Serum Sclerostin and Risk of Hip Fracture in Older Caucasian Women

Aarthi Arasu, Peggy M. Cawthon, Li-Yung Lui, Thy P. Do, Puneet S. Arora, Jane A. Cauley, Kristine E. Ensrud, Steven R. Cummings, and The Study of Osteoporotic Fractures Research Group

Department of Medicine (A.A.), University of California, San Francisco, and San Francisco Coordinating Center (P.M.C., L.-Y.L., S.R.C.), and California Pacific Medical Center Research Institute (P.M.C, L.-Y.L., S.R.C.), San Francisco, California, 94107; Amgen Inc., (T.P.D., P.S.A.) Thousand Oaks, California 91320; Department of Epidemiology (J.A.C.), Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15260; and Minneapolis Veterans Affairs Medical Center (K.E.E.), University of Minnesota, Minneapolis, Minnesota 55417

Context: Sclerostin, a protein secreted by osteocytes, inhibits bone formation. Individuals with genetic mutations that decrease the availability of sclerostin have very high bone mass.

Objective: The aim of this study was to examine the hypothesis that elevated serum sclerostin levels are associated with increased risk of hip fracture in older women.

Design, Setting, and Participants: This was a case-cohort study of a prospective, community-based cohort of 9704 women aged 65 yr or older. Sclerostin levels were measured in serum collected in 1989–1990 in 228 women with incident hip fractures and 227 women in a randomly selected sample; average follow-up time was 9.8 yr.

Results: Serum sclerostin levels were correlated with total hip bone mineral density (BMD; $r = 0.27$, $P < 0.001$). The risk of hip fracture increased across quartiles of serum sclerostin (test for trend, $P < 0.001$) and was significantly elevated among those in the fourth quartile (hazard risk 3.4, 95% confidence interval 1.7–7.0) compared with women in the lowest quartile, after adjusting for age, body mass index, estrogen use, history of fracture since age 50 yr, and total hip BMD. When dividing the cohort into eight groups by sclerostin quartile and median hip BMD, women with lower total hip BMD in the highest sclerostin quartile had a 22.3-fold (95% confidence interval 5.8–86.3) increased risk of fracture compared with women with higher total hip BMD in the lowest sclerostin quartile.

Conclusions: We conclude that higher serum sclerostin levels are associated with a greater risk of hip fractures in older women. In addition, the risk of hip fracture is amplified when high sclerostin levels are combined with lower BMD. **(*J Clin Endocrinol Metab* 97: 2027–2032, 2012)**

Hip fractures account for a substantial part of public health burden in terms of disability, mortality, and costs (1). Although bone mass is a major determinant of fracture, many women who are on treatment or have normal bone mineral density (BMD) continue to suffer fractures, reflecting an incomplete understanding of the pathophysiology of fractures, the hallmark of osteoporosis (2, 3).

Sclerostin, a protein secreted by osteocytes, influences bone remodeling by inhibiting the Wnt pathway, which is involved in bone formation (4–7). Mutations that lead to congenital absence of or reduced sclerostin levels have

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Abbreviations: BMD, Bone mineral density; BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; HR, hazard ratio.

been identified in patients with the autosomal recessive conditions sclerosteosis and van Buchem disease (8, 9). These patients have increased bone mass. A phase I trial with a sclerostin monoclonal antibody in normal humans has shown a dramatic increase in bone formation and bone mineral density (10), although no fracture data are available for this study. Therefore, we hypothesized that elevated circulating sclerostin levels are associated with decreased BMD and increased risk of hip fracture. The aim of this study was to examine the hypothesis in the prospective Study of Osteoporotic Fractures, a large cohort of older women.

Materials and Methods

Participants

From 1986 to 1987, 9704 Caucasian women who were 65 yr old or older were recruited through mailings to women on lists such as voter-registration lists for the Study of Osteoporotic Fractures baseline visit at four clinical centers in the United States. These analyses were limited to the 2458 women who were enrolled at the University of Minnesota clinical center. To be eligible for the study, women must have been able to walk without assistance and not have had previous bilateral hip replacement.

