alcune forme di mucopolisaccaridosi) ed il trattamento enzimatico della malattia di Gaucher e di Fabry permettono la sostituzione di enzimi carenti, tanto per citare alcuni esempi), mentre la terapia genica permetterà la sostituzione del gene o delle funzioni del gene mancante e l'impianto di cellule staminali o cellule fetali atte a sostituire la funzione del gene o dell'enzima carente nell'organismo. Inoltre, la genetica gioca un ruolo importantissimo per la prevenzione di svariate malattie neurologiche e per la diagnosi prenatale. Il ruolo della genetica ha inoltre radicalmente mutato lo stesso concetto di disturbo psichiatrico e comportamentale nel bambino: oggi sappiamo che la depressione, l'ansia, l'iperattività, l'autismo sono tutti favoriti da anomalie specifiche del patrimonio genetico. Ciò ha contribuito a cambiare il nostro approccio anche terapeutico a questi disturbi.

L'approccio genetico al ritardo psicomotorio ed alle malformazioni cerebellari

E' essenziale comprendere come l'approccio al bambino con ritardo psicomotorio o mentale (RM) sia cambiato negli ultimi anni. L'avanzare della genetica ci apre sempre più strade per la comprensione dei differenti profili cognitivi caratteristici delle singole anomalie genetiche, soprattutto delle malformazioni isolate (come quelle cerebellari). E' la comprensione del profilo neuropsicologico che sottende il ritardo mentale che ci permetterà di interpretare poi le basi molecolari delle diverse sindromi che coinvolgono il SNC.

In effetti, il ritardo mentale è una patologia comune, che per definizione colpisce il 3% della popolazione. Il RM non è un semplice disturbo cognitivo, ma una sindrome clinica che molto probabilmente si origina a partire da un disturbo cognitivo. In una fase cruciale dello sviluppo, il disturbo cognitivo condiziona l'evoluzione complessiva del soggetto, della sua personalità, del suo comportamento adattivo e delle sue relazioni sociali (Masi e Stella G, 1995).

Il solo criterio del quoziente intellettivo non è quindi sufficiente per la diagnosi di RM, e tre sono i criteri diagnostici (APA, 1994): 1. disturbo intellettivo, con caduta di almeno due deviazioni standard del quoziente di intelligenza (QI) rispetto alla norma Questo corrisponde ad un QI < 70, misurato con i più comuni test di valutazione dell'intelligenza, come le scale Wechsler (Wechsler 1974, 1984, 1991); 2. disturbo significativo delle capacità di adattamento alle esigenze di un ambiente sociale normale; 3. insorgenza prima dei 18 anni.

Sulla base di evidenze cliniche, il ritardo mentale può venire caratterizzato secondo due assi: il primo individua i gruppi fondamentali di sintomi e comprende il disturbo cognitivo, i disturbi linguistici, psicomotori, affettivi e comportamentali fino ai disturbi dell'adattamento sociale ed dell'autonomia personale.

Il secondo differenzia le diverse forme in rapporto al livello di gravità. In generale distinzioni vengono fatte sulla base del QI e vengono distinti 4 livelli: le forme con RM lieve (QI da 50-55 a 70) e con RM medio (QI da 35-40 a 50-55) sono le più frequenti. Molto più rari sono i RM grave (QI da 20-25 a 35-40) e gravissimo o profondo (QI inferiore a 20-25). Anche gli altri sintomi però spesso variano conseguentemente. Ad esempio nel RM grave, il livello cognitivo non supera quello

di un bambino di 2-3 anni ma anche il linguaggio espressivo è assente o limitato alla parola-frase. Lo sviluppo psicomotorio è grossolano. Il livello di autonomia è molto limitato. La struttura affettiva può essere armonica, ma più spesso sono presenti difficoltà relazionali importanti, con chiusura semi-autistica, stereotipie gestuali, autoaggressività.

Il RM si può presentare in forme molto diverse e le cause sono sconosciute in circa il 30% dei ritardi gravi e nel 50% di quelli lievi. Ci sono diversi fattori non-genetici (infezioni, nascita prematura, anossia perinatale ed altre) che possono causare il RM (Kyllermann, 1989). Molte e diverse sono le cause genetiche. Tra queste, possiamo distinguere tre gruppi di pazienti. Il primo gruppo comprende disordini in cui si presenta un'alterazione di una funzione ubiquitaria importante per funzioni metaboliche comuni, come potrebbe essere una deficienza in un enzima lisosomale o difetti nel metabolismo di acidi organici. Questo gruppo include un'enorme varietà di patologie in cui il RM è spesso accompagnato da altri sintomi neurologici e da tutta una serie di sintomi di valore diagnostico.

Il secondo gruppo comprende tutte quelle patologie associate a difetti dello sviluppo del sistema nervoso e in cui quindi il RM può essere in linea di massima attribuito ad alterazioni delle interazioni tra i neuroni che si instaurano durante lo sviluppo pre e postnatale. Tra queste sono le importanti alterazioni dello svi-luppo della corteccia responsabili delle lissencefalie e delle eterotopie, spesso associate a RM (Guerrini et al, 2001) .

L'ultimo gruppo è quello dei pazienti in cui il solo sintomo è il RM, che è definito RM non-sindromico o non-specifico. Una percentuale non ben determinabile ma sicuramente rilevante dei casi di RM non sindromico sembra avere origine genetica e molti studi negli ultimi anni sono stati rivolti all'analisi genetica di questo tipo di RM. Questi studi hanno il duplice scopo di migliorare la diagnostica di questa patologia così comune e di porre le basi della genetica delle funzioni cognitive. Si pensa infatti che questo tipo di RM sia causato da alterazioni in *pathways* molecolari importanti per lo sviluppo delle funzioni cognitive. Il RM non-specifico ha avuto alcuni importanti sviluppi negli ultimi anni, con l'identificazione di nuove metodologie di analisi, di loci e soprattutto di geni responsabili di alcune forme di questa condizione.

Un recente approccio genetico allo studio del RM, sindromico o non, si basa sull'osservazione di riarrangiamenti cromosomici abbastanza piccoli, al di sotto della sensibilità delle tecniche citogenetiche tradizionali (5 Megabasi, Mb) (Knight e Flint, 2001). Tali riarrangiamenti sono stati trovati per primi nelle regioni subtelomeriche, associati a sindromi specifiche, come l'ATR-16, alfa talassemia associata a RM (Wilkie et al, 1990) . Generalizzando dal caso di queste sindromi, è stato suggerito che nelle regioni subtelomeriche, la presenza di numerose sequenze ripetute potrebbe essere la causa di una maggiore frequenza di riarrangiamenti cromosomici, come traslocazioni bilanciate e non, di piccole delezioni, e di duplicazioni (associate o meno a disomia uniparentale) che non coinvolgendo un grande numero di geni non dovrebbero causare sintomi complesse ma avere un effetto fenotipico più modesto. Sonde specifiche sviluppate per tutti i telomeri umani e usate per identificare riarrangiamenti subtelomerici inferiori alle 5 megabasi (Mb) hanno dimostrato in diverse occasioni la presenza di riarrangiamenti criptici in pazienti affetti da RM idiopatico (Knight e Flint, 2001). La frequenza di riarrangiamenti è abbastanza variabile nei diversi gruppi studiati, con valori tra lo 0,5% e più del 20% (Van Karnebeek et al, 2005). Questi sono rari tra i casi di RM lieve, mentre si trovano più frequentemente quando oltre a un RM più grave sono presenti lievi dismorfie non classificabili in nessuna sindrome specifica.

Come detto, le malformazioni cerebellari isolate rappresentano una stimolante area di ricerca in neurologia pediatrica per la possibilità di comprendere i meccanismi che regolano lo sviluppo del sistema nervoso centrale. Alcuni geni responsabili sono stati individuati recentemente, come nella sindrome di Joubert (Louie e Gleeson, 2005), mentre per altri lo studio di modelli animali permette la selezione di geni candidati da esplorare nei pazienti affetti (Wang e Zoghbi, 2001).

Molti sono gli sforzi della comunità scientifica per definire i criteri di un corretto approccio alla diagnosi genetica del RM (McDonald et al, 2006 ; Moeschler et al, 2006), primo passo verso l'analisi del fenotipo cognitivo molecolare.

Lo sviluppo neurocognitivo

Prima di analizzare i test neuropsicologici correntemente usati per la diagnosi del ritardo psicomotorio, passiamo in rassegna le tappe fondamentali dello sviluppo cognitivo del bambino. La trattazione si focalizzerà soprattutto sul primo anno di vita poiché, in genere, le alterazioni genetiche colpiscono precocemente le diverse fasi di maturazione delle aree di sviluppo cerebrali, e poiché il primo anno rappresenta un periodo di crescita che non ha pari in altre età della vita. Questa crescita è notevolissima soprattutto grazie alla formazione di centinaia di miliardi di sinapsi che sono la chiave dell'apprendimento e della memoria. Esse formano un complesso centro di controllo per la percezione del mondo circostante e per l'acquisizione di funzioni specifiche: non solo vedere, ascoltare, muoversi, ma anche pensare, percepire le sensazioni, e comportarsi in un determinato modo. Lo sviluppo della vista, del linguaggio, delle attività motorie e la loro progressiva integrazione con il controllo da parte delle funzioni esecutive sono la base del funzionamento cognitivo di ogni individuo.

Gli input neurosensoriali

La visione è una delle maggiori fonti d'informazione del neonato sul mondo esterno. Prima dei tre mesi i bambini vedono con la visione periferica. Notano più facilmente i movimenti e i contrasti (ad es. il chiaro/scuro). Poi, gradualmente sviluppano la visione centrale e a partire dai tre mesi cominciano a guardarsi le mani e possono seguire con lo sguardo un oggetto in movimento circolare. Diversi studi hanno dimostrato che la parte visiva del cervello del bambino è quella maggiormente attiva. Tra i due e i sei mesi i bambini sviluppano sempre maggiori capacità di visualizzare un oggetto o un volto nel dettaglio, di seguire un oggetto con lo sguardo e di mettere a fuoco. Dai quattro mesi la visione del bambino è simile a quella dell'adulto. Durante questo periodo, inoltre, il bambino comincia a integrare ciò che vede con ciò che gusta, con ciò che sente e con le sensazioni che prova. Questa è la cosiddetta integrazione sensoriale.

I neonati sentono una grande varietà di suoni e sono particolarmente sensibili al suono della voce umana e preferiscono la voce della mamma a quella di qualsiasi

altra persona. Dai tre mesi il cervello del bambino è in grado di distinguere diverse centinaia di parole del linguaggio parlato, molte di più di quante non siano presenti nella sua lingua madre. Il cervello si organizza poi intorno alle parole che ascolta più spesso e inizia a creare una mappa uditiva per poter organizzare il linguaggio in modo efficiente. Lo sviluppo del bambino in questo periodo è particolarmente rapido e riguarda tutte le funzioni sia cognitive che motorie. Si possono notare notevoli cambiamenti sia nella comprensione verbale sia nello sviluppo motorio che nell'apprendimento e nelle relazioni sociali. Prima dei sei mesi i bambini sono in grado di riconoscere parole familiari ma la comprensione del significato è limitata a poche parole. Tra i sei e i dodici mesi i bambini cominciano a perdere la capacità di cogliere le differenze nei suoni di una lingua straniera ma allo stesso tempo migliora la loro abilità nel distinguere i vari suoni della loro lingua madre. Il legame tra suono e significato inizia al sesto mese. Dagli 8-10 mesi, mostrano d'iniziare a comprendere le parole, rispondendo a ordini semplici come fare "ciao" con la mano.

Il controllo motorio

Alla nascita, le aree del cervello deputate al controllo e al coordinamento del movimento volontario non sono ancora ben sviluppate. Tali aree motorie cerebrali maturano in una sequenza cranio-caudale, il che vuol dire che le aree cerebrali che controllano i movimenti del capo e del collo maturano prima di quelle che controllano i muscoli degli arti superiori e del tronco che, a loro volta, maturano prima di quelle che controllano gli arti inferiori. La corteccia cerebrale forma gradualmente connessioni con parti del corpo più lontane. Questa progressiva maturazione ha inizio alla nascita e, nell'arco di 6/12 mesi, raggiunge le aree che controllano i muscoli del tronco e delle gambe che sono d'importanza critica per la maggior parte delle fasi di crescita di questo periodo. Gradualmente, gli scatti incontrollati e i riflessi precoci lasciano il posto al controllo del movimento. Tuttavia i gruppi muscolari non maturano allo stesso tempo e il bambino passa attraverso diversi stadi, dalla posizione seduta alla marcia autonoma. In ogni momento, il cervello automaticamente rifinisce i circuiti deputati a un particolare movimento, dall'afferrare un oggetto alla corsa. Alla fine di questa fase il bambino è in grado di stare in piedi per qualche istante da solo o con sostegno o anche di spostarsi con

appoggio laterale. Per quanto riguarda la coordinazione motoria, questa fase inizia con la capacità di eseguire semplici azioni motorie come afferrare goffamente un oggetto con cui giocare, per arrivare fino alla capacità di usare la pinza formata dal pollice e uno o due dita per prendere piccoli oggetti e sviluppare la motricità fine.

Socialità e funzioni esecutive

Tra i nove e i dodici mesi, il bambino continua a raccogliere una gran quantità d'informazioni e utilizza ciò che impara per ottenere le cose. Impara a "leggere" le parole, i gesti e le espressioni del volto di chi si occupa di lui. La sua memoria s'incrementa e compare una nuova abilità nel ricordare esperienze passate. Man mano che il suo cervello cresce in dimensioni e complessità, il bambino sviluppa un controllo sempre maggiore sugli oggetti e le persone del suo ambiente. La corteccia frontale - l'area associata con la capacità di regolare ed esprimere le emozioni, i pensieri e i progetti - mostra d'incrementare la sua attività. Il bambino é maggiormente in grado di regolare il suo livello di benessere e di calmarsi con l'aiuto di una figura di riferimento o di un oggetto. Il suo interesse nell'esplorazione del mondo e nell'affermare la propria indipendenza è reso possibile dal suo sviluppo fisico che gli permette di muoversi, allontanandosi e poi riavvicinandosi alle persone care. Situazioni spiacevoli che un tempo avrebbero provocato automaticamente il pianto, ora possono tradursi nel voltare il capo e imbronciarsi per mostrare così la sua disapprovazione, frustrazione o stress. Si sviluppano quindi le funzioni esecutive che regolano i processi di pianificazione, controllo e coordinazione del sistema cognitivo e che governa l'attivazione e la modulazione di schemi e processi. Fra queste troviamo:

- l'organizzazione delle azioni in sequenze gerarchiche di mete;
- lo spostamento flessibile dell'attenzione sulle informazioni rilevate;
- l'attivazione di strategie appropriate e l'inibizione di risposte non adeguate.

L'impiego delle funzioni esecutive è indispensabile in tutti i tipi di *problem solving,* non solo in quelli più complicati ed astratti, come la soluzione di problemi matematici, ma hanno un ruolo importante anche nell'acquisizione delle abilità

sociali. La comprensione delle persone (metacognizione) per esempio è una di queste, perché la sensibilità ad obiettivi, emozioni o desideri altrui richiede uno sganciamento dell'attenzione dai propri stati mentali. Si pensa che molti disturbi mentali siano associati a questo tipo di deficit, anche se in ogni disturbo è probabile che cambi il grado in cui ciascuna componente delle funzioni esecutive è colpita. Nelle funzioni esecutive, infatti, si possono individuare tre componenti principali: la memoria di lavoro, la capacità di inibizione e l'abilità di generare nuove soluzioni.

È importante comunque tener presente che lo sviluppo del singolo bambino può essere più o meno avanzato rispetto a un altro bambino della stessa età. I bambini infatti si sviluppano alla loro propria velocità piuttosto che seguire uno schema temporale fisso. Su tali basi, si verifica la comparsa di determinate funzioni e abilità nell'arco di un determinato arco di tempo, e non in un preciso momento. Si ritiene anche che le diverse parti del cervello del bambino maturino a differenti velocità. Di conseguenza, è facile osservare dei "picchi" di sviluppo improvvisi per esempio nell'area del controllo fisico, o del pensiero, o della comunicazione o della relazione con gli altri. Da qui la necessità di individuare mezzi oggettivi di misurazione dello sviluppo psicomotorio normale.

Diverse scale sono disponibili per tale sviluppo ed individuarne eventuali devianze dalla norma. La più usata nella pratica clinica è quella di Denver II (Frankenbourg et al, 1992), che esplora socialità, motricità grossolana e fine, e linguaggio. Peraltro, questa scale consente solo un orientamento iniziale, e uno studio cognitivo dettagliato é quindi indispensabile. Molti sono i test disponibili per ricercare alterazioni nelle diverse funzioni cerebrali, e questi sono selezionati caso per caso per poter trarre il maggior numero di informazioni.

A scopo esemplificativo, le scale sono divise in quelle per la valutazione generale delle capacità cognitive e quelle che valutano il comportamento.

Le prime si eseguono secondo l'età ed il livello intellettivo dei pazienti, attraverso le classiche Brunet-Lezine, WPPSI, WISC o WAIS (Baron, 2005). Sono in genere completate dalla batteria NEPSY per gli items delle funzioni esecutive nei bambini tra 3 e 12 anni.

La Valutazione comportamentale è di solito stabilita grazie all'utilizzo di questionari consegnati ai genitori (De Bildt et al, 2005). Tra queste si segnalano:

- Kiddie Schedule for affective Disorders and Schizophrenia (Kiddie-SADS): questionario semi strutturato che esplora un ampio gruppo di disordini psichiatrici, basato sulla DSM4
- Child Behaviour Check List (CBCL): questionario compilato dai genitori che valuta in particolare i disordini comportamentali

- Scale di Conners: orientate in particolare verso la valutazione dei disordini dell'attenzione e dell'iperattività
- Scala Emotivity Activity Sociability (EAS)
- Scala Dimension of Temperament (DOT): permette una valutazione dell'umore dei bambini
- Questionario Children Social Behaviour (CSBQ): questionario generale che esplora il comportamento sociale dei bambini
- Scala d'adattamento di Vineland (VABS): questionario compilato con i genitori, che valutano le capacità in 4 settori: socializzazione, comunicazione, capacità e motricità

L'analisi dei profili cognitivi e le patologie neurogenetiche

L'avanzare delle conoscenze tecniche nel campo della neuroradiologia (RMN funzionale, fiber tracking, spettroscopia) e della neurofisiologia (potenziali evocati event-related) ha permesso di studiare approfonditamente la morfologia ed il metabolismo del cervello, e si iniziano a correlare specifiche funzioni cognitive a specifiche aree corticali (Thomas, 2003). Dati recenti indicano come le funzioni cerebrali superiori siano finemente modulate nel corso dello sviluppo, con un controllo sempre più importante delle aree associative col progredire dell'età del bambino (Casey et al, 2005). Emerge cosi' l'interesse per la neuropsicologia, che permette delle misure accurate delle differenti funzioni cognitive anche in età pediatrica (memoria, apprendimento, processi emozionali, attenzione). Mentre la neuroradiologia e la neurofisiologia da sole possono fornire delle informazioni utili alla diagnosi, se associate a dati neuropsicologici possono permettere una comprensione fine delle aree cerebrali coinvolte.

L'analisi dei profili cognitivi di specifiche sindromi genetiche rappresenta un altro approccio alla conoscenza delle funzioni cerebrali. Comprendere le basi molecolari delle patologie di sviluppo del SNC e correlarle con uno specifico profilo cognitivo fornirà un approccio nuovo e affascinante allo studio delle funzioni cerebrali superiori. Ciò fornirà verosimilmente anche delle indicazioni diagnostiche e terapeutiche, apportando in un futuro che si spera prossimo, dei benefici diretti anche per i pazienti affetti da tali patologie.

OBIETTIVI DELLA TESI

Gli scopi principali della tesi sono stati:

- la valorizzazione dell'impiego diagnostico delle sonde subtelomeriche
- la ricerca di anomalie genetiche nelle patologie di sviluppo del SNC, in particolare delle malformazioni cerebellari
- lo studio neuropsicologico di base ed il raffronto post-intervento terapeutico in casistiche di pazienti
- la programmazione di una ricerca multicentrica, clinica e molecolare, per la fenotipizzazione cognitiva delle microcefalie genetiche primarie.

SEZIONE 1

RICERCA DI ALTERAZIONI GENETICHE NELLE ANOMALIE DI SVILUPPO DEL SNC

Pazienti e metodi

La selezione dei pazienti è stata effettuata con la revisione delle banche dati dell'Area funzionale di Genetica Medica e del Settore di Neuropsichiatria Infantile del Dipartimento di Pediatria della Università Federico II e dei Dipartimenti di Genetica Clinica e Neurologia Pediatrica dell'Università Parigi VII .

Per l'individuazione delle anomalie genetiche si sono utilizzate tre approcci:

1. tecniche citogenetiche (sonde subtelomeriche), soprattutto per i pazienti con anomalie di sviluppo del SNC e note dismorfiche o altre malformazioni associate. Ci si è avvalsi della collaborazione con i dipartimenti di Citogenetica della Università Federico II (Prof. Lucio Nitsch) e della Università Parigi VII (Prof. Jacques Elion). Sono stati selezionati i pazienti con ritardo mentale idiopatico sulla base di uno score clinico proposto da De Vries et al (2003), che include la familiarità, le dismorfie, le altre malformazioni associate.
2. approccio per geni candidati nei pazienti con anomalie isolate dello sviluppo cerebellare. Ci si è avvalsi della collaborazione con il Baylor College of Medicine, Houston, USA (Dr. Nicola Brunetti-Pierri).
3. inquadramento dal punto di vista nosologico per le altre rare anomalie di sviluppo cerebrale, al fine di porre le basi di una futura ricerca molecolare.

I dettagli relativi ai metodi di genetica molecolare impiegati, sono riportati nelle relative referenze.

Risultati

Alterazioni subtelomeriche

Sono stati selezionati in totale 134 pazienti.

Dallo studio mediante FISH si sono diagnosticati 16 casi, alcuni dei quali di notevole interesse scientifico:

1) E' stato osservato un bambino di 11 mesi con un quadro clinico caratterizzato da: distrofia, microcefalia, ipertelorismo, orecchie a coppa, filtro allungato ed appiattito, labbra sottili, ponte nasale prominente, capezzoli invertiti, pectus excavatum, ipospadia balanica, metatarso varo-supinato, camptodattilia alle mani ed ai piedi. L'esame clinico mostrava, inoltre, un soffio proto-mesosistolico con quadro ecocardiografico di coartazione aortica di grado lieve, associata a persistenza di dotto arterioso pervio ed ipertrofia ventricolare destra (Abstract: a).
Il paziente presentava un esame neurologico caratterizzato da ipotonia generalizzata movimenti spontanei ripetitivi e grossolani, risposta lenta e graduale agli stimoli esterni, difficoltà ad agganciare lo sguardo ed una RMN dell'encefalo che evidenziava un assottigliamento del corpo calloso ed una ipoplasia del cervelletto. Ad un successivo controllo si segnalava la comparsa di episodi critici tipo assenze complesse, con quadro elettroencefalografico di encefalopatia epilettogena. Un cariotipo standard risultava nella norma, mentre l'analisi delle regioni subtelomeriche mostrava una trisomia 2p33>2qter e una monosomia 6q27>6qter. La monosomia 6q27>6qter è stata recentemente riportata in un grande pedigree e non risulta associata ad un fenotipo clinico (Kraus et al, 2003). Pertanto, il fenotipo del nostro paziente appare come risultante completamente dalla trisomia parziale del cromosoma 2. I casi sinora riportati di trisomia 2p33>2qter isolata sono estremamente rari (Aviram et al, 2000). La ulteriore definizione della regione cromosomica coinvolta, tuttora in corso, potrà permettere un mappaggio delle diverse manifestazioni cliniche e di eventuali geni coinvolti.

2) Si è osservata una paziente con tremore essenziale, ritardo di sviluppo psicomotorio, anemia microcitica, piede torto bilaterale, note dismorfiche, malrotazione intestinale (Fig 1, Abstract: b). Attraverso l'analisi subtelomerica si é diagnosticato un riarrangiamento cromosomico coinvolgente i cromosomi 16 e 2. Oltre la delezione terminale del cromosoma 16, che si associa a ritardo mentale e ad alfa-talassemia (Daniels et al, 2001), si è trovata una trisomia terminale del

cromosoma 2q, che è raramente riportata in letteratura (Bonaglia et al, 2000). Tale associazione non è stata sinora riportata in letteratura.

3) Di particolare interesse un caso di sindrome di Wolf, causata dalla delezione terminale del cromosoma 4p (ref. 5, Abstract c). La sindrome di Wolf presenta delle caratteristiche facciali peculiari (elmetto di guerriero greco), e si associa a ritardo di crescita intrauterino, bassa statura, ritardo mentale ed epilessia (Rodriguez et al, 2005). Il nostro paziente presentava una epilessia generalizzata, ritardo mentale lieve, sclerosi ippocampale sinistra e deficit parziale di GH. L'analisi citogenetica ha messo in evidenza una delezione 4p16.2-pter di 4.5 Mb, confermando la diagnosi clinica. Il deficit di GH non è stato mai ricercato in tale sindrome e la evidenza di tale deficit, seppur parziale, apre la strada ad un eventuale trattamento ormonale sostitutivo, almeno per quei pazienti che si presentano con un deficit intellettivo lieve.

4) E' stata infine osservata una bambina nata da genitori consanguinei, con ano imperforato, agenesia sacrale parziale, dismorfie facciali (epicanto, ipertelorismo, microftalmia, radice nasale piatta) e ritardo mentale moderato (ref. 12, Abstract: d). L'esame RMN cerebrale ha evidenziato una pachigiria unilaterale ed una displasia biopercolare, quello midollare una tethered cord. Lo studio con sonde subtelomeriche ha ritrovato una delezione pura, *de novo*, 6q25.3 --> qter. L'analisi dei microsatelliti ha ristretto la regione deleta tra i markers D6S363 e D6S446. Il confronto con i dati della letteratura relativi alle descrizioni cliniche delle delezioni pure terminali 6q ci ha consentito di individuare una regione cromosomica, compresa tra i microsatelliti D6S959 and D6S437. Si é quindi ipotizzato che uno dei 4 geni mappati in questo locus di 0.3 Mb sia responsabile delle malformazioni sacroanali, agendo probabilmente sulla fase di sviluppo della notocorda.

Anomalie di sviluppo del cervelletto

Sono stati selezionati in totale 14 pazienti. Sono stati quindi analizzati i pazienti affetti da malformazioni cerebellari non sindromiche, isolate, le cui basi molecolari sono ancora sconosciute. Nello specifico si sono individuati pazienti affetti da

agenesia cerebellare isolata e ipoplasia del verme cerebellare autosomica dominante. Tali patologie sono molto rare e rivestono un particolare interesse per le possibili implicazioni teoriche sulla morfogenesi del cervelletto e di conseguenza del SNC.

1) L'agenesia cerebellare totale o parziale (CA) è un difetto congenito estremamente raro ed è sempre associata ad un grave deficit del movimento (Glickstein, 1994). Può a volte presentarsi in associazione ad altri disturbi dello sviluppo nei bambini con sindromi malformative. Reperti autoptici di cinque individui con completa o quasi completa CA sono stati descritti dal 1965. Sono stati riportati i risultati di RMN in solo 6 casi (van Hoof Wilmink, 1996; Van Coster et al, 1998; Velioglu et al, 1998). Il nostro paziente è il settimo caso di CA nel quale una valutazione neurologica e studi RMN sono stati possibili nel corso della vita. Inoltre, riportiamo il primo studio molecolare in CA non associata ad altre malformazioni (ref. 7, Abstract: e).
M.A. è nato a termine da parto spontaneo. Non si riferiscono problemi di rilievo perinatali. Valutato a pochi mesi di vita per ipotonia e tremore, è stata riscontrata una agenesia completa cerebellare non associata ad altre malformazioni. All'osservazione clinica a 17 anni il piccolo si presenta in buono stato di salute. L'esame neurologico evidenzia uno strabismo convergente, ROT rotulei vivaci. L'andatura e la stazione eretta sono a larga base di appoggio, con lieve atassia nel movimento. Sorprendentemente il piccolo non presenta dismetria, né tremori, né nistagmo. Ha un comportamento scolastico e sociale generalmente adeguato. Occasionalmente presenta crisi di eteroaggressività secondarie a provocazioni o ad atteggiamenti da lui ritenuti tali. Pratica psicomotricità e logopedia. Ha praticato indagini per la ricerca di alterazioni subtelomeriche, potenziali evocati visivi ed uditivi nella norma.
La CA è stata ampiamente riesaminata da Macchi e Bentivoglio (1987). La presentazione clinica della CA è variabile, andando dalla morte precoce ai vari gradi di disfunzione cerebellare. Pazienti con CA mostrano una sindrome cerebellare non progressiva, con ritardo dello sviluppo neuromotorio e variabile inabilità nei movimenti di coordinazione, normalmente associati a ritardo mentale. La descrizioni dei pazienti in vita ha portato a chiedersi se la CA sia compatibile con uno sviluppo motorio funzionale. Nel nostro paziente si osserva un lieve ritardo mentale con una

funzionalità motoria sufficiente a garantire una pressochè normale vita sociale. E' probabile che altre aree cerebrali suppliscano alla capacità di apprendimento motorio generalmente di pertinenza cerebellare. Le basi patogenetiche e molecolari della CA restano ancora sconosciute. L'ulteriore conoscenza sui precursori neuronali implicati nello sviluppo cerebellare in relazione all'espressione genica temporo-spaziale e di modelli murini selettivamente inattivati potrebbero portare nel prossimo futuro all'identificazione delle basi molecolari della CA e altre sindromi malformative cerebellari.

2) L'ipoplasia del verme cerebellare è una atassia congenita non progressiva, geneticamente trasmessa, la cui forma più raramente riportata è quella a trasmissione autosomica dominante. Abbiamo descritto una famiglia in cui sia il padre che il figlio sono affetti da ipoplasia del verme cerebellare, evidenziata alla RMN dell'encefalo, senza altre malformazioni associate (ref. 8, Abstract: f).

Il padre ha mostrato una atassia di grado lieve, associata a tremori del tronco, sin dall'infanzia. La sintomatologia neurologica è progressivamente migliorata sino a scomparire in età adulta. Si segnala la comparsa di crisi comiziali tonico-cloniche generalizzate dall'età di 23 anni, attualmente ben controllate dalla terapia antiepilettica.

Nel bambino la sintomatologia neurologica è esordita a 7 anni e 10/12 ed é caratterizzata da episodi frequenti di perdita dell'equilibrio durante le sue normali attività. La sintomatologia è progressivamente migliorata ed attualmente persiste soltanto un lieve tremore al tronco.

Dal punto di vista clinico, sono state sinora riportate solo 4 famiglie con tale patologia (Rivier e Echenne, 1992). In tutte è stato descritto uno spontaneo e lento miglioramento delle prestazioni motorie, facendo ipotizzare l'intervento compensatorio di altre aree cerebrali.

Sulla base dello studio del fenotipo di topi knock-out in banca dati, è stato possibile pertanto selezionare alcuni geni candidati. Sono stati selezionati En2 e Zic-1, per il loro ruolo nella fase iniziale di sviluppo del cervelletto (Wang e Zoghbi, 2001). L'analisi molecolare di tali geni, condotta in collaborazione con il dr. Nicola Brunetti-Pierri ci ha permesso di escluderli come causa della patologia.

L'individuazione dei geni responsabili di tali patologie potrà essere un modello per lo studio della proliferazione e della migrazione neuronale nei diversi compartimenti del sistema nervoso centrale.

3) E' stato inoltre descritto il caso di un bambino di 3 anni con sindrome di Prader-Willi e ipoplasia cerebellare unilaterale (ref. 13, abstract: g). L'analisi FISH della microdelezione ha permesso di ritrovare una delezione interstiziale 15q11.2-q13.1, di circa 3 Mb. La constatazione di alterazioni cerebellari nella sindrome di Prader-Willi è descritta sinora solo in rarissimi rapporti autoptici (Hayashi et al, 1992). La nostra osservazione apre la strada alla comprensione della fisiopatologia della sindrome, quale, ad esempio, quella relativa alla ipotonia neonatale.

Altre anomalie di sviluppo del SNC

Sono stati identificati 32 pazienti con patologia rara di sviluppo del SNC.

Tra le differenti patologie neurologiche osservate, alcune hanno meritato la pubblicazione su riviste internazionali per l'interesse clinico, la loro rarità e il contributo a una migliore definizione nosografica.

1) E'stata discussa l'ereditarietà della sindrome di Myhre, patologia rara di cui lo stesso dottorando ha riportato in letteratura un caso peculiare associato con macrocefalia ed autismo (Titomanlio et al, 2001) (ref. 2). Tale sindrome é caratterizzata da ritardo mentale, bassa statura, blefarofimosi e pseudoipertrofia muscolare. Si è sottolineato che l'ereditarietà sia X-linked piuttosto che autosomica dominante, prendendo spunto da ulteriori osseervazioni cliniche di Burglen et al (2003).

2) E' stata descritta una famiglia con due fratelli affetti da sindrome di Alstrom (ref. 4, Abstract: h). Questa é una rara patologia che associa sordità neurosensoriale, retinite pigmentosa, obesità e diabete mellito. La presenza di deficit cognitivo é incostante. Ci si è interessati soprattutto alla correlazione genotipo-fenotipo, in quanto i due fratelli esaminati presentavano un decorso clinico differente. L'analisi mutazionale ha permesso di ritrovare una nuova alterazione nonsense nell'esone 16.

L'omozigosità ha permesso di concludere che la sindrome di Alstrom sia in realtà una patologia eterogenea, con una importante influenza sul fenotipo da parte di fattori genetici e ambientali.

3) E' stata osservata una bimba di 3 anni nella quale è stata posta la diagnosi di sindrome di Michels (ref. 9, Abstract: i). Questa è una rarissima patologia che associa, tra i segni clinici principali: craniostenosi, blefarofimosi, ptosi, epicanto inverso e ritardo mentale (Fig. 5). La revisione della letteratura sull'argomento ha permesso di inquadrare meglio tale patologia, e di classificarla in un gruppo diagnostico nuovo, chiamato 3MC syndrome. Questa nuova entità diagnostica raggruppa la sindrome di Michels e tre altre patologie sindromiche (Malpeuch, Carnevale, OSA), che sono parte di uno stesso spettro clinico.

4) E' stata anche riportata una nuova sindrome caratterizzata dall'associazione osteosclerosi-polimicrogiria (ref. 10, Abstract: l). Si è osservato un feto di sesso maschile, di 29 settimane di età gestazionale, da genitori consanguinei. Le caratteristiche cliniche peculiari (osteosclerosi congenita diffusa, anomalie dismorfiche cranio facciali –ipertelorismo, micrognazia, agenesia dell'epiglottide, riduzione del numero dei denti- allargamento delle falangi terminali). La scoperta di una polimicrogiria bilaterale fronto-parietale ha permesso di descriverla come una nuova sindrome, di etiologia sconosciuta e di probabile ereditarietà autosomica recessiva.

SEZIONE 2

INDIVIDUAZIONE DI ALTERAZIONI COGNITIVE, E MODIFICAZIONE CON LA TERAPIA, IN PATOLOGIE NEUROLOGICHE NOTE

Dal punto di vista biologico, l'apprendimento puo' essere considerato come un processo di modulazione nella formazione di specifici circuiti neuronali in risposta agli stimoli esterni (Koizumi, 2004). Lo studio neurocognitivo, associato alle tecniche neuroradiologiche di base e a quelle più recenti di tipo funzionale, permette di localizzare le aree cerebrali coinvolte nei vari processi fisiologici. Lo studio delle alterazioni di sviluppo cerebrale, soprattutto quando si conoscono i meccanismi genetici che regolano la formazione dei differenti circuiti, e la correlazione con le alterazioni cognitive è un campo in rapida espansione e che promette di dare un notevole impulso alle neuroscienze nei prossimi decenni.

Pazienti e metodi

Si sono selezionate delle casistiche di pazienti già seguiti presso il Dipartimento di Pediatria della Federico II (Genetica Medica e Neuropsichiatria Infantile) per poterle analizzare dal punto di vista neurocognitivo.

In un secondo tempo si è proceduto all'analisi statistica delle differenze post-intervento.

I dettagli relativi all'analisi cognitiva e statistica sono riportati nelle relative referenze.

