Differences in Extracellular Matrix Production and Basic Fibroblast Growth Factor Response in Skin Fibroblasts from Sporadic and Familial Alzheimer's Disease

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Extracellular matrix (ECM) molecules and growth factors, such as fibroblast growth factor (FGF), play a crucial role in Alzheimer's disease (AD). The purpose of this investigation was to determine whether phenotypic alterations in ECM production are present in non-neuronal AD cells associated with different FGF expression and response. Synthesis of glycosaminoglycans (GAG) and collagen were measured in skin fibroblasts from patients with familial, sporadic AD (FAD and SAD respectively), and from age-matched controls by radiolabeled precursors. Proteoglycans (PG), metalloprotease (MMP)-1, and FGF gene expressions were measured by reverse transcription-polymerase chain reaction. The results showed different ECM neosynthesis and mRNA levels in the two AD fibroblast populations. FAD accumulated more collagen and secreted less GAG than SAD. Biglycan PG was upregulated in FAD while betaglycan, syndecan, and decorin were markedly downregulated in SAD fibroblasts. We found a significant decrease of MMP1, more marked in FAD than in SAD fibroblasts. Constitutive FGF expression was greatly reduced in both pathological conditions (SAD > FAD). Moreover, an inverse high affinity/low affinity FGF receptor ratio between SAD and FAD fibroblasts was observed. FGF treatment differently modulated ECM molecule production and gene expression in the two cell populations. These observations in association with the changes in FGF gene expression and in the FGF receptor number, suggest that cellular mechanisms downstream from FGF receptor binding are involved in the two different forms of AD.

INTRODUCTION

Alzheimer's disease (AD) accounts for about 50 to 70 percent of dementia disorders. It is pathologically characterized by intracellular neurofibrillary tangles as the result of hyperphosphorylated microtubule-associated protein tau accumulation, as well as extracellular amyloid deposits, called senile plaques (1), with a progressive accumulation of insoluble amyloid in brain parenchyma and vasculature. Senile plaques are mainly composed of β-amyloid (Aβ) peptide which is generated by the anomalous endoproteolytic processing of the amyloid precursor protein (APP). The characteristic extra-cellular histopathological lesions due to the amyloid fibrils are the main cause of neurodegeneration. Only about ten percent of AD cases appear to be related to mutations in the APP gene or in the presenilin genes, PS1 or PS2 (2). The majority of adult onset AD cases are not genetically transmitted and are considered multifactorial sporadic type (SAD).

Great attention has been focused on the extracellular matrix (ECM) components present in the amyloid deposits. ECM is a complex network composed of an array of macromolecules important for the regulation of cell functions and tissue architecture. Its constituents such as laminin, collagen, fibronectin, glycosaminoglycans (GAG), proteoglycans (PGs), and metalloproteases (MMP) are involved in the formation of neuritic plaques because they regulate the amount of the amyloid precursor protein and its amyloidogenic fragments (3–7). In particular, fibronectin enhanced APP secretion, whereas laminin and collagen caused a decrease of accumulation of cellular mature APP and APP fragments (8). Laminin and type IV collagen have all been localized within the senile plaque as punctuate deposits (9). Several studies report that sulphated glycosaminoglycans also may be a key factor in the formation of neurofibrillary lesions in AD. Heparan sulphate PGs (HSPGs), in fact, act as nucleating agents in the formation of the diffuse amyloid deposits involved in the formation of the neuritic plaque in the brain of AD patients (10). HSPG and hyperphosphorylated tau co-exist

in neurons at the earliest stages of neurofibrillary pathology. Furthermore, amyloidoses have been correlated with disordered processing of basement membrane heparan sulphate proteoglycans (11–13) and neuritic plaques were shown to be associated with immunoreactivity for dermatan sulphate, keratan sulphate, and chondroitin sulphate (14,15).

In the brain of AD patients, neurons and glial cells participate in a local inflammatory cascade by releasing cytokines such the tumor necrosis factor (TNF)- α, interleukin (IL)-1, transforming growth factor (TGF)- β, fibroblast growth factor (FGF) and other molecules such as metalloproteases, complement proteins, acute phase reactants, and oxygen radicals (16,17). An aberrant production of cytokines and growth factors by activated glial cells might result in facilitating the amyloidogenic pathway, leading to an overproduction of β-amyloid peptide (Aβ) in AD brains (18).