Selection of case and cohort samples

Of the 2458 women from the Minnesota clinic recruited at baseline visit, 2387 returned for a second clinic visit (1989–1990). Due to stored serum availability, the second clinic visit was used as the baseline visit for this study. None of the participants were maintained on bisphosphonate therapy at any time before serum collection. Of the 2387 who returned for the second visit, 2142 had available serum samples and hip dual-energy x-ray absorptiometry (DXA) scans. Using a case-cohort approach (11), we identified 230 participants who suffered their first hip fracture from this group excluding traumatic fractures, and we also randomly selected 227 women without regard to fracture status; follow-up time averaged 9.8 yr. Because case-cohort sampling was used, this random sample also included participants with first hip fractures. Thus, the number of women without hip fracture was 204 of 227 women in the cohort sample. There were two hip fracture cases with inadequate serum for sclerostin measurement and therefore were excluded for analysis.

Women with available serum approximately 4–6 yr after the first sclerostin measurement (1992–1994; visit 4), which included 173 women of the random cohort and 131 women who suffered hip fractures subsequent to this time point, were identified for repeat sclerostin measurement.

Measurements

Serum samples were stored in liquid nitrogen at −190 C. Samples were sent to a laboratory (Pharmaceutical Product Development, Richmond, VA) at which sclerostin was measured by a validated electrochemiluminescence-based ELISA sandwich assay (Amgen Inc., Thousand Oaks, CA). The assay has a detectable range of 50–50,000 pg/ml. The interassay and intraassay coefficients of variation for the assay are less than 10%. The results have been shown to be accurate up to four freeze/thaw cycles. None of the study's serum samples have been thawed more than twice.

Women were surveyed every 4 months with a telephone call or mailed questionnaire to ask about fractures that occurred in the prior 4-month period (99% of these contacts were completed). Reported fractures including anatomical location were centrally adjudicated by physician review of radiographic reports. Areal BMD was measured in the proximal femur by DXA (Hologic 1000, Bedford, MA) at the second clinical visit. All hip scans were performed on the right hip unless the subject reported a hip prosthesis or metal object(s) on the right side of the leg in which case the left hip was scanned.

Demographic, anthropometric, lifestyle, physical function, medications, and medical history data were obtained at the second clinical visit. Body mass index (BMI) was calculated as weight in kilograms (measured on a balance beam scale) divided by height in meters (measured using a wall mounted stadiometer) squared. Smoking and alcohol history, history of falling in the past year, parental history of fracture, and history of fractures since age 50 yr were ascertained through a self-administered questionnaire. During a clinic interview, self-reported health, number of years since menopause, history of thyroid disease, and current bone-altering medication use were assessed. Participants were also asked to rise from a chair five times without using their arms.

Statistical analysis

Characteristics of participants in the random sample were compared across quartiles of serum sclerostin at the following cutpoints: quartile 1 (≤209 pg/ml), quartile 2 (210–267 pg/ml), quartile 3 (268–353 pg/ml), and quartile 4 (>353 pg/ml). We used ANOVA for normally distributed continuous variables, Kruskall-Wallis tests for skewed continuous variables, and χ^2 tests for categorical variables to test for significant differences across quartiles. Student's *t* tests were used to determine whether mean serum sclerostin levels differed between hip fracture cases and the random sample.

Cox proportional hazards models that account for the case-cohort design were used to analyze the risk of hip fracture by quartile and per SD increase of serum sclerostin. Results were reported as hazard ratios (HR) with estimation of 95% confidence intervals (CI). Potential confounders were selected on the basis of biological plausibility or a strong bivariate association with serum sclerostin and hip fracture ($P < 0.05$). Models were adjusted for age and were further adjusted for BMI, fracture since age 50 yr, and current estrogen use. Tests for trend were performed by including serum sclerostin quartiles as an ordinal variable with four levels. To determine the association between sclerostin and hip fracture among women not taking estrogen, a sensitivity analysis was run excluding women reporting estrogen use. To determine whether the increased risk for fracture in women with high serum sclerostin levels was mediated by BMD, we examined the effect of further adjusting the final multivariate model for total hip BMD. We also adjusted the multivariate model for femoral neck BMD instead of total hip BMD and found that the results remained similar. To determine whether the association between higher serum sclerostin levels and risk of hip fracture varied by hip fracture location, we analyzed the

TABLE 1. Baseline demographic characteristics of random sample by sclerostin quartile