Risultati

Sono state analizzate le casistiche di bambini con epilessia rolandica (effetti farmaci anti-epilettici), malattia di Gaucher (terapia enzimatica sostitutiva) e sindrome di Down (terapia neuroablitativa).

1) E' stata effettuata una valutazione neuropsicologica approfondita in pazienti con epilessia rolandica per valutare possibili deficit minori, usando una batteria

computerizzata (FePsy) (ref. 3). Questo tipo di epilessia, caratteristica dell'età scolare, è considerata benigna, in quanto guarisce spontaneamente all'adolescenza e può anche non essere trattata farmacologicamente se le crisi sono rare. I farmaci di comune impiego sono il valproato e la carbamazepina. Abbiamo esaminato dal punto di vista cognitivo 16 pazienti senza crisi da almeno 2 anni, trattati con carbamazepina, appaiati con controlli sani.

I pazienti con epilessia rolandica hanno mostrato risultati peggiori nei seguenti test: 1) Binary Choice Reaction Test ($p < 0,01$), che valuta la capacità decisionale e 2) Recognition Tasks che valutano la memoria a breve termine: a) riconoscimento simultaneo di parole ($p < 0,01$) e figure ($p < 0,001$) e b) riconoscimento seriale di parole ($p < 0,001$) e figure ($p < 0,01$). L'analisi di regressione multipla lineare ha mostrato correlazioni significative a) tra il punteggio al Binary Choice Reaction Test ed il sesso e la localizzazione del focolaio; b) il riconoscimento simultaneo di parole e la durata della terapia con carbamazepina ed il tempo trascorso dalla sospensione del farmaco; c) riconoscimento simultaneo di figure e sesso.

I nostri dati sottolineano l'utilità di valutare accuratamente dal punto di vista neuropsicologico tutti i pazienti con epilessia rolandica, a prescindere dalla durata del trattamento con carbamazepina.

2) Sono state ricercate delle eventuali alterazioni neurologiche in pazienti affetti da malattia di Gaucher, una patologia metabolica dovuta al deficit di glucocerebrosidasi (ref. 6). E' una delle poche malattie lisosomali per le quali una terapia enzimatica specifica con enzima ricombinante sia disponibile ed efficace. Tra i tre sottotipi descritti, il tipo 1 non comporta anomalie neurologiche, mentre i tipi 2 e 3 sono quelli neuronopatici e rispondono male al trattamento. Sono stati analizzati 17 pazienti con malattia di Gaucher tipo 1 attraverso analisi cognitiva e studio dei potenziali evocati multimodali (motori, visivi, acustici e somatosensoriali). Mentre non sono state riportate anomalie del profilo neuropsicologico, i potenziali evocati motori sono risultati alterati nel 69% dei casi, quelli uditivi nel 31%, i visivi nel 25% e i somatosensoriali nel 19%. I nostri risultati suggeriscono che delle alterazioni subcliniche siano presenti anche nel sottotipo di malattia di Gaucher ritenuto non-

neuronopatico. Questo potrà avere delle implicazioni per la sorveglianza e le modificazioni del trattamento enzimatico sostitutivo.

3) Infine, è stato realizzato uno studio cognitivo per la valutazione di interventi neuroabilitativi precoci nella sindrome di Down (ref. 11, Abstract: m). Infatti, la trisomia 21 si caratterizza per un ritardo di sviluppo psicomotorio che sembra essere migliorabile con interventi precoci (Ulrich et al, 2001). Lo scopo era di valutare se il Carolina Curriculum for Infants and Toddlers with Special Needs (CCITSN) potesse essere di maggior beneficio per bambini con sindrome di Down rispetto all'approccio standard offerto dalla regione Campania. Il CCITSN è un programma di abilitazione neurologica e di apprendimento che utilizza degli schemi di sviluppo alternativi allo sviluppo fisiologico. Questi schemi, possono essere insegnati anche dai genitori, sono utilizzati dal bambino per supplire ai suoi deficit e permettere uno sviluppo psicomotorio quanto più prossimo alla norma. Un totale 47 bambini con sindrome di Down sono stati assegnati in modo *random* ad uno dei trattamenti e seguiti per 12 mesi. Al termine, il quoziente di sviluppo medio del gruppo CCITSN è stato 12.31 punti più alto dell'altro gruppo (95%CI: 1.53 to 23.57; $p<0.05$). L'uso dei genitori come terapisti può essere pertanto efficace e probabilmente più economico rispetto allo standard di trattamento abilitativo.

SEZIONE 3

STUDIO CLINICO E GENETICO DELLE MICROCEFALIE PRIMARIE

Le microcefalie genetiche sono un modello estremamente utile per la comprensione dello sviluppo normale del cervello e per la neuronogenesi e rappresentano dunque un settore di ricerca appassionante in biologia dello sviluppo e nelle neuroscienze. Questo progetto di ricerca, ancora in corso, è stato iniziato negli ultimi due anni del dottorato, in coincidenza con il periodo di soggiorno all'estero. Ne vengono pertanto riportati solo i risultati preliminari, inerenti alla parte di ricerca clinica e neuropsicologica (Abstract: n).

Microcefalie genetiche isolate

La microcefalia e definita da una circonferenza cranica (CC) inferiore a 2DS per l'età ed il sesso. Le forme severe corrispondono a CC inferiori a -3DS. La crescita del cranio e determinata dall'espansione del cervello che prende posto durante tutta la vita fetale e durante l'infanzia e le microcefaliee si verificano generalmente a causa di un difetto di crescita del cervello. Qualsiasi condizione che influisce su questa crescita causerà dunque un microcefalia, che si tratti di patologia acquisita (post anossica, post ischemica, lesione cerebrale d'origine malnutrizionale, embriofoetopatie infettive o da teratogeni...) o a seguito di un'anomalia dello sviluppo cerebrale. Quest'ultimo gruppo può essere diviso in modo semplice in forma isolata (microcefalia primaria) ed in una forma sindromica nella quale la microcefalia coesiste con altre anomalie dello sviluppo, nel quadro d'anomalia cromosomica o di sindrome polimalformativa.

Il nostro studio riguarda le microcefalie isolate di origine genetica. La microcefalia primaria può essere divisa in 2 categorie: quella che deriva da un disordine della proliferazione neuronale e/o gliale a livello della zona germinativa, e quella che deriva da un disordine della migrazione neuronali della zona germinativa verso la loro posizione definitiva nella corteccia cerebrale. Essendo differenziazione e migrazione dei processi sincroni, si può osservare una combinazione dei 2

meccanismi patogenetici (Barkovich et al, 1998; Barkovich et al, 2001)

Le anomalie della proliferazione neuronogliale conducono alla microcefalia vera (MV) ed alla microcefalia a girazione semplificata (MSG) (con uno strato di sostanza grigia di spessore normale o ridotto.) Quando la proliferazione normale si associa con un disordine della migrazione, si parla di microlissencefalia (MLIS) (caratterizzata da una corteccia di spessore aumentato). In tutte queste forme il CC alla nascita (a termine) si situa tra 24 e 29 cm (valore normale superiore a 32 cm). Attualmente, la classificazione clinica e neuroradiologica di queste diverse entità non ha ancora raggiunto un consenso.

MV: Microcefalia Vera

La MV e una patologia genetica nella quale il cervello è di piccole dimensioni a causa di un numero diminuito di neuroni, ma conserva una girazione quasi normale. Non si dispone di dati precisi sulla frequenza della MV. Cifre riportate nella letteratura valutano questa frequenza tra 1/25000 e 1/50000 nascite. Il peso del cervello non supera in generale 500 g (cioe 1/3 del peso normale). Il ritardo mentale è di solito moderato. Non esiste alcuno studio sistematico sul piano neuropsicologico o cognitivo per questa patologia nella letteratura medica (fonte: NCBI PubMed). Attualmente 8 loci autosomici ed un locus gonosomico distinti (Shripton et al, 1999) sono stati identificati per la MV. Cinque geni sono stati clonati: ASPM (Bond et al, 2002), MCPH1 (Jackson et al, 2000; Jackson et al 2002), CDK5RAP2 e CENPJ (Bond et al, 2005), DNC (Rosenberg et al, 2002). Le 5 affezioni presentano un'eredità autosomica recessiva. Il gene ASPM sembra essere responsabile almeno della metà dell'insieme dei casi di MV. Gli altro due contribuiscono in modo marginale all'insieme dei casi. Per gli altri 3 loci, solo la localizzazione cromosomica è conosciuta.

Geni della MV (Tabella)

<u>Microcephalin (MCPH1)</u>

Questo gene e stato clonato per *homozygosity mapping* in 2 famiglie pakistane consanguinee. Struttura: 14 esoni, proteina di 835 aminoacidi (microcefalina). Presenza di 3 domini BRCT omologhi al settore C-terminale di BRCA1. Il locus

MCPH1 contiene il gene dell'angiopoietina 2 sul segmento inverso dello introne 12. La microcefalina potrebbe svolgere un ruolo nella riparazione del DNA o nella regolazione del ciclo cellulare dei progenitori neuronali. E espressa in modo preferenziale nel cervello, fegato e rene. Nel topo, è espressa nel cervello fetale durante la neuronogenesi (al livello del cervello anteriore, nella zona germinale). Questo gene è mal conservato fra i vertebrati: il 57% della sequenza è identico tra l'uomo ed il topo (generalmente l'identità media uomo/topo e pari all' 85%). Un cambiamento unico S25X nel 1motivo BRCT e stato descritto in una popolazione pakistana consanguinea. Nessun cambiamento di MCPH1 e stato trovato in altri gruppi etnici.

ASPM (MCPH5)

Corrisponde al gene ASPM, uno ortologo umano del gene "abnormal spindle" di Drosophila (*asp*). Questo gene è stato identificato con *homozygosity mapping* ed è responsabile di circa il 50% delle MV in tutti i gruppi etnici. Struttura: 62kb, 28 esoni, 3478 aminoacidi. Il gene contiene ripetizioni multiple di una sequenza di 20 aminoacidi che cominciano con l'isoleucina (I) e la glutammina (Q). Questa sequenza e stata dunque denominata "repeat IQ". Tutte le mutazioni pubblicate conducono ad una perdita di funzione. Nella Drosophila *asp* è essenziale per la funzione normale del fuso mitotico nei neuroblasti embrionali. La proteina codificata dal gene ASP e dai suoi ortologhi mostra un aumento regolare di dimensione che è parallelo all'albero evolutivo (connessione fisiologica?). La differenza principale tra *asp* ed ASPM è l'inserzione di molti repeats IQ. Il numero di repeats sembra legato alla complessità del SNC: nel nematode si contano 2 IQ, in Drosophila 24 IQ, nel topo 61 IQ, nell'uomo da 72 ad 80 IQ.

CDK5RAP2 (MCPH3) e CENPJ (MCPH6)

Questi geni sono stati recentemente clonati e la loro funzione biologica non è ancora conosciuta. Sembra comunque che rientrino nel meccanismo di divisione cellulare e quindi nella proliferazione neuronale. Sono responsabili di MV in due soli gruppi familiari consanguinei (Moynihan et al, 2000; Leal et al, 2003).

SLC25A19

Questo gene è responsabile del sottotipo di MV che si osserva esclusivamente nella popolazione Amish. Si tratta di una patologia autosomica recessiva che si presenta con una microcefalia estrema (da -6 a -12 DS), associata con una aciduria alfa-chetoglutarica. SCL25A19 codifica per un trasportatore di desossinucleotidi (DNC).
Altri loci responsabili di MV/MSG sono mappati: MCPH2 (19q13.1-13.2) - (Roberts et al, 1999), MCPH4 (15q) (Jamieson et al, 1999), e MRXS9 (Xq12-q21.31) (Shrimpton et al, 1999). Circa il 20% delle famiglie studiate non ha linkage ad alcun locus conosciuto.

MSG: microcefalia a girazione semplificata

Questa anomalia eccezionale è caratterizzata da un microcefalia associato ad una rotazione incompleta (numero di giri insufficiente; profondità dei solchi < al 50% del valore normale), una corteccia non ispessita ed una sostanza bianca (SB) normale o alterata. Barkovich et al (2001) hanno proposto una classificazione in 5 tipi, basata su elementi radiologici e clinici. Questa classificazione deve essere considerata come un orientamento nosologico, poiche la validità e l'eterogeneità di queste 5 differenti forme non e certa. In particolare, i tipi 1, 2 e 4 formano una continuum di gravità. La classificazione di Barkovich e riassunta in tabella:

Tipo	*Neuroimaging*	*Clinica*
Tipo 1	Solchi poco profondi (50%) Corteccia di spessore normale Giunzione corteccia / SB normale SB: normale	Gravidanza e parto normali Esame clinico normale alla nascita eccetto la microcefalia Danno corticospinale: spasticità con/senza Babinski, progressiva Ritardo variabile, che può peggiorare con il tempo
Tipo 2	Solchi poco profondi (30%) Corteccia di spessore normale Giunzione corteccia / SB normale SB: ritardo di mielinizzazione	Gravidanza e parto normali Alla nascita: spasticità, riflessi anormali, disordini alimentari Epilessia generalizzata molto precoce Ritardo severo, prognosi riservata
Tipo 3	Solchi molto poco profonda (<<30%) Corteccia di spessore normale Giunzione corteccia / SB normale Eterotopie sottoependimali SB: normale	Gravidanza e parto normali Alla nascita: spasticità, riflessi assenti, disordini alimentari Epilessia generalizzata neo-natale Ritardo severo, prognosi riservata

Tipo 4	Solchi poco profondi (50%) Corteccia di spessore normale Giunzione corteccia / SB normale SB: normale	Gravidanza anormale (oligoidramnios, artrogriposi...) Alla nascita: spasticità, riflessi assenti, nistagmo optocinetico Epilepsia generalizzata neonatale Ritardo severo, prognosi riservata
Tipo 5	Cervello molto piccolo con distensione degli spazi subaracnoidei Meno di 5 solchi, molto poco profondi (<<30%) Corteccia di spessore ridotto Giunzione corteccia / SB normale Eterotopie sottoependimali SB: ritardo di mielinizzazione	Gravidanza e parto normali Alla nascita: spasticità, riflessi assenti, disordini alimentari. Epilessia mioclonica neonatale Ritardo severo, prognosi riservata (quaod vitam).

MLIS: microlissencefalia

Questo termine era precedentemente utilizzato per designare le MSG. E' caratterizzata dall'associazione di microcefalia, di girazione semplificata e di un'anomalia di migrazione che conduce ad un addensamento dello strato corticale (lissencefalia). Il tipo A era precedentemente chiamato sindrome di Norman-Roberts ed il tipo B sindrome di Barth. Si distinguono 3 forme di MLIS:

Tipo	*Neuroimaging*	*Clinica*
Tipo A	Solchi molto poco profondi/assenti (lissencefalia grado 1) Corteccia ispessita Cervelletto e tronco cerebrale normali	Ritardo severo Dismofia facciale?
Tipo B	Solchi molto poco profondi/assente (lissencefalia grado 1) Cortecce ispessita Cervelletto e tronco cerebrale severamente ipoplasici	Microcefalia estrema Decesso neonatale
Tipo C	Solchi molto poco profondi (lissencefalia grado 3) in frontale Girazione semplificata in posteriore Corteccia ispessita Cervelletto e tronco cerebrale normali	Ritardo severo

Obiettivi principali sul piano clinico

- Reclutare un gruppo di pazienti con MV, con MSG e MLIS
- Raffinare la descrizione clinica e di *neuroimaging* di queste malattie, e le correlazioni fenotipo/genotipo nei pazienti portatori di tali patologie
- Precisare le correlazioni neuro-anatomiche grazie a studio di morfometria in 3D
- Migliorare la conoscenza sul piano neurologico e cognitivo in ogni sotto tipo di

microcefalia

- Sviluppare la diagnosi molecolare delle mutazioni di MCPH5 e MCPH1 (e di altri geni d'interesse, in funzione dell'evoluzione delle conoscenze)
- Identificare famiglie consanguinee suscettibili di potere essere studiate con homozygosity mapping sul piano sperimentale
- Sviluppare un'analisi funzionale del gene ASPM in modelli animali (topo, xenopus)
- Sviluppare un topo transgenico che esprima il gene ASPM umano

PAZIENTI E METODI

Protocollo d'indagine clinica

Criteri d'inclusione

La selezione dei pazienti è effettiva dopo completamento del bilancio che è parte integrante della diagnosi eziologica di microcefalia congenita. Sono eleggibili per lo studio i pazienti che presentano:

1. microcefalia inferiore a -3DS alla nascita (valore di riferimento: 31 cm a termine). La presenza di una malformazione del corpo calloso, di un'anomalia infratentoriale (ipoplasie del tronco cerebrale e/o del cervelletto), di un'epilessia o di un deficit sensoriale non sono criteri di esclusione.
2. assenza di ritardo di crescita pre o postnatale (-2DS)
3. RMN compatibile con MV, MLIS o MSG.
4. altre cause di microcefalia escluse

Non c'è limite d'età per l'inclusione. Il bilancio neuropsicologico e cognitivo varierà tuttavia in funzione dell'età all'inclusione.

Criteri d'esclusione

I pazienti non sono eleggibili se uno dei criteri qui di seguito è soddisfatto:

1. Anamnesi:

a. esposizione a tossici I.U. (tra cui alcool e fenilchetonuria materna)

b. Irradiazione I.U.

2. Fetopatia infettiva:
a. elementi sierologici o virologici a favore di una embriofetopatia da rosolia, CMV, toxoplasma, HSV
b. segni indiretti di una embriofetopatia virale: calcificazioni intracraniche, cataratta, corioretininte... nei bambini per i quali le sierologie sono indisponibili o ininterpretabili

3. anomalia genetica
a. sindrome polimalformativa identificabile conosciuta o sconosciuta (in particolare: nanismo di Seckel)
b. anomalia cromosomica ricercata almeno su un cariotipo postnatale standard (400 bande)
c. anomalia metabolica cercata con almeno un bilancio standard che comprenda AAs, AAu, AOu, 7OHcolesterolo)
d. Fenilchetonuria (PKU) materna

4. assenza di immagini di risonanza magnetica utilizzabili

Preselezione dei candidati all'inclusione
La valutazione comporta:
1) un esame clinico e neurologico dettagliato, con misurazione dei parametri standard (peso, altezza, CC). La CC dei genitori é misurata.
2) la raccolta di dati anamnestici (secondo un formulario prestabilito), e la realizzazione di un albero genealogico
3) la raccolta specifica di informazioni riguardanti i precedenti medici del paziente
4) la verifica dei criteri d'esclusione.

I pazienti che soddisfanno le condizioni d'inclusione sono inclusi dopo raccolta di un consenso specifico firmato dal paziente o dal suo rappresentante legale.

Protocollo d'indagine neuropsicologica

Valutazione generale delle capacità cognitive

Secondo l'età ed il livello intellettivo dei pazienti, è realizzata per mezzo delle scale classiche Brunet-Lezine, WPPSI, WISC o WAIS, che permettono un confronto con i dati della letteratura. E' completata dalla batteria NEPSY per gli items delle funzioni esecutive nei bambini tra 3 e 12 anni.

Valutazione comportamentale

E' stabilita grazie all'utilizzo di questionari consegnati ai genitori. Le valutazioni proposte non saranno applicabili in tutto i casi, a causa delle differenti età. Al massimo, essa comporta 7 indagini:

- Kiddie Schedule for affective Disorders and Schizophrenia (Kiddie-SADS)
- Child Behaviour Check List (CBCL)
- Scale di CONNERS
- Scala Emotivity Activity Sociability (EAS)
- Scala Dimension of Temperament (DOT)
- Questionario Children Social Behaviour (CSBQ)
- Scala d'adattamento di Vineland (VABS)

Protocollo d'indagine neuroradiologica

Lo studio neuro-anatomico 3D é realizzato a posteriori a partire dalle acquisizioni RMN realizzate, in bambini selezionati, nel bilancio diagnostico di pazienti con microcefalia vera.

Per i pazienti nei quali quest'esame non è realizzabile, è utilizzata la RMN convenzionale.

Analisi statistica

L'analisi statistica è effettuata con la comparazione delle medie dei valori di CC, alla nascita (CCN) e al momento dell'esame (CCA), espresse come deviazioni standard, e dei valori di QI verbale e QI di performance.

Si valuta anche la correlazione tra QI totale, QI verbale e QI di performance con i due

valori di CC (CCA e CCN), attraverso il metodo di Pearson.

RISULTATI

Sono stati sinora reclutati e completamente analizzati dal punto di vista neurologico, cognitivo e comportamentale 11 pazienti (7 M, 4 F. Età media : 9.7 anni, range 4.2-16.1)

L'esame neurologico approfondito non ha ritrovato alterazioni in alcun paziente. Un paziente (VA) ha presentato, anamnesticamente, delle convulsioni febbrili semplici.

Una sordità neurosensoriale bilaterale, di intensità moderata, è stata ritrovata nel paziente BS e una iperplasia del corpo vitreo primitivo nel paziente VA.

Il valore medio del QIT è di 52 (range : 26-104) ; le medie dei valori di QIV e QIP sono rispetivamente di 59 e 55.

Tutti i pazienti presentano una microcefalia vera all'esame di RMN cerebrale (fig. 7).

Per quanto riguarda l'analisi statistica dei risultati dello studio neuropsicologico, abbiamo trovato una differenza statisticamente significativa tra CCN e CCA (media -1.3, 95%CI –0.3 à -2.3, p=0.01). Non c'è invece differenza tra QIV e QIP (-1.4, p=ns).

Il QIT é correlato significativamente solo con il CCA ($r=0.6$, $p<0.05$) (Fig. 8a). Non c'è correlazione invece tra QIT e CCN.

Il QIV non è correlato statisticamente alla severità della microcefalia. Peraltro, il QIP correla sia con il CCN ($r= 0.7$, $p<0.05$) (Fig. 8b) che con il CCA ($r= 0.7$, $p<0.05$) (Fig. 8c).

L'analisi dei risultati dell'evaluazione comportamentale con la scala di Vineland ha ritrovato un deficit più marcato nella sfera della comunicazione (39% del valore atteso), in confronto alle aree della vita quotidiana (43%), della motricità (49%) e della socialità (54%).

DISCUSSIONE

Nell'ambito delle microcefalie genetiche, sono state sinora intraprese solo poche

indagini neuropsicologiche. La letteratura mette in evidenza la presenza di un ritardo mentale variabile, di gravità da lieve a severa [Woods et al, 2005].

Seconso la definizione classica, la diagnosi di ritardo mentale si basa sulla duplice costatazione di un funzionamento intellettivo inferiore al normale e di un deficit delle condotte adattative. Così, una performance significativamente bassa a un test di livello intellettivo generale costituisce un elemento diagnostico fondamentale, ma non rappresenta in sé la diagnosi di ritardo mentale. E' per questo che sono state incluse nel protocollo neuropsicologico delle prove cognitive globali e variate, ed anche una scala di valutazione del comportamento adattativo (VINELAND), realizzata con la cooperazione dei genitori.

La valutazione del livello intellettivo (QIT) è strettamente dipendente dalle valutazioni del QIV e del QIP. La scala di QI verbale raggruppa tutte le prove che esigono una risposta verbale del bambino. Queste prove non necessitano di alcuna manipolazione di materiale e si presentano sotto forma di domande: informazioni, similitudini, aritmetica, vocabolario, comprensione, memoria immediata delle cifre (rappresentativa della memoria di lavoro). La scala di QI di performance raggruppa tutte le prove che richiedono la manipolazione di materiale e che non necessitano di alcuna partecipazione verbale: completamento di immagini, ricostruzione di immagini, cubi, assemblaggio di oggetti, codici.

La serie di 11 pazienti con microcefalia vera, completamente analizzati dal punto di vista neurocognitivo, ci ha permesso di mettere in evidenza che generalmente non ci sono segni neurologici all'esame clinico, e che il ritardo mentale è di solito leggero e omogeneo. Peraltro, ci sono dei bambini che sono gravemente affetti ed altri che rientrano nel *range* della normalità.

Per quanto concerne il pronostico cognitivo, si osserva generalmente col tempo un aggravamento significativo della circonferenza cranica. E' la CC al momento dell'esame che si correla meglio alla severità del ritardo mentale, piuttosto che la CC alla nascita. Inoltre, la misura della CC (CCN e CCA) è significativamente correlata alle performance (QIP), e non alle capacità verbali. La comunicazione è l'handicap più importante dei pazienti con MV.

Una conferma di questi risultati con la valutazione di un numero superiore di pazienti e l'individuazione della alterazione molecolare responsabile caso per caso ci

permetterà di comprendere il rapporto tra funzioni congnitive superiori e le anomalie della proliferazione neuronale.

CONCLUSIONI

L'approccio utilizzato per l'individuazione delle alterazioni genetiche nelle patologie di sviluppo del SNC e per l'analisi dei rispettivi fenotipi cognitivi può essere un modello valido di ricerca clinica e molecolare.

Le diverse pubblicazioni prodotte nel corso degli anni del dottorato su importanti riviste internazionali testimoniano anche dell'importanza della integrazione delle conoscenze biologiche di base con le conoscenze che scaturiscono dall'esame cognitivo del paziente. E' facile prevedere che una sempre maggiore comprensione dei meccanismi che regolano la fisiologia neuronale porteranno anche ad una migliore comprensione dello sviluppo psicomotorio normale. L'analisi dei difetti molecolari, ovviamente, porterà alla comprensione degli effetti di tali alterazioni su questo sviluppo e quindi alle conseguenze sul comportamento dell'individuo.

Le prospettive terapeutiche, neuroabilitative o farmacologiche, sono enormi. Poter intervenire nell'area cerebrale malfunzionante, dopo averne elucidato i meccanismi fisiopatologici, è la sfida che le neuroscienze sapranno certamente cogliere nel prossimo futuro.

Per ciò che riguarda i risultati preliminari presentati sulle microcefalie primarie, sono il frutto di una riuscita collaborazione europea.

E' attualmente in corso l'analisi molecolare dei geni ASPM e Microcephalin in tutti i pazienti.

Inoltre, stiamo sviluppando un modello animale che permetta di studiare la funzione di ASPM. Questo gene presenta delle caratteristiche particolari, tra cui la presenza di un motivo ripetitivo IQ la cui grandezza cresce con la progressione dell'evoluzione. Non si conoscono né il ruolo preciso né la regolazione della sua espressione nell'uomo. L'ipotesi seconda la quale questo gene regola la proliferazione dei neuroni primitivi nel corso dello sviluppo cerebrale normale é dedotta dal fenotipo della MV.

Anche in questo caso, la determinazione dei fattori implicati nella crescita del volume cerebrale sembra essere fondamentale per comprendere i meccanismi che portano

all'handicap mentale di origine genetica. Il fatto di avere un gene (ASPM) che, se mutato, determina una riduzione drammatica del numero di neuroni senza altre modificazioni dell'architettura cerebrale, può senz'altro rappresentare un punto fondamentale nella comprensione della fisiologia dei processi cognitivi e dei meccanismi che conducono alla microcefalia in altre patologie genetiche.

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FIGURE

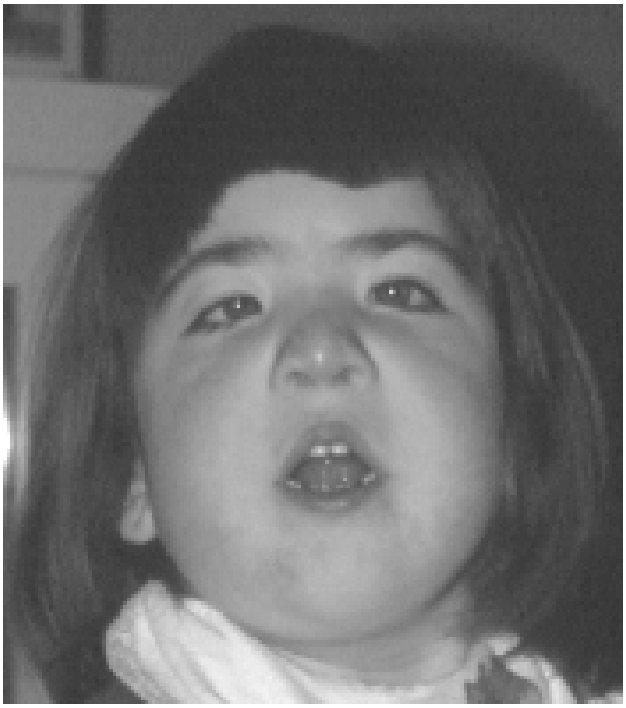
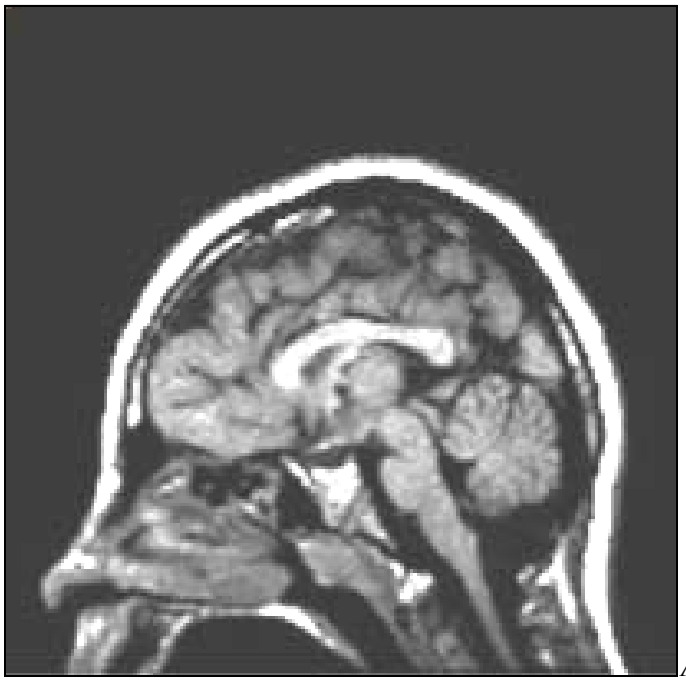
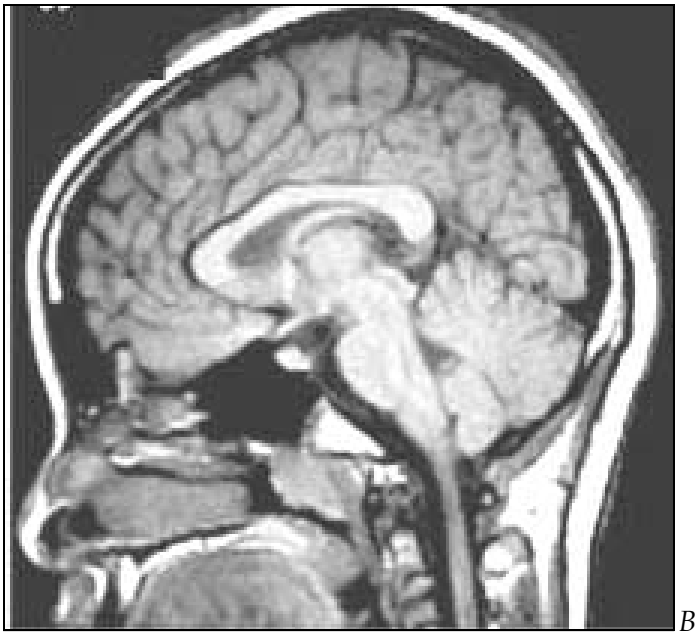


Fig. 1: il paziente PM all'età di 5 anni. Notare l'ipertelorismo e le rime palpebrali rivolte verso il basso, la radice nasale piatta, le narici anteverse, il filtro lungo, la bocca "carp-shaped", le orecchie a basso impianto.



A



B

Fig. 2: RMN cerebrale del paziente LM (A) : notare la severità della microcefalia in confronto alla RMN di un paziente normale della stessa età (B).

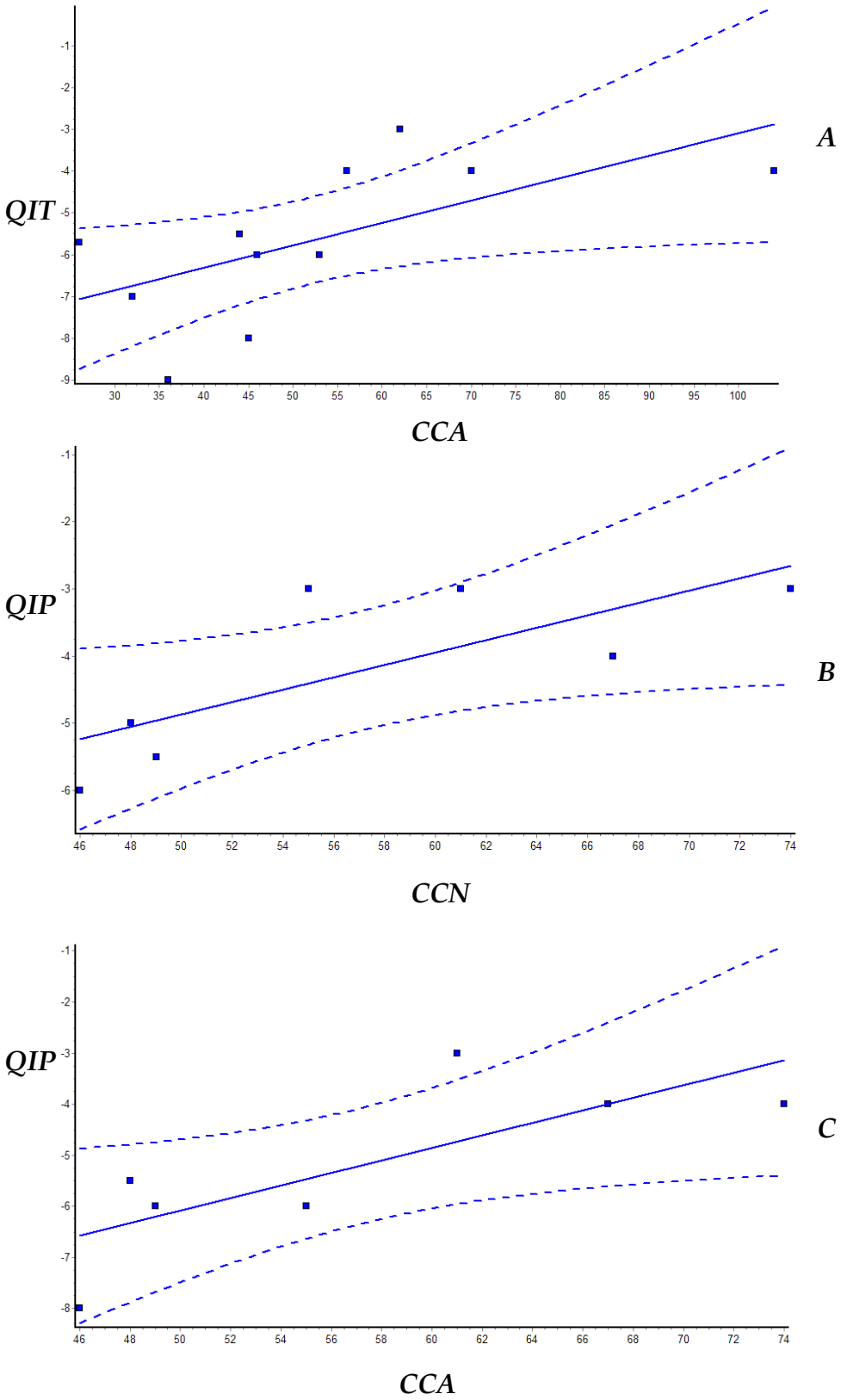


Fig. 3: Correlazione tra severità della microcefalia al momento dell'esame e il QI totale (A), tra il QI di performance e la CC alla nascita (B), tra il QI di performance e la CC attuale (C).

TABELLA

Gene	*Locus*	*Proteina*
MCPH1	8p22-pter	Microcephaline
MCPH2	19q13.1-13.2	-
MCPH3	9q34	CDK5RAP2
MCPH4	15q	-
MCPH5	1q31	ASPM
MCPH6	13q12.2	CENPJ
MRXS9	Xq12-q21.31	-
ALM	17q25	SLC25A19

Geni delle microcefalie congenite primarie.

Appendice

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Summary

The analysis of cognitive profiles in specific genetic syndromes is an alternative approach to the understanding of normal and abnormal physiology in developmental neurosciences. Correlating the molecular basis of developmental CNS anomalies with specific neuropsychological phenotypes, in association with neuroradiological (functional MRI, fiber-tracking) and neurophysiological (EEG, EP, cognitive potentials) data, would help determining localisation and function of the altered cerebral region. This is an important step in future diagnostic and therapeutical approaches to human pathology.