The basic fibroblast growth factor (bFGF or FGF2) possesses several effects on nervous and glial cells and it is presumed to play an important role in the development, maintenance, and wound healing in the brain (19). It activates the glial reaction in the injured brain, thus influencing the regulation of ECM components in the brain inflammatory process (20,21)

In the central nervous system, FGF is found in the cytoplasm and nucleus of neurons and astrocytes (22,23) and exerts its biological action through the binding with FGF receptors (FGFRs), identified as low- and high-affinity receptors (24). FGF2 was shown to be elevated in Alzheimer's disease and localized in the plaques and neurofibrillary tangles (25). It enhances the basal level of promoter activity of the APP gene (26), moreover, FGF-HSPG binding plays a role in transforming normal soluble tau protein into insoluble paired helical filaments present in the neurofibrillary tangles (27), thus confirming the importance of the interaction between FGF and ECM in AD physiopathology.

Altered metabolic behavior in non-neuronal cells also has been demonstrated in AD patients (28). High levels of APP, for example, are found to be expressed in non-neural cell types, including skin fibroblasts, suggesting that a wide variety of non-neuronal cells are also capable of processing APP to produce β-amyloid (29). As a result of interaction between APP and ECM components, it has been suggested the possibility that the nucleation process in amyloid genesis might disturb the process of basement membrane formation, a phenomenon which is consistently found in amyloid deposits of non-nervous tissues such as skin (30), hepatic and splenic vasa (31), cardiac tissue, and medullary carcinoma (32), where abnormalities in ECM structure have occurred.

In addition, it has been suggested that amyloid fibril accumulation is only in part responsible for the neurodegeneration observed in AD. Other disease mechanisms include in fact apoptosis, aberrant calcium signaling and other disordered intracellular signaling such as the catenin/Wnt pathway in somatic cells from AD patients (33–37). For example, cultured skin fibroblasts from PS1 and PS2 mutation carriers showed altered calcium signaling (38), a greater DNA damage induced by oxidative injury (39), and higher spontaneous and chemically induced cytogenetic alterations (40) when compared with the control group.

In the light of these data, it was of interest to investigate whether AD may be associated also with changes in components of peripheral connective tissue. For this reason we performed an in vitro study on skin fibroblasts obtained from SAD and FAD Alzheimer patients and evaluated alterations in neosynthesis of ECM components (GAG, collagen, and PGs) and their phenotype, according to FGF2 treatment.

Quantitative monitoring of levels of mRNA encoding for FGF2, proteoglycans and metalloprotease (MMP)-1 was performed by RT-PCR analysis. The data were discussed in relation to the high-

and low-affinity FGF receptor numbers determined in both cell populations by a binding assay.

METHODS

Cell Cultures

Primary human fibroblast cultures were obtained from skin biopsies of AD patients and from normal subjects at the Department of Neurology, University of Florence after informed consent was given from all subjects or, where appropriate, from their caregivers. Clinical assessment was done according to published guidelines (The Dementia Study Group of the Italian Neurological Society). AD patients included ten FAD patients (age range 60-75 years) bearing the PS-1 (Leu392Val) missense mutation and ten age-matched SAD subjects. As controls, skin biopsies obtained from ten age-matched normal subjects, carefully assessed to exclude any neurological disorder, were analyzed (41).

Primary cultures were performed in Department of Neurological and Psychiatric Sciences (Florence). Briefly, skin biopsies (3 mm punch) were collected from the volar side of the forearm. Each biopsy was cut into small pieces (around 0.5 mm) and seeded in 25 cm² flasks. When the flasks became confluent, subcultures were made by seeding 150,000 cells/T25 flask (42).

For experimental procedure, human fibroblasts were cultured in serum free Dulbecco's modified Eagle's medium for 24 h (a culture interval needed to reach approximately 70 to 80 percent of confluence), with and without 20 ng/mL basic FGF (FGF2, Sigma Chemical Company, St. Louis, MO, USA).

The viability of the cells was estimated by examining their ability to exclude Trypan Blue (0.1 percent Trypan Blue in 0.9 percent NaCl).

Cell Number

To determine cell number, fibroblasts were harvested with PBS, sedimented by centrifugation at 450g and resuspended in 1 mL of medium. Tripan Blue was

Table 1. Oligonucleotides Used for Real Time RT-PCR

MRNA	Sequence (5'-3')	Product (bp)	GenBank Accession No.
betaglycan (sense)	CCTGTCATTCCCAGCATACAAT	363	XM001924
betaglycan (reverse)	ATCACCTGACTCCAGATCTTCATA		
Decorin (sense)	GGCCACTATCATCCTCCTT	270	NM133503
Decorin (reverse)	TTGTTGTTTTGCAGGTCTAGC		
syndecan (sense)	TCTGACAACTTCTCCGGCTC	210	NM001006946
syndecan (reverse)	CCACTTCTGGCAGGACTACA		
biglycan (sense)	GCTCCGACCTGGGTCTGCTGAA	306	NM001711
biglycan (reverse)	ACTCCCTTGGGCACCTTGC		
MMP-1 (sense)	CTCATGCTTTTCAACCAGGC	370	X05231
MMP-1 (reverse)	GTGCGCATGTAGAATCTGTC		
FGF2 (sense)	AGTGTGTGCTAACCGTTACC	214	NM002006
FGF2 (reverse)	AAGTATAGCTTTCTGCCCAGG		
β-actin (sense)	ACCTTCTACAATGAGCTGCG	197	NM001101
b-actin (reverse)	TCCATCACGATGCCAGTGGTA		

added to the cell suspension to obtain a final concentration of 2 mg/mL. Cells were incubated for five minutes at room temperature and the number of stained cells determined by counting with Burker's camera.