Characteristics	Sclerostin quartile 1 (≤ 209 pg/ml) (n = 57)	Sclerostin quartile 2 (210–267 pg/ml) (n = 57)	Sclerostin quartile 3 (268–353 pg/ml) (n = 58)	Sclerostin quartile 4 (>353 pg/ml) (n = 55)	*P* value for χ^2 tests
Age (yr)	74.5 ± 5.8	72.3 ± 4.8	74.6 ± 5.3	75.7 ± 6.2	0.01
Height (m)	158.4 ± 6.7	160.3 ± 6.7	161.2 ± 5.2	159.2 ± 6.0	0.08
Weight (kg)	63.5 ± 9.1	63.9 ± 9.9	64.1 ± 9.8	64.3 ± 10.6	0.98
BMI (kg/m^2)	25.4 ± 3.8	24.9 ± 3.8	24.7 ± 3.7	25.3 ± 3.4	0.73
BMD (g/cm^2)					
Total hip	0.69 ± 0.12	0.73 ± 0.13	0.73 ± 0.14	0.79 ± 0.13	0.0007
Femoral neck	0.61 ± 0.10	0.64 ± 0.10	0.64 ± 0.11	0.68 ± 0.11	0.02
Lumbar spine	0.79 ± 0.16	0.79 ± 0.15	0.87 ± 0.15	0.96 ± 0.19	<0.0001
Lifestyle					
Smoker	10.5%	8.9%	6.9%	3.7%	0.56
Alcohol use	71.9%	75.4%	84.5%	83.6%	0.27
Medication use					
Hormone therapy	26.3%	16.1%	15.5%	3.7%	0.01
Corticosteroid use	5.3%	1.8%	6.9%	3.7%	0.59
Ca	40.4%	37.5%	46.6%	18.5%	0.01
Vitamin D	42.1%	42.9%	48.3%	27.8%	0.15
Fracture since age 50 yr	61.4%	47.4%	48.3%	32.7%	0.03
Fallen in past year	54.4%	33.9%	19.0%	35.2%	0.001
Hx of hyperthyroidism	5.4%	9.1%	5.3%	13.0%	0.40
Parental Hx of hip fracture	20.9%	10.4%	17.0%	16.7%	0.59
Number of years since menopause	27.4 ± 8.1	24.2 ± 6.8	28.3 ± 9.3	27.8 ± 9.6	0.05
Self-assessment of health					
Excellent/good	86.0%	98.3%	84.5%	87.3%	0.08
Ability to rise from chair	95.8%	95.9%	94.6%	94.4%	0.98

Hx, History.

association between serum sclerostin and risk of intertrochanteric fracture and between serum sclerostin and risk of femoral neck fracture.

To assess whether measurement of serum sclerostin nearer to the hip fracture event influenced the association, we used serum sclerostin levels at visit 2 and visit 4 treated as a time-dependent variable. This allowed for the participant to have updated information about serum sclerostin level over time within the model. If serum sclerostin data were not available at visit 4, the serum sclerostin level from visit 2 was used. Using visit 4 serum sclerostin as a time-dependent covariate did not alter our findings; therefore, we have reported results using only visit 2 serum sclerostin. When examining sclerostin levels in those participants with both visit 2 and visit 4 serum sclerostin levels, the mean value for visit 2 (299 ± 7.1 pg/ml) did not differ significantly from the mean value for visit 4 (302 ± 6.7 pg/ml; $P = 0.55$).

To understand the joint effect of serum sclerostin and total hip BMD on hip fracture risk, we divided the cohort into eight mutually exclusive groups. First, we divided the cohort into higher and lower BMD groups, split at the median (0.74 g/cm^2; T-score = −1.6) for sample size considerations. We then divided these two groups by serum sclerostin quartile such that there were higher BMD/quartile 1 serum sclerostin, higher BMD/quartile 2 serum sclerostin, higher BMD/quartile 3 serum sclerostin, higher BMD/quartile 4 serum sclerostin, lower BMD/quartile 1 serum sclerostin, lower BMD/quartile 2 serum sclerostin, lower BMD/quartile 3 serum sclerostin, and lower BMD/quartile 4 serum sclerostin. This Cox proportional hazards model was not adjusted for other covariates due to sample size limitations. We also tested for the possibility of an interaction between total hip BMD as a dichotomous variable split at the median BMD and serum sclerostin quartile as an ordinal variable with four levels for the risk of hip fracture.