Aims of the 4-years doctoral study were the classification and identification of the genetic bases of the developmental pathologies of the CNS, with a special focus on the cerebellum (section 1) and the utilisation and validation of cognitive tests in developmental neurosciences (section 2). Final goal was to plan a multicentric project on the genetical and cognitive aspects of primary microcephalies. Yet, analysing the complex relations among genetic factors that lead to a malfunctioning small brain will pave the way to the understanding of mental retardation, when intergrated with cognitive data. So, the implementation of laboratory and clinical research tools in the field of pediatric neurosciences has allowed the planification of a post-doc program in developmental neurobiology, in collaboration with the Inserm unit U676 (dr. P. Gressens, Paris, France). The european project on primary microcephalies is still ongoing, and perliminary results are resported in the 3rd section of the doctoral thesis.

A 2-years period has been spent in France to establish a continuative cooperation in pediatric research, basic and clinical (Pr. P. Evrard; Pr. A. Verloes; Pr. J.-C. Mercier – Robert Debré Hospital, APHP, Paris VII University).

CASE REPORT
CASO CLINICO

Chronic Inflammatory Demyelinating Polyradiculoneuropathy with Central Nervous System involvement

Poliradicoloneuropatia cronica infiammatoria demielinizzante con coinvolgimento del sistema nervoso centrale

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Summary

We report the case of a 12-year-old child with a relapsing/remitting form of Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP), with Magnetic Resonance Imaging (MRI) evidence of Central Nervous System (CNS) involvement during a relapse. We critically review the literature on this atypical form of Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP).

Riassunto

Riportiamo il caso di un bambino di 12 anni affetto da una forma recidivante/remittente di Poliradiculoneuropatia Demielinizzante Infiammatoria Cronica (PDIC), che ha presentato durante un episodio di riacutizzazione un coinvolgimento del Sistema Nervoso Centrale (SNC) documentato in Risonanza Magnetica (RM). Viene revisionata criticamente la letteratura riguardante questa forma atipica di PDIC.

Key words

Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) • Guillan-Barré Syndrome (GBS) • Albuminocytological dissociation

Parole chiave

Poliradicolonevrite Demielinizzante Infiammatoria Cronica (CIDP) • Sindrome di Guillan-Barré (GBS) • Dissociazione albumino-citologica

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Introduction

Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) is an immune-mediated motor and sensory neuropathy with predominant motor involvement in some patients. Diagnostic criteria were established in 1984 by Dick and Arnason [1].

A symmetrical involvement of the upper and lower limbs with marked weakness and hypo/areflexia are the rule. The clinical course may either be monophasic or relapsing/remitting. Cerebral spinal fluid (CSF) findings usually show elevated protein values despite a normal cell count (albuminocytological dissociation). The clinical diagnosis is supported by electrophysiological studies, which evidence a segmental demyelination with variable involvement of the different nerves. Nerve biopsy shows moderate reduction of myelinated fibers, demyelination and remyelination features and sometimes the presence of endoneurial and/or epineurial mononuclear inflammatory cells.

Little is known on the onset, clinical course, treatment and prognosis of CIDP in paediatric age, given its rare occurrence in this age range [2]. The central nervous system (CNS) is involved in a small number of cases. We report on the clinical course and treatment of a case of pediatric CIDP with clinical CNS involvement, as confirmed by MRI.

Case report

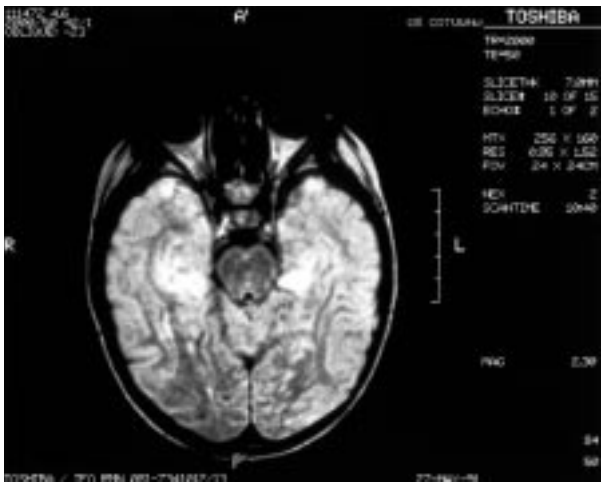
B.G. is a 13-year and 8 month-old boy, born from a normal delivery and with a negative family history for neuromuscular disease. The patient had a normal psychomotor development until the age of four years, when he was hospitalised because of fever, dysequilibrium and paresthesias of the upper and lower limbs. A few days later he showed a mild impairment of consciousness, progressive muscle weakness starting at the distal lower limbs. Deep tendon reflexes were absent in the lower limbs. A lumbar puncture showed an abuminocytological dissociation with sterile CSF. MRI of the brain revealed small hypersignal abnormalities on T2-weighted images at the level of the temporal lobes (Fig. 1). A diagnosis of encephalomyelitis was then entertained and treatment with corticosteroids (metilprednisolone, 1 mg/kg/die per os for five weeks) was prescribed. The patient completely recovered within 40 days.

Five years later, at the age of nine the patient was again hospitalised because of vertigo, malaise, confusional state, paresthesias, generalized flaccid paresis and areflexia. His CSF was sterile, with albuminocytological dissociation. Brain MRI was normal. Treatment with metilprednisolone, similar to that already reported, was started and the patient recovered in four weeks. Three years later, at the age of 12 years and 10 months he was admitted to hospital because of generalized discomfort, fatigability and paresthesias. A neurological examination showed an ataxic gait that worsened when the patient closed his eyes, horizontal nystagmus, severe weakness and hypotonia of the lower limbs, distal hypoesthesia and paresthesias. Deep tendon reflexes were absent in the lower limbs. Haematological parameters and serum inflammatory tests were negative. EEG and brain MRI were normal. The child's parent refused to have him undergo lumbar puncture and CSF investigation. A motor nerve conduction study was performed on the median and peroneal nerves, which.showed prolonged distal latencies, a severe reduction in conduction speeds and a partial block of conduction in the right median nerve (Fig. 2). F-wave latencies were prolonged in the right median nerve; motor action potentials presented abnormal temporal dispersion and a slightly reduced amplitude. A sensory conduction study was performed on sural and median nerves where it was impossible to record any potential. The confirmation of a proximal and distal demyelinating neuropathy together with a relapsing and remitting course suggested the diagnosis of CIDP.

A five days cycle of I.V. immunoglobulins (400 mg/kg/day) was given, followed by prednisone 1 mg/kg/day for two weeks, which was gradually discontinued within four weeks.

A follow-up after one month showed an improvement of the sensory and motor deficits, whereas eight months later the recovery was clinically and electrophysiologically complete. The results of a subsequent clinical examination at the age of 13 years and 8 months was normal.

Fig. 1 (a, b, c). Proton density weighted magnetic resonance images, showing a diffuse and inhomogeneous area of hyperintense signal encompassing the mesial aspect of both temporal lobes. The hyperintensity includes the head of the hippocampus and the parahyppocampal gyrus in both sides (a, b), the entorhinal area and, partially, the amigdala of the right side (b). T2 weighted magnetic resonance image (c) showing multiple small and confluent areas of hyperintense signal in the retrotrigonal white matter of both sides.



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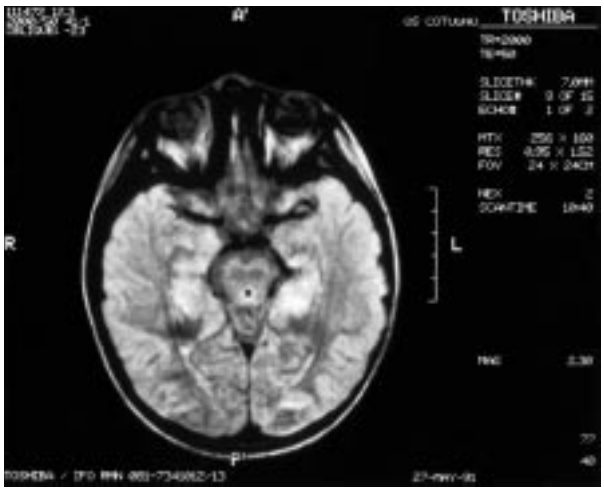

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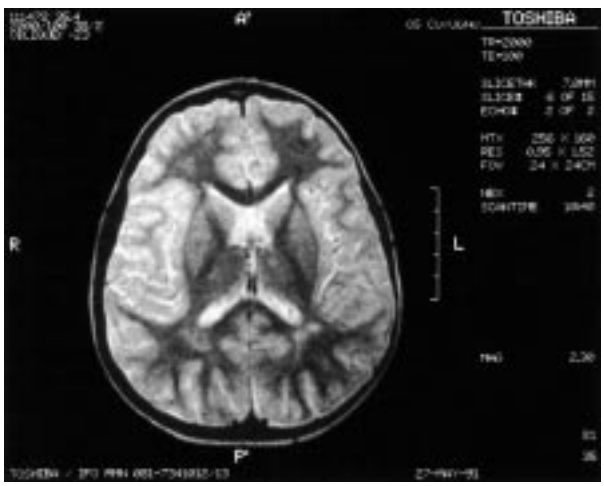

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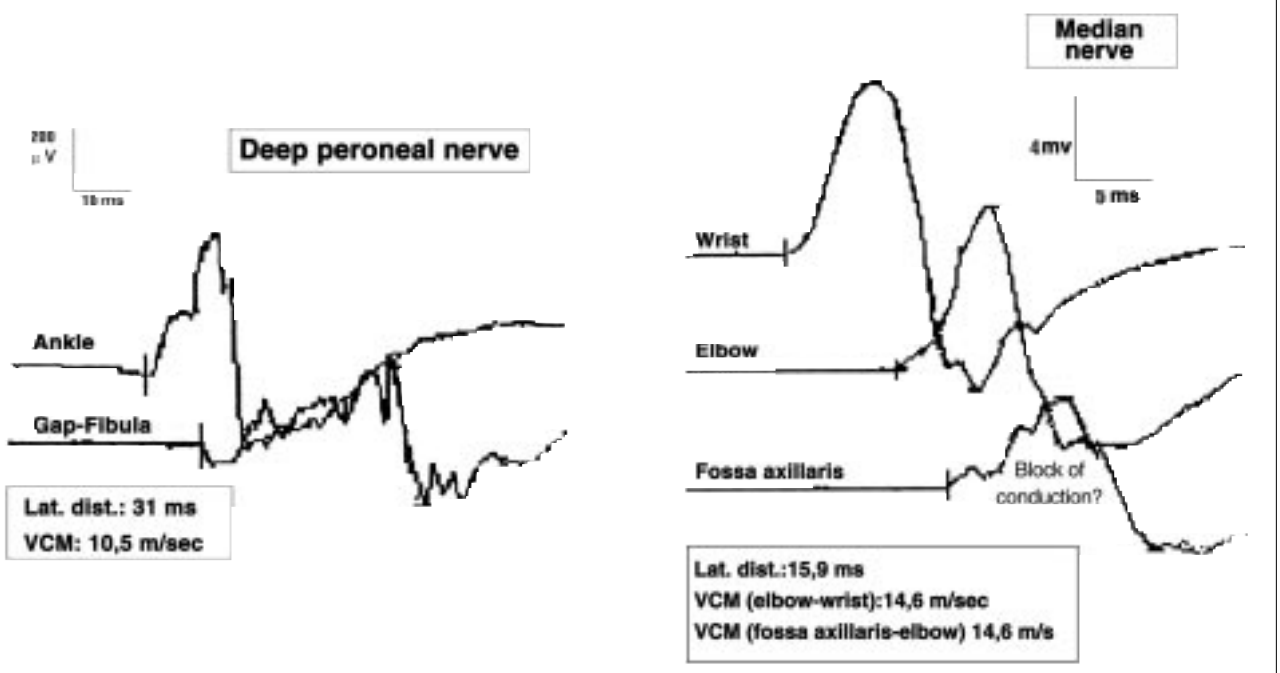
Fig. 2. Motor nerve conduction study on the peroneal (left) and median (right) nerves showing prolonged distal latencies (Lat. dist.) and a severe reduction in conduction speeds (VCM), with a partial block of conduction in the right median nerve.



Discussion

The term «inflammatory demyelinating polyradiculoneuropathies» indicates a group of diseases characterized by progressive and symmetrical muscle weakness mostly involving the lower limbs, associated with hypo/areflexia. The clinical course may be either acute (AIDP), subacute or chronic (CIDP). An immune-mediate basis of uncertain etiology is suspected in all these forms [3].

A classic example of AIDP is the Guillain-Barrè syndrome (GBS), which is usually characterized by a rapid course that lasts no more than four weeks. Two/third of the patients report a preceding event, such as an upper respiratory or gastrointestinal infection. The disease is usually self-limited and monophasic. GBS is more frequent than CIDP in both children and adults.

In the chronic form (CIDP) a preceding event is rare, weakness must be present for at least two months and the clinical course may be either progressive or relapsing/remitting. A hereditary predisposition has been proposed for this form. Only 93 cases of paediatric CIDP have been reported to date [3-10]. The male-to-female ratio is 1.32. Antecedent events are reported in 30% of children (more frequently than in adults). The age at onset is below 10 years in 36 patients (39%). At referral all children usually have a severe neurological impairment and gait abnormalities are common. Seventy-eight children out of 93 (84%) present with severe weakness ranging from the inability to stand up to a complete flaccid paresis with diffuse hypo/areflexia. Pain and/or paresthesiae are often associated. Diplopia and tremor are less common than in adults. A benign outcome is the rule, even if treatments are heterogenous (corticosteroids, plasma exchange, intravenous immune globulin (IVIG). The first episode in the relapsing forms usually lasts less than three months. CSF generally shows albuminocytological dissociation. Neurophysiological studies exhibit slower conduction velocities (in at least two motor nerves); partial block of conduction or abnormal temporal dispersion (in at least one motor nerve); prolonged distal latencies (in at least two motor nerves); absent F-wave or prolonged F-wave latencies (in at least two motor nerves). Sural nerve biopsy shows signs of demyelination (thinly myelinated axons and onion bulb formations) and remyelination, usually with infiltration by mononuclear cells [2 3].

Our patient had three relapses, at the ages of four, nine and twelve years. In the first two episodes there was impaired consciousness. In both cases the patient's CSF was sterile with albuminocytological dissociation. During the first episode, brain MRI gave abnormal hypersignals at the level of the temporal lobes. Cortical involvement was so severe that a diagnosis of encephalomyelitis was hypothesized. In a few hours consciousness returned to normal, whereas limb weakness and peripheral nerve involvement lasted for about 40 days. The second episode was similar to the first one, but consciousness was less severely affected and the

MRI was normal. In the third episode there was no impairment of consciousness but only prolonged weakness. MRI was normal. Neurophysiological studies performed at that time demonstrated a demyelinating polyneuropathy.

Some investigators have reported an initial atypical presentation in children with GBS consisting in drowsiness, headache, irritability and meningismus, suggesting CNS involvement. Bradshaw and Jones noticed that in children with GBS the presence of meningismus, severe muscle pain and irritability, either alone or in combination, can delay the diagnosis in some cases [11]. Meningeal signs and symptoms are probably the equivalent of proximal nerve roots inflammation. Garson et al. have found lombosacral nerve root enhancement with gadolinium on MRI in some patients affected by GBS who showed severe back or leg pain [12].

Only few cases of CIDP with clinical evidence of CNS involvement – confirmed by MRI – have been reported to date [13]. Our patient exhibited bilateral temporal lobe involvement associated with meningismus at the age of 4 years. The pathophysiology of CNS involvement in CIDP is difficult to explain but could be the same proposed for GBS, especially in relapse/remitting forms. An autoimmune inflammatory process involving the proximal nerve roots might determine the diffusion of degradation proteins in the CSF, with alteration of the blood-brain barrier and consequent CNS involvement (meningismus, irritability, drowsiness). Yan et al. [14] have recently provided evidence that antibodies to P0 glycoprotein are present in the serum of about 30% of patients with CIDP, which have the potential to induce demyelination. Similar data have been described in relation to the peripheral myelin protein 22 (PMP22) [15].

A differential diagnosis with the Hereditary Motor and Sensory Neuropathy (HMSN) should be considered in all cases of childhood polyneuropathy. In HMSN the clinical course is slower and progressive and a family history is usually present. One of the parents is often infraclinically affected, due to which it is necessary to perform neurophysiological studies in the relatives.

As far as treatment of paediatric CIDP is concerned, available data suggest that prednisone, plasma exchange and intravenous immunoglobulin (IVIG) are usually effective in children. Plasma exchange therapy is based on the hypothesised role for autoantibodies in the pathogenesis of CIDP at least in some patients [16], but it has some technical limitations in childhood. In our case a combined therapy with IVIG and prednisone was successful. In particular, IVIG therapy is effective, easy to administer and well tolerated, and could be also considered in patients with GBS, in whom corticosteroids are ineffective.

In conclusion, although CIDP in paediatric age is rare, it is important to know about this condition in order to provide children and their families with the adequate treatment and a correct long-term prognosis.

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Letters to JMG:
L Burglen, D Héron, A Moerman, A Dieux-Coeslier, J-P Bourguignon, A Bachy, J-C Carel, V Cormier-Daire, S Manouvrier, and A Verloes

Myhre syndrome: new reports, review, and differential diagnosis
J Med Genet 2003; 40: 546-551 [Full text]

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▼ **X-linked inheritance of Myhre syndrome is more likely than autosomal dominant.**
Luigi Titomanlio, Nicola Brunetti Pierri (24 July 2003)

X-linked inheritance of Myhre syndrome is more likely than autosomal dominant.

24 July 2003

Luigi Titomanlio, Pediatrician
Department of Pediatrics, Federico II University, Naples, Italy,
Nicola Brunetti Pierri

Send letter to journal: Re: X-linked inheritance of Myhre syndrome is more likely than autosomal dominant.

Email Luigi Titomanlio, et al.

Dear Editor

We read with interest the work by Burglen *et al*.[1]

We really appreciate their attempt to provide a careful differential diagnosis with other rare syndromes whose pathogenesis is still unknown. We observed one patient with Myhre syndrome [2] and another one affected by geleophysic dysplasia [3] and find their table 2 very useful in everyday clinical practice.

However, after their report of 4 more male patients with Myhre syndrome, we do not agree with their conclusion about the inheritance of the disease. They observe that paternal age was increased in half of the reported cases, suggesting a new mutation of an autosomal dominant gene. They also state that X linked transmission cannot be excluded since all reported cases (11/11) were males. The probability to observe 11 consecutive male patients is 1/2064, e.g. less than 5%, that is the usual value indicating statistical significance. Moreover, mean paternal age at birth is 35 years but the range is wide, varying from 23 to 43 years. So, we think that a X-linked pattern of inheritance is more likely in Myhre syndrome. That could be an important data to plan further investigation about the molecular basis of the disease. All reported cases are sporadic. So, a candidate gene approach is the only possibility to date. Our findings of abudant close-packed collagen fibers at skin biopsy could be a key-point to identify possible candidate genes involved in collagen metabolic pathway localized on the X chromosome. Understanding the pathogenesis of Myhre syndrome could provide further clues to identify the molecular basis of similar syndromes.

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ORIGINAL ARTICLE
ARTICOLO ORIGINALE

Subtle neuropsychological alterations in children with rolandic epilepsy

Alterazioni neuropsicologiche lievi in bambini affetti da epilessia rolandica

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Summary

Objectives. Benign childhood epilepsy with centrotemporal spikes or rolandic epilepsy is an idiopathic epileptic syndrome with a favorable long-term outcome. Our study aimed at investigating possible subtle neuropsychological deficits in rolandic epilepsy patients using the computerized battery "Fepsy".
Methods. We assessed 16 rolandic epilepsy patients free from seizures and carbamazepine since at least 2 years, matched with healthy controls.
Results. Rolandic epilepsy patients performed worse than controls at the following tests: 1) Binary Choice Reaction Test ($p < 0.01$), that evaluates decision-making and 2) Recognition Tasks that evaluate short-term memory: a) simultaneous recognition of words ($p < 0.01$) and figures ($p < 0.001$) and b) serial recognition words ($p < 0.001$) and figures ($p < 0.01$). Multiple linear regression analysis showed significant correlations between: a) Binary Choice Reaction Test score and sex and localization of discharging focus; b) simultaneous presentation of words and duration on carbamazepine therapy and time since carbamazepine withdrawal; c) simultaneous presentation of figures and sex.
Conclusions. Our data strengthen the usefulness of performing an accurate neuropsychological assessment in all patients affected by rolandic epilepsy, regardless of the duration of treatment with carbamazepine.

Riassunto

Obiettivi. L'epilessia benigna dell'infanzia a punte centro-temporali o epilessia rolandica è una sindrome epilettica benigna con una prognosi favorevole a lungo termine. Scopo del nostro studio è stato di valutare possibili deficit neuropsicologici minori in pazienti con epilessia rolandica usando una batteria computerizzata.
Metodi. Abbiamo esaminato 16 pazienti senza crisi da almeno 2 anni, appaiati con controlli sani.
Risultati. I pazienti con epilessia rolandica hanno mostrato risultati peggiori nei seguenti test: 1) *Binary Choice Reaction Test* ($p < 0,01$), che valuta la capacità decisionale e 2) *Recognition Tasks* che valutano la memoria a breve termine: a) riconoscimento simultaneo di parole ($p < 0,01$) e figure ($p < 0,001$) e b) riconoscimento seriale di parole ($p < 0,001$) e figure ($p < 0,01$). L'analisi di regressione multipla lineare ha mostrato correlazioni significative a) tra il punteggio al *Binary Choice Reaction Test* ed il sesso e la localizzazione del focolaio; b) il riconoscimento simultaneo di parole e la durata della terapia con carbamazepina ed il tempo trascorso dalla sospensione del farmaco; c) riconoscimento simultaneo di figure e sesso.
Conclusioni. I nostri dati sottolineano l'utilità di valutare accuratamente dal punto di vista neuropsicologico tutti i pazienti con epilessia rolandica, a prescindere dalla durata del trattamento con carbamazepina.

Key words

Rolandic • Epilepsy • Neuropsychological • Cognitive

Parole chiave

Rolandica • Epilessia • Cognitivo • Neuropsicologia

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Introduction

Benign epilepsy of childhood with centrotemporal spikes or rolandic epilepsy (RE) has been considered for many years a benign epileptic disorder with long-term remission by mid-adolescence in almost all patients. Genetic pre-

disposition is frequent with a slight male predominance, and there is also evidence for linkage on chromosome 15q14 [1]. Treatment with antiepileptic drugs such as phenytoin or carbamazepine (CBZ) has been proposed only for patients with frequent seizures. The recent identification of subtle neuropsychological deficits in children affected by RE has suggested the need to perform specific neuropsychological tests to evaluate higher cortical functions [2].

It is still not clear whether the treatment with CBZ may have influenced the long-term outcome in such patients [3]. The Fepsy computerized neuropsychological test battery has proven effective in studying epileptic patients before and after antiepileptic drug withdrawal [4], analyzing cognitive function in newly diagnosed epilepsies [5] and, recently, in the evaluation of a possible cognitive impairment associated with interictal epileptiform electroencephalographic discharges [6]. We report and discuss the results of the Fepsy computerized neuropsychological assessment in a group of 16 children affected by RE.

Patients and methods

This study was an open controlled parallel-group and nonrandomized clinical investigation. Each patient was matched with a healthy control with regard to age (within 1 year), sex and cultural level. Sixteen children (12 males and 4 females) aged 9.8 to 16 years (mean = 13.3 years) were included based on the following criteria: a clinical and EEG diagnosis of RE with normal neuroradiologic examination; no cognitive impairment that could interfere with neuropsychological assessment; IQ within normal range; neither seizures nor EEG abnormalities since at least 2 years; withdrawal of CBZ since at least 2 years. All patients were examined with the Fepsy computerized neuropsychological test battery. The test procedure was always tutored by one of the authors. The test, the test procedure and its validation have been reported elsewhere [7 8]. From this battery we selected the following tasks: A) Speed measures: Finger tapping task for both dominant and nondominant hand, measuring motor speed and motor fluency; Simple reaction times on auditory (800-Hz tones) and visual (a white square on the screen) stimuli to evaluate alertness and motor speed. B) Attention and information processing: the Computerized Visual Searching Task (CVST), by means of a centered grid pattern and 10 surrounding patterns of which only one is identical to the target, which assesses information processing and mental strategies. C) Decision-making: the Binary choice reaction task (BCRT) in which the patient has to react differentially to a red square and a green square presented on the opposite sides of the screen: reaction time and number of correct responses represent not only motor speed but also reflect the decision-making-process. D) Memory function: Recognition tasks, in which four words and then four figures are presented first simultaneously and then serially during a learning phase. A target item has to be recognized after a delay of two seconds.

Statistical analysis was performed by comparing the child's performance with his or her own matched control (matched-pairs t test). Correlation was calculated by the use of the Pearson coefficient. A multiple regression analysis model for each test was evaluated considering sex, type of crises (simple or complex), localization of discharging focus (right or left), academic achievement, socioeconomic level, age at diagnosis, disease duration, duration of antiepileptic therapy and time since CBZ withdrawal as main variables. Univariate analysis of significant covariates were assessed to estimate marginal means. The degree of significance chosen for all the statistical tests was $p < 0.05$.

Results

The general clinical characteristics and duration of treatment with CBZ for each patient are reported in Table I. Mean age at time of diagnosis was 5.9 years, mean duration of CBZ treatment 3.8 years, and withdrawal of CBZ 3.2 years.

Table II shows the results of the Fepsy testing for the epilepsy group and the matched controls.

Speed measures: no significant differences were found in the epilepsy group versus controls in the finger tapping task and simple motor reactions. Attention and information processing: there were no significant group differences in the visual searching task. Decision-making: a statistically significant difference ($p < 0.01$, mean 9, 95% CI = 4.1-14) was found in the BCRT test. Memory function: patients achieved significantly poor-

Tab. I. Clinical characteristics in the rolandic epilepsy and control groups.

	Patients Mean	SD	Range	Controls Mean	Range
Sex	M:12 F:4			M:12 F:4	
Age (y)	13.5	2.1	9.2-16	12.6	9.8-15.3
Age at diagnosis (y)	5.9	1.8	3.1-9.1		
Carbamazepine therapy (y)	3.8	3.1	0.1-8.5		
Carbamazepine withdrawal (y)	3.2	1.1	2-6		

Tab. II. Mean (SD) neuropsychological tests results.

Domain	Measure	Patients	Controls	p
Speed measures	FINGER TAPPING RATE			
	Dominant hand	57 (13.9)	63.4 (6.9)	ns
	Nondominant hand	48.8 (12)	57.2 (11.5)	ns
	SIMPLE MOTOR REACTION			
	Auditive, dominant hand	328.9 (144.9)	294.9 (58.1)	ns
	Visual, dominant hand	325.3 (86.1)	301.4 (52.4)	ns
Attention and information	VISUAL SEARCHING TASK (CVST)			
processing	No. errors	0.9	0,4	ns
	Time (sec)	7.1 (5.2)	4.7 (0.9)	ns
Decision-making	BINARY CHOICE REACTION (BCRT)			
	No. correct responses	52 (11.6)	57.4 (1.9)	< 0.01
	Reaction time	376 (121.4)	360.3 (54.4)	ns
Memory function	RECOGNITION MOMORY TEST			
	SIMULTANEOUS PRESENTATION	21.4	23	< 0.01
	Words (no. correct responses)	13.1	15,5	< 0.001
	Figures (no. correct responses)			
	SERIAL PRESENTATION	18.9	21.1	< 0.01
	Words (no. correct responses)	14,6	17.1	< 0.001

er results in the simultaneous recognition of words ($p < 0.01$, mean 2, 95% CI = 0.6-3.4) and figures ($p < 0.001$, mean 4.4, 95% CI = 2.6-6.1), and in the serial recognition of words ($p < 0.001$, mean 4, 95% CI = 2.1-5.9) and figures ($p < 0.01$, mean 3.4, 95% CI = 1-5.8). Multiple linear regression analysis showed significant correlations: a) between BCRT score, sex, and localization of discharging focus; b) simultaneous presentation of words, duration on CBZ therapy and the time since CBZ withdrawal; c) simultaneous presentation of figures, and sex (Tabs. III and IV).

Discussion

Mild intellectual disability (IQ < 70), impaired visuomotor coordination and poor fine motor performances have all been reported in children with rolandic epilepsy [2 9]. A consistent pattern of language dysfunction, which suggests interictal dysfunction of perisylvian language areas has also been described [10]. However, verbal functions seem to be less affected than non-verbal cognitive functions [2]. Simple motor speed and visual searching measures have both been reported to be in the normal range [2], and our results seem to confirm these data.

While no involvement of immediate memory (less than 5 minutes) has been found in RE patients [2 10], short-term memory (recall after 30 minutes) was affected in the series of Weglage [9]. Our patients showed impaired short-term memory in the recognition of both figures and words. In the simultaneous recognition of words we found that a longer disease duration and a shorter interval since CBZ withdrawal were negative predictors. Males performed significantly better in the simultaneous recognition of figure task. Disease duration and sex as risk factors for memory function should be confirmed on larger series.

As far as the influence of CBZ treatment on neuropsychological profile is concerned, Aldenkamp reported

Tab. III. Multiple linear regression analysis for the altered variables: BCRT, simultaneous recognition of words and figures, serial recognition of words and figures. NS = not significant.

Dependent variable	Predictors	Adj. R^2	β	p
Binary Choice Reaction Test	Sex	0.33	0.63	< 0.05
	Discharging focus		0.72	< 0.05
Simultaneous recognition of words	Carbamazepine withdrawal	0.75	0.92	< 0.05
	Disease duration		0.7	< 0.05
Simultaneous recognition of figures	Sex	0.38	0.83	< 0.05
Serial recognition of words	–	–	–	NS
Serial recognition of figures	–	–	–	NS

Tab. IV. Analysis of variance: quantitative changes (estimated marginal means) for the altered Binary Choice Reaction Test (a), Simultaneous recognition of words (b) and Simultaneous recognition of figures (c), adjusted for independent variables and covariates.

a) Dependent variable: BCRT

	Mean	95% CI	
Sex		Lower limit	Upper limit
Male	52,447	46,876	58,018
Female	37,410	26,465	48,354

	Mean	95% CI	
Focus		Lower limit	Upper limit
Right	55,979	47,684	64,273
Left	41,396	33,102	49,691

b) Dependent variable: Simultaneous recognition of words

	Mean	95% CI	
Disease duration		Lower limit	Upper limit
< 6 yrs	22,338	21,047	23,629
> 6 yrs	19,959	18,870	21,048

	Mean	95% CI	
Carbamazepine withdrawal		Lower limit	Upper limit
< 3 yrs	19,400	18,230	20,569
> 3 yrs	22,245	21,242	23,247

c) Dependent variable: Simultaneous recognition of figures

	Mean	95% CI	
Sex		Lower limit	Upper limit
Male	12,259	10,729	13,789
Female	7,723	5,056	10,389

the impact of carbamazepine on higher-order cognitive function to be rather limited [4]. Vermeulen showed that CBZ in normal doses does not impair psychomotor functioning or memory and, if subtle differences exist, they do not appear to be clinically significant [11]. Our data seem to confirm that CBZ is associated with reduced performance on memory function tests, which steadily improves after drug withdrawal. However, the need to pharmacologically treat patients with rolandic epilepsy is still debated.

RE patients achieved lower scores in decision-making task (BCRT): RE patients make more mistakes. Likewise, females and patients with a left epileptogenic focus performed significantly worse than males and children with a right focus. Recent studies using PET and fMRI show an activation increase in the inferotemporal cortex and the ventral prefrontal cortex to which it projects (ventral visual system) in subjects performing a choice reaction time task [12]. Moreover, during a two-choice response task an activation of a limbic and frontoparietal neural system occur in normal subjects and in patients with lesions in the prefrontal cortex [13 14].Corbetta et al. [15 16] recently confirmed these data underlining how the control of goal-directed and stimulus-driven attention is functionally located at least partially in the rolandic areas. The limbic system has a central role in regulating processes such as motor and sensory functions as well as memory and cognition [17]. So, alterations in either the visual ventral system or the limbic system could be functionally epilepsy-related and may play a central role in the pathogenesis of the anomalies of cognitive and executive functions found in RE patients.

In conclusion, rolandic epilepsy should not be considered entirely free from subtle effects on cognitive functions. Decision-making alterations have to be searched and could be functionally related to alterations in the ventral visual system. In any case, from a practical point of view, these minor neuropsychological deficits do not impair learning nor quality of life in these children. So, there is the need for long-term studies of patients followed to adulthood before drawing conclusion on the possible importance of minor cognitive problems.

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CLINICAL GENETICS

Letter to the Editor

Alström syndrome: intrafamilial phenotypic variability in sibs with a novel nonsense mutation of the *ALMS1* gene

To the Editor:

Alström syndrome (ALMS, OMIM 203800) is an autosomal recessive disease whose main clinical features are pigmentary retinal dystrophy, usually occurring in infancy; sensorineural hearing loss; obesity; non-insulin dependent diabetes mellitus (NIDDM), generally developing later in childhood. Other reported clinical findings include short stature; hypogonadism; *acanthosis nigricans*; renal, respiratory, cardiac, and hepatic failure (1, 2). The gene mutated in ALMS patients, *ALMS1*, has recently been identified (3, 4).

We report clinical and molecular findings of a couple of sibs affected by ALMS. Major clinical differences are shown in Table 1. By the age of 3 months, patient 1 suffered from bronchial non-atopic asthma, which had a poor response to conventional therapy. Clinical evaluation at 9 years disclosed sensorineural hearing loss and *acanthosis nigricans* at the neck. NIDDM developed at 9 years 8 months. Cardiologic impairment has never been detected from the first clinical evaluation at 3 months of age. He is currently 10 years old.

Patient 2 was observed at 2 years 1 month because of severe dyspnea. A dilated cardiomyopathy was diagnosed. Medical treatment with saluretics and digitoxin was started and produced progressive improvement. At 4 years the therapy was interrupted. Bronchial obstruction was never evidenced. He is currently 6 years 3 months.

Molecular analysis of *ALMS1* gene (GeneBank accession number AJ417593) was performed in both sibs. As most of the reported mutations are harbored within exons 10 and 16, we first analyzed these two exons and corresponding intron–exon boundaries by direct sequencing of polymerase chain reaction (PCR) products from genomic DNA of both patients, their parents, and controls. Primers used and PCR conditions are summarized in Table 2. A C–G substitution at position 11,571 in exon 16 was found in homozygosity in both sibs. This change causes a nonsense mutation of a tyrosine residue at position 3820 (Y3820X). The mutation was present in heterozygosity in both parents and was not found in 50 healthy controls.

Sensorineural hearing impairment, NIDDM, and *acanthosis nigricans*, observed only in patient 1, are probably age-related and should not be considered as real differences in the clinical pictures of the two sibs.

Non-atopic asthma is described in a few ALMS patients and is proposed as a specific clinical sign of ALMS (2). However, further clinical data are needed to determine its real incidence and clinical progression in the syndrome.

Dilated cardiomyopathy is reported in some ALMS patients (1, 2, 5). In some cases, it represents the first evidenced and most important finding, possibly leading to heart failure [(1, 5) and patient 2 in the present report]. In other patients, cardiomyopathy is occasionally observed during routine cardiac evaluation (5).