GAG Synthesis

Control, SAD, and FAD fibroblasts were labeled with 5 μCi/mL of ^{3}H-glucosamine hydrochloride (s.a. 29 Ci/mmol, Amersham Biosciences, Little Chalfont, UK) after being maintained for 24 h in serum-free DMEM with and without 20 ng/mL recombinant human FGF2. At the end of incubation, media was recovered separately and processed. Aliquots of ^{3}H-labeled GAG from media were applied to a DE-52 cellulose anion exchange column. Individual GAGs were identified by their enzymatic susceptibility (43). Testicular hyaluronate lyase (beef, Miles Italiana S.p.A., Milano, Italy), streptomyces hyaluronate lyase (Streptomyces hyalurolyticus) (Seikagaku Kogyo Company, Tokyo), chondroitin AC-II lyase (Arthrobacter aurescens) (Seikagaku Kogyo Company) digestions were performed. Standard GAG (Sigma) were then added and precipitated with three volumes of five percent potassium acetate in ethanol. Radioactivity was measured in both supernatants (digested GAG) and pellet. Results are expressed as cpm/10^5 cells.

Collagen Synthesis

Control, SAD, and FAD fibroblasts were cultured for 24 h in serum-free DMEM with 50 μg/mL ascorbic acid and 50 μg/mL β-aminoproprionitrile fumarate with and without 20 ng/mL recombinant human FGF2 in the presence of 2 μCi/mL of ^{3}H-labeled proline (s.a. 29 Ci/mmol, Amersham Biosciences). Collagen was extracted as described elsewhere (44). Results are expressed as cpm/10^5 cells. The specificity of the assay was verified by monitoring the susceptibility of precipitates to purified

bacterial collagenase (Calbiochem-Novabiochem Co., CA, USA).

Detection of mRNA Levels for Betaglycan, Decorin, Syndecan, Biglycan, MMP1, and FGF2 by Real-Time PCR Analysis

Total RNA was isolated from control, SAD, and FAD fibroblasts by lysing the cells with Trizol reagent (Invitrogen, Paisley, Great Britain). The RNA was treated immediately with DNAse I (Invitrogen) and the integrity of the treated RNA was examined by detection of ribosomal RNA bands (28S and 18S) in ethidium bromide stained agarose gels. RNA was quantified by reading the optical density at 260 nm. One microgram of total RNA was subjected to reverse transcription (RT) in a final volume of 50 mL. Real-time PCR was performed using 1 mL of the cDNA prepared by the RT reaction (Table 1).

Real-time PCR was carried out in an Mx3000P cycler (Stratagene, Amsterdam, Netherlands) using FAM for detection and ROX as a reference dye. One step PCR was performed in 25 μl of Brilliant SYBR Green QPCR Master Mix (Stratagene) according to the manufacturer's instructions. Product formation was monitored continuously with the fluorescent double-stranded DNA binding dye SYBR Green I at each annealing step. Immediately following the PCR, a

Table 2. Summary of the Results

	Control	SAD	FAD	Control +	SAD +	FAD +
Cell number	18,300	27,100	39,350	27,800	35,500	37,380
c.p.m./cell number x 10^5						
Total GAG	30,700	8,420	8,350	33,230	8,808	3,050
Total Collagen	1,790	1,196	3,718	1,406	1,370	4,976
fold change						
MMP1	1	0.31	0.058	0.505	1.12	0.059
Biglycan	1	1.07	1.38	0.69	0.533	1.88
Decorin	1	0.48	0.911	1.37	0.278	0.385
Betaglycan	1	0.63	0.932	0.63	0.69	0.33
Syndecan	1	0.401	0.57	1.12	0.495	0.455
FGF2	1	0.139	0.417	0.239	0.178	0.268
FGF2 receptor number						
Low Affinity Receptor	308,781	396,178	625,076	378,663	483,859	422,697
High Affinity Receptor	5,435	11,640	7,476	7,267	8,920	5,980

