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Letter to Editor Open Access

First Skin Biopsy Reports in Children with Autism Show Loss of C-Tactile Fibers

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Abbreviations

ASD: Autism Spectrum Disorder; CARS: Childhood Autism Rating Scale; ATSC: Autism Touch and Self-regulation Checklist. IENF: Intraepidermal Nerve Fibers; SFN: Small-Fiber Neuropathy

Introduction

Autism is the most common developmental disability, affecting 1/45 children [1]. Its cause remains unknown. Clinically, it is defined by the appearance of social/language delay, unusual, repetitive behavior, and abnormal sensory responses by the age of 3 [2]. Abnormal responses to touch are amongst the earliest and most universally prevalent of abnormal sensory symptoms [3]. In 2013, when abnormal sensory responses were reclassified from co-morbid autism symptoms to core diagnostic symptoms, the need for definitive evaluation of the sense of touch was recognized. Children with autism are distinguished both by lack of interest in affective and affiliative touch [4] and by a generalized pattern of allodynia and hypoesthesia affecting multiple areas including the face, mouth, hands and feet [5]. Pain, temperature and affective/affiliative touch are mediated by small unmyelinated fibers known as C-tactile fibers [6]. Small fiber loss is diagnosed by skin biopsy and specialized staining [7]. Quantitative sensory testing is invalid in children with autism, and skin biopsies have not yet been reported. This is an exploratory study to determine whether C-tactile fiber loss is present in four autistic children with signs of hypoesthesia and allodynia.

Materials and Methods

Subjects

Children were recruited from the mid-Willamette valley in Oregon. Inclusion criteria were

- Medical diagnosis of ASD.
- Age between 8 and 12 years.

Exclusion critierion was another chronic medical/neurological condition that would have a significant impact on small fibers (e.g. diabetes). The study was conducted with Institutional Review Board approval, and parents of all participants signed informed consent on their behalf. Four male children aged 8, 9, 9 and 11 met study criteria. We used published normative data for biopsies in the distal leg in children aged 10-19 as the comparison group [8,9]. Normal distal leg intraepidermal nerve fiber (IENF) density for 10-19 year olds is Mean/SD 20.3 ± 7.4; range is 12.7-36.5. No published normative IENF data are available for children younger than 10.

Verification of autism diagnosis

Diagnostic records were reviewed: all children had received the autism diagnosis by a neurodevelopmental paediatrician. A Childhood Autism Rating Scale (CARS) [8] was administered and confirmed the diagnosis. The CARS is a widely used rating scale for the diagnosis of autism and a reliable measure of autism severity [10]. A score of 25.5 is the cut-off for a diagnosis of autism on the mild end of the spectrum; 30 to 36 is the moderate range; and > 36 is the severe range.

Assessment of tactile responses

In the absence of children's ability to cooperate with neurological exam, tactile responses were evaluated with the Autism Touch and Self-regulation Checklist (ATSC) [11]. The ATSC is a validated parent/caregiver questionnaire that assesses abnormal tactile responses by location and severity in the context of daily life. Higher scores reflect greater abnormality. Questions from the tactile section of the ATSC were used to identify hypoesthesia (e.g. "Does not cry tears when hurt") and allodynia (e.g. "Face-washing is difficult," "Haircuts are difficult"). Mean tactile scores for autistic children are 28 ± 8; and for typically developing children are 11 ± 7.

Skin biopsy procedure

Following local anesthestic, a 3 mm punch biopsy specimen was taken from the distal leg, 10 cm above the lateral ankle. Biopsy samples were fixed in Zamboni's solution, cryoprotected and submitted to the Carlo Besta Neurological Institute in Milan for evaluation. Cryosections were cut at a thickness of 50 µm and stained by an immunoperoxidase method using polyclonal antibodies directed against the panaxonal marker protein gene product 9.5. Using bright field microscopy, total intraepidermal nerve fibers (IENF) were counted in 3 different tissue sections and expressed as the number per millimeter of length of the epidermis.