Molecular characterization led to the identification of a novel mutation (11571C > G) in *ALMS1* gene, determining a nonsense change (Y3820X) in exon 16. This mutation represents the closest to the C-terminal described so far and produces a

Table 1. Clinical features of ALMS patients from present report

Patient	1	2
Sex	Male	Male
Age at first observation	4 years	2 years 1 month
Generalized obesity	+	+
Short stature	–	–
Pigmentary retinopathy	+	+
Dilated cardiomyopathy	–	+
Non-insulin dependent diabetes mellitus	+	–
Acanthosis nigricans	+	–
Sensorineural hearing impairment	+	–
Asthma	+	–
Renal involvement	–	–
Hepatic involvement	–	–
Psychomotor/mental retardation	–	–

Table 2. Exon 10 and 16 primers and PCR amplification conditions used to perform molecular analysis of *ALMS1* gene

Name	Exon	Sequence	Annealing temperature
ALMS1–10aF		GTTTATAACTACTTGGACTAC	
ALMS1–10aR	10	CACAGATGCTCCAGTACACTG	53 °C
ALMS1–10bF		CTGTTGTTAAGGTTGGTGTTAC	
ALMS1–10bR	10	CATTTGATGATCTACATATG	52 °C
ALMS1–10cF		CGAGAACTCTTTGAACAGTGC	
ALMS1–10cR	10	CAAAGTCCATCCAGCTTGCTTG	59 °C
ALMS1–16aF		GTATTTCTAAACAGAATGC	
ALMS1–16aR	16	GAGACCTGGAGAGAATGTG	52 °C
ALMS1–16bF		GAACTGTCCTTGGTGGACCG	
ALMS1–16bR	16	GCAGTCACATTGCCAGATG	56 °C

truncated protein lacking the last part of C-terminus. Other nonsense mutations in exon 16 have been described (3, 4), thus indicating that the carboxyl-terminal portion represents a functionally important region of the protein.

As to genotype–phenotype correlation in ALMS, there are presently few clinical data available from molecularly characterized patients (3). A different disease progression was observed only in two unrelated patients, both homozygous for the 10775delC mutation. The first presented with recurrent episodes of dilated cardiomyopathy occurring in infancy, without signs of hepatic dysfunction at 18 years, and the second developed hepatic failure at age 20 and suffered from a single episode of infantile dilated cardiomyopathy. Our sibs show a difference both in their clinical presentation and course, suggesting that genetic and/or non-genetic factors probably interact with the *ALMS1* gene. Since both sibs always shared the same environment, genetic modifiers are likely to make the greatest contribution to phenotypic differences.

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Clinical Report

Mild Wolf–Hirschhorn Phenotype and Partial GH Deficiency in a Patient With a 4p Terminal Deletion

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Wolf–Hirschhorn syndrome (WHS) is caused by a variably-sized deletion of chromosome 4 involving band 4p16 whose typical craniofacial features are "Greek warrior helmet appearance" of the nose, microcephaly, and prominent glabella. Almost all patients show mental retardation and pre- and post-natal growth delay. Patient was born at term, after a pregnancy characterized by intra-uterine growth retardation (IUGR). Delivery was uneventful. Developmental delay was evident since the first months of life. At 2 years, he developed generalized tonic-clonic seizures. Because of short stature, low growth velocity and delayed bone age, at 4 years he underwent growth hormone (GH) evaluation. Peak GH after two provocative tests revealed a partial GH deficiency. Clinical observation at 7 years disclosed a distinctive facial appearance, with microcephaly, prominent eyes, and beaked nose. Brain MRI showed left temporal mesial sclerosis. GTG banded karyotype was normal. Because of mental retardation, subtelomeric fluorescence in situ hybridization (FISH) analysis was performed, disclosing a relatively large deletion involving 4p16.2 → pter (about 4.5 Mb), in the proband, not present in the parents. The smallest deletion detected in a WHS patient thus far includes two candidate genes, *WHSC1* and *WHSC2*. Interestingly, that patient did not show shortness of stature, and that could be due to the haploinsufficiency of other genes localized in the flanking regions. Contribution of GH alterations and possible GH therapy should be further considered in WHS patients.

KEY WORDS: GH; 4p deletion; Wolf–Hirschhorn

INTRODUCTION

Variably-sized deletion of the distal portion of the short arm of chromosome 4 usually results in Wolf–Hirschhorn syndrome (WHS). WHS is characterized by typical craniofacial features consisting of "Greek warrior helmet appearance" of the nose, microcephaly, prominent glabella, ocular hypertelorism, epicanthus, micrognathia, and poorly formed ears [Wilson et al., 1981; Battaglia et al., 2000]. Mental retardation of variable degree is always present [Battaglia et al., 2001]. Seizures occur in the majority of children and are either unilateral clonic or tonic, or generalized, frequently triggered by fever and usually associated with distinctive electroencephalographic abnormalities [Battaglia et al., 2001]. Structural central nervous system defects are also reported [Lazjuk et al., 1980; Battaglia et al., 2000]. Skeletal anomalies, hearing loss, heart defects, and urinary tract malformations have been reported only in a part of the patients, but all patients present with pre- and post-natal growth deficiency, despite adequate energy and protein intake [Estabrooks et al., 1995; Battaglia et al., 2001]. WHS could be clinically misdiagnosed because it partially overlaps with other disorders, including CHARGE association, and Smith–Lemli–Opitz [Battaglia et al., 2001]. The similarity in the size of the critical regions between Pitt–Rogers–Danks (PRD) syndrome and WHS in combination with the phenotypic similarities of the two syndromes suggested that they represent the clinical spectrum of the same condition [Wright et al., 1998]. A critical region, namely WHS critical region (WHSCR), has been considered responsible for the WHS phenotype. Recently, a new critical region (WHSCR-2, more terminal than WHSCR) has been identified and suggested to contribute to the basic WHS phenotype, consisting of typical facial appearance, mental retardation, seizures, congenital hypotonia, and growth delay [Zollino et al., 2003]. Diagnosis of WHS by conventional cytogenetic analysis (routine and high-resolution) is successful in only 60–70% of patients whereas fluorescence in situ hybridization (FISH) using a WHSCR probe detects more than 95% of deletions in WHS [Battaglia et al., 2001]. We report on a patient carrying a 4p terminal deletion presenting with a mild WHS phenotype and partial GH deficiency.

CLINICAL REPORT

The patient, B.G., is a male, born at term, after a pregnancy characterized by intra-uterine growth retardation (IUGR). Delivery was uneventful. Developmental delay was evident since the first months of life. He sat at 13 months and walked alone at 22 months. Parents reported beginning of language at about 3 years and 6/12. At 2 years, he developed generalized tonic-clonic seizures. At the age of 4 years, height was 91.3 cm (less than 5th centile), weight was 10.900 g (less than 5th centile), head circumference was 46 cm (less than 5th centile). Because of short stature quite below his target height (175 ± 5 cm), delayed bone age (corresponding to 2 years), and impaired growth velocity (4.8 cm/year, at the 3rd centile for age), an endocrine evaluation was performed. Growth hormone (GH) reserve was investigated by both arginine and clonidine GH provocative tests. Both tests showed partial GH deficiency, GH

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peak being 6.1 and 7.8 ng/ml, respectively (normal range: more than 10 ng/ml). Serum IGF-I level was also low, being 35 ng/ml (less than 2 SD). Other pituitary hormones as well as thyroid and adrenal hormones were within normal limits. No feeding difficulties occurred. Brain MRI, before and after gadolinium administration, showed left temporal mesial sclerosis. The results of heart and kidney ultrasonographic evaluations were normal.

The child's mental and psychomotor development had been assessed at 5 9/12 years using the Griffiths mental development scales [Griffiths, 1996]. The Griffiths is divided into five subscales (locomotor, personal–social, hearing and language, eye–hand coordination, and performance). His general developmental quotient was 73 (mean 100.18, SD 12.76) with worse performances in personal–social, coordination, and expressive language areas. Clinical observation of the patient at 7 years disclosed a typical facial appearance, with microcephaly, prominent eyes, and beaked nose (Fig. 1). Despite the GH deficiency, parents refused medical treatment with GH.

CYTOGENETIC ANALYSIS

Cytogenetic investigation by standard and high-resolution G banding on peripheral blood lymphocytes from the patient and his parents was performed, following standard procedures. FISH was performed on metaphase spreads from peripheral blood lymphocytes using standard techniques with co-denaturation. Chromosomes were counterstained with DAPI (Vysis, Downers Grove, IL). The Multiprobe T (Cytocell, Celbio, Milan, Italy) was used to investigate the subtelomeric regions of all the chromosomes. A commercial DNA probe wcp4 (Bouty, Milan, Italy) for the whole four chromosome was used to detect rearrangements involving chromosome 4. Specific regions of chromosome 4 were hybridized with the commercial probe LSI WHS (Vysis), for the Wolf–Hirschhorn critical region that maps in 4p16.3 and with a probe from the BAC RP11-323F5 (rocchi@biologia.uniba.it), which maps in 4p16.2 at 4.5 Mb from the telomeric end (http://www.ensembl.org). The probe was extracted from BACs with standard methods [Birnboim, 1993] and then labeled by nick translation with FluorX-dCTP (Amersham Pharmacia Biotech, Milan, Italy). Image acquisition was performed using a Leica Aristoplan Microscope with a Photometrics CCD camera. Images were elaborated using Power Gene McProbe software version. 4.3 (Applied Imaging Corporation, Santa Clara, CA).

RESULTS

Standard cytogenetic analysis at 500 banding resolution did not show chromosome anomalies in both the proband and his parents. Subtelomeric FISH analysis in the patient was requested because of the presence of mental retardation, facial dysmorphisms, IUGR, and post-natal growth retardation. The FISH with Multiprobe T demonstrated that the subtelomeric region of the short arm of one chromosome 4 was missing in the

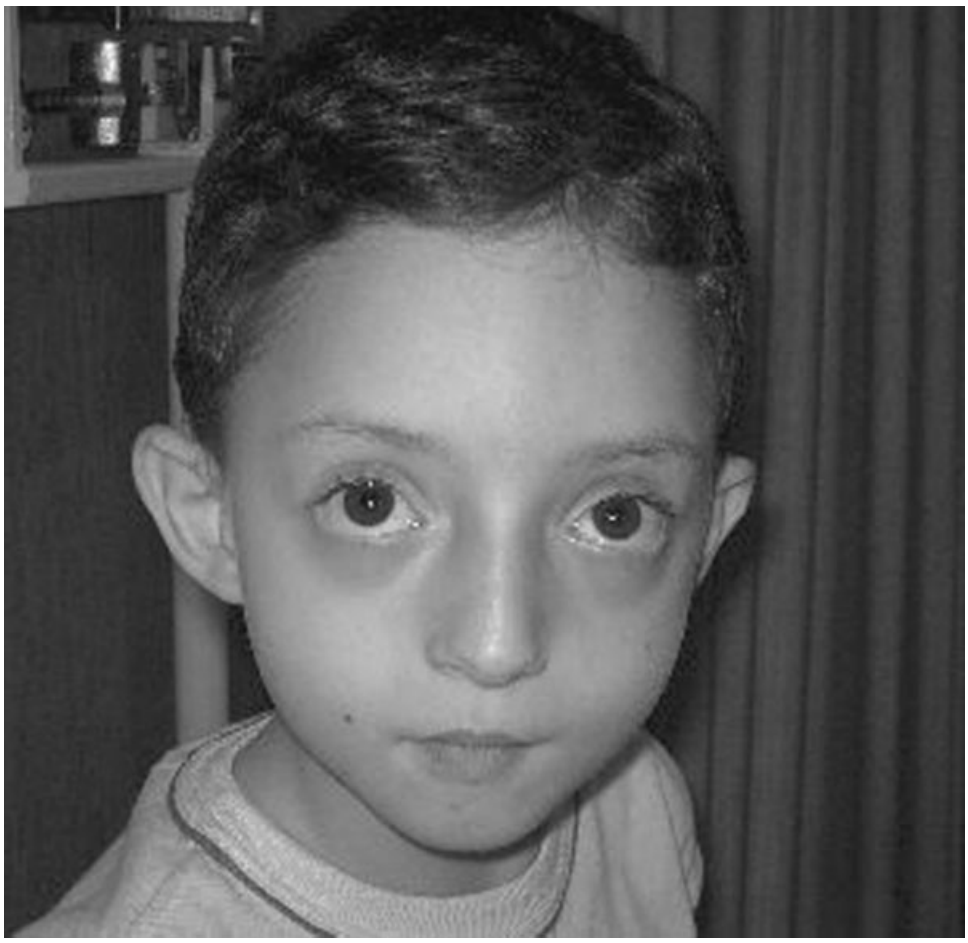

Fig. 1. The patient at 7 years: note the facial appearance with microcephaly, protruding eyes, and prominent nose.

patient. Painting of chromosome 4 demonstrated that the 4p deleted region was not translocated to any other chromosome. The hybridization with the LSI WHS probe showed that the WHSCR was deleted. FISH analysis with the probe from the BAC RP11-323F5 demonstrated that the deletion involved the band 4p16.2 (Fig. 2), thus extending at least 4.5 Mb at 4p telomere (http://www.ensembl.org). Complete patient's karyotype was: 46,XY, del(4)(qter → p16.2:). Parents' karyotype was normal, indicating that the deletion was "de novo."

DISCUSSION

The patient described in the present report had been referred because of psychomotor retardation, seizures, short stature, and mild dysmorphic features, compatible with a Wolf–Hirschhorn/PRD phenotype. FISH analysis unraveled the presence of a 4p terminal deletion of about 4.5 Mb involving WHSCR and WHSCR-2. Although terminal 4p deletions are usually associated with a clearly recognizable WHS phenotype, variability of clinical signs associated with this condition has been reported [Battaglia et al., 2001]. Also the Seckel phenotype presents some clinical features overlapping in part WHS, in particular IUGR, post-natal growth deficiency, microcephaly, and beaked nose. A single patient with 4p deletion and Seckel-like phenotype has been described so far [Anderson et al., 1997].

WHSCR has been narrowed to 165 kb on 4p, flanked by the markers D4S166 and D4S3327, containing two genes of unknown function, *WHSC1* and *WHSC2* [Wright et al., 1997, 1999; Stec et al., 1998]. *WHSC1* spans a 90 kb genomic region and is widely expressed in mouse embryos. Its product is a DNA binding protein, most likely a transcription factor [Stec et al., 1998]. *WHSC2* spans a 26.2 kb genomic region and is ubiquitously expressed. Its function is unknown [Wright et al., 1999]. Recently, Zollino et al. [2003] described a patient with a preserved WHSCR in spite of a typical WHS phenotype thus defining a new critical region for the WHS phenotype, WHSCR-2.

In order to explain the wide phenotypic variability of WHS, investigators have searched for correlations between size of the 4p deletion and severity of clinical manifestations. Recent evidence from the eight patients reported by Zollino et al. [2003] suggest that small deletions tend to be associated with a milder phenotype with respect to both the degree of mental retardation and the presence of major malformations. They suggest that a division of WHS into a classical and a mild form, strongly correlated to the size of the deletion, could help proper genetic diagnosis and counseling. Our patient, showing a 4p deletion (at least 4.5 Mb), presented with relatively mild developmental delay and no major malformations. Thus, it seems that the extension of the deletion does not always correlate with the severity of the phenotype and that probably other genetic and environmental factors do contribute to the phenotype of this contiguous gene syndrome.

Our patient exhibited short stature and linear growth failure due to GH deficiency documented by appropriate stimulation tests. Notwithstanding this, he did not get GH treatment because of parents' refusal. GH secretion had already been evaluated in two patients previously diagnosed as PRD phenotype [Lindeman-Kusse et al., 1996]. The first patient

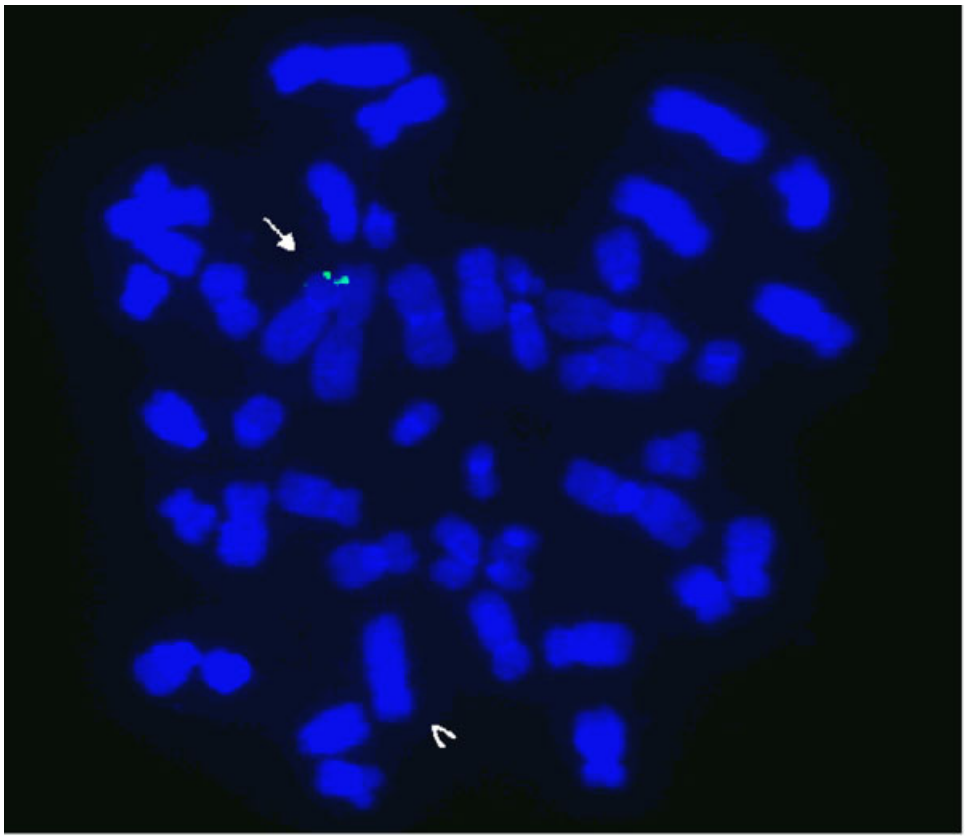

Fig. 2. Fluorescence in situ hybridization (FISH) analysis with the probe from the BAC RP11-323F5 (mapping to 4p16.2): double signal is present on normal 4p only (arrow) and is absent on the rearranged chromosome 4 (arrowhead). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

had been treated with GH in spite of a normal response to stimulation tests (arginine, peak GH 16 mU/L; L-Dopa propranolol, peak 93 mU/L) and normal serum IGF-I levels. In this patient, height increased from −5.3 to −1.8 Standard Deviation Score (SDS) after 4 years of GH treatment. In the second patient of Lindeman-Kusse et al. [1996], no evidence for endocrine dysfunction was detected. However, in an attempt to stimulate growth, he was treated with thyroxine without apparent effects.

The smallest deletion detected in a patient with WHS is 191.5 kb and includes *WHSC1* and *WHSC2* [Rauch et al., 2001]. Interestingly, the patient did not present with microcephaly, seizures, mental retardation, and shortness of stature. Neurological signs, supposed to be due to the haploinsufficiency of *LETM1* (coding for a putative EF-hand Ca^{2+}-binding protein [Burgess et al., 1997; Endele et al., 1999] and localized within the WHSCR flanking region), are not present in this patient, who preserved *LETM1* gene. He also presented with normal stature. We hypothesize that shortness of stature could be due to the haploinsufficiency of other genes localized in the regions flanking the WHSCR. Detailed breakpoint analysis in more patients with small deletions may allow further understanding of the role that other genes outside the candidate regions may play in the disease. GH pathway alterations should be further evaluated in WHS patients, also if they lack typical GH deficiency clinical features (i.e., delayed bone age, impaired growth velocity), because of possible benefits from GH therapy. Further studies are needed to better evaluate this issue.

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Original Article

Study of Multimodal Evoked Potentials in Patients With Type 1 Gaucher's Disease

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ABSTRACT

To detect early subclinical nervous dysfunction in Gaucher's disease type 1, we carried out motor, brainstem auditory, visual, and somatosensory evoked potentials in 17 patients with Gaucher's disease type 1. Central motor evoked potential abnormalities were found in nine patients (69.2%), consisting of an increased motor threshold in all, with prolonged central motor conduction time in two patients. Brainstem auditory evoked potentials were abnormal in five patients (31.2%), and the most frequent abnormality was a bilateral increased I–III interpeak latency. Visual evoked potentials showed a delayed latency of the P100 wave in four patients (25%). Somatosensory evoked potential abnormalities were found in three patients (18.7%), consisting of an increased N13–N20 interval in two patients and a not reproducible N13 wave in one patient. Our findings suggest that the multimodal evoked potential approach provides information about nervous subclinical damage in Gaucher's disease type 1; transcranial magnetic stimulation proved to be the most sensitive tool. Early detection of subclinical neurologic dysfunction can be useful in view of more effective therapeutic strategies. (*J Child Neurol* 2005;20:124–128).

Gaucher's disease is a lipid storage disorder caused by the deficiency of the lysosomal enzyme β-glucocerebrosidase. The phenotypic spectrum of the disease is characterized by extreme variability. The presence of neurologic involvement is considered of major importance, together with the age at onset of symptoms and the disease course, in classifying patients with Gaucher's disease into different clinical categories, namely the non-neuronopathic (type 1) and the acute (type 2) or chronic (type 3a, -b, and -c) neuronopathic forms.[1] The molecular bases of clinical variability in Gaucher's disease have been extensively studied.[2] It has been shown that the most common mutation, N370S, is protective against neurologic involvement and is associated with Gaucher's disease type 1, whereas homoallelism for other mutations, such as the L444P, is generally associated with the neuronopathic forms of the disease.[2,3] However, atypical central nervous system disease was recently described in older patients with Gaucher's disease type 1,[4,5] pointing to the possible occurrence of neurologic involvement also in association with genotypes not considered at risk of this manifestation.

Gaucher's disease is the first lysosomal storage disorder for which enzyme replacement therapy has been developed using extractive (alglucerase) or recombinant (imiglucerase) β-glucocerebrosidase.[6] The effectiveness of enzyme replacement therapy in reverting hematologic, skeletal, and visceral symptoms has been clearly demonstrated. On the other hand, the efficacy of enzyme replacement therapy in patients with neurologic involvement is still debated,[7–9] and therapeutic regimens using higher doses of β-glucocerebrosidase are commonly required in these patients.

The detection of neurologic involvement in patients with Gaucher's disease is therefore crucial to determine the prognosis of patients and to establish the appropriate therapeutic regimen. Besides a careful clinical evaluation, approaches such as the study of saccadic movements have been used to detect signs of neurologic disease.[10] Evoked potentials have been considered a sensitive and noninvasive approach to detect subclinical neurologic involvement in other lysosomal diseases, such as mucopolysaccharidoses[11] or mucolipidoses,[12] and in a few studies in Gaucher's disease.[8,13–18] These studies have generally been performed in single patients and have evaluated only one evoked potential modality.

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In the present study, for the first time, a group of patients with Gaucher's disease type 1 was investigated by a multimodal evoked potential approach, including transcranial magnetic stimulation of the motor cortex, brainstem auditory, visual, and somatosensory evoked potentials. Early detection of subclinical nervous dysfunction may have implications for the treatment of these patients, and may represent an indication for the increase of enzyme replacement dosage.

PATIENTS AND METHODS

Patients

To evaluate whether patients with Gaucher's disease type 1 have a subclinical central nervous dysfunction, 17 patients, 12 adults (8 females and 4 males; mean age 31.2 years; range 17–48 years) and 5 children (3 females and 2 males; mean age 7 years; range 3–12 years), affected by Gaucher's disease type 1 were examined (Table 1). The diagnosis was performed by enzymatic assay in all patients and confirmed by molecular analysis of the β-glucocerebrosidase gene in 15 patients. Three patients were homoallelic for the L444P mutation. Fourteen patients were treated with enzyme replacement therapy (imiglucerase mean dosage 74.0 U/kg/month; range 48–130 U/kg/month). All patients were classified on clinical grounds as having non-neuronopathic (type 1) Gaucher's disease. The neurologic examination was normal in all patients. No abnormalities of eye movements were detectable in patients homoallelic for the L444P mutation.

Methods

Motor Evoked Potential Recording

A magnetic stimulator (Dantec Mag Pro, Skovlunde, Denmark) producing a monophasic transient magnetic field of 1.9 Tesla at maximum field strength was used for transcranial magnetic stimulation of the motor cortex. The circular coil (outer diameter 14 cm) was placed tangentially on the skull, centered over the vertex; an anticlockwise current circulation in the coil was used to stimulate the left hemisphere. For recording muscle evoked potentials, the transcranial magnetic stimulation intensity used was 20% above motor threshold (the threshold was defined as the stimulus intensity required to produce a motor evoked potential at rest of at least 100 μV amplitude in about 50% of 10 consecutive stimuli). To obtain motor evoked potentials in contracted right abductor pollicis brevis muscle (active electrode placed on the muscle belly and reference electrode on tendon), adhesive disposable surface electrodes (Medtronic 13L0202, Skovlunde, Denmark) were used. During recording by Dantec Counterpoint electromyograph, active muscular contraction (20% of maximum voluntary contraction) was maintained with the help of auditory and visual electromyographic feedback. Nine patients were stimulated at 100% of the stimulator output because in these patients the motor threshold was higher than 80% of the maximum output. Filter bandpass was 10 to 2000 Hz. The shortest onset latency and the largest peak-to-peak amplitude of four consecutive motor evoked potentials were measured. Radicular responses were obtained in the abductor pollicis brevis muscle at rest, stimulating cervical roots by positioning the magnetic coil in the midline over the lower cervical vertebrae until the longest latency. Central motor conduction time was the difference between cortical motor evoked potentials and radicular responses latencies.

Brainstem Auditory Evoked Potential Recording

Brainstem auditory evoked potentials were performed in a soundproof room, and each ear, in turn, was stimulated using condensant clicks resulting from a 100 μs monophasic square electrical pulse, at 65 dB above the individual click hearing threshold, at a stimulation rate of 11 Hz. The responses were recorded, via a surface disk electrode, 11 mm in diameter (Medtronic 13L29), filled with conductive Electro-Gel electrode paste (Electro-Cap International, Inc, Eaton, OH), with impedance kept below 5000 ohms, placed on the scalp vertex and referred to the ipsilateral and contralateral earlobes to the stimulation site electrodes; the contralateral ear was stimulated by a white masking noise. Two consecutive sets of 2000 responses were averaged and superimposed for each ear using a conventional Dantec Counterpoint electromyograph with a bandpass of 100 to 2000 Hz. The latency of the first five positive peaks and the I–III, I–V, and III–V interpeak latencies were measured.

Visual Evoked Potential Recording

Visual evoked potentials were obtained, stimulating each eye, in turn, with a checkerboard reversal pattern, produced by a Dantec Counterpoint electromyograph, at a frequency of 1.5 Hz. The visual angle subtended by the single checks and by the whole monitor measured 30 minutes and 18 degrees, respectively. One uncooperative patient (case 17) underwent white flash visual evoked potentials, obtained by a stimulator (Biomedica Mangoni, Pisa, Italy, Flashtube power supply BMI 1621) at an intensity of 5 J and a frequency of 1 Hz. Visual evoked potentials were recorded by surface disk electrode (Medtronic 13L29), 11 mm in diameter, filled with electrogel (impedance kept below 5000 ohms), placed on Oz and Fpz of the International 10-20 system. Two consecutive sets of 100 responses were averaged and superimposed. The filter bandpass was 0.5 to 100 Hz. The P100 latency of the negative-positive-negative complex (N75-P100-N145) was measured, and the amplitude was calculated peak to peak from the N75 to the P100 wave. The P2 latency of the white flash visual evoked potentials was measured.

Somatosensory Evoked Potential Recording

Somatosensory evoked potentials to electrical stimulation of the right median nerve at the wrist were recorded by Dantec Counterpoint electromyograph, via surface disk electrode (Medtronic 13L29), 11 mm in diameter, filled with electrogel. Recordings were obtained from the Erb's point ipsilateral to stimulation (Erb's potential) referred to contralateral Erb's point, the seventh cervical spine (N13 wave) referred to an anterior cervical electrode (AC), ipsilateral centroparietal (CPi) (P14 and N18 waves) referred to contralateral Erb's point, and contralateral centroparietal (CPc) (N20 wave) referred to CPi of the International 10-20 system. Electrode impedance was below 5000 ohms. The pulse duration was 0.2 milliseconds, and the stimulation rate was 3 Hz. The bandpass was 10 to 2000 Hz. Two sets of 500 responses were averaged and superimposed. The latency and amplitude of the Erb's potential; N13, P14, N18, and N20 waves; and N13–N20 interval (sensory central conduction time) were measured. Motor, brainstem auditory, visual, and somatosensory evoked potential findings were compared with age-matched control data obtained by the same techniques in our laboratory. All of the latency values deviating by more than 2.5 SD from the mean control value were considered abnormal, as were amplitude values below the minimum control value and threshold values of motor evoked potentials above the maximum control value.

RESULTS

Motor, brainstem auditory, visual, and somatosensory evoked potentials findings are summarized in Table 1. Motor evoked potentials were performed in 13 patients (9 adults, 4 children) (Table 2) and were found to be abnormal in 9 of them (69.2%). Of the nine adult patients, four showed normal motor evoked potential findings (cases 1, 7, 8, and 9), whereas the other five patients (cases 2, 5, 6, 10, and 12) showed an increased motor threshold with associated prolongation of the central motor conduction time in two of them (cases 6 and 12) (Figure 1). In all four children (cases

Table 1. Clinical and Electrophysiologic Findings in 17 Patients With Gaucher's Disease Type 1

	Clinical Findings				*Electrophysiologic Findings*			
Case No.	*Age (yr)*	*Sex*	*Genotype*	*ERT*	*MEP*	*BAEP*	*VEP*	*SEP*
Adults								
1	37	F	N370S/L444P	+	N	A	A	N
2	20	F	NP	+	A	NP	NP	N
3	17	F	N370S/IVS2G-A	+	NP	N	A	N
4	27	F	R353G/R353G	−	NP	N	A	A
5	26	F	R353G/R353G	+	A	N	N	N
6	27	M	1053T/?	+	A	A	N	N
7	26	F	N370S/L444P	+	N	A	N	N
8	38	M	N370S/L444P	−	N	A	N	N
9	36	F	NP	+	N	N	N	A
10	30	M	L444P/?	+	A	N	N	N
11	48	F	L444P/?	−	NP	N	N	N
12	42	M	N370S/N370S	+	A	N	N	A
Children								
13	6	F	L444P/L444P	+	A	N	N	N
14	8	M	L444P/L444P	+	A	N	N	N
15	6	M	N370S/IVS2G-A	+	A	N	N	N
16	12	F	N370S/del	+	A	N	N	N
17	3	F	L444P/L444P	+	NP	A	A	NP

A = abnormal; ERT = enzyme replacement therapy; MEP, BAEP, VEP, SEP = motor, brainstem auditory, visual, and somatosensory evoked potentials; N = normal; NP = not performed; + = on ERT; − = without ERT.

13, 14, 15, and 16), we could not obtain motor evoked potentials at rest, whereas central motor conduction time was normal.

Brainstem auditory evoked potentials were performed in 16 patients (11 adults and 5 children) (see Table 1) and were found to be abnormal in 5 of them (31.2%). Of the 11 adult patients, 4 showed electrophysiologic abnormalities consisting of bilateral increased I–V interpeak latency (case 1), bilateral increased I–III interpeak latency (cases 6 and 7), and bilateral increased I–III interpeak latency and monolateral I–V interpeak latency (case 8). Of the five children studied, one patient (case 17) showed an irregular waveform potential with a bilateral increased I–III interpeak latency (Figure 2).

Visual evoked potentials were performed in 16 patients (11 adults and 5 children) and found to be abnormal in 4 of them (25%). Electrophysiologic abnormalities in adults consisted of an increased interocular P100 latency difference in one patient (case 1) and prolongation of P100 latency to stimulation of both eyes in two patients (cases 3 and 4) (Figure 3). A prolongation of P_2 latency to stimulation of both eyes was found in one pediatric patient (case 17).

Somatosensory evoked potentials were performed in 16 patients (12 adults and 4 children) and were abnormal in 3 patients, all adults (18.7%). Two of them (cases 4 and 9) showed an increased N13–N20 interval, with absence of P14 and N18 waves in case 4 (Figure 4); in the latter patient (case 12), the N13 wave was not reproducible.

DISCUSSION

We used a multimodal evoked potential approach to study a series of patients with Gaucher's disease type 1 without overt neurologic symptoms. Until now, only a single evoked potential modality was applied to patients with Gaucher's disease type 2 or 3, showing clinically evident central nervous system involvement of a variable degree.[8,13,14,16]

Progressive deterioration of auditory evoked potentials at the brainstem level was observed in a patient with acute Gaucher's disease.[13] In another patient with acute Gaucher's disease, a correlation between brainstem auditory evoked potential abnormalities and brainstem pathology was found.[14] More recently, an improvement in brainstem auditory evoked potentials was observed in a patient with Gaucher's disease type 3b after a year of enzyme replacement therapy.[8] Another study showed brainstem auditory evoked potential alterations in nine patients with Gaucher's disease type 3.[15]

Somatosensory evoked potential abnormalities, consisting of giant potentials, were observed in a case with Gaucher's disease type 3a.[16] A recent study showed in 18 patients with Gaucher's disease type 3 enlarged stretch somatosensory evoked potentials (both in patients with and without progressive myoclonic epilepsy) compared with normal stretch somatosensory evoked potential findings in six patients with Gaucher's disease type 1; furthermore, in the same study, a significant negative correlation between stretch somatosensory evoked potential amplitude and the IQ in the patients with Gaucher's disease type 3 was found.[17] In a pre-

Table 2. Motor Evoked Potential Findings in 13 Patients With Gaucher's Disease Type 1

Case No.	*Age (yr)*	*MEP Threshold (%)*	*CMCT (ms)*
Adults			
1	37	70	8.0
2	20	**ND**	6.2
5	26	**95**	7.0
6	27	**90**	**9.8**
7	26	70	8.0
8	38	80	5.2
9	36	80	7.8
10	30	**90**	6.2
12	42	**ND**	**9.8**
Children			
13	6	**ND**	7.8
14	8	**ND**	7.0
15	6	**ND**	5.1
16	12	**ND**	8.0
Normal values			
Adults		≤ 80	6.1 ± 0.77
5–8 yr		≤ 100	
11–13 yr		≤ 80	
6–12 yr			6.4 ± 1.2

CMCT = central motor conduction time; MEP = motor evoked potentials; ND = not detectable MEP at rest.
Abnormal values are in boldface.

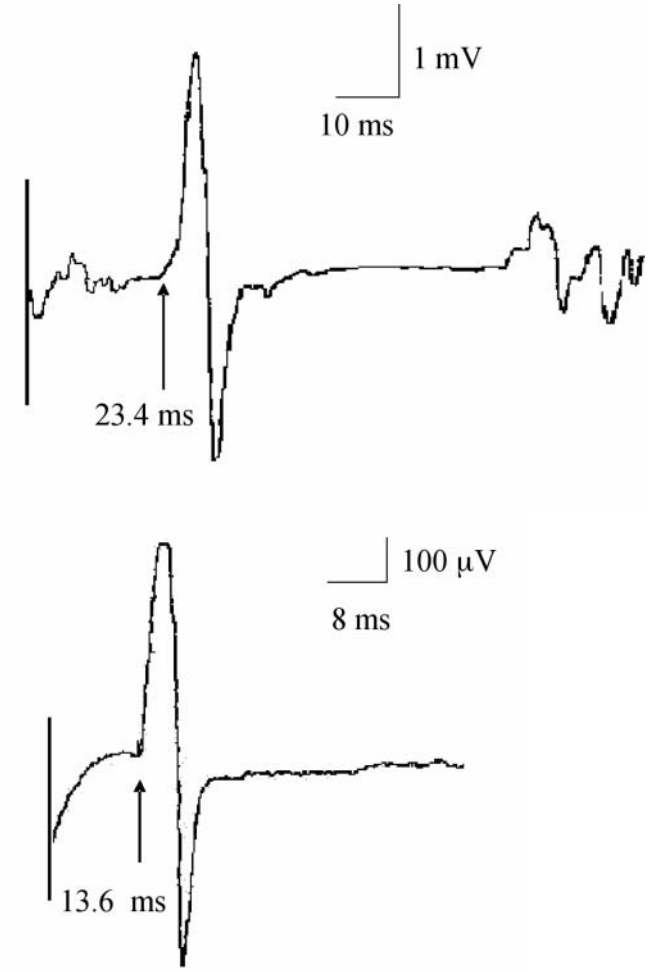

Figure 1. Motor evoked potentials obtained from abductor pollicis brevis muscle by cortical magnetic stimulation (*upper trace*) and cervical magnetic stimulation (*lower trace*) in patient 12, with N370S/N370S mutation. Note the increased latency of the cortical potential with consequent prolonged central motor conduction time (9.8 milliseconds; upper normal value = 8.0 milliseconds).

vious article, we described the presence of electrophysiologic abnormalities in two sibs with Gaucher's disease, homoallelic for a novel mutation of the β-glucocerebrosidase gene.[18]

Multimodal evoked potentials allow us to investigate, by means of noninvasive techniques, different nervous pathways. In view of the therapeutic possibilities, evoked potential investigation can be advantageous for early detection and monitoring of subclinical neurologic dysfunction.[11,12,18]

Transcranial magnetic stimulation of the motor cortex, which explores the pyramidal pathways,[19] was particularly sensitive in our patients because it showed an altered central motor excitability (increased motor threshold) in about 70% of the patients examined. In addition, two patients showed a prolonged central motor conduction time, indicating a slowed conduction along the central motor pathway. The increased motor threshold found in our patients can be the result of an altered excitability of the corticospinal motor system, which might be due to a dysfunction of the motor cortex or to an altered modulation of the motor cortex by basal ganglia. The postnatal maturation process of the corticospinal system, which continues until puberty,[20,21] and the more considerable fluctuation of the threshold for cortical activation in childhood[22] could also be implied in the absence of motor evoked potentials at rest in our pediatric patients. There are some observations against these last hypotheses: first, the persistence of absent motor evoked potentials or of a higher motor threshold, at the follow-up, in cases 3 and 14 (personal unpublished data, 2002); second, the presence of an abnormal motor threshold also in some of our adult patients. In conclusion, we think that motor threshold abnormalities and the prolongation of central motor conduction time, found in our patients, can represent a dysfunction of or damage to the motor corticospinal system.