The scheme summarizes the overall results discussed in the text.
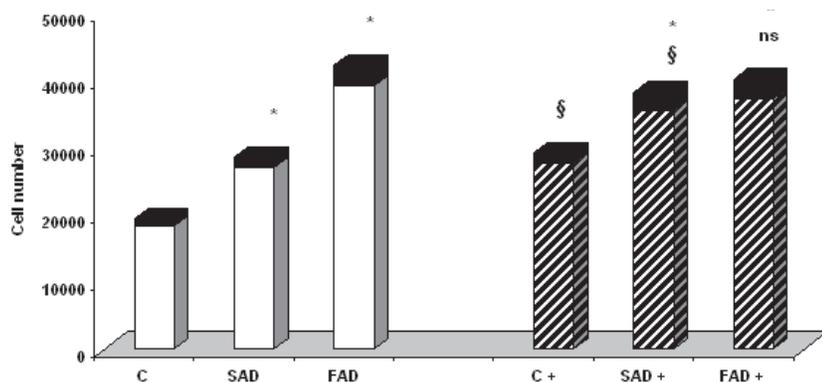
Figure 1. Cell number and FGF2 effects (+) on control (C), sporadic (SAD) and familial (FAD) Alzheimer's disease fibroblasts. Fibroblasts were mantained in serum–free MEM for 24h with and without FGF2 (20 ng/ml). The values were the mean ± SD of three independent experiments each performed in quintuplicate for n=10 subjects for group. Data were analyzed by ANOVA. Differences vs. respective control: *F–test significant at 99%. Differences vs. respective untreated group: §F–test significant at 99%; ns = not significant.

melting curve was undertaken by raising the incubation temperature from 55° to 95°C to confirm amplification specificity. All samples and the templates for the standard curves were run in triplicate. The mRNA level for each sample was normalized against β-actin mRNA and expressed as fold changes versus the level observed in untreated control fibroblasts.

FGF2 Binding Assay

FGF2 receptor binding was performed as described elsewhere (45,46). Briefly, control, SAD, and FAD fibroblasts were incubated for two hours at 37°C in serum free DMEM with 0.15 percent gelatin before each experiment. Cells then were washed twice with cold phosphate-buffered saline (PBS) and 200 mL of cold MEM containing 25 mM Hepes pH 7.5 0.15 percent gelatin. Increasing concentration of [^{125}I] FGF2 (0.1–10 ng/mL, Amersham Biosciences) were added to each well. Cells were incubated for two hours at 4°C on an orbital shaker.

To determine low affinity binding of FGF2, the cells were incubated twice for five minutes with cold PBS pH 7.5 containing 2 M NaCl and the cell extract was counted in a γ counter (Canberra Packard Central Europe GmbH, Schwadorf, Austria). Non-specific binding was estimated in the presence of 100-fold excess of unlabeled recombinant human FGF2 and subtracted from all data.

High affinity bound FGF2 was determined by sodium acetate 20 mM, NaCl 2 M pH 4.0 extraction. Receptor binding was normalized with respect to cell number, and analyzed with Scatchard method (47).

Statistical Analysis

Results reported in figures were the mean ± SD (standard deviation) of three independent experiments each performed in triplicate or quintuplicate (see legends) for n = 10 subjects for group. Statistical analysis was performed by analysis of variance (2-WAY ANOVA) followed by the Sheffe F-test.

RESULTS

Cell Count

After 24 h of in vitro maintenance, there was an increase of cell number both in SAD and FAD fibroblasts (FAD > SAD) with respect to the controls (Figure 1). FGF treatment significantly enhanced cell number only in control and SAD fibroblasts (+52 percent and +31 percent respectively).