Results

The baseline characteristics of the study participants in the random cohort across quartiles of serum sclerostin concentrations are shown (Table 1). Age and total hip BMD differed by serum sclerostin quartile such that women in the highest quartile tended to be older and have higher total hip BMD. Total hip BMD was correlated with serum sclerostin concentrations ($r = 0.27$; $P < 0.001$). Other characteristics that differed by sclerostin quartile included hormone therapy, calcium use, history of fracture since age 50 yr, and falls. Individuals with hip fractures, including 104 intertronchanteric and 114 femoral neck fractures, tended to have higher mean serum sclerostin levels (314 ± 130.8 pg/ml) compared with controls (291 ± 119.1 pg/ml; $P = 0.06$, Table 2).

In the multivariate-adjusted Cox proportional hazards model, there was a trend toward increased risk across quartiles (test for trend, $P = 0.04$; Table 3) with women in quartile 4 having the highest risk (HR 1.9, 95% CI 1.0–3.5) of hip fracture compared with women in quartile 1 after adjusting for age, BMI, estrogen use, and history of

TABLE 2. Baseline demographic characteristics of hip fractures and controls

Characteristics	Hip fractures (n = 228)	Controls (n = 204)	*P* value
Sclerostin (pg/ml)	314.2 ± 130.8	291.3 ± 119.1	0.06
Age (yr)	76.6 ± 6.3	73.9 ± 5.5	<0.0001
Height (m)	158.8 ± 6.5	159.8 ± 6.4	0.11
Weight (kg)	61.5 ± 10.3	64.1 ± 10.0	0.01
BMI (kg/m^2)	24.4 ± 3.7	25.1 ± 3.8	0.04
BMD (g/cm^2)			
Total hip	0.67 ± 0.12	0.74 ± 0.13	<0.0001
Femoral neck	0.58 ± 0.09	0.65 ± 0.11	<0.0001
Lumbar spine	0.80 ± 0.16	0.86 ± 0.18	0.0008
Lifestyle			
Smoker	7.5%	6.8%	0.85
Alcohol use	72.6%	79.6%	0.09
Medication use			
Hormone therapy	8.3%	16.0%	0.02
Corticosteroid use	7.9%	3.9%	0.10
Ca	36.1%	38.4%	0.69
Vitamin D	40.4%	41.3%	0.92
Fracture since age 50 yr	61.4%	47.4%	0.03
Fallen in past year	33.8%	35.9%	0.69
Hx of hyperthyroidism	9.2%	8.4%	0.86
Parental Hx of hip fracture	28.0%	12.7%	<0.0001
Number of years since menopause	29.4 ± 8.4	26.3 ± 8.6	0.0001
Self-assessment of health			
Excellent/good	86.5%	88.8%	0.56
Ability to rise from chair	92.7%	95.7%	0.28

Hx, History.

fracture since age 50 yr. This trend toward increased risk across quartiles was substantially strengthened (test for trend, $P < 0.001$) after adjusting for total hip BMD, with women in the fourth quartile having significantly higher risk (HR 3.4, 95% CI 1.7–7.0) of hip fracture compared with those in quartile 1. The results were similar for both femoral neck and intertrochanteric hip fractures; women in the highest serum sclerostin quartile had a significantly increased risk for femoral neck (HR 2.9, 95% CI 1.4–6.2; test for trend across quartiles, $P < 0.001$) and intertrochanteric (HR 4.0, 95% CI 1.5–10.6; test for trend across quartiles, $P = 0.005$) hip fractures compared with those in the lowest quartile. For each increase of 1 SD in serum sclerostin concentration, the risk of hip fracture increased by 51% (HR 1.51; 95% CI 1.18–1.93) when adjusting for age, BMI, total hip BMD, estrogen use, and history of fracture since age 50 yr. Results were essentially unchanged when women taking estrogen were excluded (HR 1.54; 95% CI 1.19–2.00).