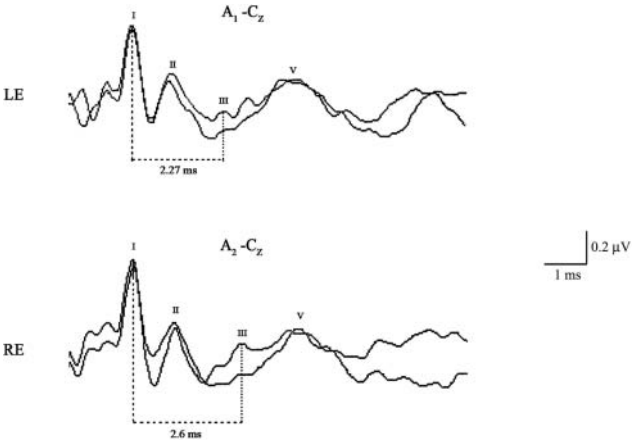

Figure 2. Brainstem auditory evoked potentials from patient 17, with L444P/L444P mutation. Note a bilateral increasing of the I–III interpeak latency (upper normal value = 2.20 milliseconds) and the irregular morphology of the potentials owing to a poor definition of III and V waves. LE = left ear; RE = right ear.

About 30% of the patients examined showed brainstem auditory evoked potential abnormalities of a moderate degree. The most frequent finding was an increased I–III interpeak latency, which suggests a conduction defect in the brainstem auditory system, from the cochlear portion of the eighth nerve across the subarachnoid space into the lower pons.[23] The concomitant presence of a normal wave I in all of our patients excluded damage to the eighth nerve close to the cochlea.[23] Visual and somatosensory evoked potential abnormalities were present but less frequent in our patients (25% and 18.7%, respectively). Visual evoked potential findings suggest a slowed conduction along the visual pathways, but we could not define the level of damage. The increased N13–N20 interval, found in two patients, indicates a slowed conduction in the somatosensory central pathway.

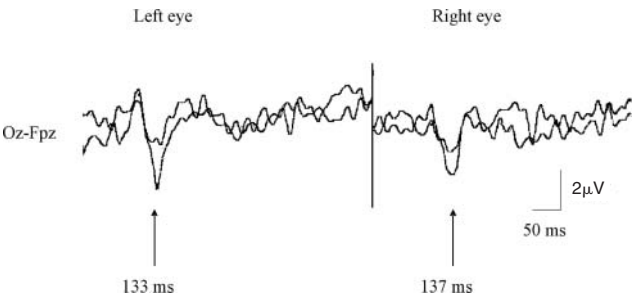

Figure 3. Reversal pattern visual evoked potentials from patient 3, with N370S/IVS2G-A mutation. Note the delay of the P100 wave to stimulation of both eyes (upper normal value = 113.0 milliseconds).

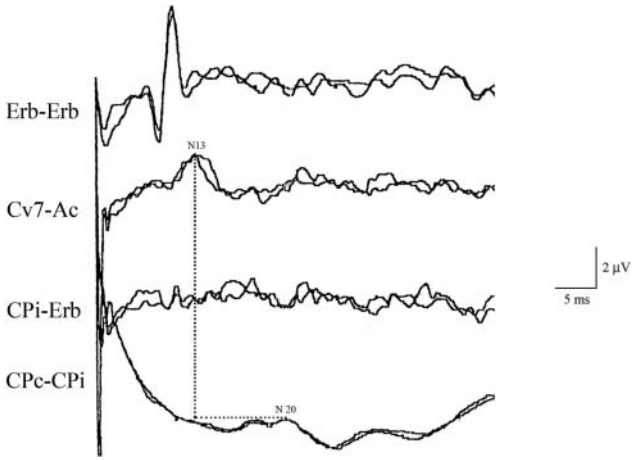

Figure 4. Somatosensory evoked potentials from patient 4, with R353G/R353G mutation. Note the increased N13–N20 interval (10.9 milliseconds; upper normal value = 7.4 milliseconds) and the absence of the P14–N18 components.

Among our patients, electrophysiologic abnormalities were found both in patients homoallelic for the "neuronopathic" L444P mutation and in patients with different genotypes. The presence of abnormalities in almost all patients carrying the N370S mutation, which is considered protective against central nervous system damage, is of particular interest. The recent finding of atypical neurologic disease (parkinsonism) in patients with Gaucher's disease type 1 might suggest that neurologic involvement is not confined to the neuronopathic types 2 and 3 and that the patient's age might contribute to the development of neurologic disease.[24] The higher incidence of neurophysiologic abnormalities in the central motor system is in agreement with the above-mentioned clinical observations; moreover, the higher sensitivity of transcranial magnetic stimulation could be explained by the different site of stimulation. In fact, the motor cortex, which is particularly sensitive to metabolic damage, is directly involved by the magnetic stimulus; in contrast, when we explored the other pathways, the stimulus was applied on the peripheral receptors, which are less vulnerable to a metabolic insult. In conclusion, motor evoked potentials might be a highly sensitive technique in revealing subclinical involvement of the central nervous system in Gaucher's disease type 1.

The presence of electrophysiologic abnormalities also has implications for enzyme replacement therapy. Increased dosages of recombinant β-glucocerebrosidase are recommended for the treatment of patients with the chronic neuronopathic Gaucher's disease type 3.[9] If electrophysiologic abnormalities indicate an increased risk of the development of clinically evident neurologic disease, the use of a high-dose regimen could also be considered in patients with Gaucher's disease type 1 showing such abnormalities. Alternatively, substrate reduction with miglustat, which crosses the blood–brain barrier and is potentially useful in patients with neurologic involvement, might be used in combination with enzyme replacement therapy to prevent the progression of central nervous system disease.

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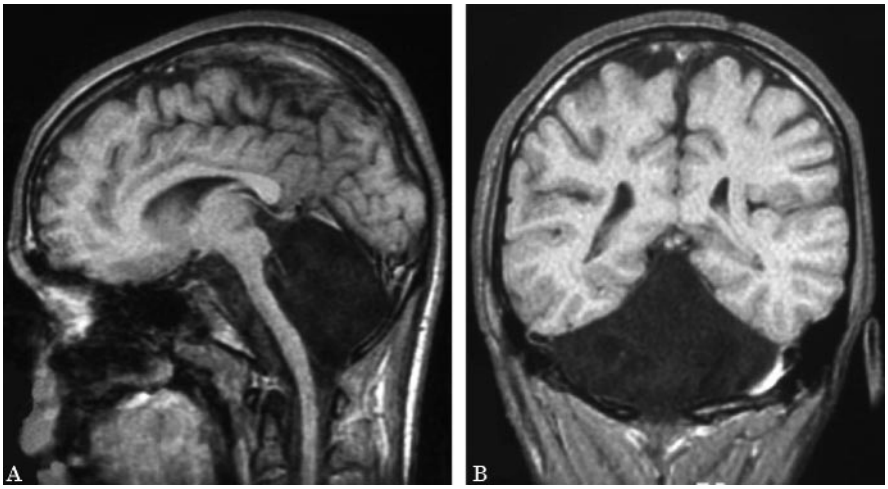

Figure. T1-weighted sagittal (A) and coronal (B) brain MRIs showing total cerebellar agenesis.

Cerebellar agenesis

Luigi Titomanlio, MD; Alfonso Romano, MD; and Ennio Del Giudice, MD, Naples, Italy

A 17-year-old boy with a history of neonatal hypotonia was first observed by us at age 4 years because of persistent ataxia. Brain MRI revealed isolate cerebellar agenesis (CA) (figure), the empty cerebellar space having its signal similar to CSF in all performed sequences. At age 17 years he showed moderate ataxia (video 1), mild dysmetria (video 2), and no nystagmus. Neuropsychological evaluation evidenced mild mental retardation. Further examination was normal. He attends a normal school, to which he gets by bicycle.

Total or subtotal CA is an extremely rare congenital defect and is thought to be associated with profound deficits in movement.[1] Clinical presentation ranges from early death to variable degrees of cerebellar dysfunction.[2]

Reports of living patients address the question of whether CA is compatible with functional motor development. Cerebellar development occurs early during embryogenesis, so that plasticity of the remaining brain could explain functional compensation.

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Clinical Report

Cerebellar Vermis Aplasia: Patient Report and Exclusion of the Candidate Genes *EN2* and *ZIC1*

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Cerebellar vermis aplasia (ACV, OMIM 117360) is a rare malformation of the cerebellum, with only few familial patients reported so far. Main clinical features of this rare disorder include floppiness and delayed milestones in early infancy, preceding mild cerebellar ataxia, non-progressive clinical course, normal or slightly delayed intelligence, and occasional nystagmus. Neuroimaging reveals selective involvement of the cerebellum, which is prominent in the vermis. Because of the large preponderance of female patients, X-linked dominant transmission was suggested by [Fenichel and Phillips (1989); Arch Neurol 46:582–583], and subsequent reports only concern female patients. Only one family with male-to-male transmission presenting with a generalized atrophy of the cerebellum rather than a more localized vermis aplasia has been reported so far. We report on a family in which father and son are affected by a mild form of ACV, thus confirming an autosomal mode of inheritance of the disease. Our patients showed a progressive improvement of their motor abilities, neurological examination of the father being actually normal except for a mild mental retardation. We also evaluated the potential role of two candidate genes, *EN2* and *ZIC1*, responsible for abnormal cerebellar development in murine knock-out models. However, molecular analysis failed to reveal any causative mutation in the coding sequence of the two genes in our patients. The understanding of the genetic basis of autosomal dominant ACV would allow a better classification of isolate cerebellar malformations and might permit to understand cell differentiation and migration in the developing central nervous system.

KEY WORDS: cerebellar vermis aplasia; *EN2*; *ZIC1*; Joubert syndrome; orthosympathetic dysfunction

INTRODUCTION

Recent availability of high-resolution MRI allows cerebellar malformation to be recognized with increasing frequency. ACV, or early-onset non progressive cerebellar ataxia (OMIM 117360), is a rare isolate cerebellar malformation, with only few patients reported [Kattah et al., 1983; Furman et al., 1985; Tomiwa et al., 1987; Fenichel and Phillips, 1989; Kornberg and Shield, 1991; Rivier and Echenne, 1992; Imamura et al., 1993] and is characterized by a selective involvement of the cerebellar vermis. Clinical features include floppiness and delayed acquisition of milestones, preceding early onset cerebellar ataxia, with a non-progressive clinical course and an occasional nystagmus. Intelligence is reported as normal or slightly delayed. Inheritance is supposed to be X-linked dominant or autosomal dominant.

We report on a family in which father and son are affected by a mild form of ACV. Moreover, we evaluated the potential role of two candidate genes, engrailed 2 (*EN2*) and zinc finger protein of cerebellum 1 (*ZIC1*) in the pathogenesis of ACV. *EN2* has been implicated in the control of pattern formation during CNS development [Kuemerle et al., 1997]. *ZIC1* encodes a member of C_2H_2-type zinc finger proteins, which play an important role during CNS development. En2 and Zic1 have been recently excluded as a causative gene of Joubert syndrome (JS, MIM 213300), a rare neurological disorder presenting with agenesis or dysgenesis of the cerebellar vermis [Blair et al., 2002; Bennett et al., 2004].

PATIENTS AND METHODS

Patient 1

SP, the father of patient 2, was born at term after a normal pregnancy. Delivery was uneventful. Developmental milestones were delayed, particularly for gross motor functions. In infancy, he showed truncal ataxia and difficulty in manipulating small objects because of intentional tremor. Cerebellar signs progressively disappeared in the following years. At the age of 23 years he developed generalized tonic-clonic seizures (grand-mal) that required treatment with phenobarbital. Cranial MRI disclosed an isolate cerebellar vermis hypoplasia. At the age of 35 years, neurological examination and EEG were normal. Cranial MRI disclosed an isolate cerebellar vermis hypoplasia, without morphometric changes when compared with the precedent one. Other CNS structures were normal (Fig. 1). Neuropsychological evaluation disclosed mild mental retardation (IQ: 67) with similar values of verbal and performance IQs (VIQ: 69, PIQ: 70) but with a greater involvement of praxic-constructive abilities. He also suffered from orthostatic hypotension. The autonomic nervous system was evaluated by means of cardiovascular

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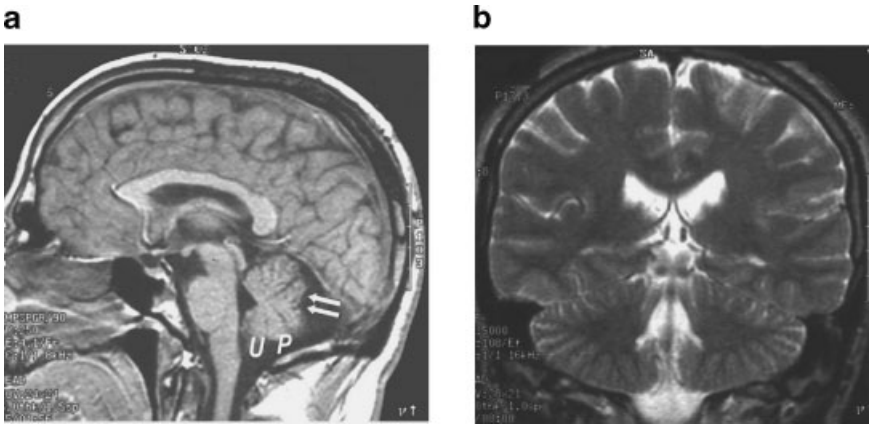

Fig. 1. Representative sagittal T1-weighted (**a**) and coronal T2-weighted (**b**) MRI images from patient 1, showing an enlarged basal cistern due to a postero-inferior cerebellar vermis hypoplasia. Folium and tuber are most obviously affected (arrowheads) than pyramid (P) and uvula (U).

tests (variation of blood pressure and heart rate in response to postural changes, Valsalva maneuver, hyperventilation, and isometric muscular contraction) and cold presser test. Blood pressure changes during isometric muscular contraction and cold test were altered, revealing a mild orthosympathetic dysfunction.

Patient 2

SR, the son of patient 1, was born at term after a normal pregnancy. He suffered from mild asphyxia at birth. Milestones were delayed. He could walk without support at the age of 18 months. At the age of 7 years he was observed because of frequent falls and rare syncopal episodes. Clinical evaluation disclosed moderate gait and truncal ataxia, incoordination and mild dysmetria. Ophthalmological evaluation evidenced mild deficit of convergence, prevalent at the left side. EEG was normal. Cranial MRI at 7 years of age showed cerebellar vermis hypoplasia (Fig. 2). In the following years, a slowly progressive improvement of coordination and ataxia was scored. At the age of 10 years he showed only mild truncal tremor. Neuropsychological evaluation revealed an IQ of 87 (VIQ 89; PIQ 88). The boy underwent the same autonomic function test administered to his father with normal overall results. On the contrary, a tilt test showed an exaggerated vagal response that could explain the syncopal episodes sometimes reported in his medical history.

Both patients were tested for trinucleotide repeat expansions causing SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA8, SCA12, and DRPLA. No mutations were found.

Methods

Candidate genes have been be selected by evaluating genes involved in cerebellar development as found in mutant mice (Mouse Genome Database: http://www.informatics.jax.org/). Genomic structure of the genes *ZIC1* and *EN2* were obtained by the analysis of public databases. Each exon was amplified by PCR performed on genomic DNA from our patients and controls. Each PCR product was sequenced by automatic system and the corresponding sequence compared with the published genomic DNA sequence of the candidate genes.

RESULTS

No mutations were found in the coding sequence of *EN2* and *ZIC1* genes.

DISCUSSION

ACV is a rarely reported cerebellar malformation [Kattah et al., 1983; Furman et al., 1985; Tomiwa et al., 1987; Fenichel and Phillips, 1989; Kornberg and Shield, 1991; Rivier and Echenne, 1992; Imamura et al., 1993]. The first report of ACV

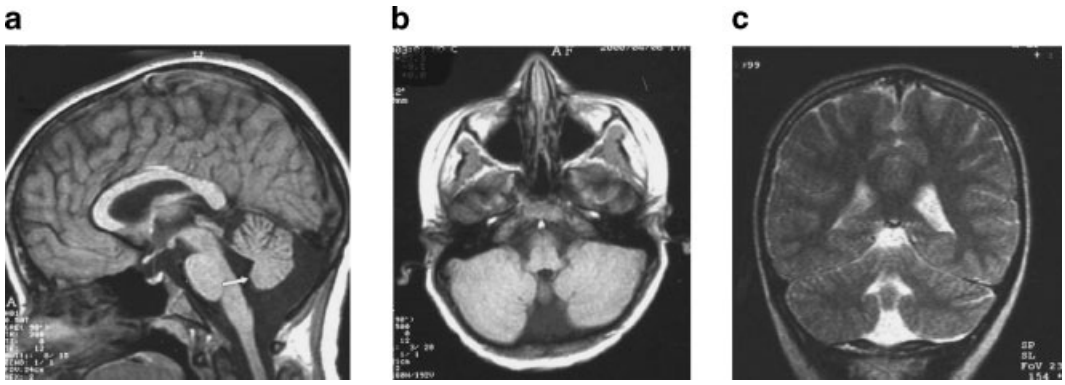

Fig. 2. Brain MRI from patient 2: Sagittal T1-weighted (**a**), axial T1-weighted (**b**), and coronal T2-weighted (**c**) images showing a marked dilatation of the liquoral spaces delimiting posterior and inferior vermis, with a large communication between fourth ventricle and basal cistern because of nodular lobule hypoplasia (arrow). Pyramidal lobule and uvula are also hypoplastic.

is attributed to Kattah et al. [1983] that described five family members presenting with primary position vertical nystagmus. Hereditary cerebellar ataxia was clinically evaluated in only three members of the family. Furman et al. [1985] described a mother and two daughters with an early-onset, non-progressive syndrome including truncal ataxia, mild limb dysmetria, upbeating nystagmus, and gaze-provoked horizontal nystagmus. MRI showed localized atrophy of the cerebellar vermis in all three affected females. Tomiwa et al. [1987] reported affected mother and daughter showing congenital cerebellar ataxia and normal intelligence. Computed tomography revealed localized atrophy of the cerebellar vermis. Fenichel and Phillips [1989] described a family in which four persons in three generations had congenital non-progressive ataxia. MRI in 1 child showed hypoplasia of the cerebellar vermis. Because of 12 out of 14 reported individuals were females, and the 2 affected males were more severely affected, they suggested an X-linked dominant pattern of inheritance. To date, only one family with male-to-male transmission of ACV is reported [Kornberg and Shield, 1991]. However, these patients showed a generalized atrophy of the cerebellum, rather than a more localized vermis aplasia, thus probably representing a type of cerebellar hypoplasia (OMIM 213000). A slowly progressive improvement of motor abilities in ACV was observed in the clinical course of an affected mother and her two affected daughters [Rivier and Echenne, 1992].

We report father and son affected by a mild form of ACV, thus suggesting an autosomal dominant rather than an X-linked pattern of inheritance of the disease. Furthermore, in patient 1, who showed early-onset cerebellar ataxia and truncal tremor, we recorded a slowly progressive improvement of motor abilities. At the age of 35 years he shows mild mental retardation but no clinical neurological signs are found. We also observed progressive improvement of motor abilities in patient 2, showing mild truncal tremor at 10 years of age. A slowly progressive improvement of motor abilities has been reported by Rivier and Echenne [1992] in one family. Thus, we hypothesize the intervention of still unidentified cerebral areas that integrate motor coordination and supply deficient cerebellar vermis activity. The mild sympathetic dysfunction present in our patient 1 does not seem to be correlated with cerebellar symptoms and signs but awaits further follow up. It is not possible to anticipate the appearance of a similar problem in his son later in life.

As to the pathogenesis of ACV, we considered *EN2* and *ZIC1* as attractive candidates for autosomal dominant ACV. Mice homozygous for a null mutation in EN2 exhibit absence of the normal process of foliation of the cerebellar cortex [Millen et al., 1994]. Heterozygous $En2^{-/+}$ mice display a less severe cerebellar phenotype [Gerlai et al., 1996]. *EN2* has been proposed as a candidate gene in JS, but molecular analysis in 26 patients did not found any mutation [Blair et al., 2002]. *ZIC1* encodes a putative nuclear factor involved in early cerebellar differentiation in the mouse [Aruga et al., 1994]. During postnatal development, the *ZIC1* knockout mice are markedly ataxic and their cerebellar vermis is hypoplastic [Aruga et al., 1998]. Heterozygous mice demonstrate hypoplasia of the cerebellar anterior vermis and significant abnormalities in locomotor tests reminiscent of the deficits seen in patients with ACV [Aruga et al., 1998; Ogura et al., 2001]. *ZIC1* has been excluded as a causative gene of JS [Bennett et al., 2004]. However, we did not find mutations in *EN2* and *ZIC1* by direct sequencing analysis in our patients.

Further clinical and radiological reports are needed to better classify isolate cerebellar malformations and allow the identification of causative genes. Identifying the underlying genetic mechanism of ACV will not only permit a better knowledge of the disease, but would also allow understanding of cell differentiation and migration in the developing CNS.

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Clinical Report

Michels Syndrome, Carnevale Syndrome, OSA Syndrome, and Malpuech Syndrome: Variable Expression of a Single Disorder (3MC Syndrome)?

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We report on a 3-year-old girl with Michels syndrome, a rare condition characterized by craniosynostosis, blepharophimosis, ptosis, epicanthus inversus, cleft lip/palate, abnormal supra-umbilical abdominal wall, and mental deficiency. The phenotypic findings are compared with the six previously reported Michels cases, and with patients referred to as Carnevale, OSA, and Malpuech syndromes. Michels syndrome is characterized by cleft lip and palate, anterior chamber anomalies, blepharophimosis, epicanthus inversus, and craniosynostosis. Carnevale syndrome shows hypertelorism, downslanting palpebral fissures, ptosis, strabismus synophrys, large and fleshy ears, and lozenge-shaped diastasis around the umbilicus. OSA syndrome resembles Carnevale, with humeroradial synostoses, and spinal anomalies as extra features. Malpuech syndrome shows IUGR, hypertelorism, cleft lip and palate, micropenis, hypospadias, renal anomalies, and caudal appendage. All are autosomal recessive. Despite the presence of apparently distinctive key features, it appears that these four entities share multiple similarities in the facial Gestalt and the pattern of MCA. Those similarities lead us to postulate that they belong to the same spectrum, which could be referred to as "3MC syndrome" (Malpuech-Michels-Mingarelli-Carnevale syndrome).

KEY WORDS: Michels syndrome; Carnevale syndrome; OSA syndrome; Malpuech syndrome; BPES

INTRODUCTION

Michels et al. [1978] and De la Paz et al. [1991] described three brothers and a sister with the triad of blepharophimosis, blepharoptosis, and epicanthus inversus (BPE triad), plus a developmental defect of the anterior segment of the eye, a cleft lip-palate, and some minor skeletal abnormalities. Subsequent reports of Cunniff and Jones [1990] and Guion-Almeida and Rodini [1995] reinforced the assumption that a similar malformative pattern represents a distinct syndrome, the so called Michels or oculo-palato-skeletal syndrome (MIM: 257920). An autosomal recessive inheritance is supported by the report of healthy consanguineous parents by Guion-Almeida and Rodini [1995]. We report an affected female, and try to delineate the key features of this syndrome and the relationships with closely related entities.

CLINICAL REPORT

Our female proband was born at 38 weeks of gestation to healthy non consanguineous Chinese parents. IUGR was evidenced during the third trimester. Her birthweight was 1,940 g (<3rd centile), length was 43 cm (<5th centile), and cranial circumference (OFC) was 31 cm (<5th centile). She was admitted to a tertiary care center for the first step of a surgical correction of a bilateral cleft lip/palate at the age of 4 months.

At clinical evaluation (7 months) she showed a weight of 5,680 g (−2.6 SD), a length of 61.5 cm (−2.6 SD), and an OFC of 43.5 cm (+ 0.3 SD). Facial dysmorphism included blepharophimosis, blepharoptosis, epicanthus inversus, telecanthus, and a bilateral cleft lip and palate (Fig. 1). The anterior fontanel was extremely large (Fig. 2). She also showed low-set ears, two accessory nipples, a tuberous angioma on the thorax, and a peculiar supra-umbilical depression of about 1×2 cm. Hands were small, with a bilaterally short fifth finger, and feet were broad. A severe axial hypotonia was also scored. At 19 months, she could walk alone, and needed nocturnal gavage feeding. At the last clinical assessment, 2 and 10/12 years, weight was 9,100 g (−2.9 SD), height 81.5 cm (−2.7 SD), and OFC 47.7 cm (−0.6 SD). Facial dysmorphism included an extremely large anterior fontanelle (9×4 cm), eyelid triad with telecanthus, unilateral ptosis, low-set ears, and micrognathia (Fig. 3). The median, supra-umbilical depression was of about 2×4 cm (Fig. 4). Her psychomotor development was mildly retarded, except for language that was completely absent. Audiometric evaluation disclosed a moderate, bilateral conductive loss. Results of routine biochemical investigations, high-resolution karyotype (600 bands), metaphasic FISH analysis for 22q11 deletion, Wolff-Hirschhorn critical region, subtelomeric rearrangements (ToTelVysion® kit, Abbott, Downers Grove), serum transferrin isoelectric focusing, cranial ultrasonography, and radiological skeletal studies were otherwise normal. Brain CT scan, echocardiography, abdominal ultrasound scan, FISH analysis of chromosomal regions were normal.

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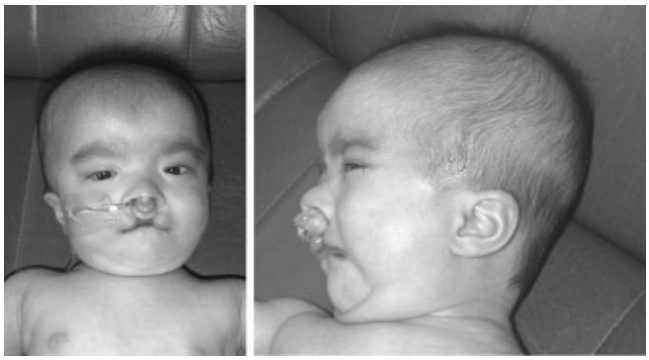

Fig. 1. Patient at age 7 months: note blepharophimosis, blepharoptosis, epicanthus inversus, telecanthus, low-set ears, and a bilateral cleft lip and palate.

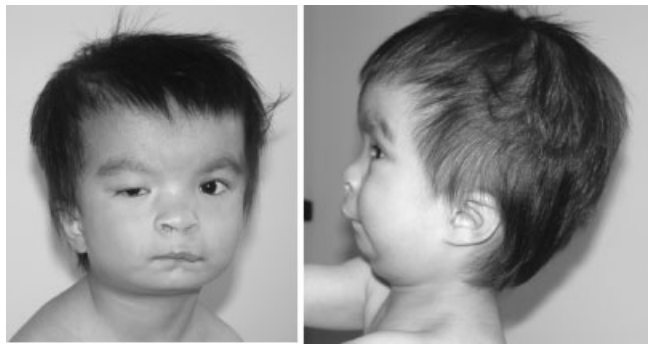

Fig. 3. Patient at 2 years 10 months of age: eyelid triad with telecanthus, right ptosis, low-set ears, and micrognathia.

DISCUSSION

Based on seven reported patients (Table I), key features of Michels syndrome consist in BPE triad and hypertelorism (inner intercantal distance is larger than expected for a telecanthus secondary to the narrow width of the palpebral fissure). The simultaneous presence of these findings allows the clinician to differentiate it from a limited number of syndromes. Blepharophimosis, ptosis, and epicanthus inversus syndrome (MIM: 110100), caused by mutations in the forkhead transcription factor *Foxl2* [Crisponi et al., 2001] is an autosomal dominant disease in which fifth finger anomalies, umbilical depression, short stature, and cleft lip have not been reported to date. Blepharophimosis is often much more severe, and there is no apparent hypertelorism (i.e., the telecanthus is secondary to the narrow width of the palpebral fissure) Kaufman oculo-cerebro-facial syndrome (MIM: 244450) could be easily distinguished for the whole clinical picture. Single reports of syndromes by Jones and Smith [1973] and by Coppeto and Monteiro [1984] could be excluded because of peculiar associated findings (ear abnormalities and over-riding toes in the former, opsoclonus in the latter.) Similarly, the four related patients reported by Al Gazali et al. [1994] with prenatal and postnatal growth retardation, anterior segment defects of the eye have distinctive features as arachnodactyly and other skeletal abnormalities, congenital heart disease and early lethality which make them easily distinguishable from the Michels-Carnevale spectrum.

There is a striking phenotypic overlap between Michels syndrome and two "private" entities. In two patients, Carnevale et al. [1989] delineated a syndrome consisting of developmental delay, ptosis of eyelids, diastasis recti, and hip dysplasia (MIM: 265050). Mingarelli et al. [1996] described "ocular-skeletal-abdominal (OSA)" syndrome in two patients. The presence of the eyelid anomalies and of an unusual and very characteristic supra-umbilical navicular depression, probably due to a limited hypoplasia of abdominal muscles in Michels and Carnevale syndrome, favor the hypothesis that these syndromes may be clinical variations of a unique disease. Indeed, the follow-up of our patient illustrate the changing phenotype, and indicate that Carnevale syndrome could represent the elder appearance of Michels syndrome, or an attenuated expression of it. At this point, the variability of this syndrome has to be stressed. This is not only true between

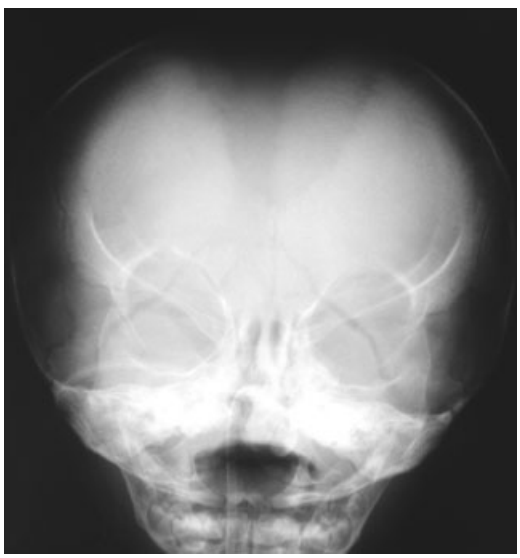

Fig. 2. Cranial X-ray at birth showing an extremely large anterior fontanelle.

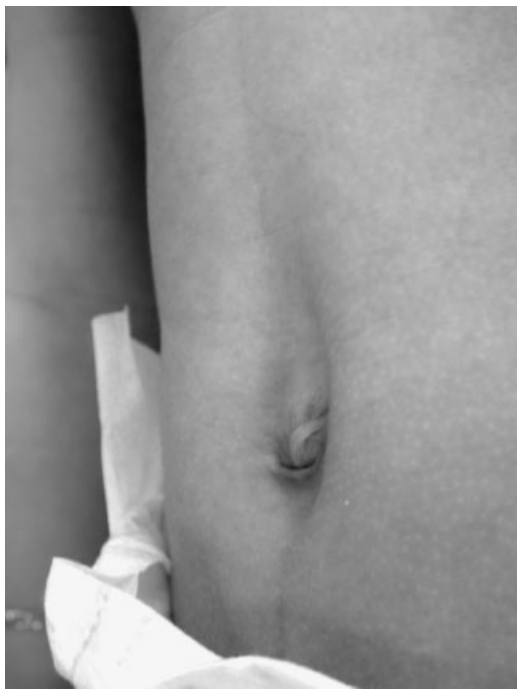

Fig. 4. Patient's peculiar umbilical depression (2×4 cm).

TABLE I. Clinical Manifestations in Reported Cases of Michels Syndrome

Patients	Michels et al. [1978] and De la Paz et al. [1991]				Cunniff and Jones [1990]	Guion-Almeida and Rodini [1995]	Present case	
Sex	M	M	F	M	F	F	F	4F/3M
Consanguinity	−	−	−	−	−	+	−	
Age at report (years)	9.8	8.5	7.2	0.5	2	9	2.5	
			Key features					
Eyelid triad[a]	+	+	+	+	+	+	+	7/7
Telecanthus/hypertelorism	+	+	+	+	+	+	+	7/7
			Frequent findings					
Short/clinodactilous fifth finger	+	+	+	+	−	+	+	6/7
Hearing loss	+	+	+	+	−	+	+	6/7
Umbilical depression	+	+	+	−	−	+	+	5/7
Craniosynostosis	−	−	+	+	+	+	−	4/7
Corneal and/or anterior chamber defect	+	+	+	+	−	−	−	4/7
Abnormal eye motility	+	+	+	+	−	−	−	4/7
Cleft lip/palate	+	+	−	−	−	+	+	4/7
Mental retardation	+	+	−	−	+	−	+	4/7
Postnatal growth deficiency	+	+	+	−	−	−	+	4/7
			Occasional findings					
Prenatal growth deficiency	+	−	?	−	−	−	+	2/6
Microcephaly	−	+	+	−	−	−	−	2/7
Accessory nipple	−	−	−	+	−	−	+	2/7
Limited supination-pronation	−	−	+	−	−	+	−	2/7
Small hands	−	−	−	−	−	+	+	2/7
Short/broad feet	−	−	−	−	−	+	+	2/7
Flat feet	−	−	+	−	−	+	−	2/7
Sacral dimple	−	+	−	−	−	+	−	2/7
Spina bifida occulta	+	+	−	−	−	−	−	2/7
Overgrowth	−	−	−	−	+	−	−	1/7
Omphalocele	−	−	−	+	−	−	−	1/7
Hydronephrosis	−	−	−	+	−	−	−	1/7
Seizures	−	−	−	−	+	−	−	1/7

[a]Eyelid triad: blepharophimosis, blepharoptosis, epicanthus inversus.

families (Michels'patient have anterior chamber anomalies, Mingarelli's patient have normal intelligence) but also between patients within a single sibship, as illustrated by Michel's pedigree (mental delay in 2/4, cleft in 3/4, growth delay in 3/4).

This important variability makes diagnosis difficult, and raises the problem of the limits of the spectrum of Michels syndrome, in our broader sense. This point has also been raised recently by Hall [2004], who drew attention to the overlap of

TABLE II. Phenotypic Comparison of Carnevale OSA, Malpuech, and Michels Syndromes

Clinical features	Carnevale	Mingarelli (OSA)	Michels	Malpuech
Number of cases	2	2	7	>7?
AR Inheritance	+	+	+	+
Eyelid triad[a]	+	+	+	±
Hypertelorism	+	+	+	+
Arched eyebrowses	+	+	+	+
Hearing loss	+	+	+	+
Umbilical depression	+	+	+	−
Umbilical hernia	−	−	−	+
Diastasis recti	+	+	−	+
Limited supination-pronation/radioulnar synostosis.	+	+	+	+
Prominent coccyx/caudal appendage	−	−	−	+
Mental retardation	+	−	±	±
Postnatal growth deficiency	+	−	+	+
Telecanthus	−	+	+	+
Bifid tip of the nose	−	−	−	+
Dysplastic/low set ears	+	−	−	−
Down-turned corners of the mouth	+	−	−	+
Cleft lip/palate	−	−	+	+
Accessory nipple	+	−	+	−
Cryptorchid testes	+	−	−	+
Short/clinodactilous fifth finger	−	+	+	−
Lordosis/scoliosis	+	+	−	−
Spina bifida occulta	+	−	+	−
Dislocation of hip	+	−	−	−

[a]Eyelid triad: blepharophimosis, blepharoptosis, epicanthus inversus.