GAG Synthesis

Total GAG production was considerably less both in SAD and FAD fibroblasts with respect to the control group (–72 percent about in both cell lines) (Figure 2). No significant differences were observed in total GAG synthesis between the two pathological populations, but there was a different GAG redistribution between the extracellular (secreted) and cellular compartment. SAD fibroblasts showed a greater content of cellular GAG (+126
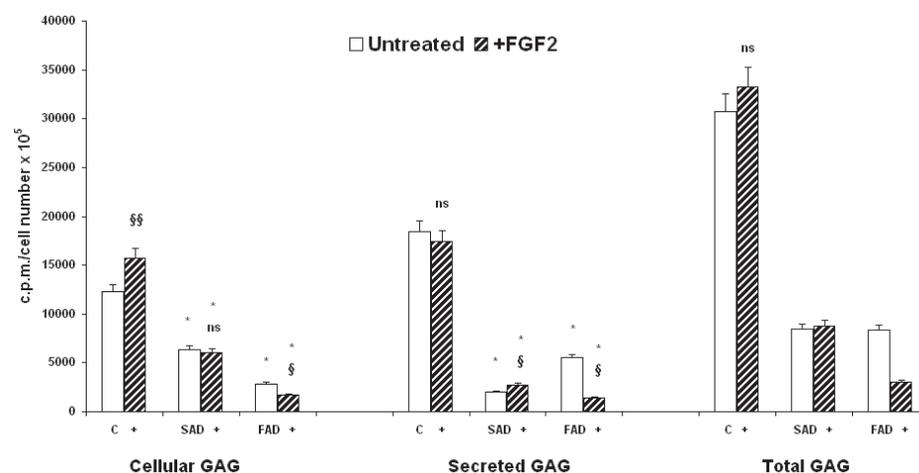
Figure 2. Cellular, secreted and total GAG production from control (C), sporadic (SAD) and familial (FAD) Alzheimer's disease fibroblasts and FGF2 effects (+). The values were the mean ± SD of three independent experiments each performed in quintuplicate for n=10 subjects for group. Data were analyzed by ANOVA. Differences vs. respective control: *F–test significant at 99%. Differences vs. respective untreated group: §F–test significant at 99%; §§F–test significant at 95%; ns = not significant.
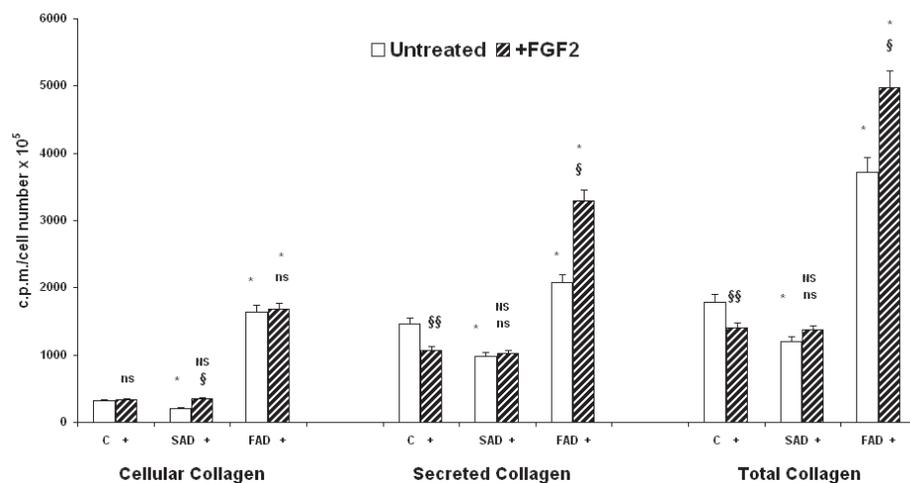
Figure 3. Cellular, secreted and total collagen production from control (C), sporadic (SAD) and familial (FAD) Alzheimer's disease fibroblasts and FGF2 effects (+). The values were the mean ± SD of three independent experiments each performed in quintuplicate for n=10 subjects for group. Data were analyzed by ANOVA. Differences vs. respective control: *F–test significant at 99%; NS = not significant. Differences vs. respective untreated group: §F–test significant at 99%; §§F–test significant at 95%; ns = not significant.

percent), while FAD fibroblasts produced greater amounts of secreted GAG (+173 percent). FGF treatment strongly decreased cellular and secreted GAG in FAD fibroblasts (–41 percent and –75 percent, respectively) and increased GAG secretion in SAD fibroblasts (+35 percent), but it did not affect their total GAG production. In control group, cellular GAG was significantly upregulated by FGF which did not induce significant differences in total GAG production.

Collagen Synthesis

FAD fibroblasts produced significantly more collagen than control (+108 percent) and SAD fibroblasts (+211 percent) (Figure 3). FGF increased only the content of cellular collagen in SAD (+64 percent) without significantly affecting total collagen production and markedly increased the content of secreted collagen in FAD fibroblasts (+59 percent).

Quantitation of Proteoglycans, MMP-1, and FGF2 Transcription by Real-Time PCR

To analyze proteoglycan, MMP-1, and FGF gene expression, total RNA samples were obtained from unstimulated and FGF-stimulated fibroblasts (see methods). Genes were analyzed by qRT-PCR and the results are shown in Figure 4.

A downregulation in expression of betaglycan and decorin was detected in SAD fibroblasts, while no significant differences were observed between FAD and control fibroblasts. Messenger RNA expression for syndecan was down regulated in both SAD and FAD fibroblasts. Biglycan expression was upregulated only in FAD fibroblasts. FGF treatment markedly decreased decorin and biglycan in SAD fibroblasts, and reduced betaglycan level in FAD fibroblasts. Syndecan expression was significantly downregulated in both SAD and FAD fibroblasts after FGF treatment.