Results

Characteristics of children and results of CARS testing, tactile assessments and skin biopsies are shown in Table 1. CARS testing confirmed previous autism diagnoses and demonstrated that all four children were in the diagnostic range for ASD; one child in the mild range, and three in the severe range. ATSC testing demonstrated elevated scores for hypoesthesia and allodynia in all four children. Skin biopsies demonstrated a mean IENF count that was less than 50% of

the mean reported for normal controls aged 10-19; morphology was normal. Results are shown in Table 1.

Age	Gender	CARS Score	ATSC Score	IENF Density	IENF Morphology
8	M	38	22	9.5	Normal
9	M	29.5	39	8.7	Normal
9	M	52	42	11.2	Normal
11	M	42.5	46	7.4	Normal
CARS scores diagnostic of autism>25.5 ATSC tactile score typical children: Mean/SD 11 ± 7; Mean/SD autistic children: 28 ± 8 Normal distal leg IENF density for 10-19 year olds: Mean/SD 20.3 ± 7.4 [8]					

Table 1: Results of CARS testing, tactile assessments and skin biopsies.

Adverse Effects

Biopsy procedures were well tolerated. Two of the four children did not appear to notice the injection of local anaesthetic. There were no adverse effects.

Discussion

This was an exploratory study to determine whether C-tactile fiber loss was present in four autistic children with signs of hypoesthesia and allodynia. Results demonstrated 50% IENF loss in all four children when compared with normative controls. Small-fiber neuropathy is difficult to diagnose in children because quantitative sensory testing is invalid and nerve conduction studies are normal; diagnosis is established by reduced IENF counts on skin biopsy [12]. Thus, based on these biopsy results and existing normative data, hypoesthesia and allodynia in these four children with autism can be explained on the basis of idiopathic small fiber neuropathy.

Even though there is only a small amount of IENF data in children aged 10-19, and even less in 3-5 year olds, what little data is available supports what is known about plasticity and pruning and the inter-relationship of the central and peripheral nervous systems during development [13]. Neuroplasticity is predicated upon the rapid rise of central synaptic connections in the first few years of life [14]. By the end of the early childhood developmental period, during which time touch has been the dominant sensory input, IENF counts are reported at levels 8-13 times higher than adults [15]. Experience-driven pruning of excessive connections occurs until adolescence, at which time IENF counts are reported at twice those of adults [8]. By adulthood, the IENF norm has drifted down to a level where it will remain, losing 1-2 fibers/mm/decade [16]. Thus, it was truly remarkable that these 8-11 year old children should have 50% loss of IENF density. From a neuroplasticity perspective alone, a 50% reduction of somatosensory input would have a dramatic effect on plasticity and pruning.

This study is too small to draw conclusions about tactile loss in autism in general, but the results are sufficiently unexpected to warrant replication with a larger number of children, and a larger case-control study is planned. Until now, it has been very difficult to obtain skin biopsies from children with autism, but these results provide justification for additional biopsies. We issue an urgent call to researchers to obtain further skin biopsy data in children with ASD, and to add to the database of normative IENF data in children.

Study Limitations

The primary limitations of this study are in the small number of participants and the application of a diagnostic technology that is highly reliable in adults to the pediatric population, where there are less normative data.

Conclusions

The clinical presentation of tactile abnormalities in children with autism fits the known presentation of loss of C-tactile fibers, both in terms of loss of the affective and affiliative properties of touch, and in terms of hypoesthesia and allodynia. This is the first study to carry out skin biopsies in children with autism and confirm small fiber loss. Not enough children were biopsied to draw conclusions about the cause of tactile abnormalities in autism in general, however the findings do account for tactile abnormalities in these first four children biopsied. This study should be urgently replicated and we issue an urgent call to clinicians and researchers to obtain further skin biopsy data in children with ASD, and to add to the database of normative IENF data for children.

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