OSA syndrome with Malpuech syndrome. Malpuech syndrome is characterized by hypertelorism, ptosis, epicanthus (sometimes inversus), mild mental retardation, prenatal growth deficiency, cleft lip and palate, urogenital anomalies (micropenis, hypospadias, hydronephrosis), and typical "caudal appendage" (in fact, prominent, raised, fleshy coccyx, with a deep groove or sacral sinus.) The tip of the nose may be bifid. Malpuech syndrome was initially observed in an inbred gypsy family [Malpuech et al., 1983]. On pictures, mild blepharophimosis may be present, although it is not mentioned in the reports. Crisponi et al. [1999] reported further sibs with convincing facial appearance and normal intelligence. Review of the literature on Malpuech is difficult, as several patients appear to have a phenotype only loosely related to the original report, making tabular analysis of signs misleading. In peculiar, the abnormal coccyx may be a false handle. Although this anomaly is uncommon, it may be non-specific, and "overweighted" as a diagnostic criteria. Guion-Almeida [1995] described three patients as Malpuech syndrome. Two of them (patient 2 and overall patient 3) have a Pitt-Rogers-Danks appearance and psychomotor delay. These patients may have a cryptic 4p deletion (already discussed by Selicorni and Faravelli [2000]). It must be stressed here that "sacral sinus" is commonly reported in 4p−. Their Gestalt is quite different from Malpuech's report. The first patient in this paper was deaf. His developmental level is not mentioned. On pictures, he looks less 4p−. Guyon-Almeida herself pointed to the similarity with Michels syndrome, but she felt that the facial appearance was not convincing. Another patient has been discussed as possibly bridging Malpuech syndrome with Juberg–Hayward syndrome [Reardon et al., 2001]. However, this patient is atypical, as he had generalized epiphyseal dysplasia. For this reason, we think that this patient should remain distinct from both Juberg–Hayward and Malpuech. Hall reported in an abstract (no clinical pictures are available) a 14-year-old male with Malpuech syndrome (including the typical caudal appendage), PDA, and radioulnar synostosis. He concluded that OSA syndrome and Malpuech syndrome were identical facially and shared enough unique non-facial features to consider them the same disorder. Although facial anomalies of Malpuech syndrome resemble those present in Mingarelli's report and in Carnevale syndrome, depressed supra-umbilical area—replaced in Malpuech syndrome by umbilical hernia or common diastasis recti, normal sacrum and absence of blepharophimosis could still allow clinical distinction between the two entities. Despite those clinical discrepancies, the overall phenotype and the Gestalt seems identical. Table II compares Michels, Malpuech, Carnevale, and Mingarelli syndromes. We agree with the conclusions of Hall, and by proxy, suggest that the four clinical entities may be allelic or represent a spectrum.

Further clinical reports are still needed to evaluate the degree of intrafamilial clinical variability of Michels syndrome, to confirm its key features and to solve the nosological problem raised by phenotypic overlaps with Carnevale, OSA, and Malpuech syndromes. One of the most puzzling features is the interfamilial heterogeneity of expression within each small group of reports; short stature and mental delay, for instance, are not constant. We have also the impression that the partition between the four entities relies artificially on the presence of a key feature (navicular supra-umbilical depression, coxxyx anomalies, . . .). In our opinion, the diagnostic value of these "key handles" remains high, but their absence does not rule out in principio any diagnosis. As long as diagnosis remains non-molecular, the clinical boundaries of each of the four syndromes will remain a matter of subjective appraisal. We feel that the similarities between the four entities are higher and more significant than their differences, and that the variability within a defined group is even higher than the variability between them. For these reasons, expanding Hall's proposal, we suggest that the four entities better fit with a single recessive spectrum than as separate disorders. We suggest referring to this clinical spectrum as 3MC syndrome (for Malpuech-Michels-Mingarelli-Carnevale).

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New Syndrome

A New Syndrome of Congenital Generalized Osteosclerosis and Bilateral Polymicrogyria

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We report a 29-week male fetus with healthy consanguineous parents. He showed a severe sclerosing bone disorder affecting all skeletal elements, resulting in insufficient modeling, generalized densification, and fragility of the skeleton. This skeletal dysplasia was associated with an abnormal craniofacial development (hypertelorism, severe microretrognathia, cleft palate, absent epiglottis, reduced number, and mineralization of teeth buds) and abnormal terminal phalanges. Neuropathologic examination showed bilateral fronto-parietal cerebral polymicrogyria. This syndrome appears to represent a new variant of congenital sclerotic bone disorder of unknown origin. Autosomal recessive inheritance is possible.

KEY WORDS: osteosclerosis; polymicrogyria; Raine syndrome; dysosteosclerosis

INTRODUCTION

Osteosclerotic bone disorders are a heterogeneous family of diseases characterized by an imbalance between osteogenesis and osteoclasis, leading to dense and often overgrown skeleton. Radiographically, osteosclerosis is suggested by the presence of an increased width of trabecular bone, contrasting with osteopetrosis, where a generalized increase in bone density with bony encroachment into the medullary cavities is observed. Chronology (congenital, infantile, and adult), topography of the modeling defect (generalized, predominating on skull basis, facial bones, and/or other parts of the body), biochemical anomalies, and associated anomalies allow the partition of sclerosing disorders into several genetic and clinical types [Spranger and Maroteaux, 1990].

Brain polymicrogyria is a malformation of cortical development characterized by an excessive number of small gyri with abnormal cortical lamination. It can appear as either a focal lesion or a more widespread cortical abnormality [Barkovich et al., 1995]. The presence of bilateral symmetric polymicrogyria is often taken to suggest a genetic etiology, and such multiple syndromes have been described. These syndromes have distinct clinical and radiological presentations and include sporadic types (bilateral frontal polymicrogyria [Guerrini et al., 2000] and bilateral para-sagittal parieto-occipital polymicrogyria [Guerrini et al., 1997]), and some familial forms, such as bilateral perisylvian polymicrogyria [Guerreiro et al., 2000; Villard et al., 2002] and bilateral fronto-parietal polymicrogyria (BFPP) [Chang et al., 2003]. In this latter, autosomal recessive form, mapped to chromosome 16q12.2-21 [Piao et al., 2002] polymicrogyria is typically present with a descending anterior–posterior gradient of severity, the fronto-parietal regions being the most significantly affected.

We report on a case of sclerosing bone disorder of very early onset associated with a bilateral fronto-parietal polymicrogyria that may represent a new radiological and clinical form within this family of disorders.

PATIENT REPORT

The patient is the second child of healthy consanguineous parents (first cousins) from Mauritania. Family history was not contributory. At 23 weeks of gestation, a routine ultrasonographic examination revealed hypertelorism, cleft palate, severe microretrognathia, abnormally shaped ribs, and a narrowed thorax. At 29 weeks of gestation, cranial MRI revealed a small brain with abnormally short corpus callosum and poorly defined sulci, suggesting an abnormal gyration pattern (Fig. 1a,b). Infectious causes were ruled out. Fetal karyotype was 46, XY. Parents elicited termination of pregnancy.

At necropsy, the boy weighted 1.340 g (75th centile), his length was 37 cm (10th–25th centile), and his head circumference was 28.5cm (90th centile). The left humerus and both femora were broken during delivery. He showed facial dysmorphism: frontal bossing, hypertelorism, downslanted palpebral fissures, upturned nose, long flat philtrum with a median groove, microstomia, and severe microretrognathia (Fig. 2a,b). Exophtalmia was not observed. Auditory meati were narrowed. The palate was cleft posteriorly and the epiglottis was absent. Gums were broad and thickened. Two para-median whitish dental cysts were present on the upper gum, and the lower gum had a median notch. Hands were slender, with long fingers ending with short and broadened, spatulate terminal phalanges (Fig. 2c). Thumbs and halluces were large.

Radiological examination showed increased biparietal diameter (>90th centile) and extreme sclerosis of the skull basis and facial bones. Mandibular dental germs were not visible. Long bones (including extremities) were irregularly shaped, with insufficient metaphyseal wedging (Fig. 3a). Diaphyseal bones were extremely dense, with thick cortex and an absent medullar canal. The upper thorax was narrow. The ribs and the clavicles were thin but regularly shaped (Fig. 3b). The vertebral

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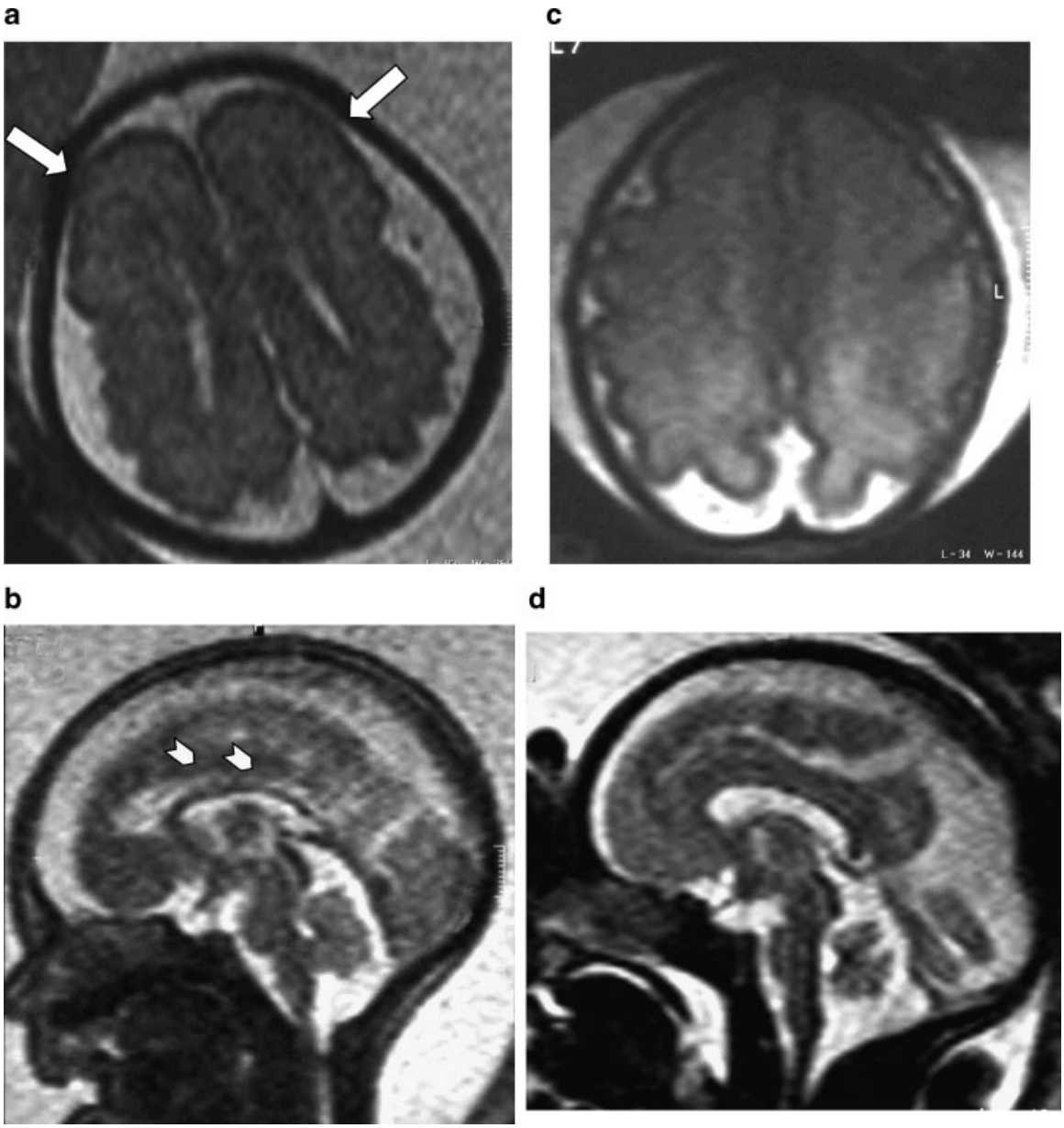

Fig. 1. MRI images of the patient's brain showing poorly defined sulci in the frontoparietal region, bilaterally (arrows) (**a**), and the corpus callosum lacking genu et splenium (arrowheads) (**b**). Comparison with brain MRI from a healthy 29 weeks-old fetus (**c**, **d**).

bodies were sclerotic and slightly flattened (Fig. 3c). The first metacarpals and the distal phalanges were short, the latter showing a terminal tufting ("mushroom profile") (Fig. 3d). Bone age was delayed, corresponding to about 23 weeks of gestation [Stempfle et al., 1995].

Macroscopic examination of the brain showed an abnormal gyral pattern and Y-shaped rolandic sulci. The corpus callosum lacked genu and splenium. Microscopically, a bilateral symmetric frontoparietal and, to a lesser extent, temporal polymicrogyria was evident (Fig. 4). The ventricular system was normal, as was the cerebellum.

Microscopic examination of the femur showed a normal architecture and cellularity of the epiphyseal cartilage and growth plate. Cortical bone was thin and irregularly lined. Trabecular bone was characterized by an irregular and insufficiently mineralized matrix containing numerous osteocytes, lined by numerous osteoblasts and osteoclasts. It was more compact than normal: the medullar cavity was not developed, and hematopoiesis was poor. Membranous ossification was studied on a fragment: the diploe. It appeared thickened and hypercellular, as was also the femoral trabecular bone (Fig. 5). Microscopic examination of the mandibula showed similar alterations of bone structure, and widened symphysis with a large median cartilaginous nucleus. There were only two dental germs, with several cuspids (primary molars?) corresponding to the gingival nodules. In the maxillary bone, six germs were found: two incisors, one canine, one medially displaced tooth (canine or incisor), and one molar. The microstructure of all dental germs was normal, but mineralization was severely delayed, and they appeared abnormally oriented.

Macroscopic and microscopic examinations of other internal organs were unremarkable, as were the placenta and cord. X-ray examination of the hands and skulls of both parents were normal.

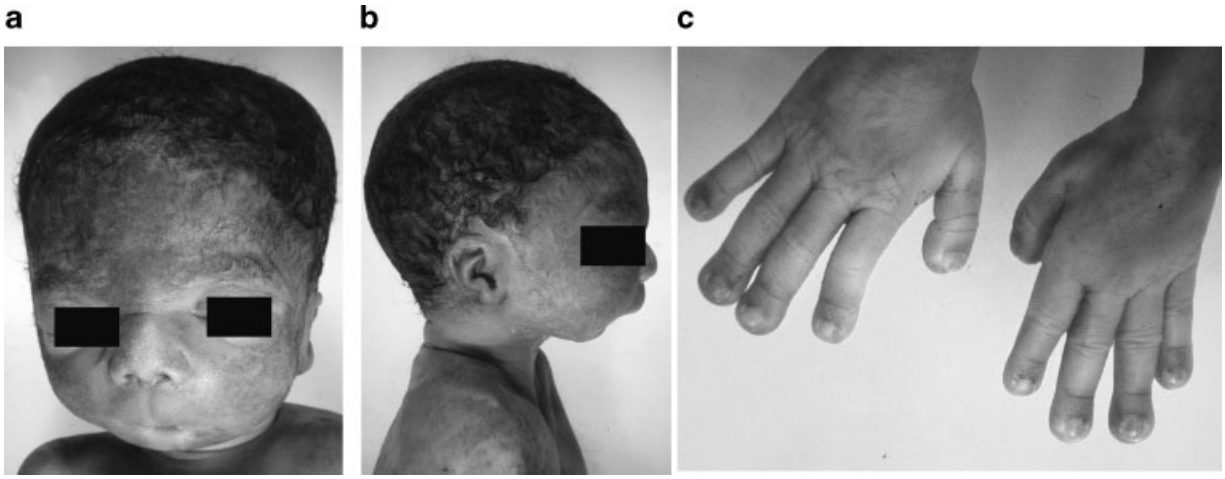

Fig. 2. External phenotype of the patient: note the frontal bossing, hypertelorism, downslanted palpebral fissures, upturned nose, long flat philtrum, microstomia (**a**), severe microretrognathia (**b**). Hands are slender, with long fingers ending with short and broadened, spatulated terminal phalanges (**c**).

DISCUSSION

The boy reported here, born to consanguineous parents, showed a severe sclerosing bone disorder affecting all skeletal elements and resulting in insufficient modeling, in generalized densification and fragility of the skeleton, and in very abnormal terminal phalanges. This skeletal dysplasia was associated with abnormal facial and dental development

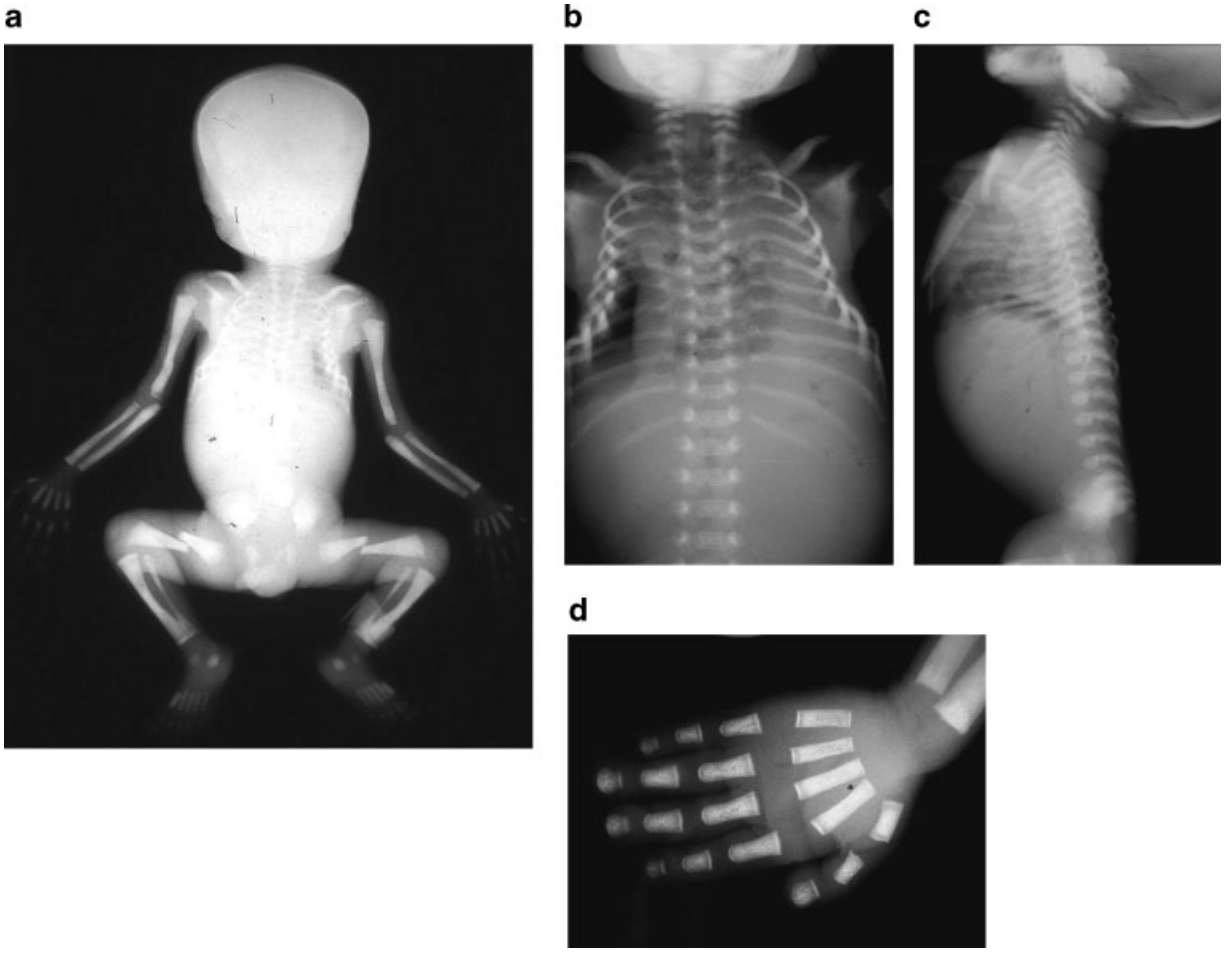

Fig. 3. X-ray examination showing: (**a**) extreme sclerosis of the skull basis and of the facial bones. **b**: Long bones and spine are irregularly shaped with insufficient metaphyseal wedging. Diaphyseal bones are extremely dense, with absent medullar canal. **c**: The chondro-costal junction is angulated. **d**: The first metacarpals and the distal phalanges are short, the latter showing a terminal tufting (mushroom profile).

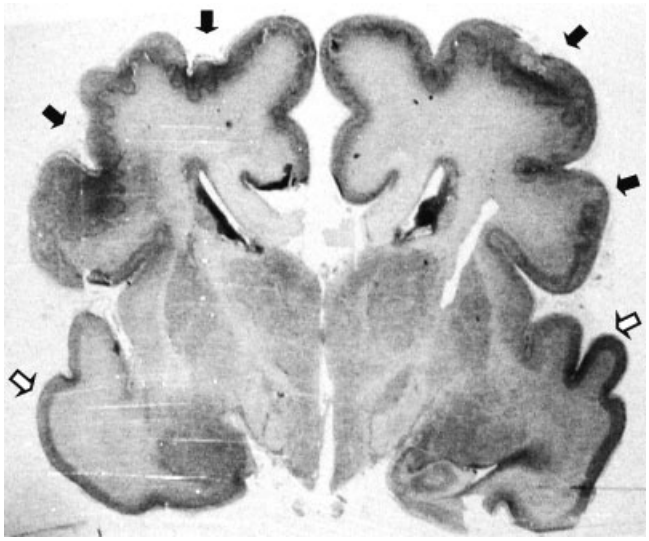

Fig. 4. Microscopic examination of a coronal section of the brain, showing bilateral parietal polymicrogyria (black arrows). The temporal cortex is normal (white arrows).

(hypertelorism, severe microretrognathia, cleft palate, absent epiglottis, and abnormal teeth buds). His brain showed bilateral fronto-parietal polymicrogyria and a hypoplastic corpus callosum, lacking genu and splenium.

Potential environmental etiologies of polymicrogyria have been reported [Barkovich et al., 1995]. A possible explanation for our patient's cortical anomalies could have been an antepartal anoxicischemic disruption, but the mild extent of laminar necrosis in the territory of the median cerebral arteries does not support this hypothesis. Furthermore, bilateral symmetric polymicrogyria present without signs of laminar necrosis in other cortical areas, and the association with a dysgenetic corpus callosum led us to hypothesize a genetic etiology for this cortical dysplasia.

Several reports may be discussed in the differential diagnosis of congenital generalized bone sclerosis and cerebral abnormalities. Lethal osteosclerotic bone dysplasia, or Raine syndrome (MIM 259775), was described by Raine et al. [1989] in a female fetus with microcephaly, exophthalmos, hypoplastic midface, gum hyperplasia, cleft palate, and osteosclerosis. To date, 14 cases of Raine syndrome have been reported, allowing a rather precise delineation of the phenotype [Al Gazali et al., 2003; Hulskamp et al., 2003]. The characteristic face of Raine syndrome consists of a narrow prominent forehead, hypertelorism, proptosis, distinct midfacial hypoplasia with downward eye slant, very short and flat nose, carp-shaped mouth, gum hyperplasia, and severe microretrognathia. The radiological findings in Raine syndrome include generalized osteosclerosis of all bones and the base of the skull with cortical hyperostosis, metaphyseal flaring, and irregular appositional bone formation along the diaphyses of the long tubular bones. These characteristic features were absent in our patient, in whom the diaphyses of the long bone and the metaphyses are regularly lined and well demarked from the surrounding soft tissues. In Raine syndrome, the ribs and clavicles are short and the vertebral bodies slightly flattened. The hands show brachytelephalangy with short terminal phalanges, quite different from the remarkably broad phalanges of our patient. Histologically, the tubular bones show normal endochondral ossification, narrow medulla, compaction and thickening of the diaphyseal cortex, and irregular trabecular bone formation with osteoblastic and osteoclastic activity around amorphous deposits and calcifications. In our case, on the contrary, the characteristic lesion was the hyperplasia of an abnormal endochondral bone, and hypoplasia of the cortical periosteal bone, without extra-osseous osteoid deposits [Kan and Kozlowski, 1992]. Desmosterolosis (MIM 602398), a metabolic abnormality resembling Raine's phenotype has been searched for and excluded. [FitzPatrick et al., 1998]. Al Mane et al. [1996] first demonstrated that intracranial calcifications of unknown etiology are a component of Raine syndrome. Widespread focal calcifications were evidenced in the periventricular white matter and basal ganglia with some meningeal calcifications as well, and these were thought to correspond to the histologic calcifications observed by Kan and Kozlowski [1992]. These features suggested the possibility of a generalized disturbance of calcium metabolism. In no instance have the calcifications been associated with polymicrogyria.

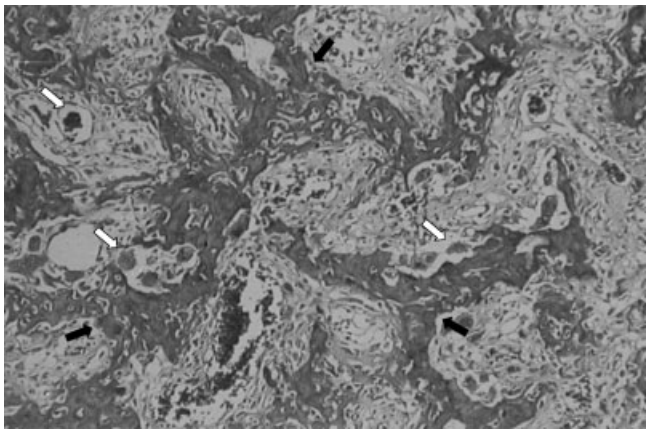

Fig. 5. Microscopic examination of diaphyseal trabecular bone showing reticular aspect of trabeculae (black arrow), hypercellularity and irregularity of the bone matrix. An increased number of osteoclasts (white arrow) and osteoblasts are observed around the trabeculae. Hematopoiesis is reduced.

Dysosteosclerosis (MIM 224300) is a rare autosomal recessive bone dysplasia associated with neurodevelopmental deterioration and optic atrophy due to cranial nerve compression. X-linked inheritance has been described [Pascual-Castroviejo et al., 1977]. In this syndrome, which expression starts in infancy, sclerosis is associated with progressive metaphyseal expansion and alteration of bone density. The early craniotubular bone modeling and clinical presentation resemble osteopetrosis. Affected individuals have dysmorphic features with a round face, sagging cheeks, and a prominent forehead. Dentition is abnormal. There is sclerosis of the skull base, the ribs (that are wide), clavicles, scapulae, and middiaphyses. The metaphyses show progressive expansion and, as in the spine, develop sclerotic islands in areas of relative radiolucency. These irregularities of bone density were not observed in our patient. There is mild platyspondyly with wide intervertebral spaces. The vertebral bodies are small with irregular end plates and pronounced anterior notches. The tubular bones are short with progressive bowing, and fractures are a complication [Elcioglu et al., 2002]. Chitayat et al. [1992] reported a girl with a clinical and radiological diagnosis of dysosteosclerosis who presented diffuse intracerebral calcifications. Although our case shows some radiographic similarities with other severe osteosclerotic dysplasias, these usually present with limb shortness and without neurological involvement, with the exception of the above mentioned Raine syndrome [see the table by Brodie et al., 1999].

Some other patients with osteosclerosis and abnormal brain structure have been recorded. Lehman et al. [1977] described a

mother and daughter with generalized osteosclerosis, multiple lumbar and thoracic meningoceles, an empty sella, hypoplasia of the cerebellar vermis, and small cerebral gyri, the socalled lateral meningocele syndrome [Gripp et al., 1997]. In the report of El Khazen et al. [1986], a lethal condensing bone disorder was detected in utero in two sibs. The bones were brittle and fractures occurred. Neuropathological examination revealed hydrocephalus and an intense gliosis throughout the cortex and white matter, with an extensive loss of neurons. Numerous axonal swellings were observed in the cortex, white matter, and brainstem. Calcifications were occasionally seen. The cerebellum was hypoplastic. Al-Gazali et al. [1998] reported a consanguineous Pakistani family where 10 infants suffered from the same condition. The only well-described child showed macrocephaly, downslanted palpebral fissures, depressed nasal bridge, and micrognathia. Brain imaging revealed a huge interhemispheric cyst communicating with the lateral ventricles and causing hydrocephalus, callosal agenesis and cerebellar hypoplasia. Skeletal survey showed sclerosis of all bones with wide metaphyses.

Our patient's phenotype is not consistent with any of these syndromes because of the peculiar brain abnormalities found in the lateral meningocele syndrome (thoracic meningoceles), and in the patients reported by El Khazen et al. [1986] and by Al-Gazali et al. [1998] (hydrocephalus and cerebellar hypoplasia).

We were unable to find any murine model showing bone sclerosis and abnormal brain structure (Mouse Genome Informatics, The Jackson Laboratory, USA, http://www.informatics.jax.org/). Interestingly, osteopetrotic (op/op) mice defective in producing functional macrophage-colony-stimulating factor (M-CSF) show abnormal brain development. The numerical density of microglial cells was found to be reduced by 47% in the corpus callosum, by 37% in the parietal cortex, and by 34% in the frontal cortex of mice mutant at the op locus, which are totally devoid of M-CSF [Wegiel et al., 1998; Sasaki et al., 2000].

These data, with the above-mentioned reports of patients presenting structural brain abnormalities and sclerosing bone disease, suggest that alterations in still unidentified (metabolic?) pathways might cause brain-and-bone anomalies, as in peroxisomal disorders, and that this association should be searched for. The combined skeletal and cerebral pattern of anomalies observed in our patient appears to be clearly different from the other osteosclerotic syndromes with CNS involvement. Because of parental consanguinity, an autosomal recessive mode of inheritance is possible. Further clinical, radiological, and histological data are needed to clarify whether the different reported patients correspond to diversely severe phenotype of a unique syndrome, or show causally distinct entities.

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Early Intervention for Children With Down Syndrome in Southern Italy

The Role of Parent-implemented Developmental Training

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The aim of this study was to assess whether parent-implemented developmental training—by means of the Carolina Curriculum for Infants and Toddlers with Special Needs (CCITSN)—could be of greater benefit to young children with Down syndrome (DS) than the standard therapist-implemented treatment provided by the National Health Service of the southern Italian region of Campania (NHST). A total of 47 children with DS were randomly assigned either to the experimental (CCITSN) or to the comparison (NHST) group. Children from both groups were tested periodically with the Brunet-Lézine Psychomotor Development Scale. After completion of the 12-month followup, children in the CCITSN group showed developmental gains over time while children in the comparison group showed a slight but not statistically significant improvement. Moreover, mean developmental quotient scores of the CCITSN group, over the entire study period, were significantly higher than those of the comparison group. A commitment to using parents as interventionists is not a common practice in Italy and many other countries, but may be the most effective and cost-efficient way of providing services to young children with DS and other developmental disabilities. **Key words:** *Carolina Curriculum for Infants and Toddlers with Special Needs*, *Down syndrome*, *early intervention*

THE recent focus on the importance of early childhood development is unprecedented and indicates that it has become a national priority for most wealthy countries (Ragalado & Halfon, 2001). Each industrialized country has acknowledged the need for early childhood intervention by providing funds for specific programs, such as Part C of the Individuals with Disabilities Education Improvement Act public law in United States (Danaher & Guadagna, 1998). The benefits of early intervention for children with developmental disabilities are widely acknowledged (Guralnick, 1997; Majnemer, 1998; Thompson, 2001). Of importance, after a period during which the interest had been centered on the individual, the intervention focus now shifted to the whole family (Committee on Children

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with Disabilities, 2001; Gillette, 1992; Guralnick, 1998), indicating that parental involvement could play a crucial role in early intervention (Hauser-Cram et al., 2001; Miedel & Reynolds, 1999).

Some evidence suggests that early intervention is effective in children with Down syndrome (DS) (Connolly & Michael, 1986; Connolly, Morgan, & Russell, 1984; Connolly, Morgan, Russell, & Fulliton, 1993), even though conflicting evidence for some domains still exists (Gibson & Harris, 1988). Down syndrome has attracted interest as a model of genetic disorder for evaluating the effectiveness of intervention in children with developmental disabilities. Guidelines for health supervision of children with DS have recently been provided by the American Academy of Pediatrics, Committee on Genetics (2001). In children with DS, numerous studies have confirmed a uniform global developmental delay across all skills, including cognition, language, and motor areas (Berglund, Eriksson, & Johansson, 2001; Henderson, 1986; Kerr & Blais, 1985; Latash, 1992; Palisano et al., 2001; Spano et al., 1999; Stoel-Gammon, 2001).

In Italy, the provision of services for children with developmental delays still lacks specific early intervention programs implemented over a regional or even national basis. Healthcare and healthcare services are provided to the entire population by the Italian National Health Service (NHS) whose financial and organizational structure is similar to that of the British NHS. Any treatments—including early intervention—provided by the Italian NHS are implemented by professional therapists and every attempt to encourage the Italian NHS to teach parents specific skills to support their child's development has been unsuccessful, at least in the Italian south or "Mezzogiorno." The main reasons suggested for this neglect have been the following: first, parents should not be asked to abandon their primary role as a parent in order to be their child's teacher and second, parents would not have sufficient skills to support the development of their children with disabilities.

This study was designed to compare the efficacy of the standard therapist-implemented program offered by the Italian NHS and a parent-implemented developmental training program in which parents were instructed in ways to incorporate therapeutic/educational activities into the daily care routines of their young children with DS. The study was conducted in the region of Campania, an area where habilitative and/or early interventions were usually left to the individual therapist's initiative. One group of children received only the standard Italian NHS therapist-implemented program. A second group received only a parent-implemented program based on the Carolina Curriculum for Infants and Toddlers with Special Needs (CCITSN) (Johnson-Martin, Jens, Attermeier, & Hacker, 1991). The CCITSN was chosen for the parent-implemented intervention because it is based on activities that can be readily incorporated into the daily care routines of infants and because of its recent translation into Italian (Johnson-Martin, Jens, Attermeier, & Hacker, 1997).

METHODS

Participants

In agreement with the regional DS association, we contacted 65 families to offer them the possibility of entering our study. Twelve families refused entry into the study and 6 were excluded because of one of the following reasons: major malformations, perinatal asphyxia, seizures, thyroid disorders, ophthalmological problems, and heart defects requiring either medical or surgical intervention. A total of 47 infants with DS were finally enrolled in the study (see Fig 1).

Computer-generated random number lists were used to assign participants to either the "new" type of intervention, that is, CCITSN, or the standard treatment program provided by the NHS, which we will call NHST (constituting the comparison group). Twenty-four children with DS were assigned to parent-implemented CCITSN and 23 to the standard

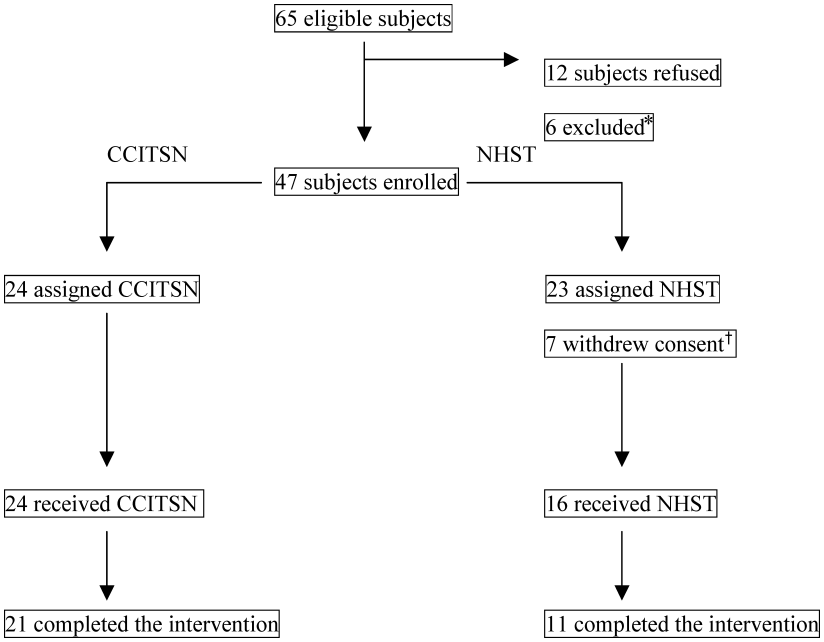

Figure 1. Sequence of assignment and subject participation. CCITSN, Carolina Curriculum for Infants and Toddlers with Special Needs; NHST, National Health Service treatment. Astrisk (*) indicates 2 congenital heart disease requiring medical treatment, 3 congenital heart disease requiring surgical intervention, and 1 seizure. Single dagger (†) indicates subjects refusing further clinical evaluation after random assingment to NHST.