MMP-1 expression was detectable in lower concentration in FAD fibroblasts when compared with control and SAD fibroblasts. FGF treatment induced a decrease of MMP-1 signal in control and FAD fibroblasts, and a three-fold increase of the MMP1 transcript level in SAD fibroblasts.

FGF2 mRNA showed a maximal downregulation in SAD fibroblasts when

compared with control and FAD fibroblasts, being markedly upregulated by the addition of exogenous FGF On the contrary, FGF expression was significantly decreased in FGF-treated control and FAD groups.

### Quantitative Analysis of FGF Receptors by Binding Assay

The Scatchard analysis shows the presence of a high affinity and a low affinity binding site. The low affinity receptor number (Figure 5) was +26 percent in SAD and about two-fold in FAD fibroblasts compared with control. FGF treatment induced an increase of the low affinity receptor density in the control and SAD fibroblasts, while it led to a clear decrease of these receptors' density in FAD fibroblasts.

Accordingly, the high affinity receptor/cell ratio showed a different trend (Figure 6). It was strongly enhanced in both pathological cell populations (SAD > FAD), increased by FGF in control group and decreased in both SAD and FAD fibroblasts.

DISCUSSION

ECM components and FGF have been implicated in the formation of neurite plaques in Alzheimer's disease (48,49). Different components of the ECM, such as HSPGs (9,10), laminin, fibronectin (50), and collagen (10) bind in fact secreted APP stimulating fibril nucleation and, acting as "seed molecules" (3), promote senile plaque formation. In addition, FGF-ECM binding regulates the amount of APP and its processing to amyloidogenic fragments in neuronal and non-neuronal cells (51).

Previous reports showed that lysosomal alterations were detectable not only in neurons but also in skin fibroblasts and lymphoblasts obtained from AD patients (41,52,53). In this report we carried out an investigation on ECM components using as cell model primary fibroblast cultures established from FAD patients mutated on PS1 gene, SAD cases, and cognitively normal controls.
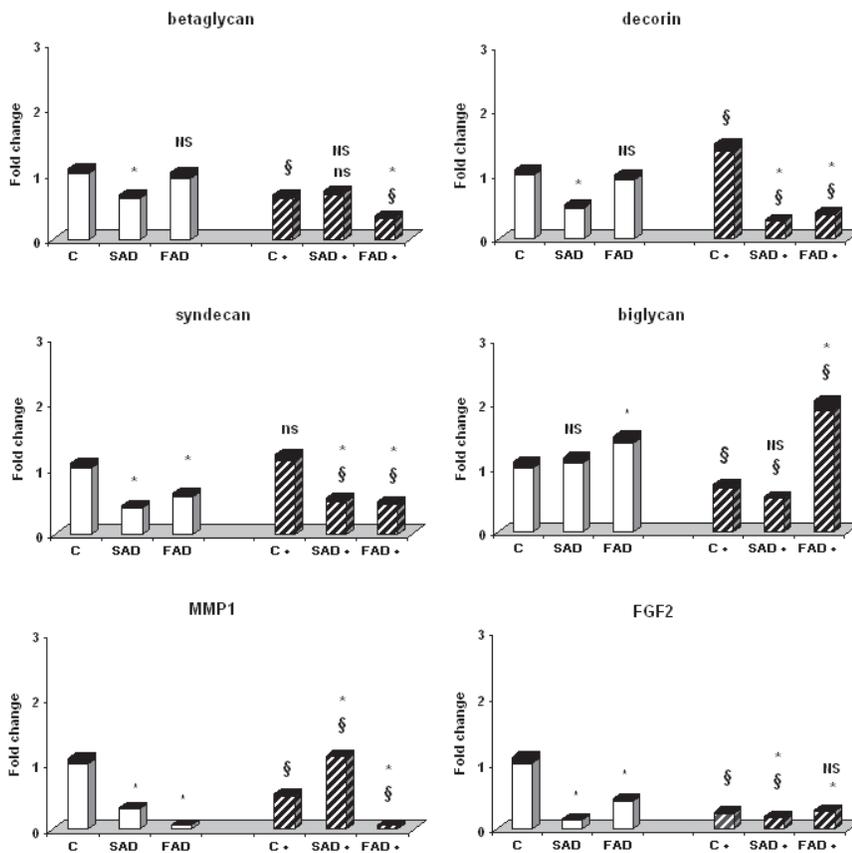
Figure 4. Expression data (mRNA levels) for betaglycan, decorin, syndecan, biglycan, MMP1 and FGF2 obtained from control (C), sporadic (SAD) and familial (FAD) Alzheimer's disease fibroblasts treated or not with FGF2 (+). The mRNA levels were quantified by real–time quantitative PCR. Values were the mean ± SD of three independent experiments each performed in triplicate for n=10 subjects for group. Data were analyzed by ANOVA. The results were expressed as fold change in β–actin normalized mRNA values. Differences vs. mRNA levels in each respective control: *F–test significant at 99%; **F–test significant at 95%; NS = not significant. Differences vs. mRNA levels in each untreated group: §F–test significant at 99%; ns = not significant

The observed differences between each patient group and FGF effects are reported in the schematic Table 2.