When analyzing by serum sclerostin quartile and total hip BMD level concurrently to assess the risk of hip fracture unadjusted for other covariates, women in the highest serum sclerostin quartile who had lower BMD (Fig. 1) had the highest risk of hip fracture (HR 22.3, 95% CI 5.8–86.3) compared with women with higher BMD in the lowest serum sclerostin quartile. Women in the highest serum sclerostin quartile who had higher BMD also had an increased risk of hip fracture (HR 7.3, 95% CI 2.0–26.3) compared with women with higher BMD in the lowest serum sclerostin quartile. The test for interaction for the risk of hip fracture between BMD and serum sclerostin, however, did not quite reach significance ($P = 0.07$).

TABLE 3. Hazard ratio for hip fracture by sclerostin level

	Unadjusted HR (95% CI)	Age-adjusted HR (95% CI)	Multivariate HR (95% CI)[a]	Multivariate HR with BMD (95% CI)[b]
Quartile 1 (≤209 pg/ml)	1.00	1.00	1.00	1.00
Quartile 2 (210–267 pg/ml)	1.05 (0.61–1.81)	1.45 (0.78–2.71)	1.39 (0.74–2.62)	1.33 (0.55–2.64)
Quartile 3 (268–353 pg/ml)	1.47 (0.86–2.50)	1.71 (0.96–3.07)	1.72 (0.95–3.11)	2.07 (1.13–3.81)[c]
Quartile 4 (>353 pg/ml)	1.92 (1.12–3.29)[c]	1.82 (1.00–3.32)	1.86 (0.99–3.49)	3.41 (1.66–6.99)[c]
P for trend	0.008	0.04	0.04	<0.001
Per SD	1.22 (1.03–1.46)[c]	1.15 (0.95–1.39)	1.22 (0.99–1.51)	1.51 (1.18–1.93)[c]

[a] Multivariate covariates are age, BMI, fracture since age 50 yr, and current estrogen use.

[b] BMD indicates the total hip BMD.

[c] Significant values at $P < 0.05$.

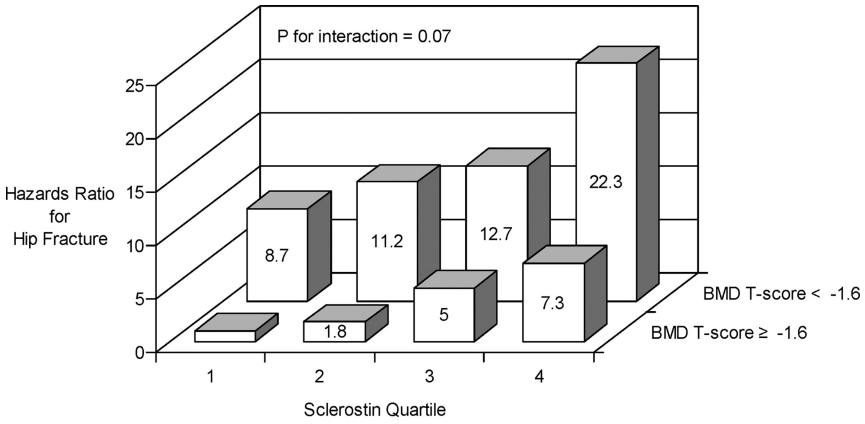

FIG. 1. Risk of hip fracture according to total hip BMD and sclerostin quartile. The median BMD (0.742 g/cm^2; T-score = −1.6) was chosen as a cutoff point. Lower BMD is defined as less than 0.742 g/cm^2, and higher BMD is defined as 0.742 g/cm^2 or higher. Serum sclerostin quartiles are as follows: quartile 1 (≤209 pg/ml); quartile 2 (210–267 pg/ml); quartile 3 (268–353 pg/ml); and quartile 4 (> 353 pg/ml). The HR for the risk of hip fracture with 95% CI as compared with higher BMD/quartile 1 are as follows: higher BMD/quartile 2 HR 1.8 (0.4–7.3); higher BMD/quartile 3 HR 5.0 (1.3–18.7); higher BMD/quartile 4 HR 7.3 (2.0–26.3); lower BMD/quartile 1 HR 8.7 (2.4–31.3); lower BMD/quartile 2 HR 11.2 (3.1–41.0); lower BMD/quartile 3 HR 12.7 (3.5–45.9); and lower BMD/quartile 4 HR 22.3 (5.8–86.3).