NHST program. Of the 47 initially randomized subjects, 7 withdrew consent immediately after assignment to NHST; 3 subjects withdrew from the CCITSN group because parents had to go to live in another region for family reasons. Five additional subjects, with few from NHST, joined during the course of the study. Consequently, 32 subjects, 21 in the CCITSN group and 11 in the NHST group, completed the study.

Baseline characteristics for the full groups at enrollment are shown in Table 1. Subjects had an average age of 4.5 and 5.9 months in the CCITSN and NHST groups, respectively. More than a half were females and almost all showed a trisomy 21 at karyotype. At baseline, there were no significant differences between the 2 study groups in terms of social and environmental data, pregnancy and delivery, breastfeeding, and age at the beginning of intervention ($P > .05$). Children in the 2 groups had comparable mean developmental levels at the beginning of the study. An analysis of the subsets of children who completed the study found the groups to be equally similar. An analysis comparing the group of 19 children whose families refused entry into the study, either prior to ($n = 12$) or after ($n = 7$) random assignment (for the NHST group), found no significant differences between these latter subsets and the full groups ($P > .05$). Moreover, the comparison between dropouts versus participants in the NHST group ($n = 5$) as well as in the CCITSN group ($n = 3$) was not statistically significant at least on these baseline measures. Final comparisons therefore were made between 21 CCITSN and 11 NHST subjects.

Procedures

Children in the CCITSN group received developmental training at home from their parents. The CCITSN consists of 26 areas or sequences of development

Table 1. Baseline characteristics of the full groups*

	CCITSN	NHST
Sex		
Male	8 (33)†	7 (30)†
Female	16 (67)†	16 (70)†
Karyotype		
Trisomy	19 (79)‡	20 (87)‡
Mosaic	2 (8)‡	1 (4)‡
Balanced translocation	3 (13)‡	2 (9)‡
Maternal education, y (SD)	7.8 (3)	7.5 (3.7)
Paternal education, y (SD)	8.1 (2.6)	7.5 (2.9)
Number of cohabitants (SD)	4.0 (0.9)	4.1 (1)
Maternal age at birth, y (SD)	29.6 (5.3)	30.5 (6.3)
Gestational age, wk (SD)	37.4 (2.1)	36.3 (8)
Birthweight, g (SD)	2790 (543.4)	2664 (825.3)
Breastfeeding, d (SD)	64.2 (108.0)	36.8 (69.8)
Mean age at beginning therapy, mo (SD)	4.5 (2.3)	5.9 (3.2)
DQ (SD)§	55.4 (30.4)	53.2 (14.8)

*CCITSN denotes Carolina Curriculum for Infants and Toddlers with Special Needs; NHST, National Health Service Treatment.
†Number of subjects of the specified sex, with values in parentheses indicating percentages unless indicated otherwise.
‡Number of subjects of the specified karyotype, with values in parentheses indicating percentages unless indicated otherwise.
§Based on Brunet-Lézine Psychomotor Development Scale.

representing 5 main developmental domains: cognition, communication/language, social skills/adaptation, fine motor skills, and gross motor skills. Each area of development contains a variable number of items or skills arranged in a logical—but not chronological—sequence. A baseline profile of each individual child is obtained on the basis of the mastered skills, and from this starting point it is possible to move onwards. The theoretical background of CCITSN is based on Piagetian concepts for cognitive development, and strategies of intervention rely on behavioral methodology. In this study, parent-implemented CCITSN was used as the sole method of intervention. This does not necessarily mean that CCITSN excluded other professional therapists.

After collection of baseline data, including a psychomotor assessment, a professional team at the Department of Pediatrics of "Federico II" University completed the initial intervention plan for each subject in the CCITSN group. A worksheet containing the selected items from the abovementioned sequences and domains of the CCITSN was then handed out to each parent. Parents were taught to administer each item and were helped in their understanding of the objectives underlying each individual skill to be acquired by their children. They were advised that the implementation of the program was their responsibility and that they should attempt to practice each item with their child at least twice a day between meetings with the tutoring professionals. These meetings were scheduled every 3 weeks for the first 6 months and every 4 weeks thereafter. At each meeting, progression through the curriculum was noted and new items were added after in-depth discussion with the parents about all aspects of possible concern. The professionals adapted the general behavioral techniques described in the CCITSN manual to make them

appropriate for each child before providing the parents with a revised program. All parents were able to carry out the intervention. Both parents were involved in training and intervention delivery, even though it was the mothers who usually implemented the program.

Treatment of children in the NHST group took place at the NHS rehabilitation centers and was administered by therapists belonging to local teams. Therapy sessions, each lasting 50 minutes, were carried out 3 times weekly for each child. These children received no additional intervention other than their weekly involvement in developmental training. Parents were allowed to observe the therapy sessions but were never encouraged to do so. Also, they were not directly instructed in specific techniques to be reproduced at home. While each child received individual treatment based on the specific objectives decided after the initial baseline testing, the common goal for all was the enhancement of normal developmental milestones in different areas. The most widely preferred method of treatment used by physiotherapists was the neurodevelopmental treatment approach developed by the Bobaths (Bobath, 1980; Bobath & Bobath, 1976). Although originally developed for use with children having cerebral palsy, elements of the Bobath method have been recommended for a wide variety of children with developmental disabilities. The essence of the instruction then was to stimulate, as far as possible, the most important abilities in the main developmental domains, that is, gross motor and fine motor skills, cognition, communication/language, and social skills. In each case, the training was based systematically on the normal developmental pattern of children without disabilities. For each developmental area, therapists made a plan of the normal developmental milestones during the first 2 years of a child's life, and they checked the child's functional level against this at regular intervals in order to make a decision about further training.

To evaluate the effects of the 2 training approaches, all children from both groups were tested periodically with the Brunet-Lézine Psychomotor Development Scale—a French adaptation of the Gesell Scale (Brunet & Lézine, 1955). The test was composed of 4 subscales: gross motor skills (designated as Posture), fine motor skills/eye-hand coordination (designated as Coordination), communication/language (designated as Language), and social/self-help skills (designated as Socialization). A general developmental quotient (DQ) and 4 partial quotients (Posture, Coordination, Language, Socialization) were obtained. The choice of the Brunet-Lézine Developmental Scale as the assessment instrument was an obligatory one because this is the only scale for pychomotor assessment for which an Italian standardization is available (Brunet & Lézine, 1967). In any case, the Brunet-Lézine Psychomotor Development Scale is a widely accepted tool for measuring the developmental levels of infants and toddlers (Malvy et al., 1999).

The intervention period lasted 12 months, and both groups were tested every 3 months. All children were assessed for DQ at the Department of Pediatrics of "Federico II" University. Besides a developmental assessment, our protocol included a thorough history and physical examination and a semistructured interview with parents about children's behavior and parental feelings. Developmental assessments were performed by a clinical psychologist and a child neuropsychiatrist. These independent testers were not informed about group assignment. The Brunet-Lézine Psychomotor Development Scale had an interrater reliability of 0.84. The primary endpoint of the study was the general DQ of the Brunet-Lézine Developmental Scale.

RESULTS

Before going into details, it is important to note that the full groups at randomization showed many dropouts: that is, only a smaller subset of children (21 in the CCITSN group and 11 in the NHST group) completed the intervention program as intended (Fig 1). We then decided to carry out a direct comparison

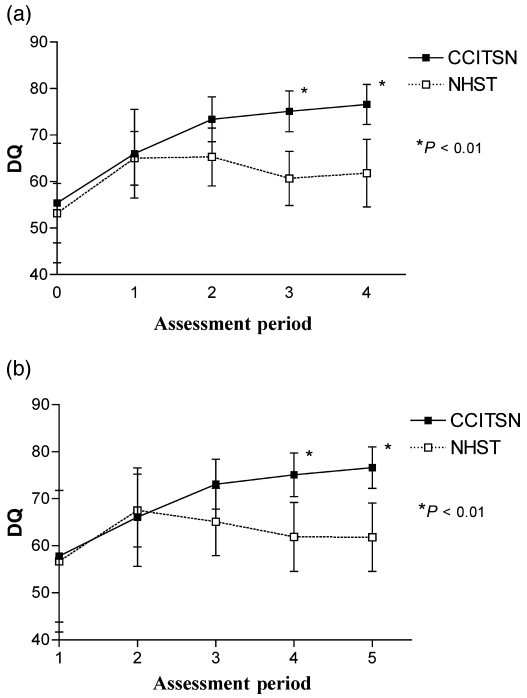

Figure 2. Mean values of DQ in the 2 groups at each assessment (0 = baseline, 1 = 3 months, 2 = 6 months, 3 = 9 months, 4 = 12 months). (a): full groups at randomization; number of subjects for comparisons at each timepoint (CCITSN: 0 = 24; 1 = 24; 2 = 24; 3 = 23; 4 = 21. NHST: 0 = 23; 1 = 16; 2 = 16; 3 = 16; 4 = 11). (b): subsets of children who completed the intervention. CCITSN: experimental group ($N = 21$); NHST: comparison group ($N = 11$). Bars represent 95% confidence intervals.

of outcomes in the 2 groups of children who actually completed the 2 interventions, alongside an intention to treat analysis (analyzing all subjects available over time).

Figure 2 shows the overall results of the study. Figure 2a refers to the full groups at randomization, with numbers of subjects varying over time (CCITSN: baseline = 24; 3 months = 24; 6 months = 24; 9 months = 23; 12 months = 21. NHST: baseline = 23; 3 months = 16; 6 months = 16; 9 months = 16; 12 months = 11), whereas Figure 2b takes into account only the subsets of children who completed the 2 interventions. Inasmuch as the 2 curves nearly overlap, we will discuss data pertaining only to Panel A. Children in the CCITSN group clearly exhibited a developmental gain over time, as shown by the difference between the first and the endpoint assessment: final DQ = 76.56, SD = 9.7, $P <$ .001. In contrast, children in the comparison group did not show a significant gain: final DQ = 61.76, SD = 10.8, $P >$ .05. Asterisks in the figure also indicate differences between the groups at the indicated assessment period. The results of a multivariate linear regression for the full groups at randomization revealed that only one predictor (from baseline measures) contributed significantly to the primary endpoint: the type of intervention (accounting for 7.7% of the variance).

DISCUSSION

Italy is a heterogeneous country where there are marked regional differences in income level and rates of unemployment as well as infant mortality, neonatal and perinatal care, and the general quality of health services. The differences are particularly evident between the north and the south of the country, with the southern regions

being more disadvantaged (Corchia et al., 1995; Piperno & Di Orio, 1990; Ulizzi, San Martini, & Terrenato, 1979). Following the introduction of the NHS in 1978, there has been an overall improvement in the provision of health services throughout the country, but regional differences still persist. With time the southern regions are bridging the gap but this kind of social differentiation is one of the most persistent epidemiological characteristics of the Italian population (Bollini, Reich, & Muscettola, 1988; Tamburlini, Ronfani, & Buzzetti, 2001).

This study has been carried out on the premise that a commitment to using parents as interventionists is not a common practice in many countries, such as Italy—and particularly the Italian south. Yet, it may be the most effective and cost-efficient way of providing services to young children with DS and those with other developmental disabilities, even in difficult sociocultural contexts. There are other philosophical and developmental reasons for pursuing this approach as well (see Guralnick, 1998). The scope of our study was then to provide evidence for the effectiveness of our model of early intervention, one that entailed direct parental involvement in the developmental support of children with disabilities in a sociocultural context characterized by uneven standards of healthcare and sometimes by difficult-to-reach health facilities. It is a relatively common practice in countries such as the United States to have parents involved in early intervention. In contrast, most southern Italian regions having families of children with special needs, such as children with DS, have to deal with the exact opposite situation, where professional therapists do tend to be the only providers of all kinds of intervention. As a consequence, parents are almost excluded from specific developmental care of their children.

A relevant ethical issue relates to the fact that by accepting to be enrolled in our study some families could be asked to take on the role of therapist and at the same time to renounce the standard treatment offered by the Italian NHS. It should be understood that such a choice sounded quite "revolutionary" at the time we started our project: in fact, most parents were aware of only one kind of intervention, that is, the one provided by professional therapists at NHS rehabilitation centers. Some considerations are now in order. First, parents willing to participate in the study were probably more inclined to be involved in delivering intervention and second, we were convinced that many parents in our region—though not all of them—could be successfully trained to act as interventionists for their children. There was sufficient evidence provided by the existing literature on parent training and education on the effectiveness of teaching parents specific strategies to support their children's development (Bidder, Bryant, & Gray, 1975; Kaiser & Hancock, 2003; Reid, Webster-Stratton, & Beauchaine, 2001; Stormshak, Kaminski, & Goodman, 2002; Wagner & Clayton, 1999). Without any doubt, it was a challenge for our work group, inasmuch as it was very difficult to fully engage a wide range of parents in our training program: our region encompasses rural, urban, middle-class, and low-income families with variable degrees of education. We were strongly motivated in carrying out our project because we hoped to change the widely held belief that parents do not have sufficient skills to support the development of their children with disabilities.

Unfortunately, the overall results of the study did not enable us to state that parent-implemented therapy was more effective than therapist-implemented treatment, inasmuch as the content, among other factors, also differed between the 2 types of interventions. Consequently, it must be remembered that at least 2 factors contributed to the developmental gains documented by our results: first, the therapist (parent vs professional therapist) and second, the type of intervention program (CCITSN vs NHST). The main determinants of the developmental score were not only the parents as teachers but also the intervention program used by interventionists. Nevertheless, a key contribution of our study is that a parent-implemented approach appears to be feasible in this context. The usual decline in

cognitive development often found for children with DS not receiving early intervention (see Guralnick, 1998) was not evident for the CCITSN group but was increasingly apparent for the NHST group. Clearly, longer term follow-up is needed as the effectiveness of early intervention should be evaluated in terms of enduring and sustained effects.

At this point, we would like to stress the importance of the dropouts. First, we should consider the original refusals, that is, families who refused entry into the study prior to random assignment. Even though no explanations were given by parents, we believe that we were not able to persuade them to fully engage themselves. As far as the dropouts after random assignment are concerned, and specifically those in the NHST group, we believe that families withdrew for practical reasons or because of a lack of interest in the type of treatment provided. The smaller number of dropouts from the CCITSN group further suggests that this is a feasible approach to early intervention.

Accordingly, our work suggests that, in our sociocultural context, early intervention provided by early education specialist–tutored parents might be an alternative to traditional intervention based solely on professional involvement. Likewise, the CCITSN is one of the structured curriculum programs that can be adapted to rely entirely on parental involvement for teaching children with disabilities developmental skills embedded in daily routines. Our data also confirm the flexibility of CCITSN and at the same time strengthen the idea that direct parental involvement, whenever possible, should be taken into account in planning early intervention programs for children with disabilities (Bidder et al., 1975; Kaiser & Hancock, 2003; Wagner & Clayton, 1999). It is worth remembering however that parental involvement in administering items of CCITSN is never as imposing as, for instance, the Doman-Delacato patterning method (American Academy of Pediatrics, 1982), because, as already mentioned, teaching skills become part of the parents' everyday routines with their children. Another strength of the CCITSN, in our opinion, resides in the number of items devoted to cognitive development, which focuses parents' attention on relevant abilities to further cognitive development. Finally, we believe that, having documented the usefulness of both parent-mediated early intervention and CCITSN even in quite difficult sociocultural contexts, it will prompt other countries with similar problems to take parent-based programs for early intervention into proper consideration.

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SHORT REPORT

A locus for sacral/anorectal malformations maps to 6q25.3 in a 0.3 Mb interval region

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Partial absence of the sacrum is a rare congenital defect that also occurs as an autosomal-dominant trait, whereas imperforate/ectopic anus is a relatively common malformation, usually observed in multiple congenital anomalies syndromes. We report on a girl born to healthy consanguineous parents (first cousins once removed) with anal imperforation and associated rectovaginal fistula and partial sacral agenesis. Facial dysmorphism included a high forehead, epicanthic folds, downslanting palpebral fissures, hypertelorism and a depressed nasal root. Brain MRI showed a bilateral opercular dysplasia with a unilateral (right) pachygyria; MRI and X-ray imaging of the spine disclosed a tethered cord associated with partial sacral agenesis. She showed a moderate developmental delay. Ophthalmologic examination evidenced bilateral microphthalmos and relative microcornea. Cytogenetic studies in our patient disclosed a pure *de novo* 6q25.3→qter deletion. By genotype analysis, we detected in our patient a maternal allele loss encompassing D6S363 and D6S446. Pure distal 6q deletion is a rare anomaly, reported in association with sacral/anorectal malformations (sacral agenesis, anal imperforation/ectopia) and never with cortical dysplasia. Pooling deletion mapping information in patients with pure terminal and interstitial 6q deletion allowed us to define a critical region spanning 0.3 Mb between the markers D6S959 and D6S437 for sacral/anal malformations. We hypothesize that haploinsufficiency for a gene within the deleted region may impair normal development of caudal structures, possibly acting on the notochordal development.
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Keywords: 6q deletion; sacrum agenesis; pachygyria; anal atresia; genotype/phenotype correlation; opercular dysplasia

Introduction

Terminal deletions of 6q (6q25→6qter) have been rarely reported in the literature and have been associated to a specific phenotype. Findings in a group of 26 patients[1] included mental retardation (100%), ear anomalies (88%), hypotonia (86%), microcephaly (82%), limb anomalies (71%), brain anomalies (67%), eye anomalies (50%), cardiac defects (48%), genital anomalies (48%) and seizures (38%). However, only in the patient by McLeod *et al*,[2] a pure deletion was found. To date, subtelomeric deletion of 6q have been reported in three patients,[3–5] and patients carrying a pure terminal or interstitial deletion of 6q have been rarely observed.[6–12]

Partial absence of the sacrum is a rare congenital defect, which also occurs as an autosomal-dominant trait; association with anterior meningocoele, presacral teratoma and

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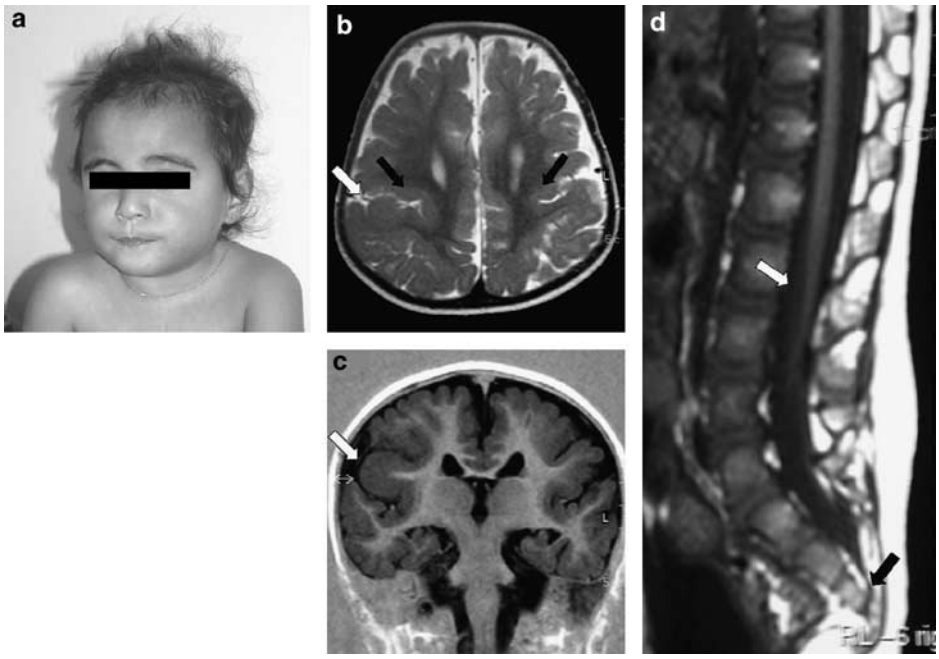

Figure 1 Clinical and neuroradiological aspects. (**a**) Facial appearance at age 3. (**b**) MRI: coronal section (T2), note normal gyral pattern. (**c**) MRI: frontal section (T1), bilateral opercular dysplasia and unilateral (right) pachygyria. (**d**) Sagittal MRI section of dorso-lumbar spinal canal disclosing tethered spinal cord.

anorectal abnormalities constitutes the Currarino triad (MIM 176450).

Imperforate/ectopic anus is a relatively common malformation. It has been rarely reported in familial cases (MIM 207500, MIM 301800), but no mapping data are available.

Patient report

The girl was born at 35 weeks of gestation to healthy consanguineous Malagasi parents (first cousins once removed). Family history and gestation were unremarkable. Birth weight was 2500 g (10th–25th centile), length was 48 cm (25th–50th centile) and cranial circumference was 32 cm (5th–10th centile). She was immediately admitted to a tertiary care center for surgical correction of an anal imperforation with an associated rectovaginal fistula. Imaging studies showed partial sacral agenesis and a tethered cord. Echocardiographic evaluation revealed atrial and ventricular septal defects that were corrected at the age of 12 months. Clinical evaluation at 24 months showed a severe developmental delay, microcephaly, short stature and minor anomalies. Weight was 10 kg (−1.3 SD), height was 80 cm (−1.4 SD) and head circumference was 44 cm (−2.8 SD). Facial dysmorphism included a high forehead, epicanthal folds, downslanting palpebral fissures, hypertelorism and a depressed nasal root (Figure 1a). Brain MRI showed a bilateral opercular dysplasia with a unilateral (right) pachygyria (Figure 1b and c); MRI and X-ray imaging of the spine disclosed a tethered cord associated with a partial sacral agenesis (Figure 1d).

At 3 years, 2/12 of age ophthalmologic examination evidenced bilateral microphthalmos (diameter of the globe <20 mm) and relative microcornea. Visual-evoked potentials and electroretinogram were normal.

Methods

Cytogenetic analysis

Subtelomeric FISH probing was performed with Cytocell Chromoprobe Multiprobe kit.

Genotype analysis

DNA from leukocytes of patient and their parents were used for genotyping. Simple fluorescent PCR assays were performed using polymorphic markers: D6S292, D6S308, D6S441, D6S1577, D6S415, D6S959, D6S363, D6S437, D6S1614, D6S1581, D6S264 and D6S446 (http://www.gdb.org/). PCR reactions were performed following standard procedures. After denaturation, each sample was loaded for electrophoresis on an Applied Biosystems model 3100 automated sequencer (PE Applied Biosystems, Perkin-Elmer). Data were analyzed using the Gene Scanner Model 3.7 Fluorescent Fragment Analyzer (PE Applied Biosystems, Perkin-Elmer) and electropherograms were generated for each sample. The samples from the patient and from his parents were processed for each marker. Data were analyzed using the Gene Scanner Model 3.7 Fluorescent Fragment Analyzer (PE Applied Biosystems, Perkin-Elmer).

Results

Metaphase cells analyzed from cultures of peripheral blood on the patient revealed a normal female chromosome complement at the 650-band level. A terminal 6q deletion was found by subtelomeric FISH. The patient's karyotype was designated as 46, XX, del (6)(q25.3qter). In both parents, FISH using identical probe for the subtelomeric region of 6p and 6q yielded normal result. There was no evidence for a balanced rearrangement in the parents. By genotype analysis, we detected in our patient a maternal allele loss for the D6S363, D6S1581, D6S264 and D6S446 markers (Figure 2).

Discussion

Present patient shows a multiple congenital anomalies syndrome owing to a pure *de novo* 6q23qter deletion. A peculiar finding is the presence of a bilateral opercular dysplasia associated to a unilateral pachygyria at the brain MRI. These brain malformations are probably consequent to the chromosomal deletion, although they have never been reported to date in other patients who carry an isolate interstitial or terminal 6q25 deletion. Moreover, no locus for brain malformation has been mapped in this region, with the exception of corpus callosum agenesis (6q25). Because of the presence of parental consanguinity, a recessive phenotype compounded by a homozygote mutation is also possible. A long-range effect of the deletion on gene expression outwith the deletion is another possibility to be considered.

On the other hand, distal 6q deletions have been frequently reported in association with sacral/anorectal malformations (sacral agenesis, anal imperforation/ectopia).

Our patient showed anal imperforation with associated rectovaginal fistula and partial sacral agenesis. Probably, a common pathogenetic mechanism is involved in producing both malformations. At an early stage of development, the notochord is known to organize normal development of central axial structures, such as the spinal cord, vertebral column and anorectum. However, its role has not been completely elucidated.[13] Recently, Qi *et al*[14] suggested that an alteration in sonic hedgehog signaling may be pivotal in producing abnormal notochord development and consequently sacral/anorectal malformations.

By comparing the reported cases of affected patients carrying a pure deletion, we delimited a critical region of 0.3 Mb for sacral/anorectal malformations, lying between the markers D6S959 and D6S437 (see Table 1). The patient by Pirola *et al*[10] shows an ectopic anus, which can be considered as a mild form of anorectal malformation. Our patient's contribution to the definition of a critical region should be cautiously considered, because of the simultaneous presence of another midline defect (agenesis of the corpus callosum).

We hypothesize that there is a gene in the deleted region whose haploinsufficiency impairs the normal development of these structures, possibly acting on the notochordal development or interfering with SHH signaling.

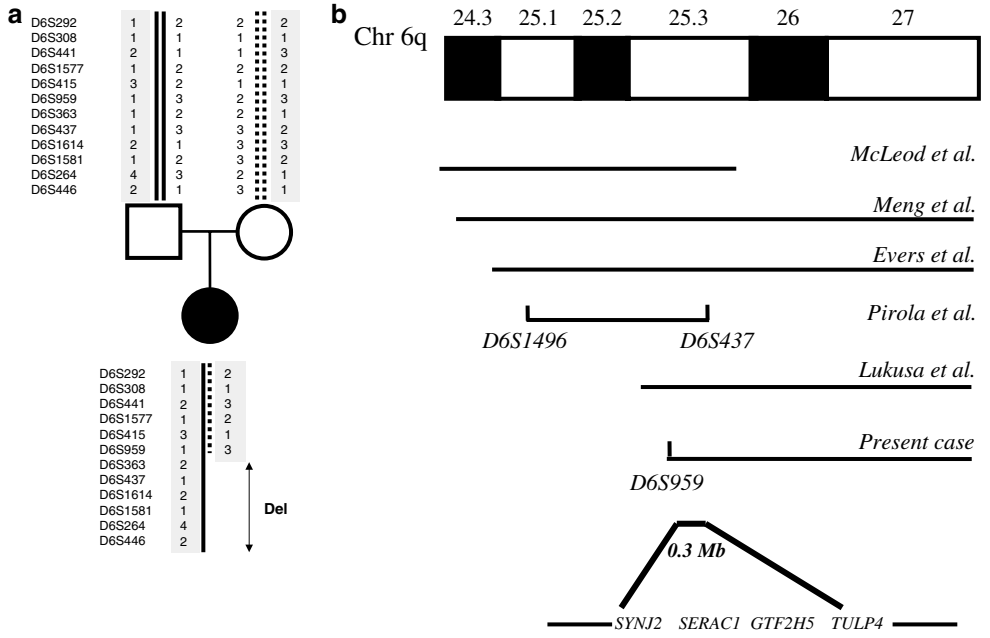

Figure 2 (**a**) Microsatelitte analysis of the deletion. Dotted arrow is used for noninformative markers. (**b**) Comparative deletion mapping in patients with 6q deletion and anorectal malformations. The overlapping segment between Pirola's case and our patient is depicted in the bottom line, with the four known genes.

Table 1 Clinical findings in selected cases with pure interstitial or terminal 6q deletions

	Present case	*Evers et al*[8]	*McLeod et al,*[2] *case 1*	*Meng et al,*[7] *case 1*	*Pirola et al*[10]	*Lukusa et al*[12]
Deletion	q25.3qter	q25.1qter	q23q25	q24.3qter	q25.1q25.3	q25.3qter
Sex	F	M	M	M	F	M
Age at report	3y 2m	2y	1m	4m	9m	10y
Microcephaly	+	+	–	+	–	–
Hypotonia	+	–	–	+	+	+
Seizures	–	–	–	–	–	–
Developmental delay	+	+	NM	NM	+	+
Brain neuroimaging	Bilateral opercular dysplasia pachygyria	Hydrocephalus	NM	Agenesis of corpus callosum	Agenesis of corpus callosum	Hydrocephalus
Retinal abnormalities	–	NM	–	–	–	+
Cardiac malformation	+	+	–	+	–	–
Sacral/anorectal malformation	Imperforate anus partial sacral agenesis	Bony appendix of the coccyx	Imperforate anus sacral agenesis	Imperforate anus	Ectopic anus	Spina bifida bony appendix of the coccyx

+, characteristic present; –, characteristic absent; NM, not mentioned.

To date, four genes are positioned in the deleted region: *SYNJ2* (synaptojanin 2), *SERAC1* (serine-active site containing 1), *GTF2H5* (general transcription factor iih, polypeptide 5) and *TULP4* (Tubby-like protein 4) (UCSC Genome Browser, http://genome.ucsc.edu/ and Ensembl Genome Browser, http://www.ensembl.org). *GTF2H5* mutations are responsible for trichothiodystrophy group A, a DNA repair syndrome, and for a form of ichthyosiform erythroderma with hair abnormality, and mental and growth retardation.[15] Sacral/anorectal malformations have not been observed in the reported patients. The Synj2b protein isoforms are localized in nerve terminals in rat brain and at spermatid manchette in rat testis. In glioblastoma cell lines, Synj2b seems implicated in the regulation of the formation of invadopodia and lamellipodia.[16] Mutations in *SERAC1* or *SYNJ2* cause male mouse sterility.[17] *TULP4* is a putative transcription factor of unknown function. No role has been attributed for the latter three genes in human developmental anomalies and/or diseases.

Other genes mapping outside the reported critical region could be involved, because of possible modifications on gene expression.

Report of further patients is needed to evaluate these genes as candidates in sacral/anorectal malformations, and their hypothetical role in notochordal development.

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CLINICAL OBSERVATIONS

Partial cerebellar hypoplasia in a patient with Prader-Willi syndrome

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Abstract
We report a 3-y-old male infant with Prader-Willi syndrome (PWS) caused by a *de novo* interstitial deletion of 15q11-q13. Additional features included a right cerebellar hemisphere hypoplasia. The extent of deletion was determined by FISH analysis using an SNRPN PW/AS probe that maps in the PWS/AS critical region (CR) and with specific 15q BACs. We unravelled an interstitial 15q11.2-q13.1 deletion spanning about 3 Mb.

Conclusion: To date only a few other PWS patients—including autopsy cases—with CNS structural anomalies have been described. Our case report adds knowledge to the issue of brain involvement in Prader-Willi syndrome. Further MRI studies of PWS patients will be helpful to clarify a correlation between PWS and brain abnormalities.

Key Words: *Cerebellar hypoplasia, NDN, Prader-Willi syndrome, SNRPN, snoRNAs*

Prader-Willi syndrome (PWS) is a neurobehavioral disorder that is due to the loss of multiple, paternally expressed, imprinted genes on human chromosome 15q11-q13. The most frequent pathogenetic mechanism (about 70% of all cases) is represented by a *de novo* interstitial deletion of paternal origin. Several genes have been mapped to the PWS critical region. The precise role of these genes in determining a specific PWS feature is still under debate. Cerebellar anomalies have been rarely reported in PWS and, moreover, molecular data are not available for all these cases.

Case report

We recently observed a 2-y-old Caucasian male because of mental retardation and dysmorphic features. Moderate hypotonia and feeding problems were noticed during the first months of life. Physical examination at 2.6 y revealed height 89.5 cm (−0.2 SD), weight 17.5 kg (+3.4 SD), occipitofrontal circumference 47.5 cm (−1.5 SD), facial dysmorphism (over-folded ear helix, convergent strabismus and pointed nose), a mild degree of right-convex thoracic scoliosis, and bilateral cryptorchidism. His general developmental quotient was 74 (Griffiths Mental Development Scales; normal range: 75–125). EEG showed a paroxysmal sharp-wave activity over the occipitoparietal regions, but no seizures were observed. Brain MRI disclosed a thin corpus callosum and a severe right cerebellar hemisphere hypoplasia (Figure 1). Auditory and visual evoked potentials were normal.

Molecular cytogenetic studies

Standard and high-resolution (HR) 650 G-banding karyotypes on peripheral blood lymphocytes from the patient and his parents were performed following standard procedures. Fluorescent *in situ* hybridization (FISH) was performed using standard techniques. The commercial DNA probe wcp15

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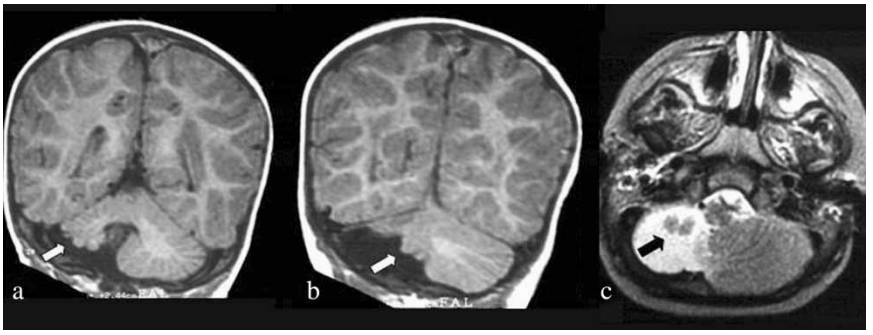

Figure 1. Brain MRI from the patient. a, b) Representative coronal T1-weighted images showing a marked hypoplasia of the right cerebellar hemisphere, associated with a partial hypoplasia of the inferior vermis (arrows), causing a right shift of the cerebellar midline. c) Axial T2-weighted image showing a dilatation of the liquoral spaces delimiting the inferior vermis. The liquor fills the right side of the posterior fossa because of cerebellar hemisphere hypoplasia (arrow).

(Bouty, Milan, Italy) for the whole chromosome 15 was used to detect rearrangements. Selected regions of chromosome 15 were hybridized with specific probes (Figure 2).

HR karyotype unravelled a 15q interstitial deletion. Painting of chromosome 15 did not show translocation to other chromosomes. FISH analysis by using polymorphic 15q probes showed a deletion of 3.07 Mb, flanked by RP11-218m19 on 15q11.2 and RP11-37J13 on 15q13.1 (Figure 2). The patient's karyotype was 46, XY, del(15) (pter→q11.2::q13.1→qter), *de novo*.

Discussion

Our patient presented with neonatal hypotonia, a low developmental quotient and a quite typical phenotypic appearance. On the other hand, he showed a structural cerebellar abnormality. To date only a few other PWS patients—including autopsy cases—with CNS structural anomalies have been reported [1–3]. Damage to the lateral cerebellar hemispheres and dentate nucleus certainly has a causative role in determining hypotonia, particularly in the neonatal period [4]. On the other hand, neonatal hypotonia represents one of the hallmark features of PWS and is a valuable clue to start PWS diagnostic testing. By using molecular cytogenetics, we unravelled an interstitial 15q11.2-q13.1 deletion spanning about 3 Mb. PWS arises from loss of paternally expressed genes in this region, including small nuclear ribonuclear protein N (SNRPN)/SNRPN upstream reading frame (SNURF), necdin (NDN), MAGE-like 2 (MAGEL2), makorin 3 (MKRN3)/zinc finger protein 127 (ZNF127), and genes for small nucleolar RNAs (snoRNAs). Gallagher et al. suggested that the minimal critical region for PWS is approximately 121 kb within the paternally expressed SNRPN locus [5]. This region also contains Prader-Willi chromosome region 1 (PWCR1)/HBII-85 and HBII-438 snoRNAs. The lack of expression of both genes is probably the cause for the neonatal lethality in the PWS model mice [6]. Recently, Schule et al. reported that PWCR1/HBII-85 has a major role in determining the human PWS phenotype [7]. Other potential candidate genes include NDN (OMIM 602117) and MAGEL2 (OMIM 605283). NDN encodes for a nuclear protein (necdin) having a role in determining brain developmental abnormalities [8,9]. MAGEL2, equally expressed in many parts of the brain, shows only a weak expression in the cerebellum, suggesting a general role in neural differentiation and maintenance rather than a specific role in cerebellar development [10]. Interestingly, our patient's deletion does not involve MKRN3/ZNF127 (OMIM 603856), an imprinted gene expressed in the cerebellum [11].