Both pathological groups showed a greater proliferative rate compared with the control fibroblasts, but FAD evidenced about two-fold increase of cell number with respect to control and +45 percent than SAD fibroblasts. Total GAG were strongly decreased in SAD and FAD cells when compared with controls, with a cellular/secreted GAG ratio in favor of cell accumulation in SAD fibroblasts.

Collagen production was different in mutant and sporadic AD fibroblasts: SAD cells produced significantly less collagen, while FAD accumulated and secreted more collagen than controls.

MMP1 expression was decreased in both pathological populations but the decrease was more evident in FAD cells.

A different regulation in PG expression also was detected and measured by real-time PCR. In SAD fibroblasts, decorin, betaglycan, and syndecan PGs appeared downregulated. Biglycan expression was not affected in SAD, but resulted upregulated in FAD fibroblasts.

We also provide evidence that mutant and wild type AD fibroblasts react differently to FGF treatment. FGF exerted a stimulating proliferative effect only in SAD fibroblasts, significantly reduced GAG production, and favored collagen synthesis only in FAD fibroblasts.
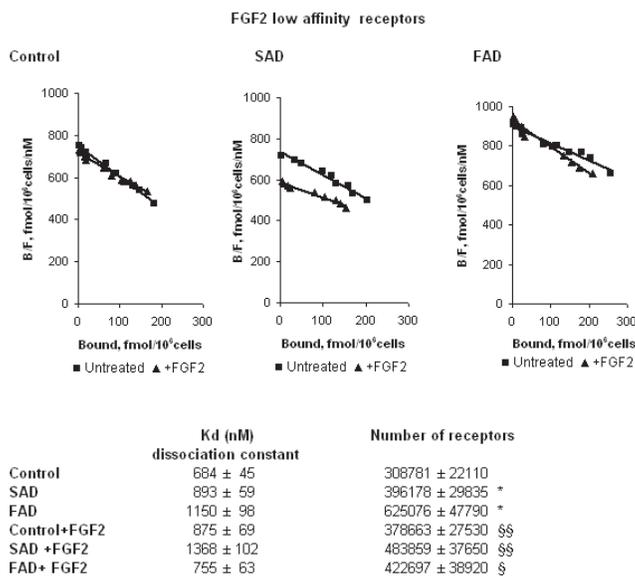
In light of our data, we suggest that changes in GAG production not only cause a reduced adhesiveness of skin fibroblasts (54), but also modulate APP effects on neuronal plasticity and Aβ neurotoxicity (4). The greater collagen production by FAD is another finding of interest because it has been reported that collagen can cause a decrease in secretion and accumulation of cellular mature APP (10).

Because biglycan and decorin are two PGs strictly related to collagen fibrillogenesis (55), the increase in collagen production that we observed in FAD fibroblasts could be mainly due to two mechanisms: the reduced expression of MMP-1 collagenase and biglycan upregulation. On the contrary, the decrease in collagen production observed in SAD fibroblasts might be associated with the reduced decorin expression.

Syndecans and betaglycan are cell surface HSPGs capable of binding ECM molecules via their covalently attached heparan sulfate chains. The different expression of syndecan and betaglycan in SAD and FAD cells also could be related to severity of AD. It has been suggested that sulphated PGs might have a neuroprotective effect, because it has been demonstrated that neurons and cortical areas show low susceptibility to neurofibrillary changes if high proportions of aggregating chondroitin sulphate proteoglycans are present in the neural microenvironment (4). The CSPGs content, for example, is inversely correlated with the amount of hyperphosphorylated tau in the brains of AD patients (56).

The above findings may be related to the different endogenous FGF expression and to FGF receptor number in the two pathological cell populations.