Discussion

We found women in the highest serum sclerostin quartile had a more than 3-fold increased risk for hip fracture compared with women in the lowest serum sclerostin quartile after adjustment for traditional risk factors including total hip BMD and prior fracture. Women with lower BMD in the highest sclerostin quartile had more than a 20-fold increased risk of hip fracture compared with women with higher BMD in the lowest sclerostin quartile. Ours is the first study to assess the association between circulating sclerostin concentrations and risk of incident hip fracture in older women.

Although there are few human sclerostin studies, there is strong biological plausibility that serum sclerostin is related to bone health and fracture risk. Individuals with null mutations in the *SOST* gene, resulting in undetectable sclerostin concentrations, have no reported fractures, even with severe trauma (12). Additionally, circulating levels of sclerostin have been found to be correlated with bone marrow plasma sclerostin levels and may therefore reflect sclerostin activity at the bone microenvironment level (13). In nonhuman studies, sclerostin has been shown to decrease osteoblast differentiation and mineralization and increase mature osteoblast apoptosis (4, 14, 15).

Contrary to our initial hypothesis, we found a positive correlation between serum sclerostin and BMD. Recently published studies have reported similar relationships although there is no clear explanation for these findings (16, 17). One possible explanation may be that increased bone mass may be associated with a greater number of osteocytes secreting sclerostin. Sclerostin levels may also be regulated as part of a feedback loop in response to BMD. As BMD decreases, there may be increased local mechanical strain leading sclerostin levels to be down regulated to increase bone formation and decrease bone resorption. However, pathological regulation of this feedback mechanism in some individuals may predispose to osteoporosis and fracture.

We found that women with lower BMD in the highest serum sclerostin quartile are at a greatly increased risk of fracture compared with women with higher BMD in the lowest serum sclerostin quartile. Although not statistically significant, our results suggest a possible interaction between BMD and serum sclerostin in the assessment of fracture risk, which would need to be further investigated in a larger study. Among women in the higher BMD group, those in the highest serum sclerostin quartile group have a 7-fold increased risk of fracture compared with those in the lowest serum sclerostin quartile. Among women in the lower BMD group, those in the highest serum sclerostin quartile group have a 2.5-fold increased risk of hip fracture compared with women in the lowest serum sclerostin quartile. The association of serum sclerostin levels with fracture risk increases after adjusting for BMD and is therefore independent of the effects of BMD on fracture. Serum sclerostin may be a potential marker for increased fracture risk in women, particularly if they do not meet criteria based on BMD.

Our study has a number of strengths. It is a prospective study of community-dwelling volunteers with high rates of follow-up and central x-ray validation of fractures. In addition, we measured serum sclerostin levels before the hip fracture. However, there are several limitations. First, the cohort included only Caucasian women 65 yr of age or older. Therefore, the results of the study may not apply to younger individuals, men, other ethnicities and other types of fractures. Second, we may have residual confounding related to unmeasured or poorly measured variables. A decrease in weight bearing leads to an increase in sclerostin levels (18), which may increase the risk for hip fracture. We did not have a marker for weight-bearing activity. Other known factors that regulate sclerostin levels include PTH levels (13) and estradiol levels (19, 20) for which we did not have measurements at the same time point. Third, it is possible that sclerostin levels may be associated with an increased fracture risk by affecting pa-

rameters of bone strength not assessed by DXA such as cortical porosity and cortical thickness. Future studies should investigate the association of sclerostin, other measures of bone, and fracture risk. Fourth, decay in sclerostin levels due to stability in the stored serum samples cannot be ruled out because some of our serum samples were collected more than 20 yr before the measurement of sclerostin levels. However, assuming the rate of decay is similar in both the hip fracture cases and random cohort, the observed relationship between hip fracture risk and sclerostin levels would have been biased toward the null. Furthermore, we confirmed our results by analysis of specimens closer to the time of fracture.

In conclusion, increased serum sclerostin levels are associated with greater risk of hip fracture within our study population. The reason for this association remains unclear. A combination of serum sclerostin levels and BMD may better identify women who are at higher risk of fracture and could therefore benefit from treatment.

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Address all correspondence and requests for reprints to: Aarthi Arasu, M.D., 185 Berry Street, Lobby 5, Suite 5700, San Francisco, California 94107. E-mail: aarthi.arasu@ucsf.edu.

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