It is likely that structural cerebellar involvement is more frequent than generally thought in PWS, in as

	CHROMOSOME 15	
Band	BAC	Present/Deleted
15q11.2	RP11-218m19	+
15q11.2	RP11-73C9	-
15q11.2	RP11-41H23	-
15q11.2	PWS/AS SRNPN	-
15q12	RP11-20B10	-
15q12	RP11-570N16	-
15q13.1	RP11-37J13	+
15q13.1	RP11-25D7	+

Figure 2. Schematic representation of the deleted region. Selected regions of chromosome 15 were hybridized with the RPN PW/AS probe (Q.biogene) that maps in the Prader-Willi syndrome/Angelman syndrome (PWS/AS) critical region, and with bacterial artificial chromosome (BAC) probes mapping to 15q11.2 (RP11-218m19, RP11-73C9, RP11-41H23), 15q12 (RP11-20B10, RP11-570N16) and 15q13.1 (RP11-37J13, RP11-25D7) (http://www.ensembl.org).

much as cerebellar anomalies might only be evident on neuropathological examinations. Further high-resolution brain MRI studies of PWS patients will be helpful to clarify a correlation between PWS and brain abnormalities.

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Fetal stroke and congenital parvovirus B19 infection complicated by activated protein C resistance

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Abstract

Parvovirus B19 infection in gestation has been associated with severe fetal complications such as anaemia, hydrops and fetal demise. Fetal infection in the first trimester poses the greatest risk for these complications, but infection during the third trimester is more common than previously appreciated and can be associated with severe complications, i.e. fetal death, in the absence of hydrops or classical clinical symptoms. Parvovirus B19 infection has been associated with vasculitis and pathological changes in the central nervous system, which may cause stroke. We report a newborn infant with a rare combination of a recent central nervous system infection with parvovirus B19 and a factor V Leiden mutation, who developed fetal stroke.

Conclusion: Factor V Leiden mutation leads to activated protein C resistance and increases the risk of thromboembolism. Thromboembolism occurs rarely in newborns with activated protein C resistance, but can be precipitated by dehydration,

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Neurologia
a cura di L. Titomanlio

Novità in Neurologia Pediatrica

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Riassunto

L'articolo tratta dei progressi più significativi in Neuropediatria degli ultimi due anni. Per molte patologie, l'ausilio delle tecniche molecolari ha permesso un notevole passo in avanti nella Diagnostica e nella Fisiopatologia. Molte sindromi rare sono state caratterizzate. Per alcune malattie frequenti, quali le epilessie, l'identificazione di forme familiari ha permesso di clonare i geni coinvolti. Inoltre, una grande quantità di dati viene dagli studi clinici riguardanti ad esempio i nuovi farmaci antiepilettici e le terapie neuroimmunologiche. Infine, la Neurofisiologia clinica è entrata in una nuova era grazie all'utilizzo delle recenti tecniche neuroradiologiche (RMN funzionale e muscolare, *fiber tracking*), soprattutto nel campo della Neuropsicologia dello sviluppo.

Summary

Some significant advances in the field of pediatric neurology in the last 2 years are reviewed. For many disorders, many concepts and diagnostic procedures have changed due to the use of new genetic markers. For several syndromes we have now started to have clues to understand the underlining functional abnormalities. Family studies of frequent disorders (e.g. epilepsy), have allowed definition of the involved genes. In addition, new findings come from clinical trials (antiepileptic, immunotherapy). Finally, clinical neurophysiology obtains a second wind thanks to functional imaging (funcional MRI, muscle MRI, fiber tracking), especially in the field of developmental neuropsychology.

Introduzione

La Neurologia è una delle aree della Pediatria in cui la velocità di acquisizione delle scoperte scientifiche rende difficile un aggiornamento costante.
La selezione degli argomenti da noi operata per l'articolo di revisione generale ha cercato di tener conto sia dei progressi avvenuti, sia della loro utilità per il pediatra.
Lo stesso vale per gli articoli di approfondimento. Quello di Ruggieri et al. sulle novità diagnostiche nelle sindromi neurocutanee risponde all'esigenza di aggiornamento su tali patologie, che sono sempre più frequentemente diagnosticate in Pediatria. L'articolo di Romano et al. sul trattamento delle epilessie riassume le recenti evidenze sui trattamenti farmacologici attualmente a disposizione.
Per questo articolo, abbiamo pertanto cercato di fornire i messaggi essenziali, lasciando al lettore la scelta di un eventuale approfondimento con gli articoli citati in bibliografia.

Obiettivo della revisione generale

Scopo della presente rassegna è quello di fornire una panoramica quanto più completa delle novità in Neuropediatria degli ultimi due anni. Per comodità di trattazione e di lettura, abbiamo ritenuto opportuno suddividere l'argomento in due paragrafi principali: uno dedicato alla Neurologia (a cura di L. Titomanlio) e l'altro alla Miologia (a cura di E. Bertini e S. Quijano-Roy).

Metodologia della ricerca bibliografica

Per gli argomenti di questa rassegna generale abbiamo condotto una ricerca bibliografica su Medline utilizzando come motore di ricerca Pubmed con limiti generali, *all child, English, publication date 2003-2005*. Sono state impiegate le parole chiave: "*neurology*", "*neuropediatrics*", "*muscular*", "*myology*". Inoltre, sono stati presi in considerazione gli articoli pubblicati nelle più importanti riviste internazionali di Medicina, Pediatria e Neurologia negli ultimi due anni. Sono state infine riviste le linee guida presenti sul sito www.guidelines.gov modificate nello stesso periodo.

Dati salienti emersi dagli studi considerati

Neurologia

Epilessie
La diagnosi eziologica delle epilessie, sostenuta principalmente dai progressi in Neuroradiologia e basata su criteri clinici ed elettroencefalografici che sono sempre più precisi, ha chiarito la diversità delle sindromi epilettiche infantili e ne ha migliorato il trattamento (vedi anche articolo di Romano et al.). La Genetica ci dimostra sempre più di frequente che l'epilessia è spesso dovuta a delle mutazioni dei geni di canali ionici, che conducono a una ipereccitabilità dei neuroni che è a sua volta responsabile delle depolarizzazioni di membrana che generano le crisi convulsive (Tab. I). Mentre mutazioni del gene del canale del sodio *SCN2A* sono state ritrovate nelle convulsioni benigne familiari neonatali-infantili (patologia autosomica dominante con crisi che scompaiono prima dell'anno di età), le mutazioni nel canale del potassio

calcio-sensitivo BK sono associate non solo ad epilessia generalizzata, ma anche a movimenti anormali (discinesia parossistica) (Du et al., 2005). Questo conduce ad ipotizzare che anche alcune anomalie parossistiche dei movimenti, di tipo extrapiramidale, siano in realtà delle canalopatie. Un altro meccanismo eziologico in causa nella genesi delle epilessie è un controllo anomalo dell'apoptosi, ritrovato nell'epilessia mioclonica giovanile (mutazione del gene *EFHC1*). Infatti, la presenza della proteina mutata riduce l'apoptosi nei neuroni dell'ippocampo in un modello sperimentale animale, e ciò conduce probabilmente alla formazione di circuiti neuronali aberranti (Suzuki et al., 2004).

Per quanto riguarda la terapia delle sindromi epilettiche, una recente raccomandazione dell'Accademia Americana di Neurologia sulla sindrome di West (spasmi infantili) raccomanda come trattamento di prima scelta l'ACTH, ed in alternativa il vigabatrin (soprattutto nella sclerosi tuberosa). Non ci sono invece prove sufficienti per l'utilizzo dei corticosteroidi per os (Mackay et al. 2004).

Per le epilessie farmacoresistenti, la chirurgia è considerata una possibile alternativa. Mentre gli effetti a breve termine sono eccellenti, poco

Tab. I. Geni identificati responsabili di sindromi epilettiche (da Guerrini 2006, modificata).

Gene	Funzione	Sindrome epilettica	Anno di identificazione
SCN2A	Canale del sodio	GEFS+ Convulsioni neonatali-infantili benigne familiari	2004 2006
SCN1A	Canale del sodio	GEFS+ Epilessia mioclonica severa dell'infanzia	2000 2001
SCN1B	Canale del sodio	GEFS+	1998
KCNQ2	Canale del potassio	Convulsioni neonatali benigne familiari	1998
KCNQ3	Canale del potassio	Convulsioni neonatali benigne familiari	1998
BKA	Canale del potassio	Epilessia generalizzata e diskinesia parossistica	2005
ATP1A2	Pompa NA + -K +	Convulsioni neonatali-infantili benigne familiari Emicrania emiplegica familiare	2003 2003
CLCN2	Canale del cloro	Epilessia generalizzata idiopatica	2003
GABRA1	Recettore GABA	Epilessia mioclonica giovanile AD	2002
GABRG2	Recettore GABA	Convulsioni febbrili GEFS+ Epilessia con assenze	2001 2001 2002
GABRD	Recettore GABA	GEFS+	2004
CHRNA4	Recettore acetilcolina	Epilessia notturna del lobo frontale AD	1995
CHRNB2	Recettore acetilcolina	Epilessia notturna del lobo frontale AD	2000
LGI1	Proteina tumor-suppressor	Epilessia parziale con allucinazioni uditive	2002
EFHC1	Proteina coinvolta nell'apoptosi neuronale	Epilessia mioclonica giovanile	2005
RING3	Regolatore della trascrizione nucleare	Epilessia mioclonica giovanile	2003

si conosce sui reali benefici a distanza (*seizure-free* > 5 anni). Dati recenti dimostrano che i risultati sono buoni per le resezioni temporali (66%), ma sono ancora scadenti per gli interventi di callosotomia (35%) e per le resezioni frontali (27%) (Tellez-Zenteno et al., 2005).

Cefalee

I criteri diagnostici per le cefalee sono stati recentemente modificati. Le quattro categorie principali individuate sono le emicranie, le cefalee tensive, le cefalee a grappolo e altre cefalee primarie (Lipton et al., 2004). In considerazione della frequenza di questa patologia, l'inclusione negli studi di un numero elevato di pazienti permette di giungere ad un trattamento sempre più basato sulle evidenze. Per l'attacco acuto di emicrania si consiglia attualmente in prima istanza l'ibuprofene o, per i bambini di più di 8 anni, il sumatriptan per via nasale (Ahonen et al., 2004). Un trattamento di profilassi con flunarizina (5-10 mg al giorno), quando necessario, sembra essere efficace (Lewis et al., 2004).

Neurogenetica

Se la Neuropediatria è in veloce progresso, la Neurogenetica è, assieme all'Epilettologia, la branca con il maggior numero di articoli scientifici. Tra i differenti geni e proteine individuati, sono stati selezionati quelli di maggior interesse dal punto di vista patogenetico.

Qualsiasi condizione che influisca sulla crescita del cervello durante tutta la vita fetale e durante l'infanzia causerà un microcefalia, che si tratti di una patologia acquisita (post-anossica, post-ischemica, lesione cerebrale d'origine malnutrizionale, embriofetopatie infettive o da teratogeni …) o a seguito di un'anomalia dello sviluppo cerebrale. Quest'ultimo gruppo di microcefalie può essere diviso, in modo semplice, in forma isolata (microcefalia primaria) ed in una forma sindromica nella quale la microcefalia coesiste con altre anomalie dello sviluppo, nel quadro di una anomalia cromosomica o di sindrome polimalformativa. Le microcefalie congenite isolate sono causate da mutazioni di differenti geni, molti dei quali identificati recentemente (Bond et al., 2005) (Tab. II). In tutte queste forme la circonferenza cranica alla nascita (a termine) si situa tra 24 e 29 cm (valore normale: superiore a 32 cm). Tutti i geni identificati sinora intervengono nella divisione neuronale. Il più frequentemente coinvolto (*ASPM*, che è mutato nel 50% dei casi) contiene dei motivi ripetuti "IQ" (Isoleucina-Glutamina) che sono in numero sempre maggiore seguendo la scala evolutiva. La funzione di questi geni è probabilmente importante dal punto di vista evoluzionaristico. Difatti, la proliferazione delle cellule progenitrici, che daranno origine ai neuroni del cervello adulto, è regolata da questi geni a livello del fuso mitotico. Una minore attività di questi geni conduce ad un minor numero di divisioni neuronali e quindi ad una microcefalia (Koupirina et al., 2005).

La sindrome Warburg-Micro, rara associazione di microcefalia, microftalmia e microgenitalismo, è causata da mutazioni della proteina RAB3GAP, che è una proteina attivatrice della GTPasi RAB-3. Quest'ultima è coinvolta nel rilascio di ormoni e neurotrasmettitori (Aligianis et al., 2005). È dunque ipotizzabile che mutazioni di proteine implicate in vie di controllo metabolico, in questo caso quello delle proteine Rab, conducano a deficit di fattori (neuro)trofici, a loro volta responsabili delle alterazioni ipoplasiche riscontrate nella sindrome. Un'altra alterazione nelle vie di controllo metaboliche è osservata nelle mutazioni del fattore di ADP-ribosilazione *ARFGEF2*. Questa anomalia porta ad un disturbo del trasporto di vescicole endoplasmiche verso la superficie neuronale. Che conduce ad una microcefalia associata ad anomalie di migrazione neuronali (eterotopie periventricolari) (Sheen et al., 2004).

La sindrome di Williams (SW) è una patologia caratterizzata dalla variabile associazione di stenosi aortica sopra-valvolare, stenosi delle arterie polmonari periferiche, ipercalcemia neonatale idiopatica e da un aspetto caratteristico del volto (strabismo, radice del naso piatta, labbra sporgenti e mento piccolo). Le difficoltà di apprendimento e di coordinazione motoria che si riscontrano nella SW non impediscono lo sviluppo di una capacità di

Tab. II. Geni identificati nelle microcefalie primarie isolate (da Woods et al., 2005; modificata).

Gene	Localizzazione	Proteina
MCPH1	8p22-ter	Microcephalin
MCPH2	19q13.1-13.2	–
MCPH3	9q34	CDK5RAP2
MCPH4	15q	–
MCPH5	1q31	ASPM
MCPH6	13q12.2	CENPJ
MRXS9	Xq12-q21.31	–
ALM	17q25	SLC25A19

espressione associata a socievolezza ed espansività. La SW è causata da una microdelezione di una regione che si trova sul braccio lungo del cromosoma 7 (7q11.23) e che contiene geni fondamentali nello sviluppo e nella modulazione del linguaggio espressivo. Mentre una delezione conduce alla iperfluenza verbale tipica della sindrome, una duplicazione della regione da luogo ad un fenotipo caratterizzato da deficit di espressione severo (Somerville et al., 2005). Questa affascinante scoperta ha delle ovvie implicazioni anche per la diagnosi molecolare del ritardo del linguaggio severo.

Per terminare, sono stati scoperti i primi 2 geni responsabili della sindrome di Joubert (JS), il gene della nefrocistina (*NPHP1*) (Parisi et al., 2004; Castori et al., 2005) e quello della jouberina (*AHI1*) (Ferland et al., 2004). La JS è una sindrome atassica congenita con aprassia oculomotoria, a trasmissione autosomico recessiva e caratterizzata da una malformazione caratteristica della giunzione ponto-mesencefalica ("segno del dente molare" alla RMN) ed associata frequentemente a retinopatia e nefronoftisi. Almeno altri 3 loci genetici sono associati alla JS (Valente et al., 2005).

Malattie neurometaboliche

Notevoli progressi si sono avuti con l'identificazione di svariate patologie neurometaboliche, anche nuove. Un esempio è dato dal deficit completo di glutamina nel liquor, che è causato da mutazioni della glutamina sintetasi e che è stato descritto sinora in solo due pazienti. Il deficit di glutamina provoca malformazioni cerebrali severe, che possono essere anche isolate (Haberle et al., 2005).

Sul versante terapeutico, il trapianto di cellule staminali di cordone è risultato essere efficace anche nella malattia di Krabbe infantile, patologia metabolica dovuta ad un deficit di galattocerebrosidasi che conduce ad una leucodistrofia progressiva, sino al decesso. Purtroppo i risultati sono buoni solo per i pazienti asintomatici, diagnosticati per la presenza di un affetto nella famiglia, e non per i bambini che hanno già sviluppato la malattia (Escolar et al., 2005). Da quanto detto, è ovvio che porre la diagnosi di malattia di Krabbe abbia dei risvolti nuovi ed importanti per la consulenza genetica alla famiglia.

Neurologia neonatale

Il progresso nella gestione della patologia del prematuro ha portato alla sopravvivenza di molti bambini con delle sequele neurologiche spesso di lieve entità. Uno studio recente, che ha esaminato 308 bambini di 6 anni nati molto prematuri (EG < 25 settimane), ha dimostrato che la frequenza di deficit cognitivo (21%) e neurologico (12%) in questi pazienti è ancora importante e richiede una osservazione clinica prolungata.

La ricerca in neuroprotezione è molto attiva e numerosi articoli ne trattano. Tra i meccanismi più importanti c'è l'ipotermia indotta. Ad esempio, una temperatura corporea di 33,5 °C per 3 giorni, seguita da un lento riscaldamento, è efficace nel ridurre il rischio di decesso o disabilità in neonati di età gestazionale superiore a 36 settimane, che hanno sofferto di una encefalopatia ipossico-ischemica moderata/severa (Shankaran et al., 2005).

Patologia neurovascolare

L'ictus cerebrale, ischemico o emorragico, è sempre più diagnosticato in età pediatrica. Questo si deve alla sensibilizzazione dell'ambiente medico e al diffondersi di mezzi diagnostici quali l'angio-IRM. Le cause non sono ancora ben conosciute, e molteplici fattori sono stati messi in causa (DeVeber, 2005).

Per gli infarti cerebrali perinatali di origine arteriosa, la prognosi è determinata dall'esordio dei sintomi. Quanto più questo è tardivo (> 28 giorni), maggiore è il rischio di paralisi cerebrale infantile (RR = 2,2). Il rischio è aumentato (di circa 2 volte) se si tratta di grossi infarti, o di infarti che coinvolgono le aree di Broca, Wernicke, la capsula interna o i gangli della base (Lee et al., 2005). Per il trattamento, il consenso è raggiunto solo per lo *stroke* nei pazienti con drepanocitosi (terapia trasfusionale), e per la trombosi dei seni venosi, la dissezione arteriosa e l'embolismo cardiaco (anticoagulanti o eparina di basso peso molecolare) (DeVeber, 2005).

Neuroplasticità

La neuroplasticità può essere definita come la capacità del cervello ad adattarsi in maniera ottimale in risposta ai diversi stimoli. La plasticità è maggiore nelle prime fasi del periodo prenatale, come ha dimostrato uno studio sulle emiparesi congenite. La riorganizzazione dei tratti corticospinali ipsilaterali, che vanno ad innervare in parte anche il lato paretico, e suppliscono quindi al deficit motorio, è più efficiente nel primo trimestre della gravidanza e decresce successivamente con l'avanzare della stessa (Staudt et al., 2004). Riguardo alla plasticità dell'ippocampo, è stato ipotizzato in un modello murino che questa sia

prodotta dall'azione del VEGF, attraverso un incremento della neurogenesi che migliora le capacità di apprendimento e la memoria (Cao et al., 2004). Infine, è stato recentemente dimostrato che i non-vedenti, soprattutto se per patologia congenita, hanno delle capacità di percezione uditiva superiore alla norma. Ciò serve verosimilmente a compensare il deficit visivo, ed è vero non solo per i suoni puri, ma anche per le voci umane e per la musica (Gougoux et al., 2004).

Neuroimmunologia

La patologia Neuroimmunologica probabilmente più frequente in età pediatrica è la sindrome di Guillain Barré. Si tratta di una poliradiculonevrite acuta demielinizzante che, nella sua forma motrice assonale, può essere associata ad una infezione gastrointestinale da C. Jejuni (Kuwabara et al., 2004).

Dei fattori genetici sembrano avere un ruolo in questa patologia acquisita, data la presenza di casi familiari (Geleijns et al., 2004). La severità della patologia è infatti in relazione con i polimorfismi del Fc gamma-RIII (recettore delle IgG dei leucociti) (Van Sorge et al., 2005), ma non con gli alleli HLA di classe II (Geleijns et al., 2005).

La sclerosi multipla è una patologia ad esordio anche in età pediatrica (Ruggieri et al., 2004). Sono purtroppo ancora irrisolti i problemi di diagnosi differenziale, soprattutto con le encefalomieliti acute disseminate. Sono ad oggi individuati due loci genetici di suscettibilità alla patologia, sul cromosoma 6 (associato all'HLA) e sul cromosoma 1 (Reich et al., 2005). Inoltre, sembra essere sempre più in causa una alterazione della risposta immunitaria contro l'EBV (Sundstrom et al., 2004). Per la prevenzione della sclerosi multipla, l'assunzione di vitamina D (> 400 UI/giorno) sembra avere un ruolo protettivo (Munger et al., 2004), ma questi dati vanno certamente confermati. L'avanzare delle ricerche permetterà forse un giorno di poter identificare e modulare i diversi trattamenti immunologici disponibili caso per caso.

La sindrome di Kinsbourne, caratterizzata da movimenti caotici oculari (opsoclono) e da mioclonie erratiche, può essere idiopatica o anche secondaria ad un neuroblastoma, che è sempre da ricercare nei pazienti affetti. L'etiopatogenesi è in relazione ad anticorpi contro i neuroni granulari del cervelletto (Blaes et al., 2005). Ciò causa appunto i sintomi cerebellari, le mioclonie e il movimento caotico degli occhi. Oltre la rimozione del neuroblastoma, una terapia immunologica che sopprima il clone produttore di anticorpi, ad esempio con farmaci anti-CD20 (Rituximab), può essere efficace (Pranzatelli et al., 2005).

L'encefalite di Rasmussen, patologia immunitaria ancora più rara e a causa sconosciuta, causa una atrofia progressiva soltanto di un emisfero cerebrale. Quest'ultima può essere almeno in parte migliorata dall'impiego di tacrolimus, un agente immunosoppressore. Questo farmaco non ha effetti sulla severità dell'epilessia, invariabilmente associata alla sindrome, ma può ritardare la emisferectomia, che è a tutt'oggi l'unica opzione valida per preservare la funzionalità del lato sano (Bien et al., 2004).

Autismo

Negli ultimi due anni molti studi si sono concentrati sulla patogenesi dell'autismo. In una proporzione ancora ignota di bambini con autismo, dei fattori neuroimmunologici sembrano essere in causa, attraverso un processo infiammatorio contro la corteccia, la sostanza bianca ed il cervelletto (Vargas et al., 2005). Un coinvolgimento della sostanza bianca, che aumenta di volume in tutti i lobi cerebrali e che tocca le connessioni intraemisferiche e cortico-corticali (secondario?), potrebbe anche essere un fattore importante (Herbert et al., 2004). Tra i geni che conferiscono una suscettibilità all'autismo c'è EN2, gene *homeobox* di regolazione dello sviluppo cerebrale (Benayed et al., 2005), ma anche le subunità dei recettori del neurotrasmettitore GABA sono implicati: GABRA4 è il più importante, e aumenta il rischio di sviluppare l'autismo attraverso una interazione con la subunità GABRB1 (Ma et al., 2005).

Patologie neuromuscolari

Gli avanzamenti nella Genetica degli ultimi anni hanno rivoluzionato le conoscenze sulle patologie neuromuscolari e sulla loro diagnostica. L'identificazione di nuovi geni ha aiutato a riconoscere molte di queste malattie ma ha anche rotto le frontiere nosologiche, basate finora sulla descrizione fenotipica e sui dati istologici, poiché ha rivelato una marcata sovrapposizione di genotipi e fenotipi.

Per esempio, mutazioni del gene della lamina A/C (LMNA) conducono a lesioni di tipo distrofia muscolare (Emery-Dreifuss) ma anche di tipo neuropatico (Charcot-Marie-Tooth) (Chaouck et al., 2003), o a patologie insospettabili quali la progeria (Van Esch et al., 2005) e la lipodistrofia (Vantighem et al., 2004).

D'altro canto, anche la separazione nosologica tra le distrofie muscolari congenite (DMC) e le miopatie congenite non è così netta. A titolo d'esempio i geni del colageno COL6 e il gene SEPN1 provocano lesioni miopatiche distrofiche e non-distrofiche, anche in muscoli diversi biopsiati simultaneamente.
Un altro passo in avanti è stata la identificazione dei deficit della O-glicosilazione nel cervello e nel muscolo (destroglicanopatie), che sono causate da molteplici mutazioni. Quelle identificate sinora sono:
– la forma giapponese, o DMC di Fukuyama;
– la forma finlandese, o *muscle-eye brain disease* (MEB);
– la sindrome di Walker-Warburg;
– la DMC di tipo 1C, per mutazioni del gene FKRP;
– la DMC di tipo 1D, per mutazioni del gene LARGE.

L'alterazione di questi geni può condurre a fenotipi estremamente variabili, da un aumento isolato delle CPK (asintomatico) a differenti forme di distrofie muscolari (congenite, dei cingoli) con o senza ritardo mentale e con o senza anomalie strutturali del SNC.
Per definire al meglio la storia naturale delle differenti patologie muscolari, si stanno compiendo degli sforzi per validare le scale di funzione motoria a livello internazionale, come ad esempio per la amiotrofia spinale (Swoboda et al., 2005).
Inoltre, nuove tecniche radiologiche, quali la RMN muscolare, cominciano ad essere di grande aiuto per la diagnosi ed il follow-up di alcune di queste patologie (Mercuri et al., 2005).

Malattie del motoneurone
La amiotrofia spinale infantile prossimale (Werdnig-Hoffmann), causata da alterazione del gene SMN1, è la patologia neurodegenerativa autosomica recessiva che causa la maggiore mortalità infantile. La sua incidenza è infatti elevata (1:25.000 nati vivi in Italia). Numerosi studi sono in corso per cercare di comprendere la fisiopatologia molecolare. Vi sono normalmente 2 copie di geni SMN in ogni cromosoma, il gene primario chiamato SMN1, ed un gene quasi identico, SMN2, che differisce per pochi nucleotidi a livello dell'esone 7. La perdita di SMN1 è essenziale nella patogenesi della SMA, mentre la gravità della malattia è condizionata dal numero di copie di SMN2. Approssimativamente 2 copie di SMN2 (con un circa 20% di proteina residua normalmente funzionante) esitano in una SMA tipo 1, tre copie sono correlate ad una SMA tipo 2, quattro copie con una SMA tipo 3, mentre le portatrici di SMA (presumibilmente con un livello del 60% di proteina) sono asintomatiche. Il gene SMN2, a causa di uno *splicing* diverso produce una piccola quantità di proteina SMN funzionante, mentre la maggior parte del prodotto, mancante dell'apporto dell'esone 7 nel trascritto, si degrada facilmente. L'identificazione di sostanze che possano aumentare i livelli di produzione della proteina modificando lo *splicing* del gene residuo SMN2 e spingendolo a produrre una maggiore quantità di proteina SMN funzionale potrebbe rappresentare una strategia terapeutica efficace per la atrofia muscolare spinale. La programmazione di studi clinici di efficacia terapeutica con molecole quali il valproato o il fenilbutirrato (Andreassi et al., 2004; Brahe et al., 2005), che sembrerebbero aumentare i livelli della proteina SMN modificando i meccanismi di *splicing* del gene duplicato SMN2, è attualmente in corso in diversi Paesi (Germania, Stati Uniti, Canada, Francia, Italia).

Distrofie muscolari congenite (DMC)
Esistono attualmente 12 geni implicati nelle DMC (Fig. 1):
– COL6A1, COL6A2 e COL6A3 (DMC di tipo Ullrich);
– SEPN1 (DMC con sindrome "*rigid spine*" tipo I, RSMD1);
– LAMA2 (deficit primario in merosina, MDC1A);
– ITGA7 (deficit di integrina alfa);
– Fukutina (FCMD);
– FKRP (MDC1C);
– POMGnT1 (*Muscle-Eye-Brain disease*, MEB);
– POMT1 e POMT2 (*Walker-Warburg syndrome*, WWS);
– Large (MDC1D).

Sebbene le DMC fossero considerate finora un gruppo di patologie a trasmissione autosomica recessiva, è stato scoperto che i difetti dei geni del collageno di tipo VI (COL6A1, A2 o A3) possono condurre a forme congenite con trasmissione autosomica dominante o a neomutazioni nei casi sporadici (Pepe et al., 2006; Giusti et al., 2005). La fisiopatologia di queste forme è complessa e il difetto del collageno di tipo VI sembra dia origine a una anomalia mitocondriale secondaria reversibile *in vitro* con la ciclosporina A, che potrebbe dunque essere di valore terapeutico (Irwin et al., 2003).
Le scoperte riguardanti il gene SEPN1 sono state importanti soprattutto per quanto riguarda l'ampiezza dello spettro anatomopatologico, riscontrandosi mutazioni in un gran

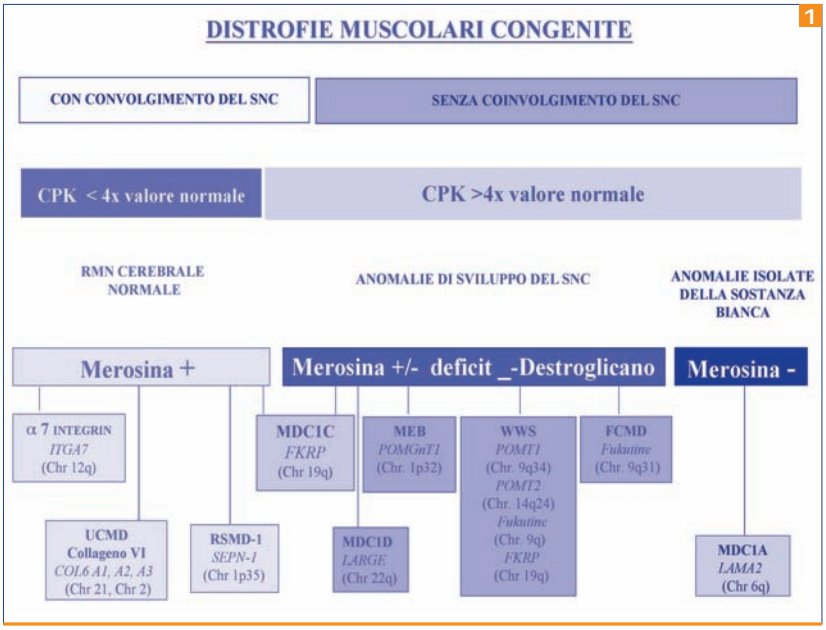

Fig. 1. Algoritmo diagnostico delle possibili mutazioni genetiche in causa nelle DMC. I geni sono in corsivo, e ne è riportata la localizzazione cromosomica. L'assenza di anomalie della merosina alla biopsia muscolare e del sistema nervoso centrale (SNC), associata a dei valori di CPK normali o poco elevati suggerisce una delle patologie a sinistra dello schema:
a) se il fenotipo è tipo Ullrich (iperlassità distale e retrazioni muscolo-tendinee assiali e prossimali), uno studio del collageno di tipo VI sul muscolo e sui fibroblasti è necessario;
b) se si ritrova una ipomobilità della colonna vertebrale associata ad una scoliosi e le retrazioni muscolo-tendinee sono poco evidenti, c'è indicazione alla ricerca di mutazioni del gene SEPN1.
Le forme con un aumento importante delle CPK ma senza ritardo mentale né malformazioni cerebrali, suggeriscono delle mutazioni del gene FKRP.
Le DMC con coivolgimento del SNC, rappresentate nella parte destra dello schema, sono: a) i diversi tipi di destroglicanopatie, che si presentano invariabilmente associate a malformazioni del SNC; b) il deficit primario di merosina, che si presenta nel primo anno di vita con una anomalia isolata della sostanza bianca senza ritardo psicomotorio.

numero di pazienti con la variante *multiminicore*, in pazienti con una desminopatia con corpi di tipo *Mallory-body like* (Ferreiro et al., 2004) e anche in alcuni pazienti con miopatia a tipo *fiber type disproportion* (Clarke et al., 2006). In tutti questi pazienti, indipendentemente dai dati morfologici, il fenotipo è sorprendentemente omogeneo: essi sviluppano una scoliosi severa e molti necessitano di una ventilazione meccanica nella prima decade di vita, conservando in generale la marcia. Questi dati hanno condotto ad utilizzare il termine di miopatia "*SENP-related*", che sembra essere più corretto di sindrome *rigid spine* di tipo I.

Le scoperte più notevoli si sono però avute nel campo delle alfa-distroglicanopatie.

La identificazione di nuovi geni responsabili di queste patologie (POMT2 e LARGE) (Van Reewijk et al., 2005; Longman et al., 2003) ha confermato che le sindromi classiche precedentemente descritte (Walker-Warburg, MEB e Fukuyama) non sono monogeniche e che inoltre uno stesso gene può produrre differenti fenotipi. Per esempio mutazioni del gene della FKRP (*fukutin-related-protein*) sono state identificate in pazienti con aumento isolato delle CPK, in una forma simil-Duchenne, con distrofia dei cingoli, fino a casi con malformazioni cerebrali severe (Beltran-Valero de Bernabe et al., 2004; Quijano-Roy et al., 2005).

Esistono infine dei pazienti con forme non ancora ben definite di distrofia muscolare, anche associate a malformazioni complesse. Tra queste ultime, sono state pubblicate le mutazioni del gene SIL1, che codifica per una proteina del reticolo endoplasmatico, nella sindrome di Marinesco-Sjögren. In questa patologia, la distrofia muscolare si associa a un ritardo mentale, una atrofia cerebellare, una insufficienza gonadica ed una cataratta (Anttonen et al., 2005; Senderek et al., 2005).

Conclusioni e prospettive per il futuro

La varietà e la vastità delle malattie neuropediatriche rende difficile una revisione esaustiva. Certo è che i progressi notevoli della genetica e delle recenti metodiche di *neuroimaging* stanno permettendo un costante avanzamento nella diagnostica e nel follow-up di queste patologie. In particolare, le immagini di risonanza magnetica ottenute con DTI (*Diffusion Tensor Imaging*, una tecnica che analizza la direzione spaziale del processo di diffusione delle molecole d'acqua nel cervello) consentono una analisi della struttura delle sostanza bianca ed una ricostruzione tridimensionale del percorso delle fibre nervose. La RMN muscolare, già utilizzata nello studio del metabolismo e della morfologia muscolare, sta diventando un prezioso orientamento diagnostico nelle DMC.

La comprensione dei meccanismi fisiopatologici alla base di molte malattie neuromuscolari permette di sperare di ottenere delle terapie efficaci, almeno parzialmente, in un futuro prossimo. Se è già vero per le epilessie, ciò si sta realizzando progressivamente nelle malattie neuroimmunologiche e non tarderà a interessare altri campi quali la Neurologia fetale e neonatale, la Miologia e le patologie genetiche e neurometaboliche.

Box di orientamento

- I progressi notevoli della genetica e delle metodiche neuroradiologiche stanno permettendo un costante avanzamento nella diagnostica e nella comprensione della fisiopatologia della patologie neuromuscolari.
- Sono stati identificati alcuni geni responsabili di sindromi epilettiche frequenti, come quello della epilessia mioclonica giovanile, e molti altri che sono implicati in patologie quali la sindrome di Joubert, le microcefalie isolate e le distrofie muscolari congenite.
- Molti studi si focalizzano sulla patogenesi dell'autismo, la cui etiologia sembra essere multifattoriale (predisposizione genetica e possibile componente immunologica).
- Le metodiche di neuroprotezione, soprattutto in Neonatologia, e le terapie neuro-immunologiche (anticorpi anti-CD20) sono oramai utilizzate nella pratica clinica e danno buoni risultati. Il trapianto di cellule staminali di cordone è stato efficacemente impiegato nei pazienti asintomatici con malattia di Krabbe infantile.
- Protocolli di terapia genica sono in corso per il trattamento di patologie severe (m. di Werdnig-Hoffmann) e lasciano ben sperare per il futuro.

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