FGF2 mRNA constitutive level was differently expressed in SAD and FAD

FGF2 low affinity receptors

	Kd (nM) dissociation constant	Number of receptors
Control	684 ± 45	308781 ± 22110
SAD	893 ± 59	396178 ± 29835 *
FAD	1150 ± 98	625076 ± 47790 *
Control+FGF2	875 ± 69	378663 ± 27530 §§
SAD +FGF2	1368 ± 102	483859 ± 37650 §§
FAD+ FGF2	755 ± 63	422697 ± 38920 §

Figure 5. Scatchard analysis of ^{125}I–FGF2 binding to low affinity receptors in control, sporadic (SAD) and familial (FAD) fibroblasts, and FGF2 effect. Specifically bound (B) anf free (F) ligands were measured as described in Materials and Methods. Values were the mean ± SD of three independent experiments each performed in triplicate for n=10 subjects for group. Difference vs. number of receptors in each respective control: *F–test significant at 99%. Differences vs. number of receptors in respective untreated group: §F–test significant at 99%; §§F–test significant at 95%.
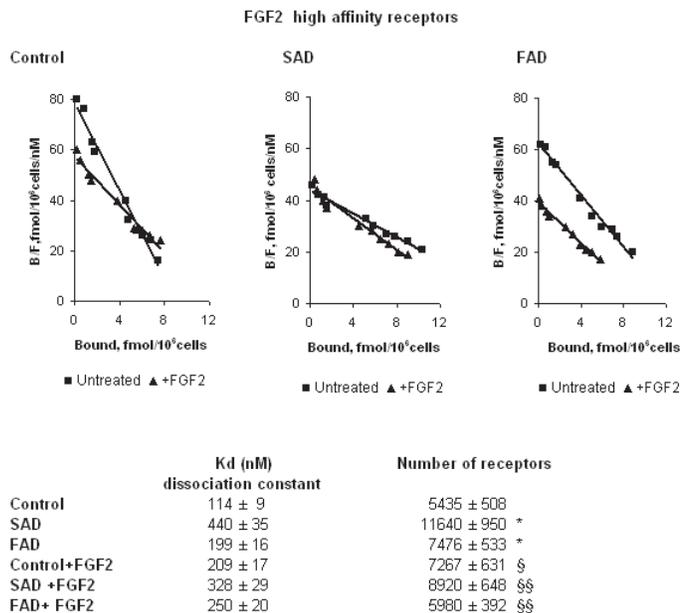
fibroblasts, being more expressed in FAD fibroblasts. The role of FGF in AD development is suggested by several contradictory reports. It has been demonstrated that FGF increases APP mRNA levels (57,58) but it protects neurons expressing mutant PS1 from exposures to excitotoxins, Aβ and reactive oxygen species (59–63). The different endogenous FGF expression that we observed in SAD and FAD fibroblasts might cause an imbalance within this protective system, thus explaining the biological differences between SAD and FAD degenerative processes (64).

Finally, while only FGF low affinity receptor numbers were more expressed in FAD cells, high affinity receptors were significantly higher in SAD and FAD cell when compared with controls. This result, in association with the finding that HSPG syndecan is reduced in SAD and FAD fibroblasts, could explain their higher proliferating state. Other authors showed a stimulating proliferative effect of HSPG on epithelial cells and a decreasing effect on mesenchymal cells (65).

Betaglycan and syndecan also are closely associated with FGF receptor. This spatial and functional relationship is very important for stabilizing FGF binding (66,67), modulating FGF activity, and transforming normal soluble proteins into insoluble paired helical filaments in AD (68,69) The different content in HSPGs that we observed in SAD and FAD fibroblasts could modulate FGF receptor binding differently and consequently could induce significant effects on neuroprotective action induced by PGs.

FGF treatment decreased both low and high affinity binding receptors in FAD fibroblasts, while it had a decreasing effect only in high affinity receptors in SAD cells. In FAD fibroblasts, a reduced neuro-protective action of FGF could be due to its parallel decreasing effect on both high and low affinity receptors.

Taken together, our data show for the first time a different ECM production in sporadic and familial Alzheimer's disease skin fibroblasts, suggesting a link with connective tissue disease. The different ef-

FGF2 high affinity receptors

	Kd (nM) dissociation constant	Number of receptors
Control	114 ± 9	5435 ± 508
SAD	440 ± 35	11640 ± 950 *
FAD	199 ± 16	7476 ± 533 *
Control+FGF2	209 ± 17	7267 ± 631 §
SAD +FGF2	328 ± 29	8920 ± 648 §§
FAD+ FGF2	250 ± 20	5980 ± 392 §§

Figure 6. Scatchard analysis of ^{125}I–FGF2 binding to high affinity receptors in control, sporadic (SAD) and familial (FAD) fibroblasts, and FGF2 effect. Specifically bound (B) and free (F) ligands were measured as described in Materials and Methods. Values were the mean ± SD of three independent experiments each performed in triplicate for n=10 subjects for group. Difference vs. number of receptors in each respective control: *F–test significant at 99%. Differences vs. number of receptors in respective untreated group: §F–test significant at 99%; §§F–test significant at 95%.

fects of FGF on FAD and SAD fibroblast phenotype might involve molecular mechanisms downstream from FGF receptor binding. Together, the data provide new insight into the complex FGF response involved in the development of AD.

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